ISSN 1948-5182 (online)

World Journal of *Hepatology*

World J Hepatol 2018 October 27; 10(10): 639-784

Published by Baishideng Publishing Group Inc

World Journal of Hepatology

Contents

Monthly Volume 10 Number 10 October 27, 2018

World Journal of Hepatology

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Monthly Volume 10 Number 10 October 27, 2018

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NAME OF JOURNAL
World Journal of Hepatology

ISSN
ISSN 1948-5182 (online)

LAUNCH DATE
October 31, 2009

FREQUENCY
Monthly

EDITORIAL BOARD MEMBERS
All editorial board members resources online at http://www.wjgnet.com/1948-5182/editorialboard.htm

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PUBLICATION DATE
October 27, 2018

INSTRUCTIONS TO AUTHORS
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World J Hepatol 2018 October 27; 10(10): 639-644

DOI: 10.4254/wjh.v10.i10.639

ISSN 1948-5182 (online)

EDITORIAL

European Association for the Study of the Liver and French hepatitis C recent guidelines: The paradigm shift

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Author contributions: Loustaud-Ratti V and Carrier P wrote the manuscript; Debette-Gratien M reread the manuscript and brought her expertise.

Conflict-of-interest statement: Dr. Loustaud-Ratti reports personal fees from GILEAD, personal fees from MSD, personal fees from ABBVIE, grants and personal fees from BMS, outside the submitted work.

Manuscript source: Invited manuscript

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Received: June 21, 2018
Peer-review started: June 21, 2018
First decision: July 9, 2018
Revised: July 17, 2018
Accepted: August 4, 2018
Article in press: August 4, 2018
Published online: October 27, 2018

Abstract

The latest Association Française pour l'Etude du Foie - French Association for Study of the Liver (AFEF) and European Association for the Study of the Liver (EASL) recommendations announce a change of paradigm, for the management of patients infected with hepatitis C virus (HCV). The AFEF recommendations focus on the elimination of HCV infection on a national level by preventing reinfection, in less than ten years. This goal involves the facilitation of patients' management in a simplified pathway by increasing screening procedures and access to pangenotypic treatments mainly in the "reservoir" population of people who inject drugs and migrants. Even in the complex pathway of patients with previous comorbidities, AFEF takes the option of a therapeutic simplification. The EASL guidelines position themselves on the state of the art with a precise description of all therapeutic options available, without separating simplified and complex pathways even if they take into account the epidemiological evolution of difficult-to-treat populations.

Key words: French; European; Hepatitis C; Guidelines; Pangenotypic; Direct acting antiviral drugs; Eradication; People who inject drugs; Migrants

Core tip: New French and European guidelines for the management of hepatitis C virus infection take into account the rapid change in the epidemiology of the infection and the arrival of short treatments, based on pangenotypic drugs with very few side effects. However, the French guidelines have a strong bias towards viral eradication with the elaboration of a simplified pathway for patients who are far from traditional healthcare structures.

Loustaud-Ratti V, Debette-Gratien M, Carrier P. European Association for the Study of the Liver and French hepatitis C recent guidelines: The paradigm shift. *World J Hepatol* 2018; 10(10): 639-644 Available from: URL: http://www.wjgnet.com/1948-5182/full/v10/i10/639.htm DOI: http://dx.doi.org/10.4254/wjh.v10.i10.639

Hepatitis C treatment history extends over approximately a quarter of a century from standard interferon for non-A non-B hepatitis, through combination with ribavirin at the end of the 1990s, to the availability of pegylated interferon in the early 2000s. It took 25 years to go from 5% to 55% of sustained virological response (SVR). The arrival of new direct-acting antiviral agents (DAAs) has revolutionized hepatitis C management in the last five years, even if the first protease inhibitors (PIs) initially associated with pegylated interferon and ribavirin greatly increased the global side effects. In fact, very quickly after just over a year, next generation DAAs in interferon-free regimen were available.

As a first step, the cost of drugs which were recommended for severe patients in most countries limited their use: In 2014-2015, sofosbuvir-based regimens combined with simeprevir, daclatasvir or ledipasvir were reimbursed by the French health insurance only for severe fibrosis, extra-hepatic manifestations, human immunodeficiency virus (HIV)-co-infected, transplanted and hemodialysis patients, and this, despite a tremendous decrease of side effects and a shortening of treatments. The extension of indications to F2 fibrosis according to the METAVIR classification, corresponded in 2016 with the marketing of ombitasvir paritaprevir/ritonavir and dasabuvir and finally, in 2017, universal treatment access in France was official. In 2018, thanks to pangenotypic associations' availability, a really ambitious median term goal of virus eradication becomes increasingly realistic.

During a 5-year period, multiple American, European and national guidelines were proposed trying to follow the tremendous therapeutic revolutions. The last 2018 recommendations that correspond to the marketing of pangenotypic associations are a real paradigm shift. We will focus on French (AFEF, Association Française pour l' Etude du Foie - French Association for Study of the Liver) and European Association for Study of the Liver (EASL) recent guidelines, highlighting the marked strategic differences.

The EASL recommendations are the state of art on hepatitis C in 2018[1]. They aim at "describing the optimal management of patients with chronic HCV infection in 2018". The French recommendations are brief, simplified, and avant-garde[2]. Their goal is "the elimination of hepatitis C virus (HCV) infection in France" before 2025 if possible (Table 1).

Of course, both guidelines highlight the epidemiological changes. In France, for example, it is estimated that the majority of HCV patients are represented by people who inject drugs (PWID, 95000 estimated patients), 46% of them being viremic and to be treated. The second most difficult population to assess is the migrant population (90035 estimated patients), with 57% of them estimated to be viremic. Today, 90% of patients transfused before 1992 are diagnosed and treated[3,4].

Among PWID, 30% attend addiction centers and the others, who are difficult to quantify, consult general practitioners who deliver opioid substitution treatments (OSTs): In the French survey HEPCORT 2011, the prevalence of HCV seropositivity was 26% in general practice on patients under OSTs with so-called "non-problematic" consumption[5]. Another important source of contamination is prison in which 70% of prisoners are PWID for whom prevalence of anti-HCV antibodies is 4.8%, 46.4% being HCV RNA (+): Thus, 2.5% of detainees are viremic for HCV[6].

According to the French recommendations, elimination of HCV infection could be possible by 2025, 2030 according to the United States Global Burden of Disease. The different guidelines advocate the eradication of the virus, made possible thanks to simple diagnostic methods and highly effective treatments, provided that screening policies are intensified and access to treatment promoted. The first proposal of AFEF recommendations is to screen every adult at least once in his life for combined HBV and HIV and HCV viruses, and a 100% reimbursement of the screening tests. Moreover, the principle of "all inclusive" in the management of particular target populations requires the use of new screening methods. In addition to the rapid diagnostic tests (RDTs) which were known to have excellent sensitivity and specificity (99%)[7,8], but only detect antibodies, EASL mentions the need for the development of Core Ag, dried blood spots, allowing HCV RNA rapid availability in patients who are difficult to collect. The principle of "reflex testing" is still in the experimental stage but is a way to obtain real time HCV RNA even if many problems remain to be solved including the cost.

The need for pre-therapeutic genotyping is addressed by AFEF and EASL. In the area of the availability of pangenotypic therapeutic associations, both guidelines consider that genotyping is not mandatory: In a "simplified pathway" for AFEF, or "in areas where genotyping is not available and/or not affordable, or simplify treatment access" for EASL. However, screening for initial fibrosis remains the key for both academic societies in simplified pathways for specific populations and in complex or specialized pathways. It determines the duration of the treatment and is essential for the follow-up especially the long-term detection of complications such as hepatocellular carcinoma or portal hypertension. FibroScan® (transient elastography) that measures liver stiffness in a non-invasive way is an educational and motivational tool for AFEF, qualities that were confirmed in several experiments available in addiction centers[9-11].

AFEF proposes FibroScan® or complex fibrosis biological tests thresholds, to rule out the diagnosis of severe fibrosis and therefore to identify patients who will not require prolonged follow-up after virological cure except for the presence of hepatic co-morbidities (Liver stiffness with FibroScan® < 10 kPa or FibroTest® ⩽ 0.58

Table 1 French and European Association for the Study of the Liver recommendations principal similitudes and differences

	French recommendations	EASL recommendations
Target audience	National	European, international
Philosophy	Goal of HCV eradication Maximum simplification of HCV management	State of art
Screening	Global "Test and treat"	Global "Test and treat"
Fibrosis	FibroScan®, FibroTest®, FibroMeter®	Enlarged to simple and accessible biological methods, APRI, Fib4
RAS screening	Only in case of previous failure to DAA treatment	May be used, in addition and if available, before treatment to optimize some non pangenotypic strategies
Prescribers	Hepatologists or general practitioners	Hepatologists
Regimens	Preferably pangenotypic associations sofosbuvir - velpatasvir 12 wk or glecaprevir - pibrentasvir 8 wk if no severe fibrosis	Pangenotypic and no pangenotypic associations according to genotype, viral load, degree of fibrosis, previous treatment, and eventual RAS No sofosbuvir - velpatasvir in case of G3 cirrhotic patients
In case of failure	RAS screening Only for first generation DAAs failures Sofosbuvir - velpatasvir - voxilaprevir 12 wk, sofosbuvir - velpatasvir - voxilaprevir with or without ribavirin 12-24 wk in G3 cirrhotic patients	RAS screening In addition, for patients with poorer prediction of response sofosbuvir - glecaprevir - pibrentasvir and sofosbuvir - velpatasvir - voxilaprevir 12-24 wk with or without ribavirin according to multidisciplinary decision
Decompensated cirrhosis	Regimen without protease inhibitors	Regimen without protease inhibitors
Renal insufficiency	Glecaprevir - pibrentasvir or, grazoprevir - elbasvir (G1) 12 wk	Glecaprevir - pibrentasvir or grazoprevir - elbasvir (G1), 8-12 wk

APRI: Aspartate aminotransférase to Platelet Ratio Index; DAA: Direct acting antiviral; EASL: European Association for the Study of the Liver; HCV: Hepatitis C virus; RAS: Resistance-associated substitutions.

or FibroMeter® $\leqslant$ 0.786). EASL retains APRI and FIB4 as an alternative in the absence of other local resources, even if the sensitivity and specificity are worse[12]. If FibroScan®, FibroMeter® and FibroTest® are easily available in France and many European countries, APRI and FIB4 can be instantly applied in all geographical area. For both academic societies, the screening strategy of particular populations in a "test and treat" goal, is therefore crucial and demonstrates an individual but also collective benefit.

The collective benefit, treating to prevent contamination in PWID has been demonstrated in various English, Australian and Icelandic experiments[13,14]. Interestingly, in several Eastern European countries, it has been shown that a global strategy - increasing screening, risk prevention with access to sterile syringes, in situ delivery of antiviral treatment associated with OSTs - reduced by almost 80% new HCV cases while the prescription of DAAs alone had an impact of only 10%[15]. Finally, one study unexpectedly suggested that accepting a diagnostic test for HCV in substitution centers, whether positive or negative, could have an impact on drug use[16].

Apart from these findings, the French recommendations commit themselves to a more proactive approach to facilitate diagnosis, treatment and eradication: "The treatment of hepatitis C must be prescribed by all doctors", "Treatment monitoring can be performed by non-medical caregivers", "Direct antiviral agents should be available in all pharmacies". Prescription by all doctors might be still a little premature and requires a culture change and systematic training. In a recent Australian experiment[17], dating from 2016, the opening of the prescription to general practitioners allowed access to treatment of rather disadvantaged populations, far from urban areas; however, much remains to be done as 58% of these prescriptions represented less than 12% of hepatitis C cases. Cost reduction and second-generation treatments generating fewer drug interactions, have allowed direct prescribing of DAAs without prior multidisciplinary consultation except in the following difficult cases: Prior DAA treatment failure, chronic renal disease, severe cirrhosis, liver cancer, co-infection with HBV or HIV, transplantation. Task delegation for therapeutic follow-up is possible as it was suggested that patients' attendance at consultations in addiction treatment centers was better with nurses than with general practitioners and specialists[18] and comparable results were experienced with the inmates[19].

Of course, according to AFEF recommendations, certain conditions are unavoidable for universal prescribing in a simplified pathway by non-specialists: Absence of HBV and/or HIV co-infection, severe renal insufficiency (eGFR < 30 mL/min per 1.73 m²), poorly controlled hepatic comorbidities (risky alcohol consumption, diabetes and obesity), severe hepatic disease, prior DAAs therapy. After ruling out the diagnosis of severe fibrosis by non-invasive methods, and in the absence of genotyping determination in the simplified pathway, the two pangenotypic therapeutic options recommended are: Sofosbuvir + velpatasvir for 12 wk and glecaprevir + pibrentasvir for 8 wk. A simple evaluation of the drug interactions is easy to do by consulting the website: https://www.hep-druginteractions.org/ or by using the smartphone app HEP iChart. Virological cure must be assessed by measuring viral load 12 wk after stopping treatment. All patients who do not meet these specifications are taken care of in a specialized pathway.

EASL guidelines do not distinguish between two types of patient pathways, even though specificities related to the management of PWID are clearly reported: Screening methods described above, *in situ* HCV RNA evaluation or easier, core antigen undetectability in serum or plasma 24 wk (SVR24) after the end of treatment, are an alternative endpoint of therapy in patients with detectable HCV core antigen prior to therapy.

In specialized patient pathways, AFEF recommendations also have a simplification bias focusing on the recommendations of pangenotypic associations. A minimal opening for non-pangenotypic options is left with the pre-requisite, of course, of systematic genotype knowledge: Sofosbuvir ledipasvir for G1 without severe fibrosis and grazoprevir elbasvir for genotype 1b, genotype 1a with an initial viral load ⩽ 800000 IU/mL and treatment naive genotype 4.

Some differences between both academic societies can be highlighted: EASL states the possibility of an 8-wk treatment with grazoprevir elbasvir for patients with genotype 1b[20] without severe fibrosis, and still finds relevant the ombitasvir paritaprevir dasabuvir combination for genotypes 1b or 4, during 8 or 12 wk whereas AFEF considers this combination obsolete. In many geographic areas however, this latter combination stays as a very good option, as studies from real life demonstrate its efficacy and safety in chronic hepatitis C, even in people with compensated liver cirrhosis[21].

A divergent point is also, according to EASL, the absence of recommendation of sofobusvir velpatasvir for G3 cirrhotic patients, the expected response being suboptimal (89% to 93% SVR)[22], while the AFEF maintains the indication of the association in this circumstance in a simplification goal.

The determination of pre-therapeutic resistance-associated substitutions (RAS) in situations that could have been demonstrated useful for some initial therapeutic options is no longer relevant for AFEF. On the other hand, EASL specifies that "in areas where only regimens that require optimization based on pre-treatment resistance testing are available, and physicians have easy access to a reliable test that evaluates HCV resistance to NS5A inhibitors", these analyses can guide decisions, as specified in the EASL recommendations for treatment of hepatitis C 2016[23].

In decompensated cirrhosis, conventionally managed by pangenotypic combinations without PIs and with ribavirin at standard doses, EASL states that increasing doses of ribavirin may be tested in terms of tolerance, and that a 24-wk regimen without ribavirin is possible in patients who have a contraindication or are intolerant to ribavirin.

AFEF only gives recommendations for patients who failed first-generation DAAs: Sofosbuvir + velpatasvir + voxilaprevir for 12 wk[24]. In genotype 3 patients without cirrhosis, the SVR was higher than in patients with cirrhosis, respectively 99% *vs* 93% and, sofosbuvir + velpatasvir + voxilaprevir with or without ribavirin for 12 to 24 wk was recommended for genotype 3 patients with cirrhosis (expert opinion). However, EASL offers solutions for patients with poorer prediction of response (several lines of treatment, advanced disease, complex RAS anti NS5a) in a multi-disciplinary decision: Sofosbuvir + glecaprevir + pibrentasvir for 12 wk[25,26] and for very difficult to retreat DAAs failures (NS5A RASs after at least two failures of a PI and an anti-NS5a): Sofosbuvir, velpatasvir, voxilaprevir + ribavirin or sofosbuvir + glecaprevir + pibrentasvir + ribavirin, and in case of intolerance to ribavirin an extension of treatment, from 16 to 24 wk (expert opinion).

For both academic societies, the post-transplantation treatment must be initiated early on stabilization (3 mo) of the patient and must include immunosuppression with therapeutic drug monitoring (TDM) of immunosuppressive (IS) treatments during DAAs treatment and after cessation. AFEF proposed sofosbuvir + velpatasvir for 12 wk or glecaprevir + pibrentasvir for 12 wk. EASL recommends mainly sofosbuvir + velpatasvir or sofosbuvir + ledipasvir without IS dose adjustments and glecaprevir + pibrentasvir only if eGFR < 30 mL/min per 1.73 m² and with IS dose adjustments. In fact, IS dose adjustment is essential regardless of the therapeutic associations used, even if the risk of imbalance of immunosuppression is greater with glecaprevir and pibrentasvir.

In case of renal insufficiency, for AFEF and EASL, if the eGFR is < 30 mL/min per 1.73 m^2, the available treatments are glecaprevir + pibrentasvir or, for genotype 1 infections grazoprevir + elbasvir. The AFEF advocates uniform treatment duration of 12 wk and EASL applies the classic rules of 8 to 12 wk even if the available clinical trials were carried out over 12 wk.

Finally, in this time of scarcity of grafts, organ transplantation from a HCV + RNA + patient to another HCV + RNA + patient is allowed. EASL offers the same option for HCV-RNA-patients provided that the patient's informed consent is obtained and that post-transplant antiviral therapy is available and very quickly proposed. In France, this possibility is not yet recognized by the official agencies but this should be the case in the near future.

In conclusion, the latest AFEF and EASL recommendations announce a change of paradigm, for the management of hepatitis C. The EASL recommendations are very detailed and describe almost all the therapeutic options. The AFEF recommendations focus on the simplification of HCV management with an eradication objective to prevent reinfection thus better taking into account the epidemiological evolution and the change of culture with respect to the disease, according to us. Patients including mainly PWID and migrants should be treated massively in a simplified way facilitated by the availability of very effective and devoid of side effects pangenotypic drugs. The philosophy of the "all inclusive" or the "talk, test and treat" will involve other actors than hepatologists in a global vision of health care of these

particular populations with a culture of task delegation.

REFERENCES

1 **European Association for the Study of the Liver**. EASL Recommendations on Treatment of Hepatitis C 2018. [cited July 11 2018]. Available from: URL: http://www.easl.eu/research/our-contributions/clinical-practice-guidelines/detail/easl-recommendations-on-treatment-of-hepatitis-c-2018

2 **AFEF**. Recommandations Textes Officiels: Recommandations. [cited July 11 2018]. Available from: URL: http://www.afef.asso.fr/RECOMMANDATIONS/recommandations_1

3 **Pioche C**, Pelat C, Larsen C, Desenclos JC, Jauffret-Roustide M, Lot F, Pillonel J, Brouardet C. Estimation de la prévalence de l' hépatite C en population générale, France métropolitaine, 2011. *Bull Epidémiol Hebd* 2016; **(13-14)**: 224-229

4 **Brutel C**. Les immigrés récemment arrivés en France. INSEE Première. 2014. Available from: URL: https://www.insee.fr/fr/statistiques/1281393

5 **Comité Scientifique de l'étude HEPCORT**. Premiers résultats: Prévalence de l'hépatite C en médecine de ville pour les patients sous TSO. Available from: URL: https://www.rvh-synergie.org/prises-en-charge-des-addictions/penser-ensemble-les-prises-en-charge/comorbidites/infections-virales-vih-vhc-vhb/650-premiers-resultats-de-letude-hepcort-prevalence-du-vhc-en-medecine-de-ville-pour-les-patients-sous-tso.html

6 **Semaille C**, Le Strat Y, Chiron E, Chemlal K, Valantin MA, Serre P, Caté L, Barbier C, Jauffret-Roustide M; Prevacar Group. Prevalence of human immunodeficiency virus and hepatitis C virus among French prison inmates in 2010: a challenge for public health policy. *Euro Surveill* 2013; **18**: pii: 20524 [PMID: 23870097 DOI: 10.2807/1560-7917.ES2013.18.28.20524]

7 **Shivkumar S**, Peeling R, Jafari Y, Joseph L, Pant Pai N. Accuracy of rapid and point-of-care screening tests for hepatitis C: a systematic review and meta-analysis. *Ann Intern Med* 2012; **157**: 558-566 [PMID: 23070489 DOI: 10.7326/0003-4819-157-8-201210160-00006]

8 **Hayes B**, Briceno A, Asher A, Yu M, Evans JL, Hahn JA, Page K. Preference, acceptability and implications of the rapid hepatitis C screening test among high-risk young people who inject drugs. *BMC Public Health* 2014; **14**: 645 [PMID: 24965699 DOI: 10.1186/1471-2458-14-645]

9 **Marshall AD**, Micallef M, Erratt A, Telenta J, Treloar C, Everingham H, Jones SC, Bath N, How-Chow D, Byrne J, Harvey P, Dunlop A, Jauncey M, Read P, Collie T, Dore GJ, Grebely J. Liver disease knowledge and acceptability of non-invasive liver fibrosis assessment among people who inject drugs in the drug and alcohol setting: The LiveRLife Study. *Int J Drug Policy* 2015; **26**: 984-991 [PMID: 26256938 DOI: 10.1016/j.drugpo.2015.07.002]

10 **Arain A**, De Sousa J, Corten K, Verrando R, Thijs H, Mathei C, Buntinx F, Robaeys G. Pilot Study: Combining Formal and Peer Education with FibroScan to Increase HCV Screening and Treatment in Persons who use Drugs. *J Subst Abuse Treat* 2016; **67**: 44-49 [PMID: 27296661 DOI: 10.1016/j.jsat.2016.04.001]

11 **Foucher J**, Reiller B, Jullien V, Léal F, di Cesare ES, Merrouche W, Delile JM, de Lédinghen V. FibroScan used in street-based outreach for drug users is useful for hepatitis C virus screening and management: a prospective study. *J Viral Hepat* 2009; **16**: 121-131 [PMID: 19175876 DOI: 10.1111/j.1365-2893.2008.01050.x]

12 **Leroy V**, Halfon P, Bacq Y, Boursier J, Rousselet MC, Bourlière M, de Muret A, Sturm N, Hunault G, Penaranda G, Bréchot MC, Trocme C, Calès P. Diagnostic accuracy, reproducibility and robustness of fibrosis blood tests in chronic hepatitis C: a meta-analysis with individual data. *Clin Biochem* 2008; **41**: 1368-1376 [PMID: 18655779 DOI: 10.1016/j.clinbiochem.2008.06.020]

13 **Martin NK**, Vickerman P, Grebely J, Hellard M, Hutchinson SJ, Lima VD, Foster GR, Dillon JF, Goldberg DJ, Dore GJ, Hickman M. Hepatitis C virus treatment for prevention among people who inject drugs: Modeling treatment scale-up in the age of direct-acting antivirals. *Hepatology* 2013; **58**: 1598-1609 [PMID: 23553643 DOI: 10.1002/hep.26431]

14 **Tyrfingsson T**, Runarsdottir V, Hansdottir I, Bergmann OM, Björnsson ES, Johannsson B, Sigurdardottir B, Fridriksdottir RH, Löve A, Löve TJ, Sigmundsdottir G, Hernandez UB, Heimisdottir M, Gottfredsson M, Olafsson S. Marked reduction in the prevalence of hepatitis C viremia among people who inject drugs during 2nd year of the Treatment as Prevention (TraP HepC) program in Iceland. *J Hepatol* 2018; **68**: S52 [DOI: 10.1016/S0168-8278(18)30325-8]

15 **Mabileau G**, Scutelniciuc O, Tsereteli M, Konorazov I, Yelizaryeva A, Popovici S, Saifuddin K, Losina E, Manova M, Saldanha V, Malkin JE, Yazdanpanah Y. Intervention Packages to Reduce the Impact of HIV and HCV Infections Among People Who Inject Drugs in Eastern Europe and Central Asia: A Modeling and Cost-effectiveness Study. *Open Forum Infect Dis* 2018; **5**: ofy040 [PMID: 29594179 DOI: 10.1093/ofid/ofy040]

16 **Farhang Zangneh H**, Eibl JK, Graham G, Pellegrini D, Feld JJ, Marsh DC, Shah HA. The impact of hepatitis C diagnosis on substance-use behaviors in patients engaged in opioid substitution therapy. AASLD Annual Meeting, 2017 Oct-22; Washington DC, US, 2017: Abstract ID: 125

17 **Scott N**, Hainsworth SW, Sacks-Davis R, Pedrana A, Doyle J, Wade A, Hellard M. Heterogeneity in hepatitis C treatment prescribing and uptake in Australia: a geospatial analysis of a year of unrestricted treatment access. *J Virus Erad* 2018; **4**: 108-114 [PMID: 29682303 DOI: 10.1016/S0168-8278(18)30505-1]

18 **Kattakuzhy S**, Gross C, Teferi G, Jenkins V, Silk R, Akoth E, Thomas A, Ahmed C, Espinosa M, Price A, Emmanuel B, Rosenthal E, Wilson E, Tang L, Masur H, Kottilil S. A Novel Task Shifting Model to Expand the HCV Care Continuum: The Ascend Investigation. *J Hepatol* 2016; **64**: S224-S225 [DOI: 10.1016/S0168-8278(16)00201-4]

19 **Lloyd AR**, Clegg J, Lange J, Stevenson A, Post JJ, Lloyd D, Rudge G, Boonwaat L, Forrest G, Douglas J, Monkley D. Safety and effectiveness of a nurse-led outreach program for assessment and treatment of chronic hepatitis C in the custodial setting. *Clin Infect Dis* 2013; **56**: 1078-1084 [PMID: 23362288 DOI: 10.1093/cid/cis1202]

20 **Abergel A**. High Efficacy and Safety of the combination HCV Regimen Grazoprevir and Elbasvir for 8 Weeks in Treatment-Naive, non-severe fibrosis HCV GT1b-Infected Patients: Interim Results of the STREAGER study. AASLD Annual Meeting, 2017 Oct-22; Washington DC, US, 2017: Abstract ID: LB-5

21 **Preda CM**, Popescu CP, Baicus C, Voiosu TA, Manuc M, Pop CS, Gheorghe L, Sporea I, Trifan A, Tantau M, Tantau A, Ceausu E, Proca D, Constantinescu I, Ruta SM, Diculescu MM, Oproiu A. Real-world efficacy and safety of ombitasvir, paritaprevir/r+dasabuvir+ribavirin in genotype 1b patients with hepatitis C virus cirrhosis. *Liver Int* 2018; **38**: 602-610 [PMID: 28816020 DOI: 10.1111/liv.13550]

22 **Foster GR**, Afdhal N, Roberts SK, Bräu N, Gane EJ, Pianko S, Lawitz E, Thompson A, Shiffman ML, Cooper C, Towner WJ, Conway B, Ruane P, Bourlière M, Asselah T, Berg T, Zeuzem S, Rosenberg W, Agarwal K, Stedman CA, Mo H, Dvory-Sobol H, Han L, Wang J, McNally J, Osinusi A, Brainard DM, McHutchison JG, Mazzotta F, Tran TT, Gordon SC, Patel K, Reau N, Mangia A, Sulkowski M; ASTRAL-2 Investigators; ASTRAL-3 Investigators. Sofosbuvir and Velpatasvir for HCV Genotype 2 and 3 Infection. *N Engl J Med* 2015; **373**: 2608-2617 [PMID: 26575258 DOI: 10.1056/NEJMoa1512612]

23 **European Association for the Study of the Liver**. EASL Recommendations on Treatment of Hepatitis C 2016. *J Hepatol* 2017; **66**: 153-194 [PMID: 27667367 DOI: 10.1016/j.jhep.2016.09.001]

24 **Bourlière M**, Gordon SC, Flamm SL, Cooper CL, Ramji A, Tong M, Ravendhran N, Vierling JM, Tran TT, Pianko S, Bansal MB, de Lédinghen V, Hyland RH, Stamm LM, Dvory-Sobol H, Svarovskaia E, Zhang J, Huang KC, Subramanian GM, Brainard DM, McHutchison JG, Verna EC, Buggisch P, Landis CS, Younes ZH, Curry MP, Strasser SI, Schiff ER, Reddy KR, Manns MP, Kowdley

KV, Zeuzem S; POLARIS-1 and POLARIS-4 Investigators. Sofosbuvir, Velpatasvir, and Voxilaprevir for Previously Treated HCV Infection. *N Engl J Med* 2017; **376**: 2134-2146 [PMID: 28564569 DOI: 10.1056/NEJMoa1613512]

25 **Wyles D**, Weiland O, Yao B, Reindollar R, Weilert F, Dufour J-F, Gordon SC, Poordad F, Stoehr A, Brown A, Mauss S, Samanta S, Pilot-Matias T, L.R, Trinh R. Retreatment of patients who failed glecaprevir/pibrentasvir treatment for hepatitis C virus infection. *J Hepatol* 2018; **68**: S23-S24 [DOI: 10.1016/S0168-8278(18)30265-4]

26 **Ledinghen VD**, Anne V, Jose U-B, Lucia P, Jean-Baptiste H, Giovanna S, Wassil M, Pageaux G-P, Helene F, Alric L, Hezode C. Sofosbuvir + Glecaprevir/Pibrentasvir in patients with difficult to treat HCV infection. Final results of the French compassionate use. *J Hepatol* 2018; **68**: S259 [DOI: 10.1016/S0168-8278(18)30732-3]

P- Reviewer: Ong J, Pallav K, Preda C **S- Editor**: Ji FF
L- Editor: A **E- Editor**: Yin SY

World Journal of Hepatology

Submit a Manuscript: http://www.f6publishing.com

World J Hepatol 2018 October 27; 10(10): 645-661

DOI: 10.4254/wjh.v10.i10.645

ISSN 1948-5182 (online)

REVIEW

Aberrant expression of alternative isoforms of transcription factors in hepatocellular carcinoma

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Author contributions: All authors equally contributed to this paper with conception and design of the study, literature review and analysis, drafting and critical revision and editing, and final approval of the final version.

Supported by Russian Foundation for Basic Research, contract No. 18-34-00816\18.

Conflict-of-interest statement: All authors declare no potential conflicts of interest related to this manuscript.

Manuscript source: Unsolicited manuscript

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Received: April 20, 2018
Peer-review started: April 21, 2018
First decision: May 8, 2018
Revised: June 8, 2018
Accepted: June 27, 2018
Article in press: June 28, 2018
Published online: October 27, 2018

Abstract

Hepatocellular carcinoma (HCC) is one of the most prevalent malignancies worldwide and the second leading cause of death among all cancer types. Deregulation of the networks of tissue-specific transcription factors (TFs) observed in HCC leads to profound changes in the hepatic transcriptional program that facilitates tumor progression. In addition, recent reports suggest that substantial aberrations in the production of TF isoforms occur in HCC. *In vitro* experiments have identified distinct isoform-specific regulatory functions and related biological effects of liver-specific TFs that are implicated in carcinogenesis, which may be relevant for tumor progression and clinical outcome. This study reviews available data on the expression of isoforms of liver-specific and ubiquitous TFs in the liver and HCC and their effects, including HNF4α, C/EBPs, p73 and TCF7L2, and indicates that assessment of the ratio of isoforms and targeting specific TF variants may be beneficial for the prognosis and treatment of HCC.

Key words: Alternative isoforms; Transcription factors; Hepatocellular carcinoma; Alternative splicing;Hepatic differentiation; Personalized treatment

Core tip: This paper aims to analyze existing data on the spectrum of isoforms of liver-specific transcription factors produced in the liver and hepatocellular carcinoma (HCC) and implicated in carcinogenesis, their distinct regulatory functions and subsequent isoform-

dependent biological effects which may be relevant for tumor progression and clinical outcomes in HCC patients.

Krivtsova O, Makarova A, Lazarevich N. Aberrant expression of alternative isoforms of transcription factors in hepatocellular carcinoma. *World J Hepatol* 2018; 10(10): 645-661 Available from: URL: http://www.wjgnet.com/1948-5182/full/v10/i10/645.htm DOI: http://dx.doi.org/10.4254/wjh.v10.i10.645

INTRODUCTION

According to the current estimations of The Human Genome Sequencing Consortium, the human genome is predicted to comprise less than 20000 protein-coding genes[1]. Due to concerted efforts of The Human Proteome Project, 85% of the predicted proteins have already been identified[2]. Multiple sources of evidence suggest that this amount may be underestimated due to the existence of protein isoforms arising from the usage of alternative promoters or translation start sites (TSSs), alternative splicing regulated by ubiquitous and tissue-specific splicing factors, which affects the transcripts of 92%-94% of genes, and alternative cleavage and polyadenylation[3-5]. Interestingly, the occurrence of both alternative promoters and multiple conserved TSSs positively correlates with alternative splicing events[4,6,7]. However, although most multi-exon genes produce several alternative isoforms, 85% of the transcriptome is generally represented by a single major gene transcript[8].

Deregulation of gene expression[9] and the generation of aberrant alternative isoforms are commonly observed in multiple cancer types[10,11]. Mechanisms underlying the misregulation of the production of alternative isoforms in cancer have been thoroughly described elsewhere[12-15] and are beyond the scope of this review.

Distinct transcriptional programs of particular cell types are principally controlled by tissue-specific transcription regulators[16]. Due to alternative promoter usage, transcription factors (TFs) produce a tissue-specific pattern of alternative isoform expression that may be altered in carcinogenesis[12], and the imbalance of isoform production in tumors contributes to a flexible context-dependent diversification of cell phenotypes[17,18]. Somatic mutations and abnormal activation of signaling pathways in cancer may cause a differential regulation of the abundance and activity of splicing factors and lead to an increase in the production of alternative transcripts, including those for TFs[14]. Additionally, the transcripts of genes encoding TFs tend to contain multiple conserved TSSs, which may contribute to the production of protein isoforms. In the case of TFs, these complex regulatory mechanisms specifying alternative isoform production frequently affect functional domains[19], and their disruption in cancer may result in the misregulation of networks of their target genes (Figure 1).

Hepatocellular carcinoma (HCC) is the most frequent type of liver cancer and the second leading cause of death among all cancer types. Although recurrent driver genes and somatic mutations have been identified in HCC, most of them cannot yet be considered as druggable targets for therapy[20]. Massive deregulation of the liver-specific TF network[16] and aberrant alternative splicing[14,21,22] have been reported in HCC. The latter arises from abnormal expression, altered transcript splicing and a high mutation rate of genes encoding splicing factors that exert an effect in hepatocarcinogenesis and result in profound changes in the isoform balance compared with the normal liver. Importantly, up to 9% of differentially spliced transcripts in HCC originate from TF-coding genes[23,24]. These profound changes in the ratios of TF isoforms should likely result in a substantial modification of gene expression programs and therefore produce tumor phenotype alterations with a distinct clinical impact. Thus, data on the induction of expression of tumor-specific TF variants may be useful for the prognosis and development of approaches to isoform-specific therapy for HCC.

Nevertheless, current data concerning the regulation of the TF network in hepatocarcinogenesis by alteration of their isoform balance are rather diverse. In this review, we consider structural properties, biological effects and the clinical impact of the most investigated TF isoforms specific to liver and HCC. Essential features of these TFs and their isoforms are summarized in Table 1.

HNF4α: DIFFERENTIATION IS THE KEY

Nuclear receptor HNF4α (NR2A1) is a key regulator of hepatocyte differentiation. Bound to DNA as a homodimer, it modulates the expression of nearly 42% of the genes expressed in hepatocytes, including a wide spectrum of hepato-specific genes, either directly or through activation of other liver-enriched TFs[25,26]. Apart from the regulation of differentiation and morphogenesis, HNF4α acts as a tumor suppressor in the liver. It has been shown to inhibit proliferation through the induction of *CDKN1A* and repression of BMP7 and MYC, to regulate the expression of the p53/p63-dependent apoptotic effector PERP and to interfere with epithelial-mesenchymal transition (EMT) *via* repression of SNAI1, SNAI2 and HMGA2 and the upregulation of CDH1[27,28].

HNF4A is transcribed from one of its alternative promoters, P1 or P2, which are regulated in a tissue- and developmental stage-specific manner. Additional exon inclusion and alternative splicing result in the generation of up to 12 HNF4α variants sharing common DNA-binding (DBD) and ligand-binding domains (LBD) and differing in the trans-activation domain (TAD) and repressor F domain[29]. The isoforms transcribed from P2 (HNF4α7-α12) are devoid of the TAD activation function (AF)-1 region involved in the interaction with co-activators, while demonstrating a significant decrease in trans-activation (TA) properties[30]. The full-length F domain of HNF4α1 and HNF4α7 variants impair their TA potential because it masks the AF-2-independent co-factor binding site. In

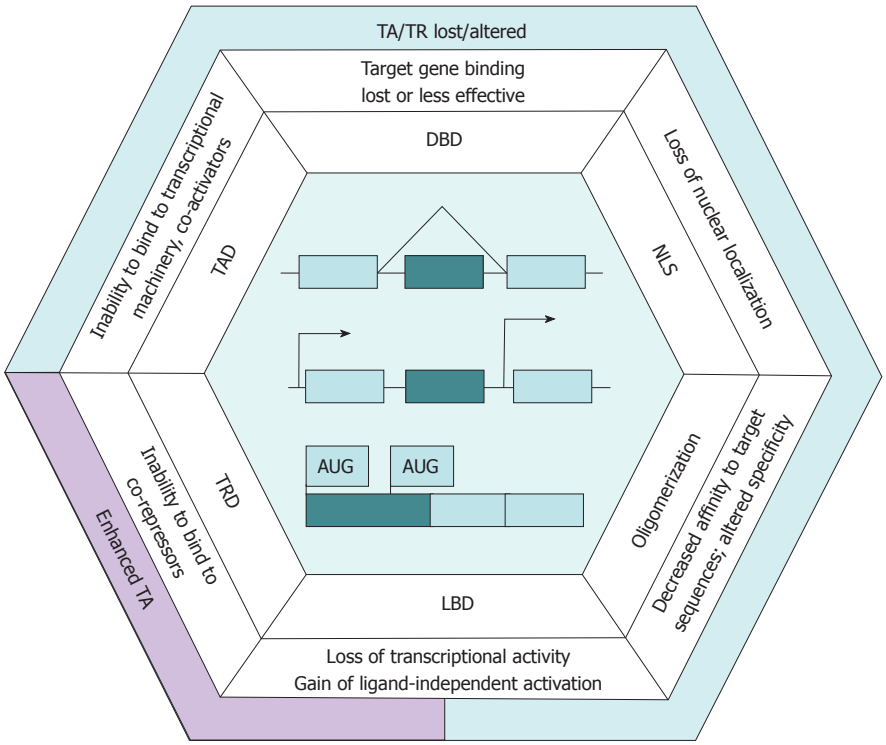

Figure 1 Alternative transcription factor isoforms possess regulatory properties distinct from those of canonical variants. Schematic representation of the common isoform formation mechanisms - alternative splicing, alternative promoter usage and alternative initiation of translation - is shown in the central part of the hexagon. Light boxes correspond to constitutive exons, and dark ones designate the exons harboring domains and regions indicated in the adjacent frame. TF isoform properties divergent from those of the canonical variants and resulting from functional domain loss are indicated at the corresponding hexagon edges on the outer side of the frame. The brace marks exon exclusion - a particular case of alternative splicing. The arrows represent transcription start sites. AUG: Start codon; TR: Transcription repression; TRD: Transcription repression domain; TF: Transcription factor; TAD: Trans-activation domain; LBD: Ligand-binding domains; DBD: DNA-binding; NLS: Nuclear localization signal.

contrast, the 10-amino-acid (AA) insert in the proline-rich F domain region of HNF4α2/α8 isoforms prevents masking, which increases the effectiveness of target gene activation[31]. Thus, the structural diversity of HNF4α variants determines a lower transcriptional activity of P2-derived isoforms (HNF4α7-α12) that lack AF-1, compared to P1-derived variants (HNF4α1-α6), and a higher transcriptional activity of isoforms that are devoid of the F domain, compared to HNF4α1/α7.

HNF4α3/α9 isoforms derive from skipping a splice site in exon 8 and contain an extended reading frame with an alternative termination site, thus encoding a protein with a completely different C-terminal F domain with undetermined function[32]. The existence of HNF4α4-α6 variants containing additional exons 1B and 1C has not been clearly proven[32]. HNF4α10-α12 isoforms bearing extended TAD due to exon 1E translation are expressed in various hepatoma cell lines, but their functions remain unclear[33].

P1 variants are predominant in the normal liver and regulate the expression of hepatocyte differentiation markers[32,34]. HNF4αP2 isoforms are prevalent at the early stages of embryogenesis; they preferentially activate promoters of early hepatic genes[35]. In the postnatal liver, the P2 promoter is repressed due to the binding of HNF4αP1 isoforms.

Deregulation of the expression of HNF4α isoforms is a frequent event in hepatocarcinogenesis. At the early stages of hepatocarcinogenesis, moderately differentiated HCC cells are characterized by activation of the expression of embryonic HNF4aP2 isoforms, which is associated with vascular invasion and poor overall survival in HCC patients[36]. In advanced dedifferentiated HCCs, which lose epithelial morphology, the expression of both P1 and P2 variants is repressed[34,37,38].

Exogenous expression of HNF4α1 in dedifferentiated HCC cells results in the restoration of epithelial morphology *via* the upregulation of epithelial markers (E-cadherin, connexin32, ZO-1), decrease in proliferation and reactivation of genes specific to differentiated hepatocytes, and decrease in *in vivo* tumor growth and metastatic potential[37,39,40]. Overall, these data indicate that HNF4αP1 reactivation might be beneficial as a therapeutic approach for HCC treatment. Further experiments are required to elucidate the role and clinical impact of particular HNF4α isoforms and to implement these findings into the development of therapeutic and prognostic approaches to HCC treatment.

ERα BALANCE IS SHIFTED TOWARDS DOMINANT-NEGATIVE VARIANTS DURING TUMOR PROGRESSION

Nuclear receptor ERα encoded by the ESR1 gene induces proliferation and exhibits anti-apoptotic and

Table 1 Transcription factors and their isoforms deregulated in hepatocarcinogenesis

TF	No. of isoforms expressed in the liver	Types of isoforms	Regulated processes (canonical isoform)	Upstream signaling pathways	Target genes expressed in the liver	Isoforms that presumably promote hepatocarcinogenesis	Isoforms that exhibit tumor-suppressive effects	Ref.
C/EBPβ	3	TA, DN	Metabolism POS: apoptosis, inflammatory response NEG: proliferation	TNF	UP(LAP1): cytochrome *p450* genes UP(LIP): GADD45B DOWN (LAP1): CCNA, CCNE, CDK2, PCNA DOWN (LIP): CLU, NUMB	C/EBPβ-LIP	C/EBPβ-LAP1, C/EBPβ-LAP2	[59,65,74,76,77, 78,139]
ERα	4	DN	POS: proliferation	Estrogen signaling	UP: CCND1, HNRNPH2, MYC, RET, WWC1	ERα-36, ERα-46, ER-αΔ5	ERα-66	[41,43,44,47,139,140]
HIF1α	3	DN	NEG: apoptosis, inflammatory response	PI3K/Akt, mTOR	UP: VEGFA	HIF1α1.1	HIF1α516, HIF1α736	[125,131,133,134,139]
HNF4α	9-12	TA	POS: angiogenesis	AMPK, Hippo, TGFβ	UP: CDH1, *CDKN1A*, HNF1a, PERP DOWN: BMP7, HMGA2, MYC, SNAI1, SNAI2	HNF4α7-α9	HNF4α1-α3	[28,29,37,139,141,142]
KLF6	3	TA, DN	POS: differentiation, morphogenesis, apoptosis	TGFβ	UP(wtKLF6): CCND1, CDH1, CDK4 UP(SV2): *CDKN1A* DOWN(wtKLF6): MDM2 DOWN(SV1): *CDKN1A*	SV1	wtKLF6, SV2	[105,108-114,143]
p73	3	TA, DN	NEG: proliferation, invasion, metastases	p53, Hippo, mTOR	UP: BCL2 family genes, caspases, CD95, TNF-R, TRAIL-R	ΔN-p73, ΔEx2p73, ΔEx2/3p73	TA-p73	[87-91,139,144]
PGC1α	3	TA, DN	POS: proliferation, differentiation, apoptosis	AMPK, Insulin signaling	UP: GLUT4, PDK4, PEPCK, PPARA, PPARG	PGC-1α1-a, L-PGC-1α, NT-Pgc 1α-a* (*promote replication and assembly of in HBV and HCV)		[49,52,54-56,139]
TCF4 (TCF7L2)	17	TA	POS: apoptosis	Wnt, Hippo	UP: AXIN2, CCND1, IRS1, JUN, MMP7, WISP1	TCF-4J, TCF-4C	TCF-4B, TCF-4K	[98-102,139,145]
WT1	4	DN	NEG: cell cycle progression		UP: BCL2 family genes, cFLIP DOWN: FADD, HNF4A, NF-κB	WT1(+/) 17AA(+)KTS	WT1(-)KTS	[119-122,146]
ZIP (ZGPAT)	2	DN	POS: gluconeogenesis		DOWN: CDC25A, EGFR, FGF5, FGF14, PDGFB, PTEN, RGS3, TBPL1, VCAM1	sZIP	ZIP(fl)	[135-137]

C/EBP: CCAAT enhancer binding protein; TF: Transcription factor; TA: Isoforms with differential transactivation properties; DN: Dominant-negative; POS: Positive regulation; NEG: Negative regulation; UP: Up regulation; DOWN: Down regulation.

anti-inflammatory activities[41] *via* estrogen-dependent binding to estrogen response elements of target genes. Non-canonical activation of ERα target genes *via* secondary messengers, activation of membrane-bound ERα isoforms or protein-protein interactions have also been described[42].

Canonical 66-kDa ERα (ERα-66) matches the conventional structure of nuclear receptors. Additionally, numerous ERα splice variants that differ in promoter usage and the presence of exons encoding TAD, DBD, LBD and the nuclear localization signal (NLS) region have been reported in normal and tumor tissues[42]. Apart from ERα-66, the expression of 3 dominant-negative (DN) isoforms has been reported in the liver, namely, ERα-46 lacking AF-1 TAD, ERαΔ5 harboring an incomplete LBD, and ERα-36, which is transcribed from an alternative promoter and is deficient in both AF-1 and LBD[41].

Investigation of the properties of truncated isoforms in various cell lines has demonstrated that the dimerization of a shorter isoform with the full-length one represses its transcriptional activity. Membrane-bound fractions of ERα-46 and ERα-36 are believed to interfere with the function of ERα-66. Moreover, ERα-36 lacks the NLS but is able to activate the MAPK/ERK cascade upon treatment

with estrogens or tamoxifen[41-45]. A constitutively active ER-αΔ5 variant exhibits approximately 10%-15% of the ligand-bound ERα-66 activity[46].

ERα-66 is highly expressed in the normal liver and, to a lesser extent, in cirrhotic tissue, while its transcription in HCC is substantially or completely suppressed. Higher ERα-66 expression is associated with a better overall and recurrence-free survival of HCC patients and is negatively correlated with the tumor size and extra-hepatic metastasis. The mRNA of DN ERα-46 is detected in non-tumor, cirrhotic and tumor liver tissues. Upregulation of ERαΔ5 in HCC causes repression of ERα-66 transcriptional activity through heterodimerization. An increase in ERα-36 isoform production has been reported in HCC and hepatoma cell lines; it correlates with the downregulation of the full-length isoform[43,44,47] and may arise from ESR1 promoter hypermethylation, which is frequently observed in HCC[48]. Thus, the shift in balance of ERα variants towards truncated isoforms with DN properties can essentially modulate the impact of the estrogen receptor signaling pathway in hepatocarcinogenesis and is likely associated with an unfavorable HCC prognosis.

PGC-1α, A CO-FACTOR OF NUCLEAR RECEPTORS THAT REGULATE METABOLISM AND INFLAMMATION

PGC-1α (PPARGC1A) is an inducible adaptor of the PPARγ co-activator 1 family. It has been shown to regulate adaptive reactions of energy metabolism in brain, cardiac and skeletal muscles, adipose tissue and liver[49]. PGC-1α is a key inducer of gluconeogenesis in the fasted liver[50]. A significant decrease in PGC-1α expression has been reported in primary human HCCs[51].

The N-terminus of PGC-1α protein contains a TAD with co-activator and nuclear receptor (HNF4α, PPARα, PPARγ, LXRα) binding sites[52] followed by phosphorylation and acetylation sites that modulate PGC-1α activity[53]. The NLS region, an arginine-serine-rich domain that enables binding to TFs and RNA-recognition motifs, is located in the C-terminal part of PGC-1α. C-terminal domains are involved in mRNA processing and splicing[53].

PGC-1α transcription is controlled by several tissue-specific promoters and is often coupled with alternative splicing. Processed alternative transcripts give rise to a set of TA-competent isoforms and DN variants lacking C-terminal domains or with truncations in their co-activator and co-repressor regions[53]. In human tissues with intense energy metabolism, the full-size PGC-1α1-a is a prevalent isoform[52]. In murine liver, mRNAs encoding *Pgc-1α1-a* and *NT-Pgc-1α-a* are transcribed from the proximal promoter. The *NT-Pgc-1α-a* variant preserves the co-activator binding domain and a portion of the co-repressor binding domain, but it lacks the C-terminal part[54]. Although less effective, *NT-Pgc-1α-a* is sufficient to stimulate gluconeogenesis in PGC-1α1-a$^{-/-}$ hepatocytes[55]. The hepato-specific promoter located downstream of the proximal one is active in human fasted liver[52] or in response to hepatitis C virus (HCV) induced endoplasmic reticulum (ER) stress. Its activation stimulates the expression of a liver-specific TA-isoform, L-PGC-1α, which is deficient in the first 127 aa but is able to co-activate most PGC-1α-interacting nuclear receptors except liver X receptor α (LXRα)[56]. LXRα has been shown to repress the FOXM1 transcriptional regulator and its target genes *CCNB1* and CCND1 in HCC cells[57]. Thus, the reduced LXRα activity may stimulate proliferation in hepatoma cell lines. The reduced LXRα activity also causes the deregulation of cholesterol metabolism, resulting in liver damage and inflammation, which facilitates HCC progression[58]. Hence, the elevated L-PGC-1α level is likely to be implicated in hepatocarcinogenesis. However, PGC-1α1-a induction occurs along with L-PGC-1α upregulation in the fasted liver and during HCV-induced ER stress. As the result, a higher PGC-1α1-a/L-PGC-1α ratio abolishes the effects of the alternative isoform. Currently, the expression of PGC-1α isoforms has been investigated in HCC cell lines but not in HCC clinical samples. Further research is required to determine a possible impact of the imbalance of PGC-1α isoforms in hepatocarcinogenesis.

INTERPLAY BETWEEN CCAAT ENHANCER BINDING PROTEINS AFFECTS PROLIFERATION AND HEPATIC DRUG METABOLISM

CCAAT enhancer binding proteins (C/EBPs) belong to a liver-enriched bZIP TF family; they regulate the expression of genes involved in proliferation, differentiation, inflammatory response and metabolism. C/EBPs bind to DNA as homo- and heterodimers, and the heterodimerization of C/EBPs can alter target site recognition[59].

Two C/EBP family members are predominant in normal liver. CEBPA is highly expressed in adult liver under normal conditions, whereas the expression of CEBPB is low in hepatocytes and can be induced by pro-inflammatory cytokines, hormones, cAMP and other agents[59,60]. The induction of hepatocyte proliferation is accompanied by C/EBPα downregulation and increases C/EBPβ levels[61]. Inactivation of the CEBPB gene significantly reduces the regenerative response in mice after a partial hepatectomy, whereas CEBPA-deficient murine hepatocytes demonstrate a high proliferative activity[59]. While CEBPA is mainly considered as a tumor suppressor that is downregulated in HCC[62], Lu *et al*[63,64] reported its upregulation in liver cancer, which was associated with escape from starvation-induced cell death and a poor prognosis. CEBPB expression is not altered in HCC, albeit the C/EBPβ protein level is decreased[65].

The N-termini of C/EBPα and C/EBPβ proteins contain a number of activation domains and negative regulatory regions that significantly differ between these TFs, while their C-termini contain a highly homologous basic

sequence-specific DNA recognition region and leucine zipper dimerization domain. Both CEBPA and CEBPB are intronless genes. Alternative protein isoforms arise due to the alternative usage of TSSs or regulated proteolysis[59].

The 42-kDa and 30-kDa C/EBPα isoforms differ in their amino termini with the p30 isoform possessing a lower activation potential than the predominant full-length variant due to the absence of two activation domains[59,62]. Apart from the DN activity, p30 has been proposed to bind and regulate a distinct set of target genes[66]. While both the p42 and p30 variants have been detected in liver, to date, the changes in the isoform ratio in HCC have not been quantitatively investigated, and reports of CEBPA upregulation leading to poor outcomes in HCC patients might arise from an isoform imbalance. Indeed, the induction of CEBPA expression in various models of liver disease leads to disease reversal and results in lower levels of liver damage markers and reduced tumor burden in rodents with cirrhotic HCC[67].

C/EBPβ isoforms are subdivided into 2 classes. Liver-enriched activating protein isoforms are able to induce the expression of target genes. C/EBPβ-LAP1 (LAP*) exhibits a stronger TA potential than C/EBPβ-LAP2 (LAP), which is deficient in the short N-terminal portion of the activation domain. C/EBPβ-LIP, a liver-enriched inhibitory protein, lacks the TAD but preserves a part of the negative regulation domain, and bZIP and acts as a DN regulator of transcription[59]. LAP1 is the major C/EBPβ variant expressed in hepatocytes[65]. According to Fang *et al*[65], LAP1 is significantly downregulated in HCC, whereas the LAP2 level is unaltered, and LIP is underrepresented. These observations are partly in line with reports on LAP1 isoform downregulation in squamous cell carcinoma[68] and breast cancer[69]. Albeit LAP1 variant expression is reduced in HCC, its expression is even weaker in liver cancer stem cells[70]. Conversely, LAP2 and LIP are induced in these tumor types, and a high LIP/LAP ratio is indicative of an advanced stage and poor prognosis in breast cancer[71].

In murine liver regeneration models, C/EBPβ regulates the proliferation of hepatocytes *via* activation of the transcription of E2F-regulated genes that are essential for DNA replication (*Mcm3, Cdc6*) and reparation (*Msh2, Msh5*) and *Cebpa* repression[72,73]. C/EBPβ-LAP expression leads to a delay of the G1/S transition, which results in the synchronization of hepatocytes after a partial hepatectomy due to the downregulation of CCNA, CCNE, PCNA and CDK2, whereas the expression of C/EBPβ-LIP induces proliferation. Intriguingly, overexpression of LAP leads to an increase in the C/EBPα p30/p42 ratio, while expression of the LIP variant upregulates both C/EBPα isoforms[74]. Exogenous expression of C/EBPβ-LAP (but not the –LIP variant) arrests cell cycle progression in HepG2 and Hep3B hepatoma cells[75,76]. C/EBPβ-LIP contributes to the survival of tumor cells. Drug-induced apoptosis is significantly reduced in Hep3B cells overexpressing the C/EBPβ-LIP but not the C/EBPβ-LAP variant[76].

Emerging data also link C/EBPβ expression to the metastatic potential of tumor cells. LAP1/2 overexpression represses Huh7 migration *in vitro* and metastasis *in vivo via* the induction of orsomucoid 2 (ORM2) gene expression, whereas LIP does not affect ORM2 levels[65]. *In vivo* experiments also demonstrate that LAP1-transfected hepatoma cells possess a reduced ability to form subcutaneous tumors in nude mice and that the expression of Ki-67 and cancer stem cell markers is reduced in tumors originating from these cells[70].

The complex impact of CEBP isoform variants on the fine regulation of liver-specific gene expression can be illustrated by the regulation of the *CYP3A4* gene encoding the most abundant type of cytochrome P450 in the liver. *CYP3A4* expression is modulated by liver-specific TFs, including C/EBPα and C/EBPβ, which have multiple binding sites in their regulatory region[77,78]. While TA-competent isoforms induce the expression of *CYP3A4*, the DN variant LIP inhibits it, and an increase in the LIP/LAP ratio results in the repression of *CYP3A4* in HepG2 cells[78]. As both TFs, including their DN variants, are able to heterodimerize, not only the levels of C/EBP proteins themselves but also the interactions between their isoforms, may affect the regulation of the metabolism of xenobiotics.

Taken together, the data on the abundance of C/EBPα and C/EBPβ variants in HCC remain limited and contradictory. CEBPA has previously been identified as a tumor suppressor that tends to be downregulated in HCC. Although there are limited data on the ratio of C/EBPα isoforms and their distinct effects in HCC, the capability of TA-competent and DN C/EBPα variants to form dimers with C/EBPβ isoforms increases the complexity of the network of target gene regulation and should be further investigated. In contrast, growing evidence of a variety of diverse effects arising from the regulation of expression by individual C/EBPβ isoforms indicates that evaluation of the ratio of LAP/LIP isoforms and their functions may be of value for HCC prognosis and treatment.

SEVERAL ISOFORMS OF p53 FAMILY PROTEINS ARE OVEREXPRESSED IN HCC AND FAVOR PROLIFERATION AND CELL DEATH EVASION

p53, p63 and p73 TFs of the p53 family regulate cell cycle progression and induce programmed cell death. Proteins of the p53 family have a similar domain structure, with TAD in their N-terminus followed by a proline-rich region, DBD and oligomerization domain. p63 and p73 possess a longer C-terminus encompassing the sterile α motif[79]. Since DBD shares substantial homology among the p53 family members, these TFs are able to regulate the expression of common target genes, including the regulation of each other's expression, although each transcription factor has specific target genes[80].

The p53 family proteins bind DNA as tetramers. The

wild-type p53 forms tetramers only with p53 variants, while mutant p53 variants can oligomerize with p63 and p73, thus reducing the activation of their target genes[81]. p63 and p73 can form mixed tetramers with varying transcriptional activity[82].

Alternative transcription and translation start sites and alternative splicing of the first exons encoding TAD result in the generation of a wide spectrum of N-terminally truncated (ΔN-) p53 family proteins that generally exert a DN effect over the full-length isoform activity. Additionally, the resultant proteins vary in their C-terminal domains due to alternative splicing[80]. p53 is frequently mutated or inactivated in cancer[80]. While p53 dysfunction is clearly associated with HCC progression, the only isoform proposed to play a significant role in hepatocarcinogenesis is the Δ40p53α variant translated from the second in-frame AUG of TP53 mRNA and lacking 39 AA of TAD. Δ40p53α has been demonstrated to be induced after doxorubicin treatment. Its exogenous expression suppresses proliferation, colony formation and induced senescence in hepatoma cells[83]. p63 is not expressed in the normal liver; however, the expression of ΔN isoforms is detected in p53-null hepatocytes. A trans-activating p63 variant (TAp63) is expressed in hepatoma cell lines irrespective of p53 status[84]. The TAp63 knockdown in HepG2 and Hep3B hepatoma cells leads to increased proliferation and colony formation. TAp63 expression negatively correlates with tumor size, intrahepatic metastasis, and distant metastasis and, according to the results of a retrospective analysis, a low TAp63 level is associated with poor survival in HCC patients[85,86].

The activation-competent TAp73 is absent in normal hepatocytes but is widely expressed in hepatoma cell cultures and human HCC samples[87-89]. Intriguingly, alternative splicing of full-length TAD-encoding transcripts originating from the first promoter (TA-promoter) is the main source of TAD-deficient pro-oncogenic p73 isoforms in HCC. The TA-promoter-driven and aberrantly spliced DN isoforms Δex2p73 and Δex2/3p73 that lack TAD, and ΔN'-p73 composed solely of TAD, are the most abundant p73 variants in HCC cells[88]. Both TAp73 and ΔTA variants including Δex2/3p73 are upregulated in hepatitis B virus-associated HCC[90]. ΔN-p73, an internal promoter-derived variant devoid of TAD, is detected both in hepatocytes and HCC and is overexpressed in 37% of tumors[87,89].

Normally, the induction of TAp73 expression inhibits cell proliferation; however, hepatoma cells tolerate its effects, presumably due to the co-expression of ΔTA variants[87]. The liver-specific expression of ΔNp73 in transgenic mice causes the formation of hepatic adenomas and subsequently to development of HCC in most animals[91]. Overexpression of the DN ΔN-p73 variant is associated with poor survival in HCC patients[89].

p63 and p73 initiate programmed cell death *via* a common mechanism. DNA damage induces TAp63 or TAp73-dependent TA of genes encoding the death receptors CD95, TNF-R and TRAIL-R, caspases and pro-apoptotic Bcl-2 proteins[91]. The expression of DN p63 and p73 variants abolishes the induction of pro-apoptotic genes and may cause resistance to chemotherapy[89,92]. Thus, since pro-oncogenic p53-family TF isoforms are generally expressed at low levels or are absent in non-transformed hepatocytes, targeting these variants may provide an option to counteract their DN effects on pro-apoptotic p53, p63 and p73 isoforms to improve the efficiency of drug therapy in HCC patients.

TCF-4 (TCF7L2) MODULATES A WIDE SPECTRUM OF TUMOR CELL PROPERTIES *VIA* INTERACTIONS WITH THE Wnt SIGNALING PATHWAY AND COMPETITION WITH HNF4α

TCF-4 (TCF7L2) is a basic helix-loop-helix (bHLH) TF of the LEF/TCF family that modulates the expression of Wnt signaling pathway target genes involved in proliferation, differentiation, apoptosis, cell polarity and motility upon β-catenin binding[93]. The conserved domain structure of TCF-4 is characteristic of the LEF/TCF family; it comprises the N-terminal β-catenin-binding domain (BCBD), followed by the context-dependent regulatory domain, high-mobility group DBD, and E-tail, which includes the second sequence-specific DBD, C-clamp and binding region for the transcriptional repressor CtBP[94].

The context-dependent regulatory domain harbors several regulatory motifs: LVPQ and SxxSS, implicated in the repression of β-catenin-mediated TA, and the Groucho/TLE co-repressor binding site. The C-clamp contains a 30 AA motif that is required for binding to weak Wnt response elements[93,95]. Approximately 20 TCF-4 isoforms generated by alternative splicing and alternative transcription start site usage have been reported[96]. Variants transcribed from the internal transcription start sites 2 and 3 lack BCBD and act as transcriptional repressors of β-catenin target genes[97]. Additionally, alternatively spliced isoforms differ by the presence of exon 4, which confers transcription repressor function, LPQV- and SxxSS motifs, and by the splicing pattern of exons 13-17 encoding a part of the C-clamp[98,99].

TCF-4 is highly expressed in hepatocytes. The pattern of expression of TCF-4 variants depends on the degree of cell differentiation[98]. The isoform TCF-4B that is predominant in normal liver lacks exon 4, the SxxSS, C-clamp and CtBP-binding region, which makes it a potent β-catenin-mediated trans-activator[98]. TCF-4B expression is maintained in HCCs and in differentiated hepatoma cell lines[98,100].

LPQV and SxxSS motif-containing variants expressed in normal liver are not substantially altered in liver tumors[100], except for a TCF-4K isoform. TCF-4K retains a portion of the C-clamp and the full CtBP-binding site[98]; it is significantly downregulated in HCC[100]. Conversely,

TCF-4C and -4J are upregulated in HCC, specifically in poorly differentiated tumors. Along with TCF-4B, TCF-4C demonstrates the highest transcriptional activity among the TCF-4 isoforms due to the lack of any repressor motifs and domains. TCF-4J, that is deficiency in most repressor units, preserves the LVPQ motif and CtBP-binding region, which results in a reduced TA ability[98,99,101].

The exogenous expression of TCF-4J in hepatoma cell cultures induces the expression of genes that are overexpressed in poorly differentiated HCCs and are distinct from SxxSS-containing TCF-4K activated targets. Additionally, TCF-4J has been demonstrated to interact with the liver-specific master regulator HNF4α, affecting its target gene expression[96]. Thus, hepatic differentiation may be affected by the balance of certain TCF-4 isoforms[102]. Alternatively, it was proposed that HNF4α competes with TCF-4 for its binding sites *in vitro* in human colorectal cancer cells in an isoform-dependent manner, where the HNF4α2, but not the HNF4α8, P2-driven variant, might displace TCF-4 in the AP-1 transcriptional complex[103]. Although there are no data on their interplay in the liver, this possible interaction may also be important for the indirect regulation of gene expression mediated by the Wnt signaling pathway and relevant for hepatocarcinogenesis.

The exogenous expression of SxxSS-deficient TCF-4B, -4C and -4J isoforms in hepatoma cell lines results in the upregulation of Wnt-responsive genes, thus promoting cell proliferation. TCF-4C and -4J overexpression also induces colony formation and promotes cell migration in hepatoma cell lines. In contrast, a SxxSS-containing TCF-4K variant reduces the proliferation rate and colony formation but stimulates cell migration[98,99,101,102].

TCF-4J-expressing hepatoma cells demonstrate higher tumorigenicity than TCF-4K-overexpressing ones. Tumors derived from TCF-4J-overexpressing cells also express high levels of HIF-2a and EGFR, which confer hypoxia resistance characteristic of an aggressive tumor phenotype[100]. Overall, these data indicate that the imbalance of TCF-4 isoforms observed in HCC contributes to multiple processes including dedifferentiation, increased proliferation, survival and metastatic potential of tumor cells and thus confers an aggressive tumor phenotype.

FULL-LENGTH KLF6 ACTS AS A TUMOR SUPPRESSOR IN LIVER AND IS DOWNREGULATED EARLY IN HEPATOCARCINOGENESIS

The tumor suppressor KLF6 belongs to the C2H2 zinc finger domain Sp1/KLF family of TFs, which are essential regulators of proliferation, differentiation and migration. Biological functions of KLFs are disrupted in a wide variety of tumors[104]. In HCC, KLF6 is frequently inactivated due to the loss of heterozygosity or point mutations[105].

The KLF6 molecule contains N-terminal TAD region, an NLS and three zinc fingers located in the C-terminus that allow DNA binding. Several KLF6 isoforms are produced *in vivo* due to the activation of cryptic splice sites[104]. In the liver, the full-length wtKLF6 isoform is prevalent, while truncated SV1 and SV2 variants are also expressed[106]. In contrast to wtKLF6, which is accumulated in the nucleus, SV1 and SV2 proteins lacking the NLS are mainly localized in the cytoplasm[107]. The SV1 variant is also defective in DNA-binding due to the absence of all zinc fingers[104]. The downregulation of wtKLF6 in dysplastic nodules is an early event in hepatocarcinogenesis, while its further decrease is observed in highly malignant HCCs and is associated with a poor prognosis[108,109]. The downregulation of wtKLF6 in HCC is often accompanied by a decrease in SV2 production and upregulation of the SV1 isoform, while higher SV1/wtKLF6 ratios correlate with an advanced tumor stage[106,108,110].

Several mechanisms regulating the balance of SV1 and wtKLF6 in HCC have been proposed. HGF- or Ras-dependent activation of the PI3K/AKT signaling pathway induces alterations of KLF6 splicing, which leads to enhanced generation of the SV1 variant[111,112]. In addition, miRNA-1301, which is abundant in HCC samples, and miRNA-210 have been found to specifically target wtKLF6 but not the SV1 variant[113].

The SV1 isoform counteracts wtKLF6 and SV2 and demonstrates obvious oncogenic properties. It has been proposed to carry out DN functions *via* the cytoplasmic sequestration of wtKLF6, which leads to its subsequent proteasomal degradation[110]. Both wtKLF6 depletion and SV1 overexpression significantly accelerate tumorigenesis in diethylnitrosamine-treated mice[110]. In human hepatoma cell lines, wtKLF6 depletion and SV1 exogenous expression enhance proliferation *via* the downregulation of *CDKN1A* and *CCNB1* target genes[110-112], whereas SV2 expression has an opposite effect[106].

KLF6 variants are implicated in the regulation of apoptosis. The exogenously expressed wtKLF6 induces transcriptional repression of the *MDM2* gene, thus securing the stabilization of the p53 level[109]. SV2 overexpression results in p53-dependent upregulation of the apoptosis inducers Bax and PUMA[106].

SV1 promotes the migration of hepatoma cell lines; it is proposed to favor metastasis *via* inhibition of the function of wtKLF6 implicated in the regulation of Rho family GTPase activity[114]. Overexpression of SV1 also upregulates the expression of mesenchymal marker genes, whereas wtKLF6 is essential for the maintenance of E-cadherin expression but does not affect other EMT-associated markers[113,114]. Thus, since the DN SV1 isoform of the KLF6 tumor suppressor performs obvious pro-oncogenic functions and is upregulated in HCC, it can be considered as a prognostic factor and candidate target for siRNA-mediated therapeutic inactivation in

liver tumors. Alternatively, targeting miRNAs implicated in wtKLF6 downregulation may also result in the restoration of the KLF6 isoform balance to reduce the pro-oncogenic effects of the SV1 TF variant.

ACTIVATION OF THE PRODUCTION OF MAJOR WT1 ISOFORMS IN HCC

WT1, a TF of the C2H2-type zinc-finger protein family, is involved in the regulation of cell differentiation, proliferation and apoptosis. Depending on the tissue-specific context, WT1 exhibits either tumor-suppressive or oncogenic properties[115], but in most solid tumors, including HCC, the upregulation of WT1 is associated with a poor prognosis[116,117]. The N-terminal proline-rich region of WT1 contains a homodimerization domain and trans-repression and trans-activation domains that are responsible for co-factor binding. The C-terminus comprises DBD with 4 C2H2-type Zn-fingers[115].

Although up to 36 alternative isoforms of WT1 produced by a combination of alternative start codon usage, alternative splicing and RNA editing have been predicted, 4 major isoforms that vary in exon 5 (17-AA insertion - "17AA") and/or exon 9 (3 AA insertion - "KTS") splicing have mostly been investigated[115,118]. The 17AA insertion located between the proline-rich region and the first zinc finger and the KTS triplet between the third and fourth Zn-fingers have been demonstrated to change the DBD conformation and diminish WT1 DNA-binding[115]. Therefore, the WT1 isoforms harboring these insertions have been suggested to be less transcriptionally active[119]. While WT1 expression in the normal liver is very low, in the cirrhotic liver and HCC tissue, all 4 major WT1 variants are upregulated[116,120,121].

The functional differences in the KTS-variable WT1 isoforms have been investigated in HCC model systems. In HepG2 and Hep3B hepatomas, exogenously expressed WT1 KTS(-) isoforms, but not KTS(+), act as tumor suppressors, triggering a p53-independent apoptotic program[119]. Conversely, in Huh7 and HLE cells, the major 17AA(+) KTS(+) isoform inhibits apoptosis *via* transcriptional activation of the cFLIP apoptotic inhibitor and repression of FADD, a caspase cascade activator[122]. These data imply that cell fate decisions may be dependent on the ratio of KTS(+)/KTS(-) isoforms in HCC cells.

The WT1 knockdown in PLC/PRF/5 HCC cells stimulates differentiation through upregulation of the key hepato-specific regulator HNF4α and leads to the loss of resistance to apoptosis[121]. In contrast, ectopic equimolar expression of 4 major WT1 variants in a primary rat hepatocytes culture induces a decrease in HNF4α expression and dedifferentiation[120]. Overall, the existing ambiguity regarding the impact of WT1 on different tumors could be explained not only by the tissue-specific context but also by a different spectrum of expressed WT1 variants. Importantly, the expression of minor WT1 isoforms, in addition to the major ones, and their impact on HCC development, progression and prognosis has not been thoroughly investigated. Thus, further investigation of WT1 isoform properties and the development of approaches for WT1 silencing or shifting the balance towards DN variants that counteract its pro-oncogenic and apoptosis resistance functions may benefit HCC prognosis and treatment. Apart from gene silencing, enforced expression of DN WT1 variants may be considered a therapeutic option for HCC to oppose the dedifferentiation and acquisition of resistance to apoptosis conferred by extrinsically expressed WT1.

MINOR ISOFORMS OF HIF1α FOUND IN HEPATOMA CELLS ARE ABLE TO COUNTERACT THE ADAPTIVE RESPONSE TO HYPOXIA

bHLH-PAS protein HIF1α promotes tumor progression through the regulation of the adaptive response to hypoxia[123]. HIF1α has been proven to be the most potent inducer of the expression of vascular endothelial growth factors and other hypoxia-responsible element-containing genes in various cell type-dependent contexts[124-126]. HIF1α is not detected in hepatocytes under normoxia; its expression is triggered by low oxygen levels in the murine liver[127]. HIF1α expression is induced in dysplastic liver nodules during malignization and is further increased in HCC[128], where its upregulation correlates with vascular invasion and a poor prognosis[129]. In Hep3B hepatoma cells, HIF1α modulates the expression and alternative splicing of its target genes to facilitate tumor cell metabolism adaptation to hypoxia[130].

The amino-terminal region of HIF1α harbors a bHLH DBD followed by the PAS domain, which enables its heterodimerization with HIF1β, a component of the TA-competent HIF1 complex, and by the oxygen-dependent degradation domain (ODDD). ODDD contains proline residues that are modified under normoxia and potentiate binding to VHL ubiquitin ligase to provide ubiquitin-dependent degradation of the TF. The C-terminal part of HIF1α contains 2 TADs and the NLS region[125,131].

HIF1α transcription is regulated by the universal I.1 promoter that drives expression of the major HIF1α1.1 isoform or by two tissue-specific promoters, I.2 and I.3, which give rise to minor HIF1α1.2 and HIF1α1.3 variants[132]. Alternative splicing of the HIF1α1.1 transcript leads to frameshifts and generates isoforms with impaired activity. DN cytoplasm-localized HIF1α516 and HIF1α557 variants are devoid of the NLS and both TADs as a result of the exclusion of exons 11/12; thus, they escape hypoxia-induced nuclear translocation and lack TA properties. The HIF1α736 variant lacks the C-terminal TAD due to the exclusion of exon 14 and demonstrates weaker TA properties compared with HIF1α1.1[123,133,134].

HIF1α1.1 is a predominant isoform generated under hypoxic conditions in hepatoma cell lines. The expression of minor fractions of HIF1α516 and HIF1α736 that inhibit the activity of HIF1α1.1 through competitive binding to HIF1β and thereby reduce the expression of HIF1 target genes has also been identified in hepatoma cells[123,134]. Although the biological role of these variants in hepatocarcinogenesis is underexplored and no survival analysis addressing their impact has been carried out to date, further investigation of DN HIF1α variants is required to define the possible utility of distinct isoforms in prognosis and therapy.

TRANSCRIPTIONAL REPRESSOR ZIP (ZGPAT)

ZIP (ZGPAT) is a DNA-binding transcriptional repressor that contains a C3H1-type zinc finger, TUDOR, G-patch, coiled-coil domains. ZIP-mediated transcriptional repression of target genes is achieved through the recruitment of the nucleosome remodeling and deacetylase (NuRD) complex[135]. Dimerization of ZIP is essential for its DNA binding ability[136]. ZIP target genes encode growth factors and their receptors, cell cycle regulators, components of the MAPK signaling pathway, actin cytoskeleton, and tight and gap junctions, particularly EGFR, PTEN, CDC25A, FGF5, FGF14, PDGFB, RGS3, and VCAM1[135].

Two ZIP isoforms have been identified. The full-length one acts as a transcriptional repressor, whereas a truncated sZIP variant deficient in DBD but capable of competitive binding with NuRD and, presumably, of dimerizing with ZIP decreases the inhibitory effect of ZIP[136,137]. Unlike most cell types, hepatocytes express both truncated and full-length ZIP variants[135]. Induced ZIP overexpression in HepG2 cells result in EGFR transcriptional downregulation, growth inhibition and a reduced clonogenic potential. In contrast, sZIP overexpression or ZIP/sZIP co-expression induce clearly opposite effects, indicating that the shortened isoform can counteract the tumor-suppressing activity of ZIP[137].

CONCLUSION

Aberrant alternative splicing has recently been proposed to be an additional hallmark of cancer[138]. We believe it reasonable to consider this hallmark in a broad sense, *i.e.*, as the generation of transcripts and protein variants that are not characteristic of a particular cell type. Such an interpretation stems from the observation that the mechanism of isoform generation is not limited to alternative splicing and can be based on additional mechanisms that are listed above or, frequently, on their combination. The production of aberrant isoforms is certainly not only a hallmark of cancer but also the cause of the development of other distinguishing characteristics of tumor cells. This statement is particularly relevant in regard to TF isoforms since TFs act as hubs that convert various incoming signals to a wide variety of target genes to modulate their expression, and the processes presumably regulated by these atypical isoforms might also be relevant for hepatocarcinogenesis (Figure 2).

The existence of TF isoform variants enables their functional diversification for the tight tissue- and condition-specific regulation of target genes. In contrast, aberrant production of TF variants with different TA properties can result in a significant alteration of the transcriptional program of the cell and, eventually, in its malignization. For instance, the overproduction of DN isoforms of TFs that are able to dimerize with functionally active variants of the corresponding TFs or compete for co-factors and/or binding to target gene promoters causes a reduction or inhibition of the functional activity of such TFs. The abnormal isoform production may result in mRNA degradation or structural changes that cause cytoplasmic localization, constitutive activation of TFs or enhanced TA ability and other alterations that eventually affect the expression of target genes.

The rapid development of transcriptome sequencing and proteomic technologies facilitates the detection, quantitative assessment and acquisition of statistically significant data on alterations of TF isoform ratios. Recently, global profiling of alternative splicing in hepatitis-associated and virus-free HCCs using a TCGA-LIHC dataset revealed deep perturbations in RNA splicing concomitant with altered expression levels and isoform patterns of splicing factors. Among the spectrum of alternatively spliced transcripts, those that are common for HCC or those that change depending on hepatitis infection status have been identified[24]. Notably, 7.6% to 9.0% of differentially spliced transcripts in HCCs originate from TF-encoding genes, including those involved in the regulation of the specification, differentiation or malignant transformation of hepatocytes (HNF1B, FOXM1, PPARA, NR1I3/CAR, NR3C1/GR)[24]. However, very few of the identified TFs have been previously investigated with regard to the biological effects or clinical implications of their isoforms. The available data on TF isoforms that are produced in the liver and HCC are summarized in Table 1.

The application of methods that allow the differential detection of TF isoforms appears to be crucial for the identification of clinically relevant alterations. Data based on the evaluation of the total level of gene expression or protein synthesis may be ambiguous, as such approaches mask the contribution of individual TF isoforms possessing distinct transcriptional activities to the overall pool of the corresponding TF. For example, several oncogenic TCF4 isoforms implicated in hepatocarcinogenesis are substantially upregulated in HCC, whereas the expression of other variants is usually decreased or unaltered.

Additionally, the ratio and functional impact of TF isoforms may significantly depend on the tissue-specific context. In the liver, HNF4a isoforms that are translated from mRNA transcribed from the P1 promoter are

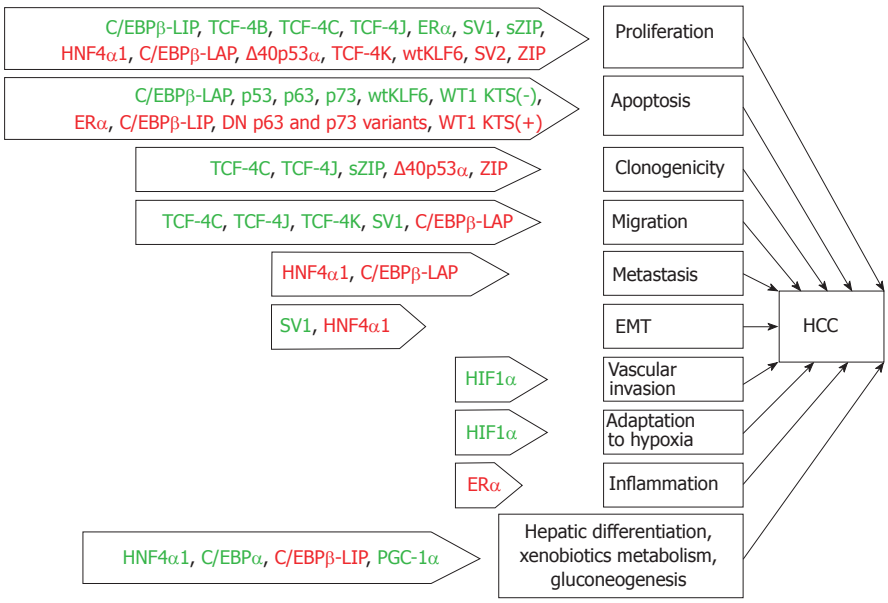

Figure 2 The key processes implicated in hepatocarcinogenesis that are regulated by transcription factors or their specific isoforms. TFs with stimulatory effects on these properties are depicted in green color, and TFs acting as repressors are colored in red. Sharp arrows indicate the stimulation of HCC development, and inhibition is indicated by blunt arrows. TF: Transcription factor; HCC: Hepatocellular carcinoma; EMT: Epithelial-mesenchymal transition.

predominant and clearly demonstrate tumor-suppressive properties[37]. In the colon epithelium, HNF4A isoforms transcribed from both promoters are normally expressed. Unlike HCC cells, non-isoform-specific HNF4A knockdown in colorectal carcinoma cell lines inhibits proliferation[147], while HNF4αP1 downregulation *in vivo* is associated with a higher metastatic potential in CRC, raising the possibility that this isoform group nevertheless implements a tumor suppressive function[148]. Thus, the isoform-discerning approach may resolve the existing contradictions in understanding the roles of certain TFs that are believed to exert opposing effects in different types of tumors.

Overall, knowledge of the effects of particular isoforms, their tissue-specific expression profiles and shifts in their ratios in disease may be of practical value for HCC prognosis and outlining potential therapeutic targets. Unfortunately, the available data on TF isoforms are mostly based on *in vitro* studies, the results of which are not to be directly extrapolated since gene expression, splicing and the effects of those variants may differ *in vivo*[149,150]. Few of the isoforms discussed above have been investigated in terms of their prognostic significance in HCC, although their aberrant production is associated with clinical features and outcomes in other types of cancer. To date, only the overproduction of HNF4αP2, ΔN-p73 and the downregulation of the TAp63 and ERα-66 isoforms have been reported to be significantly associated with the poor survival in HCC patients. A comprehensive understanding of the functions and regulation of particular isoforms may be further applied to the development of new therapeutic approaches. For instance, the upregulation of the anti-apoptotic Bcl-x(L) isoform of Bcl-2-like protein 1 is frequently observed in HCC. Its knockdown in hepatoma cell lines through RNA-interference has been demonstrated to induce apoptosis after staurosporine treatment in originally resistant cells[151]. A similar approach may be applied to inhibit isoforms that exert definite oncogenic effects, such as SxxSS-deficient TCF4 variants, which are overexpressed in HCC and possess high transcriptional activity due to the absence of co-repressor binding or posttranslational modifications that normally decrease TCF4 activity.

Identification and targeting of the regulators that control the production of particular TF variants would also be beneficial. For instance, tumor-suppressive HNF4α and KLF6 isoforms are frequently downregulated in HCC and can induce at least partial reversion of the malignant phenotype. In contrast, c-MET- and EGFR-mediated activation of signaling pathways that are essential for hepatocarcinogenesis has been demonstrated to drastically alter the expression of multiple splicing factors and induce the production of DN KLF6 and p73 isoforms[14]. Thus, targeted inhibition of the indicated signaling pathways may be considered as an additional strategy to prevent aberrant isoform synthesis. Since the expression of genes encoding splicing machinery components is altered in HCC, their inhibition may be considered as an additional way to reduce aberrant splicing. Several small molecule splicing modulators targeting basal splicing machinery or splicing factors and their regulators have been identified. Such modulators effectively inhibit tumor growth *in vivo,* but they lack specificity; in addition, they have been demonstrated to be toxic in xenograft experiments and clinical trials[152]. Alternatively, whereas some components of the splicing regulatory network are recurrently mutated in HCC[23], targeting

these mutant proteins with specific small molecules or antibodies may be relevant. Thus, further expansion of knowledge on the functions of TF variants, mechanisms of their generation and switching in HCC should shed light on additional mechanisms of hepatocarcinogenesis. Hopefully, it will also facilitate the discovery of new clinically relevant prognostic markers and therapeutic targets and contribute to the development of novel approaches to cancer treatment.

REFERENCES

1 **Ezkurdia I**, Juan D, Rodriguez JM, Frankish A, Diekhans M, Harrow J, Vazquez J, Valencia A, Tress ML. Multiple evidence strands suggest that there may be as few as 19,000 human protein-coding genes. *Hum Mol Genet* 2014; **23**: 5866-5878 [PMID: 24939910 DOI: 10.1093/hmg/ddu309]

2 **Paik YK**, Overall CM, Deutsch EW, Hancock WS, Omenn GS. Progress in the Chromosome-Centric Human Proteome Project as Highlighted in the Annual Special Issue Ⅳ. *J Proteome Res* 2016; **15**: 3945-3950 [PMID: 27809547 DOI: 10.1021/acs.jproteome.6b00803]

3 **Wang ET**, Sandberg R, Luo S, Khrebtukova I, Zhang L, Mayr C, Kingsmore SF, Schroth GP, Burge CB. Alternative isoform regulation in human tissue transcriptomes. *Nature* 2008; **456**: 470-476 [PMID: 18978772 DOI: 10.1038/nature07509]

4 **Bazykin GA**, Kochetov AV. Alternative translation start sites are conserved in eukaryotic genomes. *Nucleic Acids Res* 2011; **39**: 567-577 [PMID: 20864444 DOI: 10.1093/nar/gkq806]

5 **Jacox E**, Gotea V, Ovcharenko I, Elnitski L. Tissue-specific and ubiquitous expression patterns from alternative promoters of human genes. *PLoS One* 2010; **5**: e12274 [PMID: 20806066 DOI: 10.1371/journal.pone.0012274]

6 **Grosso AR**, Gomes AQ, Barbosa-Morais NL, Caldeira S, Thorne NP, Grech G, von Lindern M, Carmo-Fonseca M. Tissue-specific splicing factor gene expression signatures. *Nucleic Acids Res* 2008; **36**: 4823-4832 [PMID: 18653532 DOI: 10.1093/nar/gkn463]

7 **Xin D**, Hu L, Kong X. Alternative promoters influence alternative splicing at the genomic level. *PLoS One* 2008; **3**: e2377 [PMID: 18560582 DOI: 10.1371/journal.pone.0002377]

8 **Gonzàlez-Porta M**, Frankish A, Rung J, Harrow J, Brazma A. Transcriptome analysis of human tissues and cell lines reveals one dominant transcript per gene. *Genome Biol* 2013; **14**: R70 [PMID: 23815980 DOI: 10.1186/gb-2013-14-7-r70]

9 **Peng L**, Bian XW, Li DK, Xu C, Wang GM, Xia QY, Xiong Q. Large-scale RNA-Seq Transcriptome Analysis of 4043 Cancers and 548 Normal Tissue Controls across 12 TCGA Cancer Types. *Sci Rep* 2015; **5**: 13413 [PMID: 26292924 DOI: 10.1038/srep13413]

10 **Miura K**, Fujibuchi W, Sasaki I. Alternative pre-mRNA splicing in digestive tract malignancy. *Cancer Sci* 2011; **102**: 309-316 [PMID: 21134075 DOI: 10.1111/j.1349-7006.2010.01797.x]

11 **Shkreta L**, Bell B, Revil T, Venables JP, Prinos P, Elela SA, Chabot B. Cancer-Associated Perturbations in Alternative Pre-messenger RNA Splicing. *Cancer Treat Res* 2013; **158**: 41-94 [PMID: 24222354 DOI: 10.1007/978-3-642-31659-3_3]

12 **Davuluri RV**, Suzuki Y, Sugano S, Plass C, Huang TH. The functional consequences of alternative promoter use in mammalian genomes. *Trends Genet* 2008; **24**: 167-177 [PMID: 18329129 DOI: 10.1016/j.tig.2008.01.008]

13 **Chen M**, Manley JL. Mechanisms of alternative splicing regulation: insights from molecular and genomics approaches. *Nat Rev Mol Cell Biol* 2009; **10**: 741-754 [PMID: 19773805 DOI: 10.1038/nrm2777]

14 **Berasain C**, Elizalde M, Urtasun R, Castillo J, García-Irigoyen O, Uriarte I, Latasa MU, Prieto J, Avila MA. Alterations in the expression and activity of pre-mRNA splicing factors in hepatocarcinogenesis. *Hepat Oncol* 2014; **1**: 241-252 [PMID: 30190958 DOI: 10.2217/hep.13.17]

15 **Sendoel A**, Dunn JG, Rodriguez EH, Naik S, Gomez NC, Hurwitz B, Levorse J, Dill BD, Schramek D, Molina H, Weissman JS, Fuchs E. Translation from unconventional 5' start sites drives tumour initiation. *Nature* 2017; **541**: 494-499 [PMID: 28077873 DOI: 10.1038/nature21036]

16 **Lazarevich NL**, Fleishman DI. Tissue-specific transcription factors in progression of epithelial tumors. *Biochemistry* (Mosc) 2008; **73**: 573-591 [PMID: 18605982 DOI: 10.1134/S0006297908050106]

17 **Bonomi S**, Gallo S, Catillo M, Pignataro D, Biamonti G, Ghigna C. Oncogenic alternative splicing switches: role in cancer progression and prospects for therapy. *Int J Cell Biol* 2013; **2013**: 962038 [PMID: 24285959 DOI: 10.1155/2013/962038]

18 **Chen J**, Weiss WA. Alternative splicing in cancer: implications for biology and therapy. *Oncogene* 2015; **34**: 1-14 [PMID: 24441040 DOI: 10.1038/onc.2013.570]

19 **Talavera D**, Orozco M, de la Cruz X. Alternative splicing of transcription factors' genes: beyond the increase of proteome diversity. *Comp Funct Genomics* 2009; 905894 [PMID: 19609452 DOI: 10.1155/2009/905894]

20 **Llovet JM**, Zucman-Rossi J, Pikarsky E, Sangro B, Schwartz M, Sherman M, Gores G. Hepatocellular carcinoma. *Nat Rev Dis Primers* 2016; **2**: 16018 [PMID: 27158749 DOI: 10.1038/nrdp.2016.18]

21 **Berasain C**, Goñi S, Castillo J, Latasa MU, Prieto J, Avila MA. Impairment of pre-mRNA splicing in liver disease: mechanisms and consequences. *World J Gastroenterol* 2010; **16**: 3091-3102 [PMID: 20593494 DOI: 10.3748/wjg.v16.i25.3091]

22 **Liu L**, Xie S, Zhang C, Zhu F. Aberrant regulation of alternative pre-mRNA splicing in hepatocellular carcinoma. *Crit Rev Eukaryot Gene Expr* 2014; **24**: 133-149 [PMID: 24940767 DOI: 10.1615/CritRevEukaryotGeneExpr.2014007702]

23 **Cancer Genome Atlas Research Network**. Electronic address: wheeler@bcm.edu; Cancer Genome Atlas Research Network. Comprehensive and Integrative Genomic Characterization of Hepatocellular Carcinoma. *Cell* 2017; **169**: 1327-1341.e23 [PMID: 28622513 DOI: 10.1016/j.cell.2017.05.046]

24 **Tremblay MP**, Armero VE, Allaire A, Boudreault S, Martenon-Brodeur C, Durand M, Lapointe E, Thibault P, Tremblay-Létourneau M, Perreault JP, Scott MS, Bisaillon M. Global profiling of alternative RNA splicing events provides insights into molecular differences between various types of hepatocellular carcinoma. *BMC Genomics* 2016; **17**: 683 [PMID: 27565572 DOI: 10.1186/s12864-016-3029-z]

25 **Odom DT**, Zizlsperger N, Gordon DB, Bell GW, Rinaldi NJ, Murray HL, Volkert TL, Schreiber J, Rolfe PA, Gifford DK, Fraenkel E, Bell GI, Young RA. Control of pancreas and liver gene expression by HNF transcription factors. *Science* 2004; **303**: 1378-1381 [PMID: 14988562 DOI: 10.1126/science.1089769]

26 **Lazarevich NL**, Al'pern DV. Hepatocyte nuclear factor 4 (HNF4) in epithelial development and carcinogenesis. *Mol Biol* (Mosk) 2008; **42**: 786-797 [PMID: 18988528]

27 **Cicchini C**, Amicone L, Alonzi T, Marchetti A, Mancone C, Tripodi M. Molecular mechanisms controlling the phenotype and the EMT/MET dynamics of hepatocyte. *Liver Int* 2015; **35**: 302-310 [PMID: 24766136 DOI: 10.1111/liv.12577]

28 **Bonzo JA**, Ferry CH, Matsubara T, Kim JH, Gonzalez FJ. Suppression of hepatocyte proliferation by hepatocyte nuclear factor 4α in adult mice. *J Biol Chem* 2012; **287**: 7345-7356 [PMID: 22241473 DOI: 10.1074/jbc.M111.334599]

29 **Yusuf D**, Butland SL, Swanson MI, Bolotin E, Ticoll A, Cheung WA, Zhang XY, Dickman CT, Fulton DL, Lim JS, Schnabl JM, Ramos OH, Vasseur-Cognet M, de Leeuw CN, Simpson EM, Ryffel GU, Lam EW, Kist R, Wilson MS, Marco-Ferreres R, Brosens JJ, Beccari LL, Bovolenta P, Benayoun BA, Monteiro LJ, Schwenen HD, Grontved L, Wederell E, Mandrup S, Veitia RA, Chakravarthy H, Hoodless PA, Mancarelli MM, Torbett BE, Banham AH, Reddy SP, Cullum RL, Liedtke M, Tschan MP, Vaz M, Rizzino A, Zannini M, Frietze S, Farnham PJ, Eijkelenboom A, Brown PJ, Laperrière D, Leprince D, de Cristofaro T, Prince KL, Putker M, del Peso L, Camenisch G, Wenger RH, Mikula M, Rozendaal M, Mader S, Ostrowski J, Rhodes SJ, Van Rechem C, Boulay G, Olechnowicz

SW, Breslin MB, Lan MS, Nanan KK, Wegner M, Hou J, Mullen RD, Colvin SC, Noy PJ, Webb CF, Witek ME, Ferrell S, Daniel JM, Park J, Waldman SA, Peet DJ, Taggart M, Jayaraman PS, Karrich JJ, Blom B, Vesuna F, O'Geen H, Sun Y, Gronostajski RM, Woodcroft MW, Hough MR, Chen E, Europe-Finner GN, Karolczak-Bayatti M, Bailey J, Hankinson O, Raman V, LeBrun DP, Biswal S, Harvey CJ, DeBruyne JP, Hogenesch JB, Hevner RF, Héligon C, Luo XM, Blank MC, Millen KJ, Sharlin DS, Forrest D, Dahlman-Wright K, Zhao C, Mishima Y, Sinha S, Chakrabarti R, Portales-Casamar E, Sladek FM, Bradley PH, Wasserman WW. The transcription factor encyclopedia. *Genome Biol* 2012; **13**: R24 [PMID: 22458515 DOI: 10.1186/gb-2012-13-3-r24]

30 **Green VJ**, Kokkotou E, Ladias JA. Critical structural elements and multitarget protein interactions of the transcriptional activator AF-1 of hepatocyte nuclear factor 4. *J Biol Chem* 1998; **273**: 29950-29957 [PMID: 9792714 DOI: 10.1074/jbc.273.45.29950]

31 **Sladek FM**, Ruse MD Jr, Nepomuceno L, Huang SM, Stallcup MR. Modulation of transcriptional activation and coactivator interaction by a splicing variation in the F domain of nuclear receptor hepatocyte nuclear factor 4alpha1. *Mol Cell Biol* 1999; **19**: 6509-6522 [PMID: 10490591 DOI: 10.1128/MCB.19.10.6509]

32 **Huang J**, Karakucuk V, Levitsky LL, Rhoads DB. Expression of HNF4alpha variants in pancreatic islets and Ins-1 beta cells. *Diabetes Metab Res Rev* 2008; **24**: 533-543 [PMID: 18561282 DOI: 10.1002/dmrr.870]

33 **Huang J**, Levitsky LL, Rhoads DB. Novel P2 promoter-derived HNF4alpha isoforms with different N-terminus generated by alternate exon insertion. *Exp Cell Res* 2009; **315**: 1200-1211 [PMID: 19353766 DOI: 10.1016/j.yexcr.2009.01.004]

34 **Tanaka T**, Jiang S, Hotta H, Takano K, Iwanari H, Sumi K, Daigo K, Ohashi R, Sugai M, Ikegame C, Umezu H, Hirayama Y, Midorikawa Y, Hippo Y, Watanabe A, Uchiyama Y, Hasegawa G, Reid P, Aburatani H, Hamakubo T, Sakai J, Naito M, Kodama T. Dysregulated expression of P1 and P2 promoter-driven hepatocyte nuclear factor-4alpha in the pathogenesis of human cancer. *J Pathol* 2006; **208**: 662-672 [PMID: 16400631 DOI: 10.1002/path.1928]

35 **Kyrmizi I**, Hatzis P, Katrakili N, Tronche F, Gonzalez FJ, Talianidis I. Plasticity and expanding complexity of the hepatic transcription factor network during liver development. *Genes Dev* 2006; **20**: 2293-2305 [PMID: 16912278 DOI: 10.1101/gad.390906]

36 **Cai SH**, Lu SX, Liu LL, Zhang CZ, Yun JP. Increased expression of hepatocyte nuclear factor 4 alpha transcribed by promoter 2 indicates a poor prognosis in hepatocellular carcinoma. *Therap Adv Gastroenterol* 2017; **10**: 761-771 [PMID: 29051787 DOI: 10.1177/1756283X17725998]

37 **Lazarevich NL**, Cheremnova OA, Varga EV, Ovchinnikov DA, Kudrjavtseva EI, Morozova OV, Fleishman DI, Engelhardt NV, Duncan SA. Progression of HCC in mice is associated with a downregulation in the expression of hepatocyte nuclear factors. *Hepatology* 2004; **39**: 1038-1047 [PMID: 15057908 DOI: 10.1002/hep.20155]

38 **Lazarevich NL**, Shavochkina DA, Fleishman DI, Kustova IF, Morozova OV, Chuchuev ES, Patyutko YI. Deregulation of hepatocyte nuclear factor 4 (HNF4)as a marker of epithelial tumors progression. *Exp Oncol* 2010; **32**: 167-171 [PMID: 21403612]

39 **Späth GF**, Weiss MC. Hepatocyte nuclear factor 4 provokes expression of epithelial marker genes, acting as a morphogen in dedifferentiated hepatoma cells. *J Cell Biol* 1998; **140**: 935-946 [PMID: 9472044 DOI: 10.1083/jcb.140.4.935]

40 **Yin C**, Lin Y, Zhang X, Chen YX, Zeng X, Yue HY, Hou JL, Deng X, Zhang JP, Han ZG, Xie WF. Differentiation therapy of hepatocellular carcinoma in mice with recombinant adenovirus carrying hepatocyte nuclear factor-4alpha gene. *Hepatology* 2008; **48**: 1528-1539 [PMID: 18925631 DOI: 10.1002/hep.22510]

41 **Shi L**, Feng Y, Lin H, Ma R, Cai X. Role of estrogen in hepatocellular carcinoma: is inflammation the key? *J Transl Med* 2014; **12**: 93 [PMID: 24708807 DOI: 10.1186/1479-5876-12-93]

42 **Barone I**, Brusco L, Fuqua SA. Estrogen receptor mutations and changes in downstream gene expression and signaling. *Clin Cancer Res* 2010; **16**: 2702-2708 [PMID: 20427689 DOI: 10.1158/1078-0432.CCR-09-1753]

43 **Han J**, Ding L, Yuan B, Yang X, Wang X, Li J, Lu Q, Huang C, Ye Q. Hepatitis B virus X protein and the estrogen receptor variant lacking exon 5 inhibit estrogen receptor signaling in hepatoma cells. *Nucleic Acids Res* 2006; **34**: 3095-3106 [PMID: 16757575 DOI: 10.1093/nar/gkl389]

44 **Miceli V**, Cocciadiferro L, Fregapane M, Zarcone M, Montalto G, Polito LM, Agostara B, Granata OM, Carruba G. Expression of wild-type and variant estrogen receptor alpha in liver carcinogenesis and tumor progression. *OMICS* 2011; **15**: 313-317 [PMID: 21348637 DOI: 10.1089/omi.2010.0108]

45 **Wang Z**, Zhang X, Shen P, Loggie BW, Chang Y, Deuel TF. A variant of estrogen receptor-{alpha}, hER-{alpha}36: transduction of estrogen- and antiestrogen-dependent membrane-initiated mitogenic signaling. *Proc Natl Acad Sci USA* 2006; **103**: 9063-9068 [PMID: 16754886 DOI: 10.1073/pnas.0603339103]

46 **Fuqua SA**, Fitzgerald SD, Chamness GC, Tandon AK, McDonnell DP, Nawaz Z, O'Malley BW, McGuire WL. Variant human breast tumor estrogen receptor with constitutive transcriptional activity. *Cancer Res* 1991; **51**: 105-109 [PMID: 1988075]

47 **Zhang J**, Ren J, Wei J, Chong CC, Yang D, He Y, Chen GG, Lai PB. Alternative splicing of estrogen receptor alpha in hepatocellular carcinoma. *BMC Cancer* 2016; **16**: 926 [PMID: 27899088 DOI: 10.1186/s12885-016-2928-3]

48 **Hishida M**, Nomoto S, Inokawa Y, Hayashi M, Kanda M, Okamura Y, Nishikawa Y, Tanaka C, Kobayashi D, Yamada S, Nakayama G, Fujii T, Sugimoto H, Koike M, Fujiwara M, Takeda S, Kodera Y. Estrogen receptor 1 gene as a tumor suppressor gene in hepatocellular carcinoma detected by triple-combination array analysis. *Int J Oncol* 2013; **43**: 88-94 [PMID: 23695389 DOI: 10.3892/ijo.2013.1951]

49 **Finck BN**, Kelly DP. PGC-1 coactivators: inducible regulators of energy metabolism in health and disease. *J Clin Invest* 2006; **116**: 615-622 [PMID: 16511594 DOI: 10.1172/JCI27794]

50 **Yoon JC**, Puigserver P, Chen G, Donovan J, Wu Z, Rhee J, Adelmant G, Stafford J, Kahn CR, Granner DK, Newgard CB, Spiegelman BM. Control of hepatic gluconeogenesis through the transcriptional coactivator PGC-1. *Nature* 2001; **413**: 131-138 [PMID: 11557972 DOI: 10.1038/35093050]

51 **Wang B**, Hsu SH, Frankel W, Ghoshal K, Jacob ST. Stat3-mediated activation of microRNA-23a suppresses gluconeogenesis in hepatocellular carcinoma by down-regulating glucose-6-phosphatase and peroxisome proliferator-activated receptor gamma, coactivator 1 alpha. *Hepatology* 2012; **56**: 186-197 [PMID: 22318941 DOI: 10.1002/hep.25632]

52 **Felder TK**, Soyal SM, Oberkofler H, Hahne P, Auer S, Weiss R, Gadermaier G, Miller K, Krempler F, Esterbauer H, Patsch W. Characterization of novel peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) isoform in human liver. *J Biol Chem* 2011; **286**: 42923-42936 [PMID: 22009745 DOI: 10.1074/jbc.M111.227496]

53 **Martínez-Redondo V**, Pettersson AT, Ruas JL. The hitchhiker's guide to PGC-1α isoform structure and biological functions. *Diabetologia* 2015; **58**: 1969-1977 [PMID: 26109214 DOI: 10.1007/s00125-015-3671-z]

54 **Zhang Y**, Huypens P, Adamson AW, Chang JS, Henagan TM, Boudreau A, Lenard NR, Burk D, Klein J, Perwitz N, Shin J, Fasshauer M, Kralli A, Gettys TW. Alternative mRNA splicing produces a novel biologically active short isoform of PGC-1alpha. *J Biol Chem* 2009; **284**: 32813-32826 [PMID: 19773550 DOI: 10.1074/jbc.M109.037556]

55 **Chang JS**, Jun HJ, Park M. Transcriptional coactivator NT-PGC-1α promotes gluconeogenic gene expression and enhances hepatic gluconeogenesis. *Physiol Rep* 2016; **4**: [PMID: 27798359 DOI: 10.14814/phy2.13013]

56 **Yao W**, Cai H, Li X, Li T, Hu L, Peng T. Endoplasmic reticulum stress links hepatitis C virus RNA replication to wild-type PGC-1α/liver-specific PGC-1α upregulation. *J Virol* 2014; **88**: 8361-8374 [PMID: 24829353 DOI: 10.1128/JVI.01202-14]

57 **Hu C**, Liu D, Zhang Y, Lou G, Huang G, Chen B, Shen X, Gao

M, Gong W, Zhou P, Dai S, Zeng Y, He F. LXRα-mediated downregulation of FOXM1 suppresses the proliferation of hepatocellular carcinoma cells. *Oncogene* 2014; **33**: 2888-2897 [PMID: 23812424 DOI: 10.1038/onc.2013.250]

58 **Bakiri L**, Hamacher R, Graña O, Guío-Carrión A, Campos-Olivas R, Martinez L, Dienes HP, Thomsen MK, Hasenfuss SC, Wagner EF. Liver carcinogenesis by FOS-dependent inflammation and cholesterol dysregulation. *J Exp Med* 2017; **214**: 1387-1409 [PMID: 28356389 DOI: 10.1084/jem.20160935]

59 **Ramji DP**, Foka P. CCAAT/enhancer-binding proteins: structure, function and regulation. *Biochem J* 2002; **365**: 561-575 [PMID: 12006103 DOI: 10.1042/bj20020508]

60 **Takiguchi M**. The C/EBP family of transcription factors in the liver and other organs. *Int J Exp Pathol* 1998; **79**: 369-391 [PMID: 10319019 DOI: 10.1046/j.1365-2613.1998.00082.x]

61 **Rana B**, Xie Y, Mischoulon D, Bucher NL, Farmer SR. The DNA binding activity of C/EBP transcription factor is regulated in the G1 phase of the hepatocyte cell cycle. *J Biol Chem* 1995; **270**: 18123-18132 [PMID: 7629124 DOI: 10.1074/jbc.270.30.18123]

62 **Lourenço AR**, Coffer PJ. A tumor suppressor role for C/EBPα in solid tumors: more than fat and blood. *Oncogene* 2017; **36**: 5221-5230 [PMID: 28504718 DOI: 10.1038/onc.2017.151]

63 **Lu GD**, Leung CH, Yan B, Tan CM, Low SY, Aung MO, Salto-Tellez M, Lim SG, Hooi SC. C/EBPalpha is up-regulated in a subset of hepatocellular carcinomas and plays a role in cell growth and proliferation. *Gastroenterology* 2010; **139**: 632-643, 643.e1-643.e4 [PMID: 20347819 DOI: 10.1053/j.gastro.2010.03.051]

64 **Lu GD**, Ang YH, Zhou J, Tamilarasi J, Yan B, Lim YC, Srivastava S, Salto-Tellez M, Hui KM, Shen HM, Nguyen LN, Tan BC, Silver DL, Hooi SC. CCAAT/enhancer binding protein α predicts poorer prognosis and prevents energy starvation-induced cell death in hepatocellular carcinoma. *Hepatology* 2015; **61**: 965-978 [PMID: 25363290 DOI: 10.1002/hep.27593]

65 **Fang T**, Cui M, Sun J, Ge C, Zhao F, Zhang L, Tian H, Zhang L, Chen T, Jiang G, Xie H, Cui Y, Yao M, Li H, Li J. Orosomucoid 2 inhibits tumor metastasis and is upregulated by CCAAT/enhancer binding protein β in hepatocellular carcinomas. *Oncotarget* 2015; **6**: 16106-16119 [PMID: 25965830 DOI: 10.18632/oncotarget.3867]

66 **Wang C**, Chen X, Wang Y, Gong J, Hu G. C/EBPalphap30 plays transcriptional regulatory roles distinct from C/EBPalphap42. *Cell Res* 2007; **17**: 374-383 [PMID: 17242690 DOI: 10.1038/sj.cr.7310121]

67 **Reebye V**, Huang KW, Lin V, Jarvis S, Cutilas P, Dorman S, Ciriello S, Andrikakou P, Voutila J, Saetrom P, Mintz PJ, Reccia I, Rossi JJ, Huber H, Habib R, Kostomitsopoulos N, Blakey DC, Habib NA. Gene activation of CEBPA using saRNA: preclinical studies of the first in human saRNA drug candidate for liver cancer. *Oncogene* 2018; **37**: 3216-3228 [PMID: 29511346 DOI: 10.1038/s41388-018-0126-2]

68 **Anand S**, Ebner J, Warren CB, Raam MS, Piliang M, Billings SD, Maytin EV. C/EBP transcription factors in human squamous cell carcinoma: selective changes in expression of isoforms correlate with the neoplastic state. *PLoS One* 2014; **9**: e112073 [PMID: 25402211 DOI: 10.1371/journal.pone.0112073]

69 **Zahnow CA**. CCAAT/enhancer-binding protein beta: its role in breast cancer and associations with receptor tyrosine kinases. *Expert Rev Mol Med* 2009; **11**: e12 [PMID: 19351437 DOI: 10.1017/S1462399409001033]

70 **Yang LH**, Wang Y, Qiao S, Wang MJ, Chen F, Zi XY, Li JX, Zhang HB, Yu B, Hu YP. Liver-enriched activator protein 1 as an isoform of CCAAT/enhancer-binding protein beta suppresses stem cell features of hepatocellular carcinoma. *Cancer Manag Res* 2018; **10**: 873-885 [PMID: 29731667 DOI: 10.2147/CMAR.S160172]

71 **Milde-Langosch K**, Löning T, Bamberger AM. Expression of the CCAAT/enhancer-binding proteins C/EBPalpha, C/EBPbeta and C/EBPdelta in breast cancer: correlations with clinicopathologic parameters and cell-cycle regulatory proteins. *Breast Cancer Res Treat* 2003; **79**: 175-185 [PMID: 12825852 DOI: 10.1023/A:1023929504884]

72 **Wang H**, Larris B, Peiris TH, Zhang L, Le Lay J, Gao Y, Greenbaum LE. C/EBPbeta activates E2F-regulated genes in vivo via recruitment of the coactivator CREB-binding protein/P300. *J Biol Chem* 2007; **282**: 24679-24688 [PMID: 17599912 DOI: 10.1074/jbc.M705066200]

73 **Wang GL**, Salisbury E, Shi X, Timchenko L, Medrano EE, Timchenko NA. HDAC1 promotes liver proliferation in young mice via interactions with C/EBPbeta. *J Biol Chem* 2008; **283**: 26179-26187 [PMID: 18622014 DOI: 10.1074/jbc.M803545200]

74 **Luedde T**, Duderstadt M, Streetz KL, Tacke F, Kubicka S, Manns MP, Trautwein C. C/EBP beta isoforms LIP and LAP modulate progression of the cell cycle in the regenerating mouse liver. *Hepatology* 2004; **40**: 356-365 [PMID: 15368440 DOI: 10.1002/hep.20333]

75 **Buck M**, Turler H, Chojkier M. LAP (NF-IL-6), a tissue-specific transcriptional activator, is an inhibitor of hepatoma cell proliferation. *EMBO J* 1994; **13**: 851-860 [PMID: 7906646]

76 **Saint-Auret G**, Danan JL, Hiron M, Blache C, Sulpice E, Tendil S, Daveau M, Gidrol X, Salier JP. Characterization of the transcriptional signature of C/EBPbeta isoforms (LAP/LIP) in Hep3B cells: implication of LIP in pro-survival functions. *J Hepatol* 2011; **54**: 1185-1194 [PMID: 21145827 DOI: 10.1016/j.jhep.2010.09.021]

77 **Rodríguez-Antona C**, Bort R, Jover R, Tindberg N, Ingelman-Sundberg M, Gómez-Lechón MJ, Castell JV. Transcriptional regulation of human CYP3A4 basal expression by CCAAT enhancer-binding protein alpha and hepatocyte nuclear factor-3 gamma. *Mol Pharmacol* 2003; **63**: 1180-1189 [PMID: 12695546 DOI: 10.1124/mol.63.5.1180]

78 **Martínez-Jiménez CP**, Gómez-Lechón MJ, Castell JV, Jover R. Transcriptional regulation of the human hepatic CYP3A4: identification of a new distal enhancer region responsive to CCAAT/enhancer-binding protein beta isoforms (liver activating protein and liver inhibitory protein). *Mol Pharmacol* 2005; **67**: 2088-2101 [PMID: 15778453 DOI: 10.1124/mol.104.008169]

79 **Collavin L**, Lunardi A, Del Sal G. p53-family proteins and their regulators: hubs and spokes in tumor suppression. *Cell Death Differ* 2010; **17**: 901-911 [PMID: 20379196 DOI: 10.1038/cdd.2010.35]

80 **Murray-Zmijewski F**, Lane DP, Bourdon JC. p53/p63/p73 isoforms: an orchestra of isoforms to harmonise cell differentiation and response to stress. *Cell Death Differ* 2006; **13**: 962-972 [PMID: 16601753 DOI: 10.1038/sj.cdd.4401914]

81 **Ferraiuolo M**, Di Agostino S, Blandino G, Strano S. Oncogenic Intra-p53 Family Member Interactions in Human Cancers. *Front Oncol* 2016; **6**: 77 [PMID: 27066457 DOI: 10.3389/fonc.2016.00077]

82 **Natan E**, Joerger AC. Structure and kinetic stability of the p63 tetramerization domain. *J Mol Biol* 2012; **415**: 503-513 [PMID: 22100306 DOI: 10.1016/j.jmb.2011.11.007]

83 **Ota A**, Nakao H, Sawada Y, Karnan S, Wahiduzzaman M, Inoue T, Kobayashi Y, Yamamoto T, Ishii N, Ohashi T, Nakade Y, Sato K, Itoh K, Konishi H, Hosokawa Y, Yoneda M. Δ40p53α suppresses tumor cell proliferation and induces cellular senescence in hepatocellular carcinoma cells. *J Cell Sci* 2017; **130**: 614-625 [PMID: 27980070 DOI: 10.1242/jcs.190736]

84 **Petitjean A**, Cavard C, Shi H, Tribollet V, Hainaut P, Caron de Fromentel C. The expression of TA and DeltaNp63 are regulated by different mechanisms in liver cells. *Oncogene* 2005; **24**: 512-519 [PMID: 15543231 DOI: 10.1038/sj.onc.1208215]

85 **Yao JY**, Pao CC, Chen JK. Transcriptional activity of TAp63 promoter is regulated by c-jun. *J Cell Physiol* 2010; **225**: 898-904 [PMID: 20607803 DOI: 10.1002/jcp.22300]

86 **Gu H**, Fang Z, Cai X, Song R, Lin M, Ye J, Ding X, Ke Q, Chen H, Gong C, Ye M. Highly expressed histone deacetylase 5 promotes the growth of hepatocellular carcinoma cells by inhibiting the TAp63-maspin pathway. *Am J Cancer Res* 2018; **8**: 462-475 [PMID: 29637001]

87 **Sayan AE**, Sayan BS, Findikli N, Ozturk M. Acquired expression of transcriptionally active p73 in hepatocellular carcinoma cells. *Oncogene* 2001; **20**: 5111-5117 [PMID: 11526499 DOI: 10.1038/sj.onc.1204669]

88 **Stiewe T**, Tuve S, Peter M, Tannapfel A, Elmaagacli AH, Pützer

BM. Quantitative TP73 transcript analysis in hepatocellular carcinomas. *Clin Cancer Res* 2004; **10**: 626-633 [PMID: 14760085 DOI: 10.1158/1078-0432.CCR-0153-03]

89 **Müller M**, Schilling T, Sayan AE, Kairat A, Lorenz K, Schulze-Bergkamen H, Oren M, Koch A, Tannapfel A, Stremmel W, Melino G, Krammer PH. TAp73/Delta Np73 influences apoptotic response, chemosensitivity and prognosis in hepatocellular carcinoma. *Cell Death Differ* 2005; **12**: 1564-1577 [PMID: 16195739 DOI: 10.1038/sj.cdd.4401774]

90 **Knoll S**, Fürst K, Thomas S, Villanueva Baselga S, Stoll A, Schaefer S, Pützer BM. Dissection of cell context-dependent interactions between HBx and p53 family members in regulation of apoptosis: a role for HBV-induced HCC. *Cell Cycle* 2011; **10**: 3554-3565 [PMID: 22030623 DOI: 10.4161/cc.10.20.17856]

91 **Tannapfel A**, John K, Mise N, Schmidt A, Buhlmann S, Ibrahim SM, Pützer BM. Autonomous growth and hepatocarcinogenesis in transgenic mice expressing the p53 family inhibitor DNp73. *Carcinogenesis* 2008; **29**: 211-218 [PMID: 17984115 DOI: 10.1093/carcin/bgm236]

92 **Schuster A**, Schilling T, De Laurenzi V, Koch AF, Seitz S, Staib F, Teufel A, Thorgeirsson SS, Galle P, Melino G, Stremmel W, Krammer PH, Müller M. ΔNp73β is oncogenic in hepatocellular carcinoma by blocking apoptosis signaling via death receptors and mitochondria. *Cell Cycle* 2010; **9**: 2629-2639 [PMID: 20581467 DOI: 10.4161/cc.9.13.12110]

93 **Arce L**, Yokoyama NN, Waterman ML. Diversity of LEF/TCF action in development and disease. *Oncogene* 2006; **25**: 7492-7504 [PMID: 17143293 DOI: 10.1038/sj.onc.1210056]

94 **Cadigan KM**, Waterman ML. TCF/LEFs and Wnt signaling in the nucleus. *Cold Spring Harb Perspect Biol* 2012; **4**: [PMID: 23024173 DOI: 10.1101/cshperspect.a007906]

95 **Atcha FA**, Syed A, Wu B, Hoverter NP, Yokoyama NN, Ting JH, Munguia JE, Mangalam HJ, Marsh JL, Waterman ML. A unique DNA binding domain converts T-cell factors into strong Wnt effectors. *Mol Cell Biol* 2007; **27**: 8352-8363 [PMID: 17893322 DOI: 10.1128/MCB.02132-06]

96 **Neve B**, Le Bacquer O, Caron S, Huyvaert M, Leloire A, Poulain-Godefroy O, Lecoeur C, Pattou F, Staels B, Froguel P. Alternative human liver transcripts of TCF7L2 bind to the gluconeogenesis regulator HNF4α at the protein level. *Diabetologia* 2014; **57**: 785-796 [PMID: 24463962 DOI: 10.1007/s00125-013-3154-z]

97 **Prokunina-Olsson L**, Welch C, Hansson O, Adhikari N, Scott LJ, Usher N, Tong M, Sprau A, Swift A, Bonnycastle LL, Erdos MR, He Z, Saxena R, Harmon B, Kotova O, Hoffman EP, Altshuler D, Groop L, Boehnke M, Collins FS, Hall JL. Tissue-specific alternative splicing of TCF7L2. *Hum Mol Genet* 2009; **18**: 3795-3804 [PMID: 19602480 DOI: 10.1093/hmg/ddp321]

98 **Tsedensodnom O**, Koga H, Rosenberg SA, Nambotin SB, Carroll JJ, Wands JR, Kim M. Identification of T-cell factor-4 isoforms that contribute to the malignant phenotype of hepatocellular carcinoma cells. *Exp Cell Res* 2011; **317**: 920-931 [PMID: 21256126 DOI: 10.1016/j.yexcr.2011.01.015]

99 **Tomimaru Y**, Xu CQ, Nambotin SB, Yan T, Wands JR, Kim M. Loss of exon 4 in a human T-cell factor-4 isoform promotes hepatic tumourigenicity. *Liver Int* 2013; **33**: 1536-1548 [PMID: 23648141 DOI: 10.1111/liv.12189]

100 **Koga H**, Tsedensodnom O, Tomimaru Y, Walker EJ, Lee HC, Kim KM, Yano H, Wands JR, Kim M. Loss of the SxxSS motif in a human T-cell factor-4 isoform confers hypoxia resistance to liver cancer: an oncogenic switch in Wnt signaling. *PLoS One* 2012; **7**: e39981 [PMID: 22768190 DOI: 10.1371/journal.pone.0039981]

101 **Tomimaru Y**, Koga H, Shin TH, Xu CQ, Wands JR, Kim M. The SxxSS motif of T-cell factor-4 isoforms modulates Wnt/β-catenin signal activation in hepatocellular carcinoma cells. *Cancer Lett* 2013; **336**: 359-369 [PMID: 23562475 DOI: 10.1016/j.canlet.2013.03.031]

102 **Tomimaru Y**, Koga H, Yano H, de la Monte S, Wands JR, Kim M. Upregulation of T-cell factor-4 isoform-responsive target genes in hepatocellular carcinoma. *Liver Int* 2013; **33**: 1100-1112 [PMID: 23651211 DOI: 10.1111/liv.12188]

103 **Vuong LM**, Chellappa K, Dhahbi JM, Deans JR, Fang B, Bolotin E, Titova NV, Hoverter NP, Spindler SR, Waterman ML, Sladek FM. Differential Effects of Hepatocyte Nuclear Factor 4α Isoforms on Tumor Growth and T-Cell Factor 4/AP-1 Interactions in Human Colorectal Cancer Cells. *Mol Cell Biol* 2015; **35**: 3471-3490 [PMID: 26240283 DOI: 10.1128/MCB.00030-15]

104 **DiFeo A**, Martignetti JA, Narla G. The role of KLF6 and its splice variants in cancer therapy. *Drug Resist Updat* 2009; **12**: 1-7 [PMID: 19097929 DOI: 10.1016/j.drup.2008.11.001]

105 **Kremer-Tal S**, Reeves HL, Narla G, Thung SN, Schwartz M, Difeo A, Katz A, Bruix J, Bioulac-Sage P, Martignetti JA, Friedman SL. Frequent inactivation of the tumor suppressor Kruppel-like factor 6 (KLF6) in hepatocellular carcinoma. *Hepatology* 2004; **40**: 1047-1052 [PMID: 15486921 DOI: 10.1002/hep.20460]

106 **Hanoun N**, Bureau C, Diab T, Gayet O, Dusetti N, Selves J, Vinel JP, Buscail L, Cordelier P, Torrisani J. The SV2 variant of KLF6 is down-regulated in hepatocellular carcinoma and displays anti-proliferative and pro-apoptotic functions. *J Hepatol* 2010; **53**: 880-888 [PMID: 20801538 DOI: 10.1016/j.jhep.2010.04.038]

107 **Narla G**, Difeo A, Reeves HL, Schaid DJ, Hirshfeld J, Hod E, Katz A, Isaacs WB, Hebbring S, Komiya A, McDonnell SK, Wiley KE, Jacobsen SJ, Isaacs SD, Walsh PC, Zheng SL, Chang BL, Friedrichsen DM, Stanford JL, Ostrander EA, Chinnaiyan AM, Rubin MA, Xu J, Thibodeau SN, Friedman SL, Martignetti JA. A germline DNA polymorphism enhances alternative splicing of the KLF6 tumor suppressor gene and is associated with increased prostate cancer risk. *Cancer Res* 2005; **65**: 1213-1222 [PMID: 15735005 DOI: 10.1158/0008-5472.CAN-04-4249]

108 **Kremer-Tal S**, Narla G, Chen Y, Hod E, DiFeo A, Yea S, Lee JS, Schwartz M, Thung SN, Fiel IM, Banck M, Zimran E, Thorgeirsson SS, Mazzaferro V, Bruix J, Martignetti JA, Llovet JM, Friedman SL. Downregulation of KLF6 is an early event in hepatocarcinogenesis, and stimulates proliferation while reducing differentiation. *J Hepatol* 2007; **46**: 645-654 [PMID: 17196295 DOI: 10.1016/j.jhep.2006.10.012]

109 **Tarocchi M**, Hannivoort R, Hoshida Y, Lee UE, Vetter D, Narla G, Villanueva A, Oren M, Llovet JM, Friedman SL. Carcinogen-induced hepatic tumors in KLF6+/- mice recapitulate aggressive human hepatocellular carcinoma associated with p53 pathway deregulation. *Hepatology* 2011; **54**: 522-531 [PMID: 21563203 DOI: 10.1002/hep.24413]

110 **Vetter D**, Cohen-Naftaly M, Villanueva A, Lee YA, Kocabayoglu P, Hannivoort R, Narla G, M Llovet J, Thung SN, Friedman SL. Enhanced hepatocarcinogenesis in mouse models and human hepatocellular carcinoma by coordinate KLF6 depletion and increased messenger RNA splicing. *Hepatology* 2012; **56**: 1361-1370 [PMID: 22535637 DOI: 10.1002/hep.25810]

111 **Yea S**, Narla G, Zhao X, Garg R, Tal-Kremer S, Hod E, Villanueva A, Loke J, Tarocchi M, Akita K, Shirasawa S, Sasazuki T, Martignetti JA, Llovet JM, Friedman SL. Ras promotes growth by alternative splicing-mediated inactivation of the KLF6 tumor suppressor in hepatocellular carcinoma. *Gastroenterology* 2008; **134**: 1521-1531 [PMID: 18471523 DOI: 10.1053/j.gastro.2008.02.015]

112 **Muñoz Ú**, Puche JE, Hannivoort R, Lang UE, Cohen-Naftaly M, Friedman SL. Hepatocyte growth factor enhances alternative splicing of the Kruppel-like factor 6 (KLF6) tumor suppressor to promote growth through SRSF1. *Mol Cancer Res* 2012; **10**: 1216-1227 [PMID: 22859706 DOI: 10.1158/1541-7786.MCR-12-0213]

113 **Liang WC**, Wang Y, Xiao LJ, Wang YB, Fu WM, Wang WM, Jiang HQ, Qi W, Wan DC, Zhang JF, Waye MM. Identification of miRNAs that specifically target tumor suppressive KLF6-FL rather than oncogenic KLF6-SV1 isoform. *RNA Biol* 2014; **11**: 845-854 [PMID: 24921656 DOI: 10.4161/rna.29356]

114 **Ahronian LG**, Zhu LJ, Chen YW, Chu HC, Klimstra DS, Lewis BC. A novel KLF6-Rho GTPase axis regulates hepatocellular carcinoma cell migration and dissemination. *Oncogene* 2016; **35**: 4653-4662 [PMID: 26876204 DOI: 10.1038/onc.2016.2]

115 **Hohenstein P**, Hastie ND. The many facets of the Wilms' tumour gene, WT1. *Hum Mol Genet* 2006; **15** Spec No 2: R196-R201 [PMID: 16987884 DOI: 10.1093/hmg/ddl196]

116 **Sera T**, Hiasa Y, Mashiba T, Tokumoto Y, Hirooka M, Konishi I,

Matsuura B, Michitaka K, Udaka K, Onji M. Wilms' tumour 1 gene expression is increased in hepatocellular carcinoma and associated with poor prognosis. *Eur J Cancer* 2008; **44**: 600-608 [PMID: 18255279 DOI: 10.1016/j.ejca.2008.01.008]

117 **Qi XW**, Zhang F, Wu H, Liu JL, Zong BG, Xu C, Jiang J. Wilms' tumor 1 (WT1) expression and prognosis in solid cancer patients: a systematic review and meta-analysis. *Sci Rep* 2015; **5**: 8924 [PMID: 25748047 DOI: 10.1038/srep08924]

118 **Tatsumi N**, Hojo N, Sakamoto H, Inaba R, Moriguchi N, Matsuno K, Fukuda M, Matsumura A, Hayashi S, Morimoto S, Nakata J, Fujiki F, Nishida S, Nakajima H, Tsuboi A, Oka Y, Hosen N, Sugiyama H, Oji Y. Identification of a Novel C-Terminal Truncated WT1 Isoform with Antagonistic Effects against Major WT1 Isoforms. *PLoS One* 2015; **10**: e0130578 [PMID: 26090994 DOI: 10.1371/journal.pone.0130578]

119 **Menke AL**, Shvarts A, Riteco N, van Ham RC, van der Eb AJ, Jochemsen AG. Wilms' tumor 1-KTS isoforms induce p53-independent apoptosis that can be partially rescued by expression of the epidermal growth factor receptor or the insulin receptor. *Cancer Res* 1997; **57**: 1353-1363 [PMID: 9102224]

120 **Berasain C**, Herrero JI, García-Trevijano ER, Avila MA, Esteban JI, Mato JM, Prieto J. Expression of Wilms' tumor suppressor in the liver with cirrhosis: relation to hepatocyte nuclear factor 4 and hepatocellular function. *Hepatology* 2003; **38**: 148-157 [PMID: 12829997 DOI: 10.1053/jhep.2003.50269]

121 **Perugorria MJ**, Castillo J, Latasa MU, Goñi S, Segura V, Sangro B, Prieto J, Avila MA, Berasain C. Wilms' tumor 1 gene expression in hepatocellular carcinoma promotes cell dedifferentiation and resistance to chemotherapy. *Cancer Res* 2009; **69**: 1358-1367 [PMID: 19190340 DOI: 10.1158/0008-5472.CAN-08-2545]

122 **Uesugi K**, Hiasa Y, Tokumoto Y, Mashiba T, Koizumi Y, Hirooka M, Abe M, Matsuura B, Onji M. Wilms' tumor 1 gene modulates Fas-related death signals and anti-apoptotic functions in hepatocellular carcinoma. *J Gastroenterol* 2013; **48**: 1069-1080 [PMID: 23142971 DOI: 10.1007/s00535-012-0708-7]

123 **Keith B**, Johnson RS, Simon MC. HIF1α and HIF2α: sibling rivalry in hypoxic tumour growth and progression. *Nat Rev Cancer* 2011; **12**: 9-22 [PMID: 22169972 DOI: 10.1038/nrc3183]

124 **Shinojima T**, Oya M, Takayanagi A, Mizuno R, Shimizu N, Murai M. Renal cancer cells lacking hypoxia inducible factor (HIF)-1alpha expression maintain vascular endothelial growth factor expression through HIF-2alpha. *Carcinogenesis* 2007; **28**: 529-536 [PMID: 16920734 DOI: 10.1093/carcin/bgl143]

125 **Sowter HM**, Raval RR, Moore JW, Ratcliffe PJ, Harris AL. Predominant role of hypoxia-inducible transcription factor (Hif)-1alpha versus Hif-2alpha in regulation of the transcriptional response to hypoxia. *Cancer Res* 2003; **63**: 6130-6134 [PMID: 14559790]

126 **Warnecke C**, Zaborowska Z, Kurreck J, Erdmann VA, Frei U, Wiesener M, Eckardt KU. Differentiating the functional role of hypoxia-inducible factor (HIF)-1alpha and HIF-2alpha (EPAS-1) by the use of RNA interference: erythropoietin is a HIF-2alpha target gene in Hep3B and Kelly cells. *FASEB J* 2004; **18**: 1462-1464 [PMID: 15240563 DOI: 10.1096/fj.04-1640fje]

127 **Stroka DM**, Burkhardt T, Desbaillets I, Wenger RH, Neil DA, Bauer C, Gassmann M, Candinas D. HIF-1 is expressed in normoxic tissue and displays an organ-specific regulation under systemic hypoxia. *FASEB J* 2001; **15**: 2445-2453 [PMID: 11689469 DOI: 10.1096/fj.01-0125com]

128 **Nakamura K**, Zen Y, Sato Y, Kozaka K, Matsui O, Harada K, Nakanuma Y. Vascular endothelial growth factor, its receptor Flk-1, and hypoxia inducible factor-1alpha are involved in malignant transformation in dysplastic nodules of the liver. *Hum Pathol* 2007; **38**: 1532-1546 [PMID: 17640715 DOI: 10.1016/j.humpath.2007.03.002]

129 **Cao S**, Yang S, Wu C, Wang Y, Jiang J, Lu Z. Protein expression of hypoxia-inducible factor-1 alpha and hepatocellular carcinoma: a systematic review with meta-analysis. *Clin Res Hepatol Gastroenterol* 2014; **38**: 598-603 [PMID: 24835771 DOI: 10.1016/j.clinre.2014.04.004]

130 **Sena JA**, Wang L, Heasley LE, Hu CJ. Hypoxia regulates alternative splicing of HIF and non-HIF target genes. *Mol Cancer Res* 2014; **12**: 1233-1243 [PMID: 24850901 DOI: 10.1158/1541-7786.MCR-14-0149]

131 **Gothié E**, Richard DE, Berra E, Pagès G, Pouysségur J. Identification of alternative spliced variants of human hypoxia-inducible factor-1alpha. *J Biol Chem* 2000; **275**: 6922-6927 [PMID: 10702253 DOI: 10.1074/jbc.275.10.6922]

132 **Monsef N**, Soller M, Panagopoulos I, Abrahamsson PA. HIF1alpha isoforms in benign and malignant prostate tissue and their correlation to neuroendocrine differentiation. *BMC Cancer* 2010; **10**: 385 [PMID: 20663134 DOI: 10.1186/1471-2407-10-385]

133 **Chun YS**, Choi E, Yeo EJ, Lee JH, Kim MS, Park JW. A new HIF-1 alpha variant induced by zinc ion suppresses HIF-1-mediated hypoxic responses. *J Cell Sci* 2001; **114**: 4051-4061 [PMID: 11739637]

134 **Chun YS**, Choi E, Kim TY, Kim MS, Park JW. A dominant-negative isoform lacking exons 11 and 12 of the human hypoxia-inducible factor-1alpha gene. *Biochem J* 2002; **362**: 71-79 [PMID: 11829741 DOI: 10.1042/0264-6021:3620071]

135 **Li R**, Zhang H, Yu W, Chen Y, Gui B, Liang J, Wang Y, Sun L, Yang X, Zhang Y, Shi L, Li Y, Shang Y. ZIP: a novel transcription repressor, represses EGFR oncogene and suppresses breast carcinogenesis. *EMBO J* 2009; **28**: 2763-2776 [PMID: 19644445 DOI: 10.1038/emboj.2009.211]

136 **Gui B**, Han X, Zhang Y, Liang J, Wang D, Xuan C, Yu Z, Shang Y. Dimerization of ZIP promotes its transcriptional repressive function and biological activity. *Int J Biochem Cell Biol* 2012; **44**: 886-895 [PMID: 22387311 DOI: 10.1016/j.biocel.2012.02.012]

137 **Yu W**, Li R, Gui B, Shang Y. sZIP, an alternative splice variant of ZIP, antagonizes transcription repression and growth inhibition by ZIP. *J Biol Chem* 2010; **285**: 14301-14307 [PMID: 20233718 DOI: 10.1074/jbc.M110.107508]

138 **Ladomery M**. Aberrant alternative splicing is another hallmark of cancer. *Int J Cell Biol* 2013; **2013**: 463786 [PMID: 24101931 DOI: 10.1155/2013/463786]

139 **Kanehisa M**, Furumichi M, Tanabe M, Sato Y, Morishima K. KEGG: new perspectives on genomes, pathways, diseases and drugs. *Nucleic Acids Res* 2017; **45**: D353-D361 [PMID: 27899662 DOI: 10.1093/nar/gkw1092]

140 **Yamaga R**, Ikeda K, Horie-Inoue K, Ouchi Y, Suzuki Y, Inoue S. RNA sequencing of MCF-7 breast cancer cells identifies novel estrogen-responsive genes with functional estrogen receptor-binding sites in the vicinity of their transcription start sites. *Horm Cancer* 2013; **4**: 222-232 [PMID: 23526455 DOI: 10.1007/s12672-013-0140-3]

141 **Alder O**, Cullum R, Lee S, Kan AC, Wei W, Yi Y, Garside VC, Bilenky M, Griffith M, Morrissy AS, Robertson GA, Thiessen N, Zhao Y, Chen Q, Pan D, Jones SJM, Marra MA, Hoodless PA. Hippo signaling influences HNF4A and FOXA2 enhancer switching during hepatocyte differentiation. *Cell Rep* 2014; **9**: 261-271 [PMID: 25263553 DOI: 10.1016/j.celrep.2014.08.046]

142 **Lu H**. Crosstalk of HNF4α with extracellular and intracellular signaling pathways in the regulation of hepatic metabolism of drugs and lipids. *Acta Pharm Sin B* 2016; **6**: 393-408 [PMID: 27709008 DOI: 10.1016/j.apsb.2016.07.003]

143 **Botella LM**, Sanz-Rodriguez F, Komi Y, Fernandez-L A, Varela E, Garrido-Martin EM, Narla G, Friedman SL, Kojima S. TGF-beta regulates the expression of transcription factor KLF6 and its splice variants and promotes co-operative transactivation of common target genes through a Smad3-Sp1-KLF6 interaction. *Biochem J* 2009; **419**: 485-495 [PMID: 19076057 DOI: 10.1042/BJ20081434]

144 **Rosenbluth JM**, Mays DJ, Pino MF, Tang LJ, Pietenpol JA. A gene signature-based approach identifies mTOR as a regulator of p73. *Mol Cell Biol* 2008; **28**: 5951-5964 [PMID: 18678646 DOI: 10.1128/MCB.00305-08]

145 **Wands JR**, Kim M. Signaling Pathways in Viral Related Pre-neoplastic Liver Disease and Hepatocellular Carcinoma. In: Wang XW, Grisham JW, Thorgeirsson SS, editors. Molecular Genetics of Liver Neoplasia. New York: Springer New York, 2011: 103-127

146 **Wagner KD**, Cherfils-Vicini J, Hosen N, Hohenstein P, Gilson E,

Hastie ND, Michiels JF, Wagner N. The Wilms' tumour suppressor Wt1 is a major regulator of tumour angiogenesis and progression. *Nat Commun* 2014; **5**: 5852 [PMID: 25510679 DOI: 10.1038/ncomms6852]

147 **Algamas-Dimantov A**, Yehuda-Shnaidman E, Peri I, Schwartz B. Epigenetic control of HNF-4α in colon carcinoma cells affects MUC4 expression and malignancy. *Cell Oncol* (Dordr) 2013; **36**: 155-167 [PMID: 23307400 DOI: 10.1007/s13402-012-0123-3]

148 **Oshima T**, Kawasaki T, Ohashi R, Hasegawa G, Jiang S, Umezu H, Aoyagi Y, Iwanari H, Tanaka T, Hamakubo T, Kodama T, Naito M. Downregulated P1 promoter-driven hepatocyte nuclear factor-4alpha expression in human colorectal carcinoma is a new prognostic factor against liver metastasis. *Pathol Int* 2007; **57**: 82-90 [PMID: 17300672 DOI: 10.1111/j.1440-1827.2006.02061.x]

149 **Gillet JP**, Varma S, Gottesman MM. The clinical relevance of cancer cell lines. *J Natl Cancer Inst* 2013; **105**: 452-458 [PMID: 23434901 DOI: 10.1093/jnci/djt007]

150 **Katt ME**, Placone AL, Wong AD, Xu ZS, Searson PC. In Vitro Tumor Models: Advantages, Disadvantages, Variables, and Selecting the Right Platform. *Front Bioeng Biotechnol* 2016; **4**: 12 [PMID: 26904541 DOI: 10.3389/fbioe.2016.00012]

151 **Takehara T**, Liu X, Fujimoto J, Friedman SL, Takahashi H. Expression and role of Bcl-xL in human hepatocellular carcinomas. *Hepatology* 2001; **34**: 55-61 [PMID: 11431734 DOI: 10.1053/jhep.2001.25387]

152 **Salton M**, Misteli T. Small Molecule Modulators of Pre-mRNA Splicing in Cancer Therapy. *Trends Mol Med* 2016; **22**: 28-37 [PMID: 26700537 DOI: 10.1016/j.molmed.2015.11.005]

P- Reviewer: Luo GH, Roohvand F, Sukocheva OA **S- Editor**: Ji FF
L- Editor: A **E- Editor**: Bian YN

World Journal of Hepatology

Submit a Manuscript: http://www.f6publishing.com

World J Hepatol 2018 October 27; 10(10): 662-669

DOI: 10.4254/wjh.v10.i10.662

ISSN 1948-5182 (online)

REVIEW

Complements are involved in alcoholic fatty liver disease, hepatitis and fibrosis

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Author contributions: Lin CJ and Hu ZG contributed equally to this work; He SQ designed research; Lin CJ, Hu ZG and Yuan GD searched the relevant literatures; and Lin CJ wrote the review; Lei B and He SQ revised the review.

Supported by State Key Program of National Natural Science Foundation of China, No. 8143000311; National Natural Science Foundation of China, No. 81660103 and No. 81771674; 111 Project, No. D17011; Guangxi BaGui Scholars; the Natural Science Foundation of Guangxi Province, No. 2015GXNSFFA139004.

Conflict-of-interest statement: All authors have no conflicts of interest to report.

Manuscript source: Invited manuscript

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Received: April 10, 2018
Peer-review started: April 10, 2018
First decision: May 17, 2018
Revised: July 26, 2018
Accepted: August 1, 2018
Article in press: August 1, 2018
Published online: October 27, 2018

Abstract

The complement system is a key component of the body's immune system. When abnormally activated, this system can induce inflammation and damage to normal tissues and participate in the development and progression of a variety of diseases. In the past, many scholars believed that alcoholic liver disease (ALD) is induced by the stress of ethanol on liver cells, including oxidative stress and dysfunction of mitochondria and protease bodies, causing hepatocyte injury and apoptosis. Recent studies have shown that complement activation is also involved in the genesis and development of ALD. This review focuses on the roles of complement activation in ALD and of therapeutic intervention in complement-activation pathways. We intend to provide new ideas on the diagnosis and treatment of ALD.

Key words: Alcoholic liver disease; Complement system proteins; Complement regulator; Liver cells; Hepatocyte injury

Core tip: In this review, we cited evidence that the complement system is involved in the pathogenesis of each stage of alcoholic liver disease (ALD) that include fatty liver, alcoholic hepatitis, and fibrosis/cirrhosis, and we also summarized the complement regulation in ALD. We intend to provide new ideas on the treatment of ALD.

Lin CJ, Hu ZG, Yuan GD, Lei B, He SQ. Complements are involved in alcoholic fatty liver disease, hepatitis and fibrosis. *World J Hepatol* 2018; 10(10): 662-669 Available from: URL: http://www.wjgnet.com/1948-5182/full/v10/i10/662.htm DOI: http://dx.doi.org/10.4254/wjh.v10.i10.662

INTRODUCTION

Liver disease caused by long-term excessive ethanol drinking is a major cause of chronic liver disease. As the global incidence of alcoholic liver disease (ALD) increases year by year, it has become a serious threat to human health. Almost all heavy drinkers have fatty liver, 10%-20% of which develop into alcoholic hepatitis, cirrhosis, and even hepatocellular carcinoma[1]. Exploration of the mechanisms of alcohol-induced liver injury and repair is extremely important in developing methods for preventing and treating ALD.

The complement system plays an important beneficial role in the immune system: Complement activation promotes target-cell lysis, with the associated elimination of exogenous pathogens. Yet, the complement system is a "double-edged sword", as the excessive activation of complement can induce inflammation and lead to autoimmune diseases, such as autoimmune kidney disease, glomerular nephritis, acute lung injury, and others[2-5]. Most plasma complement components are synthesized in liver cells. Thus, the liver becomes the main target of damage by complement activation[6-8]. This connection is likely due to the direct effects of alcohol that activate complement, but not because the liver is a major producer of complement proteins. Several studies have illustrated that complement activation is involved in the development of ALD[8-14] (Table 1).

METABOLIC PATHWAYS OF ETHANOL

Most ingested ethanol is absorbed into the blood circulation, and soon reaches each organ of the body. About 90% of the ingested ethanol is metabolized in the liver[15], and most is metabolized by alcohol dehydrogenase and aldehyde dehydrogenase to form acetic acid, which can be used as substrate in the tricarboxylic acid cycle to produce energy. With excessive drinking, the body can activate another metabolic pathway, *i.e.*, the microsomal ethanol oxidation system (MEOS), which catalyzes ethanol mainly by cytochrome P450 2El in Kupffer cells. The MEOS can over produced reactive oxygen species and reactive nitrogen species, which may exceed the body's antioxidant capacity. Free radicals produced *via* the MEOS pathway exert a series of toxic effects: Membrane-lipid peroxidation, intracellular protease degeneration, oxidative modification of DNA, and others, which eventually lead to necrosis or apoptosis of hepatocytes[14,16-18]. A small percentage of ethanol is metabolized by fatty acid ethyl ester synthase to produce fatty acid ethyl ester through the non-oxidative pathway. Fatty acid ethyl ester has cytotoxicity, which can further injure the liver and pancreas[19]. Thus the liver becomes the main organ damaged by ethanol. Chronic ethanol exposure results in decreased protease activity in liver cells, imbalance of the liver's detoxification function, and overproduction of acetaldehyde, thus inducing hepatic oxidative stress and complement activation; all these activities can injure hepatocytes[20].

COMPLEMENT ACTIVATION PATHWAY

The complement system consists of more than 30 kinds of proteins with enzyme-like activities which are inherent components, regulatory proteins, and complement receptors. Complement regulatory proteins include plasma soluble factors, membrane binding proteins, homologous restriction factor, and membrane inhibitors of reactive lysis. Because the complement system is involved in inflammation and immune regulation, it plays an important role in regulation of pathophysiological functions[21].

Complement is activated by three pathways: The classical, mannan-binding lectin (MBL), and alternative pathways. The three pathways start with different mechanisms, but they end with a common terminal pathway, as shown in Figure 1. The classical pathway is the main mechanism of immune responses. In it, C1q identifies immune complexes, followed by the activation of C1r and C1s. Activated C1s cleaves C2 and C4 to form C3 convertase (C4bC2a), which cleaves C3 to form C5 convertase (C4bC2aC3b). In contrast to activation of the classical pathway, activation of the lectin pathway does not depend on immune complexes. In this pathway, the cascade of enzymatic reactions proceeds in this sequence: MBL identifies the pathogens to form MBL-associated serine proteases (MASP1, MASP2); MASP1 directly cleaves C3 to form C3 convertase (C3bBb), MASP2 cleaves C4 and C2 in a manner similar to that of C1s, forming C3 convertase (C4bC2a), which continues to cleave C5 to form C5 convertase (C4bC2aC3b). Thus, this pathway can cross-promote the classical and alternative pathways. The alternative pathway is activated with hydrolysis of C3 into C3(H_2O), factor B and factor D, the activation of which is also independent of immune complexes, and participates in the defense mechanisms of the early stage of inflammation[13,22-24]. The above three pathways merge into the terminal pathway, in which C5 convertase cleaves C5 to form C5a and C5b, and C5b combines with C6, C7, C8 and C9 to form the membrane attack complex (MAC). Formation of the MAC leads to cell lysis and induces cells to release inflammatory cytokines.

COMPLEMENT ACTIVATION IN ALD

ALD progresses in three distinct stages: Fatty liver, alcoholic hepatitis, and fibrosis/cirrhosis. In this review, we cite evidence that the complement system is involved

Table 1 Important publications on complement in alcoholic liver disease

Study	Complement component	Alcoholic liver disease	Species
Järveläinen *et al*[10]	C1, Crry, CD59	Alcohol-induced injury	Rat
Bykov *et al*[11]	C3	Liver steatosis	Mouse
Pritchard *et al*[8]	C3, C5, DAF	Fatty liver	Mouse
He *et al*[7]	C3	Liver steatosis	Mouse
Cohen *et al*[13]	C1q	Alcohol-induced injury	Mouse
Wlazlo *et al*[27]	C3	Liver steatosis	Human
Shen *et al*[6]	C3	Alcoholic hepatitis	Mouse
Maslowska *et al*[30]	Factor D	Alcoholic hepatitis	Mouse

Crry: Complement receptor 1-related protein y; DAF: Decay accelerating factor.

in the pathogenesis of each of these stages.

Complement activation in alcoholic fatty liver disease

The liver is the main site of fat metabolism. Disorders of fat metabolism, caused by various factors, can lead to excessive fat accumulation in the liver cells, *i.e.*, fatty liver. Long-term heavy drinking is the main independent risk factor of fatty liver disease[25], but its pathogenesis is not clearly defined. Liu *et al*[26] found that gut microbiota played a synergistic role in the liver response, and the complement system was suppressed in fatty liver which was partially due to increased blood lactic acid from enriched Lactobacillus. Abnormal complement activation reportedly enhances the sensitivity of steatotic livers to ischemia and reperfusion injury, which leads to the development of fatty liver[27,28]. Järveläinen *et al*[10] found that deposition of complement C1, C3, and C8 was increased, and the expression of membrane-binding proteins, complement receptor 1-related protein y (Crry), and CD59 was decreased in the liver cells of a mouse ALD model. These findings proved that alcohol-induced complement activation can result in ALD, at least in an experimental model. In a study in mice chronically exposed to ethanol, Cohen *et al*[13] found that lipid deposition in liver cells as well as values of liver-related serum enzymes (alanine aminotransferase and aspartate amino transferase) increased significantly; various degrees of liver cell apoptosis were also found. Moreover, with knock out of the C1q gene, hepatic steatosis in the mice was significantly decreased[13]. This study illustrated that complement activation could be associated with ethanol-induced hepatic steatosis.

Bykov *et al*[11] fed C3+/+ and C3-/- mice a high fat and high alcohol diet, respectively, and found that hepatic steatosis and significant increases in triglyceride values occurred in the C3+/+mice, whereas C3-/-mice were protected from ethanol-induced liver injury; research by Stewart *et al*[9] yielded similar results.

At the complement activation pathways, C3a converted to C3adesAg, C3adesArg which known as acylation stimulating protein had been shown to have lipogenic activity *via* its receptor C5L2, and promoted triglyceride storage in adipocytes[29,30] It was also found that C3adesArg was involved in the triglyceride metabolism[31].

Thus, activation of complement C1 and C3 appears to play a significant role in promoting fatty accumulation in the liver. Further definition of the relationship between activated complement C1, C3 and lipid metabolism in the liver may aid in the development of methods for intervention and treatment of alcoholic fatty liver disease. Besides C1 and C3, complement C5 also is involved in lipid metabolism. Bavia *et al*[32,33] found that the activation of complement C5 by high-dose ethanol exposure can affect the distribution of lipid in liver cells and serum. Less lipid and cholesterol is deposited in hepatocytes of C5- mice than in hepatocytes of C5+mice, and values of IL-17, which are involved in the synthesis and metabolism of lipid and cholesterol, are higher in C5- mice than in C5+mice[34,35]. The above-cited reports indicate that activation of C5 may play a role in the development of alcoholic fatty liver.

Complement activation in alcoholic hepatitis

ALD has many potential pathogenic factors, such as endotoxin, which may lead to complement activation and deposition in the liver cells. Shen *et al*[6] found that complement activation was involved in humans with ALD. Cohen *et al*[13] found that long-term alcohol exposure can lead to apoptosis of liver cells, and the degree of apoptosis is positively correlated with liver injury. However, whether short-term alcohol exposure can cause hepatocyte apoptosis was not known. Further research found that short-term alcohol exposure did not cause hepatocyte apoptosis, but it did promote the deposition of complement C3b and the expression of inflammatory cytokines (tumor necrosis factor and IL6). After the Cq gene was knocked out, the expression of inflammatory cytokines was significantly reduced compared to that in wild-type animals[12,13]. Experiments by Païdassi *et al*[36] and Lu *et al*[37] supported these observations.

Complement C5, a core component of the complement activation pathway, is involved in the occurrence and development of alcoholic hepatitis, in addition to fatty liver[6,8,38]. Bavia *et al*[38] documented this in a hepatitis model induced by alcohol; they found that values of pro-inflammatory cytokines (IL-6, IFN-γ, IL-1β, and others) in B6C5+ mice were significantly higher than those in B6C5- mice, and anti-inflammatory factors (IL10 and IL17) were secreted significantly more in B6C5- mice. These findings illustrated that activated C5 induced the expression of proinflammatory cytokines after alcohol exposure. Up-regulated expression of pro-inflammatory cytokines (IL-6, IFN-γ, IL-1β, and others) aids the body's defense against pathogenic microorganisms, but it also participates in the pathogenesis of alcoholic fatty liver and alcoholic hepatitis[8,39-41].

Complement activation in alcoholic hepatic fibrosis

Intrahepatic inflammatory reaction and a decrease

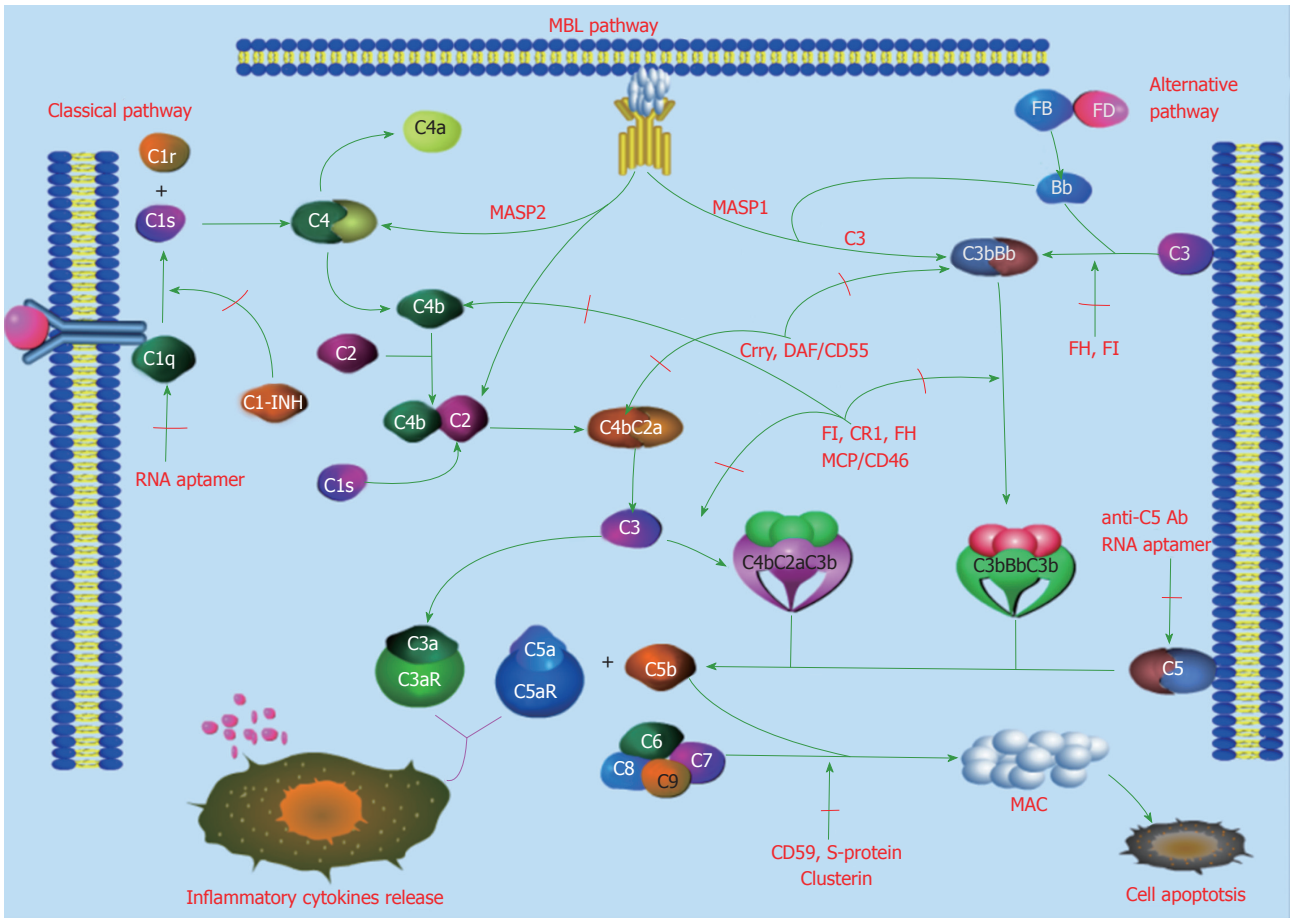

Figure 1 Complement activation pathway and regulation. Three pathways (classical, pathway, mannan-binding lectin, and alternative) are involved in complement activation. Green arrows without a red line indicate the process of complement activation. Green arrows with red line indicate the the process of inhibiting complement activation. MBL: Mannan-binding lectin; MASP: MBL-associated proteases; FB: Factor B; FH: Factor H; FI: Factor I.

in structural integrity of hepatic sinusoidal endothelial cells after long-term alcohol exposure are important inducements to liver injury. Sinusoidal endothelial cells express C5R1, which is the foundation of C5 activation-induced alcoholic hepatic fibrosis[42]. In recent years, the pathogenesis of alcoholic hepatic fibrosis has attracted worldwide attention , but the cause of the fibrosis is still not fully defined[43-45]. According to published reports[46,47], complement C3, C4 and activation of the MBL pathway are involved in the development of fibrosis. Bavia *et al*[38] using the mouse model of ALD, found that values of TGF-β, which promotes hepatic fibrosis, were significantly higher in B6C5+ mice than in B6C5- mice[38,48]. Hillebradt *et al*[49] found that the C5 gene was involved in the regulation of hepatic fibrosis on human chromosome, and further study found that C5- mice had decreased hepatic fibrosis. Thus, the evidence indicates that activation of complement C5 may promote hepatic fibrosis. Exploration of the relationship between complement activation and alcoholic hepatic fibrosis, and of possible intervention in ALD by reversing the progression of hepatic fibrosis in its early stage, seems worthwhile goals.

Complement-induced Kupffer cells activation in ALD

Kupffer cells, located in liver sinusoids, are an important part of the mononuclear phagocyte system. Alcohol exposure in the early stage can promote apoptosis of Kupffer cells, but longer exposure usually is needed[13,50,51]. Ethanol-induced activation of complement component C1q at the early stage of ALD promotes the release of inflammatory cytokines from Kupffer cells, which further promote alcoholic liver injury[51-56]. Furthermore, Kupffer cells can express C3R and C5R, then induce prostaglandin release and synthesis of pro-inflammatory cytokines[57-60]. However, in certain pathological conditions, activated C5 combines with C5R, inducing the upregulation of fibrinogen on Kupffer cells, an interaction that is believed to lead to hepatic fibrosis[22,61]. In addition, alcohol-induced upregulation of CD14 leads to Kupffer cells combining with lipopolysaccharide, which induces liver damage through the activation of TLR4 in Kupffer cells and inflammatory signaling pathways; these events can further aid in the development of hepatic fibrosis or cirrhosis[62]. Thus, Kupffer cells seem to be extensively involved in the development of ALD[63-66].

COMPLEMENT REGULATION IN ALD

Reducing inflammatory reactions by inhibiting amplification of the complement cascade and blocking the

Table 2 Complement regulators

Type of regulators	Regulators	Functions
Complement regulatory protein	DAF/CD55, Crry, FH, FI	Inhibit C3, C5 convertase
	CD59, protein S, clusterin	Inhibit MAC
Complement inhibitor	C1-INH	Inhibit C1r, C1s
Targeted inhibitor	h5G1, 1-ScFv	Inhibit C5 activation
RNA aptamer	Specifically bind C1q, C5	

DAF: Decay accelerating factor; Crry: Complement receptor 1-related protein y; FH: Factor H; FI: Factor I; MAC: Membrane attack complex.

combination of complement with the corresponding complement receptors are being pursued worldwide. Excessive activation of complement can be inhibited by self-regulation of the body (Table 2). For example, the complement regulatory protein decay accelerating factor (DAF) can inhibit C3, C5 convertase, thereby inhibiting amplification of the complement cascade. The complement regulatory protein Crry can cooperate with DAF and factor H to accelerate dissociation of C3 and C5 convertase and to cleave C3b and C4b, so that the cells avoid being attacked by autologous complement[67-69]. Deficiency of CD55/DAF and complement regulatory factors aggravate liver injury[8,11], whereas factor H can control the activity and stability of C3 convertase *via* binding with C3b[70,71]. Also, defects in the factor H gene can cause persistent activation of complement pathways and trigger various diseases[72-75]. By contrast, factor H-related proteins (FHRs), including FHR1-5, can either promote or inhibit complement activation. The degree of complement activation depends on the homeostasis between factor H and FHR[71]. However, the relationship between factor H and ALD has not been clarified and needs further research. McCullough *et al*[76] found FD-dependent amplification of complement is an adaptive response that promotes hepatic healing and recovery in response to chronic ethanol. In other complement regulatory activities, CD59, protein S and clusterin inhibit the formation of the MAC through limiting the binding of complement C9[77-81]. Membrane cofactor protein (MCP) and factor I can inhibit cells from binding with C3b and C4b[82,83].

Specific epitope structures of complement, such as anti-complement antibody, complement antisense strand, and complement mutants[84-91] have been invented, with the intent of inhibiting complement activation. In addition, complement inhibitors and RNA aptamer are being used to inhibit progression of complement-related diseases[92,93], and C1-INH and CR1 have been used in the treatment of ALD and other diseases[10].

CONCLUSION

Mounting evidence indicates that complement activation is involved in the development of ALD at all its stages - fatty liver, alcoholic hepatitis, and fibrosis/cirrhosis. Moreover, all three pathways of complement activation (classical, MBL, and alternative) promote the development of ALD. Therapeutic strategies, using various measures to inhibit complement activation, might prevent the development of ALD. Thorough understanding of the relationships between complement activation and ALD may aid in developing new approaches for the treatment of ALD.

REFERENCES

1 **Warren KR**, Murray MM. Alcoholic liver disease and pancreatitis: global health problems being addressed by the US National Institute on Alcohol Abuse and Alcoholism. *J Gastroenterol Hepatol* 2013; **28** Suppl 1: 4-6 [PMID: 23855288 DOI: 10.1111/jgh.12246]

2 **Yang Y**. Mannose Binding Lectin and kidney disease. *Int J Nephrol Urol* 2010; **30**: 241-244 [DOI: 10.3760/cma.j.issn.1673-4416.2010.02.029]

3 **Borza DB**. Glomerular basement membrane heparan sulfate in health and disease: A regulator of local complement activation. *Matrix Biol* 2017; **57-58**: 299-310 [PMID: 27609404 DOI: 10.1016/j.matbio.2016.09.002]

4 **Russkamp NF**, Ruemmler R, Roewe J, Moore BB, Ward PA, Bosmann M. Experimental design of complement component 5a-induced acute lung injury (C5a-ALI): a role of CC-chemokine receptor type 5 during immune activation by anaphylatoxin. *FASEB J* 2015; **29**: 3762-3772 [PMID: 25999468 DOI: 10.1096/fj.15-271635]

5 **Reichhardt MP**, Meri S. Intracellular complement activation-An alarm raising mechanism? *Semin Immunol* 2018 [PMID: 29631809 DOI: 10.1016/j.smim.2018.03.003]

6 **Shen H**, French BA, Liu H, Tillman BC, French SW. Increased activity of the complement system in the liver of patients with alcoholic hepatitis. *Exp Mol Pathol* 2014; **97**: 338-344 [PMID: 25217811 DOI: 10.1016/j.yexmp.2014.09.004]

7 **He S**, Atkinson C, Evans Z, Ellett JD, Southwood M, Elvington A, Chavin KD, Tomlinson S. A role for complement in the enhanced susceptibility of steatotic livers to ischemia and reperfusion injury. *J Immunol* 2009; **183**: 4764-4772 [PMID: 19752222 DOI: 10.4049/jimmunol.0900550]

8 **Pritchard MT**, McMullen MR, Stavitsky AB, Cohen JI, Lin F, Edward Medof M, Nagy LE. Differential contributions of C3, C5, and decay-accelerating factor to ethanol-induced fatty liver in mice. *Gastroenterology* 2007; **132**: 1117-1126 [PMID: 17383432 DOI: 10.1053/j.gastro.2007.01.053]

9 **Stewart S**, Jones D, Day CP. Alcoholic liver disease: new insights into mechanisms and preventative strategies. *Trends Mol Med* 2001; **7**: 408-413 [PMID: 11530336 DOI: 10.1016/S1471-4914(01)02096-2]

10 **Järveläinen HA**, Väkevä A, Lindros KO, Meri S. Activation of complement components and reduced regulator expression in alcohol-induced liver injury in the rat. *Clin Immunol* 2002; **105**: 57-63 [PMID: 12483994 DOI: 10.1006/clim.2002.5267]

11 **Bykov I**, Junnikkala S, Pekna M, Lindros KO, Meri S. Complement C3 contributes to ethanol-induced liver steatosis in mice. *Ann Med* 2006; **38**: 280-286 [PMID: 16754259 DOI: 10.1080/07853890600664608]

12 **Cohen JI**, Roychowdhury S, DiBello PM, Jacobsen DW, Nagy LE.

Exogenous thioredoxin prevents ethanol-induced oxidative damage and apoptosis in mouse liver. *Hepatology* 2009; **49**: 1709-1717 [PMID: 19205032 DOI: 10.1002/hep.22837]
13 **Cohen JI**, Roychowdhury S, McMullen MR, Stavitsky AB, Nagy LE. Complement and alcoholic liver disease: role of C1q in the pathogenesis of ethanol-induced liver injury in mice. *Gastroenterology* 2010; **139**: 664-674, 674.e1 [PMID: 20416309 DOI: 10.1053/j.gastro.2010.04.041]
14 **Orman ES**, Odena G, Bataller R. Alcoholic liver disease: pathogenesis, management, and novel targets for therapy. *J Gastroenterol Hepatol* 2013; **28** Suppl 1: 77-84 [PMID: 23855300 DOI: 10.1111/jgh.12030]
15 **Zhao J**. The metabolism and harm of alcohol. *Keji & Shenghuo* 2011; **6**: 195-198
16 **Kawaratani H**, Tsujimoto T, Douhara A, Takaya H, Moriya K, Namisaki T, Noguchi R, Yoshiji H, Fujimoto M, Fukui H. The effect of inflammatory cytokines in alcoholic liver disease. *Mediators Inflamm* 2013; **2013**: 495156 [PMID: 24385684 DOI: 10.1155/2013/495156]
17 **Cohen JI**, Chen X, Nagy LE. Redox signaling and the innate immune system in alcoholic liver disease. *Antioxid Redox Signal* 2011; **15**: 523-534 [PMID: 21126203 DOI: 10.1089/ars.2010.3746]
18 **Leung TM**, Nieto N. CYP2E1 and oxidant stress in alcoholic and non-alcoholic fatty liver disease. *J Hepatol* 2013; **58**: 395-398 [PMID: 22940046 DOI: 10.1016/j.jhep.2012.08.018]
19 **Cohen JI,** Nagy LE. Pathogenesis of alcoholic liver disease: interactions between parenchymal and non-parenchymal cells. *J Dig Dis* 2011; **12**: 3-9 [DOI: 10.1111/j.1751-2980.2010.00468.x]
20 **Nagy LE**. The Role of Innate Immunity in Alcoholic Liver Disease. *Alcohol Res* 2015; **37**: 237-250 [PMID: 26695748]
21 **Ricklin D**, Hajishengallis G, Yang K, Lambris JD. Complement: a key system for immune surveillance and homeostasis. *Nat Immunol* 2010; **11**: 785-797 [PMID: 20720586 DOI: 10.1038/ni.1923]
22 **Qin X,** Gao B. The complement system in liver diseases. Cell Mol Immunol 2006; **3**: 333-340
23 **Vieyra MB**, Heeger PS. Novel aspects of complement in kidney injury. *Kidney Int* 2010; **77**: 495-499 [PMID: 20016462 DOI: 10.1038/ki.2009.491]
24 **He W**. Medical immunology. Beijing: People's Medical Publishing House; 2010: 76-91
25 **Roerecke M**, Nanau R, Rehm J, Neuman M. Ethnicity matters: A Systematic Review and Meta-Analysis of the Non-Linear Relationship Between Alcohol Consumption and Prevalence and Incidence of Hepatic Steatosis. *EBioMedicine* 2016; **8**: 317-330 [PMID: 27428441 DOI: 10.1016/j.ebiom.2016.04.023]
26 **Liu L**, Zhao X, Wang Q, Sun X, Xia L, Wang Q, Yang B, Zhang Y, Montgomery S, Meng H, Geng T, Gong D. Prosteatotic and Protective Components in a Unique Model of Fatty Liver: Gut Microbiota and Suppressed Complement System. *Sci Rep* 2016; **6**: 31763 [PMID: 27550859 DOI: 10.1038/srep31763]
27 **Wlazlo N**, van Greevenbroek MM, Ferreira I, Jansen EH, Feskens EJ, van der Kallen CJ, Schalkwijk CG, Bravenboer B, Stehouwer CD. Activated complement factor 3 is associated with liver fat and liver enzymes: the CODAM study. *Eur J Clin Invest* 2013; **43**: 679-688 [PMID: 23586841 DOI: 10.1111/eci.12093]
28 **Copenhaver M**, Yu CY, Hoffman RP. Complement Components, C3 and C4, and the Metabolic Syndrome. *Curr Diabetes Rev* 2018 [PMID: 29663892 DOI: 10.2174/1573399814666180417122030]
29 **Gupta A**, Rezvani R, Lapointe M, Poursharifi P, Marceau P, Tiwari S, Tchernof A, Cianflone K. Downregulation of complement C3 and C3aR expression in subcutaneous adipose tissue in obese women. *PLoS One* 2014; **9**: e95478 [PMID: 24743347 DOI: 10.1371/journal.pone.0095478]
30 **Maslowska M**, Wang HW, Cianflone K. Novel roles for acylation stimulating protein/C3adesArg: a review of recent in vitro and in vivo evidence. *Vitam Horm* 2005; **70**: 309-332 [PMID: 15727809 DOI: 10.1016/S0083-6729(05)70010-8]
31 **Kalant D**, Phélis S, Fielding BA, Frayn KN, Cianflone K, Sniderman AD. Increased postprandial fatty acid trapping in subcutaneous adipose tissue in obese women. *J Lipid Res* 2000; **41**: 1963-1968 [PMID: 11108729]
32 **Bavia L**, de Castro ÍA, Massironi SM, Isaac L. Basal physiological parameters of two congenic mice strains: C5 deficient C57BL/6 and C5 sufficient A/J. *Immunol Lett* 2014; **159**: 47-54 [PMID: 24607390 DOI: 10.1016/j.imlet.2014.02.010]
33 **Bavia L**, de Castro ÍA, Isaac L. C57BL/6 and A/J Mice Have Different Inflammatory Response and Liver Lipid Profile in Experimental Alcoholic Liver Disease. *Mediators Inflamm* 2015; **2015**: 491641 [PMID: 26448681 DOI: 10.1155/2015/491641]
34 **Shi W**, Zhu Q, Gu J, Liu X, Lu L, Qian X, Shen J, Zhang F, Li G. Anti-IL-17 antibody improves hepatic steatosis by suppressing interleukin-17-related fatty acid synthesis and metabolism. *Clin Dev Immunol* 2013; **2013**: 253046 [PMID: 24396389 DOI: 10.1155/2013/253046]
35 **Hu X**, Wang Y, Hao LY, Liu X, Lesch CA, Sanchez BM, Wendling JM, Morgan RW, Aicher TD, Carter LL, Toogood PL, Glick GD. Corrigendum: Sterol metabolism controls TH17 differentiation by generating endogenous RORγ agonists. *Nat Chem Biol* 2015; **11**: 741 [PMID: 26284677 DOI: 10.1038/nchembio0915-741b]
36 **Païdassi H**, Tacnet-Delorme P, Garlatti V, Darnault C, Ghebrehiwet B, Gaboriaud C, Arlaud GJ, Frachet P. C1q binds phosphatidylserine and likely acts as a multiligand-bridging molecule in apoptotic cell recognition. *J Immunol* 2008; **180**: 2329-2338 [PMID: 18250442 DOI: 10.4049/jimmunol.180.4.2329]
37 **Lu JH**, Teh BK, Wang Ld, Wang YN, Tan YS, Lai MC, Reid KB. The classical and regulatory functions of C1q in immunity and autoimmunity. *Cell Mol Immunol* 2008; **5**: 9-21 [PMID: 18318990 DOI: 10.1038/cmi.2008.2]
38 **Bavia L**, de Castro ÍA, Cogliati B, Dettoni JB, Alves VA, Isaac L. Complement C5 controls liver lipid profile, promotes liver homeostasis and inflammation in C57BL/6 genetic background. *Immunobiology* 2016; **221**: 822-832 [PMID: 26896155 DOI: 10.1016/j.imbio.2016.01.014]
39 **Petrasek J**, Bala S, Csak T, Lippai D, Kodys K, Menashy V, Barrieau M, Min SY, Kurt-Jones EA, Szabo G. IL-1 receptor antagonist ameliorates inflammasome-dependent alcoholic steatohepatitis in mice. *J Clin Invest* 2012; **122**: 3476-3489 [PMID: 22945633 DOI: 10.1172/JCI60777]
40 **Martinon F**, Mayor A, Tschopp J. The inflammasomes: guardians of the body. *Annu Rev Immunol* 2009; **27**: 229-265 [PMID: 19302040 DOI: 10.1146/annurev.immunol.021908.132715]
41 **MacLaren R**, Cui W, Cianflone K. Adipokines and the immune system: an adipocentric view. *Adv Exp Med Biol* 2008; **632**: 1-21 [PMID: 19025110 DOI: 10.1007/978-0-387-78952-1_1]
42 **Jiang XL**, Cohen JI, Nagy LE. Pathogenesis of Alcoholic Liver Disease:Interactions between Parenchymal and Non-parenchymal Cells. *Weichangbing Xue* 2011; **16**: 131-134 [DOI: 10.3969/j.issn.1008-7125.2011.03.002]
43 **Leeming DJ**, Veidal SS, Karsdal MA, Nielsen MJ, Trebicka J, Busk T, Bendtsen F, Krag A, Møller S. Pro-C5, a marker of true type V collagen formation and fibrillation, correlates with portal hypertension in patients with alcoholic cirrhosis. *Scand J Gastroenterol* 2015; **50**: 584-592 [PMID: 25639675 DOI: 10.3109/00365521.2014.996590]
44 **Jeong WI**, Gao B. Innate immunity and alcoholic liver fibrosis. *J Gastroenterol Hepatol* 2008; **23** Suppl 1: S112-S118 [PMID: 18336653 DOI: 10.1111/j.1440-1746.2007.05274.x]
45 **Duddempudi AT**. Immunology in alcoholic liver disease. *Clin Liver Dis* 2012; **16**: 687-698 [PMID: 23101977 DOI: 10.1016/j.cld.2012.08.003]
46 **Baumann M**, Witzke O, Canbay A, Patschan S, Treichel U, Gerken G, Philipp T, Kribben A. Serum C3 complement concentrations correlate with liver function in patients with liver cirrhosis. *Hepatogastroenterology* 2004; **51**: 1451-1453 [PMID: 15362774]
47 **Chong WP**, To YF, Ip WK, Yuen MF, Poon TP, Wong WH, Lai CL, Lau YL. Mannose-binding lectin in chronic hepatitis B virus infection. *Hepatology* 2005; **42**: 1037-1045 [PMID: 16231358 DOI: 10.1002/hep.20891]
48 **Karkampouna S**, Ten Dijke P, Dooley S, Julio MK. TGFβ signaling in liver regeneration. *Curr Pharm Des* 2012; **18**: 4103-4113 [PMID:

22630085 DOI: 10.2174/138161212802430521]
49 **Hillebrandt S**, Wasmuth HE, Weiskirchen R, Hellerbrand C, Keppeler H, Werth A, Schirin-Sokhan R, Wilkens G, Geier A, Lorenzen J, Köhl J, Gressner AM, Matern S, Lammert F. Complement factor 5 is a quantitative trait gene that modifies liver fibrogenesis in mice and humans. *Nat Genet* 2005; **37**: 835-843 [PMID: 15995705 DOI: 10.1038/ng1599]
50 **Deaciuc IV**, Fortunato F, D'Souza NB, Hill DB, McClain CJ. Chronic alcohol exposure of rats exacerbates apoptosis in hepatocytes and sinusoidal endothelial cells. *Hepatol Res* 2001; **19**: 306-324 [PMID: 11251313 DOI: 10.1016/S1386-6346(00)00112-1]
51 **Miller AM**, Wang H, Park O, Horiguchi N, Lafdil F, Mukhopadhyay P, Moh A, Fu XY, Kunos G, Pacher P, Gao B. Anti-inflammatory and anti-apoptotic roles of endothelial cell STAT3 in alcoholic liver injury. *Alcohol Clin Exp Res* 2010; **34**: 719-725 [PMID: 20102572 DOI: 10.1111/j.1530-0277.2009.01141.x]
52 **Bajtay Z**, Józsi M, Bánki Z, Thiel S, Thielens N, Erdei A. Mannan-binding lectin and C1q bind to distinct structures and exert differential effects on macrophages. *Eur J Immunol* 2000; **30**: 1706-1713 [PMID: 10898508 DOI: 10.1002/1521-4141(200006)30:6<1706::AID-IMMU1706>3.0.CO;2-2]
53 **Roychowdhury S**, McMullen MR, Pritchard MT, Hise AG, van Rooijen N, Medof ME, Stavitsky AB, Nagy LE. An early complement-dependent and TLR-4-independent phase in the pathogenesis of ethanol-induced liver injury in mice. *Hepatology* 2009; **49**: 1326-1334 [PMID: 19133650 DOI: 10.1002/hep.22776]
54 **Duffield JS**, Forbes SJ, Constandinou CM, Clay S, Partolina M, Vuthoori S, Wu S, Lang R, Iredale JP. Selective depletion of macrophages reveals distinct, opposing roles during liver injury and repair. *J Clin Invest* 2005; **115**: 56-65 [PMID: 15630444 DOI: 10.1172/jci22675]
55 **Fallowfield JA**, Mizuno M, Kendall TJ, Constandinou CM, Benyon RC, Duffield JS, Iredale JP. Scar-associated macrophages are a major source of hepatic matrix metalloproteinase-13 and facilitate the resolution of murine hepatic fibrosis. *J Immunol* 2007; **178**: 5288-5295 [PMID: 17404313 DOI: 10.4049/jimmunol.178.8.5288]
56 **Nieto N**. Oxidative-stress and IL-6 mediate the fibrogenic effects of [corrected] Kupffer cells on stellate cells. *Hepatology* 2006; **44**: 1487-1501 [PMID: 17133487 DOI: 10.1002/hep.21427]
57 **Helmy KY**, Katschke KJ Jr, Gorgani NN, Kljavin NM, Elliott JM, Diehl L, Scales SJ, Ghilardi N, van Lookeren Campagne M. CRIg: a macrophage complement receptor required for phagocytosis of circulating pathogens. *Cell* 2006; **124**: 915-927 [PMID: 16530040 DOI: 10.1016/j.cell.2005.12.039]
58 **Hinglais N**, Kazatchkine MD, Mandet C, Appay MD, Bariety J. Human liver Kupffer cells express CR1, CR3, and CR4 complement receptor antigens. An immunohistochemical study. *Lab Invest* 1989; **61**: 509-514 [PMID: 2478758]
59 **Kumar V**, Ali SR, Konrad S, Zwirner J, Verbeek JS, Schmidt RE, Gessner JE. Cell-derived anaphylatoxins as key mediators of antibody-dependent type Ⅱ autoimmunity in mice. *J Clin Invest* 2006; **116**: 512-520 [PMID: 16453025 DOI: 10.1172/jci25536]
60 **Schieferdecker HL**, Rothermel E, Timmermann A, Götze O, Jungermann K. Anaphylatoxin C5a receptor mRNA is strongly expressed in Kupffer and stellate cells and weakly in sinusoidal endothelial cells but not in hepatocytes of normal rat liver. *FEBS Lett* 1997; **406**: 305-309 [PMID: 9136907 DOI: 10.1016/S0014-5793(97)00292-5]
61 **Schlaf G**, Schmitz M, Heine I, Demberg T, Schieferdecker HL, Götze O. Upregulation of fibronectin but not of entactin, collagen IV and smooth muscle actin by anaphylatoxin C5a in rat hepatic stellate cells. *Histol Histopathol* 2004; **19**: 1165-1174 [PMID: 15375759 DOI: 10.14670/HH-19.1165]
62 **Guo SP**, Zhang WF, Gong JP. Research advance in the origin and immunological function of kupffer cells. *Sheng Li Ke Xue Jin Zhan* 2016; **47**: 57-60 [PMID: 27424408]
63 **Enomoto N**, Ikejima K, Yamashina S, Hirose M, Shimizu H, Kitamura T, Takei Y, Sato And N, Thurman RG. Kupffer cell sensitization by alcohol involves increased permeability to gut-derived endotoxin. *Alcohol Clin Exp Res* 2001; **25**: 51S-54S [PMID: 11410742 DOI: 10.1111/j.1530-0277.2001.tb02418.x]
64 **Wheeler MD**, Thurman RG. Up-regulation of CD14 in liver caused by acute ethanol involves oxidant-dependent AP-1 pathway. *J Biol Chem* 2003; **278**: 8435-8441 [PMID: 12482856 DOI: 10.1074/jbc.M212076200]
65 **Schieferdecker HL**, Schlaf G, Jungermann K, Götze O. Functions of anaphylatoxin C5a in rat liver: direct and indirect actions on nonparenchymal and parenchymal cells. *Int Immunopharmacol* 2001; **1**: 469-481 [PMID: 11367531 DOI: 10.1016/S1567-5769(00)00038-2]
66 **Reid DT**, Reyes JL, McDonald BA, Vo T, Reimer RA, Eksteen B. Kupffer Cells Undergo Fundamental Changes during the Development of Experimental NASH and Are Critical in Initiating Liver Damage and Inflammation. *PLoS One* 2016; **11**: e0159524 [PMID: 27454866 DOI: 10.1371/journal.pone.0159524]
67 **Barata L**, Miwa T, Sato S, Kim D, Mohammed I, Song WC. Deletion of Crry and DAF on murine platelets stimulates thrombopoiesis and increases factor H-dependent resistance of peripheral platelets to complement attack. *J Immunol* 2013; **190**: 2886-2895 [PMID: 23390291 DOI: 10.4049/jimmunol.1202536]
68 **Laskowski J**, Renner B, Le Quintrec M, Panzer S, Hannan JP, Ljubanovic D, Ruseva MM, Borza DB, Antonioli AH, Pickering MC, Holers VM, Thurman JM. Distinct roles for the complement regulators factor H and Crry in protection of the kidney from injury. *Kidney Int* 2016; **90**: 109-122 [PMID: 27165610 DOI: 10.1016/j.kint.2016.02.036]
69 **Liu F**, Wu L, Wu G, Wang C, Zhang L, Tomlinson S, Qin X. Targeted mouse complement inhibitor CR2-Crry protects against the development of atherosclerosis in mice. *Atherosclerosis* 2014; **234**: 237-243 [PMID: 24685815 DOI: 10.1016/j.atherosclerosis.2014.03.004]
70 **Vernon KA**, Ruseva MM, Cook HT, Botto M, Malik TH, Pickering MC. Partial Complement Factor H Deficiency Associates with C3 Glomerulopathy and Thrombotic Microangiopathy. *J Am Soc Nephrol* 2016; **27**: 1334-1342 [PMID: 26374608 DOI: 10.1681/ASN.2015030295]
71 **Medjeral-Thomas N**, Pickering MC. The complement factor H-related proteins. *Immunol Rev* 2016; **274**: 191-201 [PMID: 27782332 DOI: 10.1111/imr.12477]
72 **Faber C**, Williams J, Juel HB, Greenwood J, Nissen MH, Moss SE. Complement factor H deficiency results in decreased neuroretinal expression of Cd59a in aged mice. *Invest Ophthalmol Vis Sci* 2012; **53**: 6324-6330 [PMID: 22918646 DOI: 10.1167/iovs.12-10385]
73 **Wilson V**, Darlay R, Wong W, Wood KM, McFarlane J, Schejbel L, Schmidt IM, Harris CL, Tellez J, Hunze EM, Marchbank K, Goodship JA, Goodship TH. Genotype/phenotype correlations in complement factor H deficiency arising from uniparental isodisomy. *Am J Kidney Dis* 2013; **62**: 978-983 [PMID: 23870792 DOI: 10.1053/j.ajkd.2013.05.020]
74 **Schejbel L**, Schmidt IM, Kirchhoff M, Andersen CB, Marquart HV, Zipfel P, Garred P. Complement factor H deficiency and endocapillary glomerulonephritis due to paternal isodisomy and a novel factor H mutation. *Genes Immun* 2011; **12**: 90-99 [PMID: 21270828 DOI: 10.1038/gene.2010.63]
75 **Blackall DP**, Marques MB. Hemolytic uremic syndrome revisited: Shiga toxin, factor H, and fibrin generation. *Am J Clin Pathol* 2004; **121** Suppl: S81-S88 [PMID: 15298153 DOI: 10.1309/06W402EHNGVVB24C]
76 **McCullough RL**, McMullen MR, Sheehan MM, Poulsen KL, Roychowdhury S, Chiang DJ, Pritchard MT, Caballeria J, Nagy LE. Complement Factor D protects mice from ethanol-induced inflammation and liver injury. *Am J Physiol Gastrointest Liver Physiol* 2018; **315**: G66-G79 [PMID: 29597356 DOI: 10.1152/ajpgi.00334.2017]
77 **Wu G**, Hu W, Shahsafaei A, Song W, Dobarro M, Sukhova GK, Bronson RR, Shi GP, Rother RP, Halperin JA, Qin X. Complement regulator CD59 protects against atherosclerosis by restricting the formation of complement membrane attack complex. *Circ Res* 2009; **104**: 550-558 [PMID: 19131645 DOI: 10.1161/CIRCRESAHA.108.191361]
78 **Wickham SE**, Hotze EM, Farrand AJ, Polekhina G, Nero TL,

Tomlinson S, Parker MW, Tweten RK. Mapping the intermedilysin-human CD59 receptor interface reveals a deep correspondence with the binding site on CD59 for complement binding proteins C8alpha and C9. *J Biol Chem* 2011; **286**: 20952-20962 [PMID: 21507937 DOI: 10.1074/jbc.M111.237446]

79 **Huang Y**, Fedarovich A, Tomlinson S, Davies C. Crystal structure of CD59: implications for molecular recognition of the complement proteins C8 and C9 in the membrane-attack complex. *Acta Crystallogr D Biol Crystallogr* 2007; **63**: 714-721 [PMID: 17505110 DOI: 10.1107/s0907444907015557]

80 **Podack ER**, Müller-Eberhard HJ. Binding of desoxycholate, phosphatidylcholine vesicles, lipoprotein and of the S-protein to complexes of terminal complement components. *J Immunol* 1978; **121**: 1025-1030 [PMID: 690431]

81 **McDonald JF**, Nelsestuen GL. Potent inhibition of terminal complement assembly by clusterin: characterization of its impact on C9 polymerization. *Biochemistry* 1997; **36**: 7464-7473 [PMID: 9200695 DOI: 10.1021/bi962895r]

82 **Chu Q,** Liu X, Xiang Y, Liu QW. Study on the structure and function of complement C3. *Zhongguo Mianyixue Zazhi* 2014; **30**: 549-553 [DOI: 10.3969/j.issn.1000-484X.2014.04.031]

83 **Liu Q,** Xing G. Complement regulatory proteins and IgA nephropathy. *Zhongguo Shiyong Yike* 2013; **40**: 122-124 [DOI: 10.3760/cma.j.issn.1674-4756.2013.12.059]

84 **de Haas CJ**, Veldkamp KE, Peschel A, Weerkamp F, Van Wamel WJ, Heezius EC, Poppelier MJ, Van Kessel KP, van Strijp JA. Chemotaxis inhibitory protein of Staphylococcus aureus, a bacterial antiinflammatory agent. *J Exp Med* 2004; **199**: 687-695 [PMID: 14993252 DOI: 10.1084/jem.20031636]

85 **Fujita E**, Farkas I, Campbell W, Baranyi L, Okada H, Okada N. Inactivation of C5a anaphylatoxin by a peptide that is complementary to a region of C5a. *J Immunol* 2004; **172**: 6382-6387 [PMID: 15128829 DOI: 10.4049/jimmunol.172.10.6382]

86 **Guo RF**, Ward PA. Role of C5a in inflammatory responses. *Annu Rev Immunol* 2005; **23**: 821-852 [PMID: 15771587 DOI: 10.1146/annurev.immunol.23.021704.115835]

87 **Nikiforovich GV**, Baranski TJ. Structural models for the complex of chemotaxis inhibitory protein of Staphylococcus aureus with the C5a receptor. *Biochem Biophys Res Commun* 2009; **390**: 481-484 [PMID: 19799858 DOI: 10.1016/j.bbrc.2009.09.113]

88 **Otto M**, Hawlisch H, Monk PN, Müller M, Klos A, Karp CL, Köhl J. C5a mutants are potent antagonists of the C5a receptor (CD88) and of C5L2: position 69 is the locus that determines agonism or antagonism. *J Biol Chem* 2004; **279**: 142-151 [PMID: 14570896 DOI: 10.1074/jbc.M310078200]

89 **Li BS,** Cui SH, Lv FL, Chen Y, Li YC, Hu CX, Chen CY. Protective effects of complementary peptide of anaphylatoxin C5a on lung injury of experimental sepsis mouse. *Disan Junyi Daxue Xuebao* 2006; **28**: 4 [DOI: 10.3321/j.issn:1000-5404.2006.09.020]

90 **Wu Z,** Lv F, Lv C, Wu YZ. Blocking effects of anti-sense peptide of C5a on the adhesion between pulmonary vascular endothelial cell and neutrophil. *Zhongduo Mianyixue Zazhi* 2003; **19**: 5

91 **Li L**, Chen L, Zang J, Tang X, Liu Y, Zhang J, Bai L, Yin Q, Lu Y, Cheng J, Fu P, Liu F. C3a and C5a receptor antagonists ameliorate endothelial-myofibroblast transition via the Wnt/β-catenin signaling pathway in diabetic kidney disease. *Metabolism* 2015; **64**: 597-610 [PMID: 25682062 DOI: 10.1016/j.metabol.2015.01.014]

92 **Lee JH**, Kim H, Ko J, Lee Y. Interaction of C5 protein with RNA aptamers selected by SELEX. *Nucleic Acids Res* 2002; **30**: 5360-5368 [PMID: 12490703 DOI: 10.1093/nar/gkf694]

93 **Biesecker G**, Dihel L, Enney K, Bendele RA. Derivation of RNA aptamer inhibitors of human complement C5. *Immunopharmacology* 1999; **42**: 219-230 [PMID: 10408383 DOI: 10.1016/S0162-3109(99)00020-X]

P- Reviewer: Marcos M, Metin U, Morales-González JA, Kollmann D
S- Editor: Ma YJ **L- Editor**: Ma JY **E- Editor**: Bian YN

World Journal of Hepatology

Submit a Manuscript: http://www.f6publishing.com

World J Hepatol 2018 October 27; 10(10): 670-684

DOI: 10.4254/wjh.v10.i10.670

ISSN 1948-5182 (online)

MINIREVIEWS

Era of direct acting anti-viral agents for the treatment of hepatitis C

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Author contributions: Ahmed M solely contributed to this work.

Conflict-of-interest statement: No potential conflicts of interest relevant to this article were reported.

Manuscript source: Unsolicited manuscript

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Received: April 11, 2018
Peer-review started: April 11, 2018
First decision: May 2, 2018
Revised: May 15, 2018
Accepted: May 23, 2018
Article in press: May 24, 2018
Published online: October 27, 2018

Abstract

Hepatitis C infection is universal and the most common indication of liver transplantation in the United States. The period of less effective interferon therapy with intolerable side effects has gone. Now we have stepped into the era of direct acting anti-viral agents (DAAs) against hepatitis C virus. Treatment of hepatitis C is now extremely effective, tolerable and requires a short duration of intake of oral agents. Less monitoring is required with the current therapy and drug-drug interactions are less than the previous regimen. The current treatment options of chronic hepatitis C with various DAAs are discussed in this article.

Key words: Direct acting anti-viral agents; Hepatitis C virus infection; Post-liver transplant; Hepatitis C virus/human immunodeficiency virus co-infection

Core tip: Treatment of hepatitis C has now become much easy and simple with the advent of direct acting anti-viral agents (DAAs) against hepatitis C virus (HCV). Although the DAAs are highly effective in eradicating HCV infection, they have different mechanisms of action, side effects, resistance factors and drug-drug interactions. The treatment also varies in special situations like HCV/ human immunodeficiency virus co-infection and post-liver transplant patients. Physicians treating patients with HCV infection should have a clear knowledge about the DAAs as well as the current guidelines.

Ahmed M. Era of direct acting anti-viral agents for the treatment of hepatitis C. *World J Hepatol* 2018; 10(10): 670-684 Available from: URL: http://www.wjgnet.com/1948-5182/full/v10/i10/670.htm DOI: http://dx.doi.org/10.4254/wjh.v10.i10.670

INTRODUCTION

Chronic hepatitis C infection is common in the United

States and throughout the world. About 3 million people in the United States and 177.5 million people in the world suffer from chronic hepatitis C infection[1]. Hepatitis C virus (HCV) contains structural proteins - core or C protein, E1 and E2 envelope proteins, and nonstructural proteins - P7, NS2, NS3, NS4A, NS4B, NS5A and NS5B[2]. HCV contains error prone RNA dependent RNA polymerase and mutation is present during each viral replication[3]. As a result, HCV exists as a quasispecies characterized by the presence of various genetically distinct variants which protect the HCV from host defenses as well as anti-HCV agents[4]. DAA are specific antiviral agents targeting the critical steps of HCV replication. With the development of direct acting anti-viral agent (DAA), the treatment of chronic hepatitis C has been completely revolutionized. In 1990's when we had only Interferon therapy, the sustained virological response (SVR) was 17% with many side effects[5]. Then Pegylated Interferon and Ribavirin came after few years but still the SVR was 40% to 50%[6]. In 2011, the first generation NS3/4A protease inhibitors (Telaprevir and Boceprevir) were launched to be used with pegylated interferon and ribavirin for the treatment of hepatitis C genotype 1 infection. The SVR improved to about 70% at the expense of many side effects, drug-drug interactions and complex regimen of administration of medications and cost[7]. Now we have 2nd generation NS3/4A protease inhibitors, NS5A inhibitors and NS5B inhibitors. They have markedly improved the efficacy of treatment of HCV infection. The different DAAs with their dose, efficacy, side effects, drug-drug interactions as well as specific treatment against different genotypes of HCV will be discussed in the following sections (Figure 1).

NS3/4A protease inhibitors

HCV NS3/4A is a multifunctional protein composed of a membrane-targeted serine protease domain and a helicase domain. NS3/4A protease is responsible for maturation of a viral polyprotein that cleaves and generates NS3, NS4A, NS4B, NS5A and NS5B. Thus NS3/4A protease is essential for viral replication[8]. NS3/4A protease inhibitors (PIs) are classified into first generation PIs and second generation PIs[9]. The first generation PIs include boceprevir and telaprevir. They are no longer used in clinical practice. The second generation PIs includes simeprevir, paritaprevir, grazoprevir, glecaprevir and voxilaprevir. They are highly potent DAAs but they have low barrier to resistance and limited genotypic coverage.

Simeprevir is a once daily macrocyclic 2nd - waive NS3/4A protease inhibitor approved to be used with pegylated interferon and ribavirin for the treatment of chronic hepatitis C genotype 1 patients in 2013. In QUEST-1 and QUEST-2 trials (simeprevir + PEG + RBV *vs* PEG + RBV in treatment-naïve genotype 1 patients), 80% to 81% of treatment-naïve patients achieved SVR12, *i.e.*, negative viral load 12 wk after completion of treatment[10,11]. Patients who had Q80K polymorphism had only 58% SVR12. In PROMISE trial, Simeprevir was given with pegylated interferon and ribavirin to treatment (Interferon)-experienced hepatitis C genotype 1 patients. The SVR12 was 79.2%. The SVR 12 also varied with the host IL28B genotype. Patient's IL28B genotype is involved in the host immune response to HCV infection. There are 3 IL28B subtypes: CC, CT and TT. The IL28B CC genotypes had the highest response and the IL28B TT genotypes had the lowest response[12]. In COSMOS trial, combination of simeprevir and sofosbuvir (NS5B inhibitor) with or without ribavirin were given to HCV genotype 1 infected patients - both treatment-naïve patients and non-responders to pegylated interferon and ribavirin for 12 wk and 24 wk. Ninety-two percent to 94% of patients achieved SVR12[13]. In OPTIMIST-1 trial, combination of simeprevir and sofosbuvir was given to treatment-naïve non-cirrhotic HCV genotype 1 infected patients. Ninety-seven percent of patients who received this combination achieved SVR12 in the 12 wk arm whereas only 83% achieved SVR12 in the 8-wk arm[14]. In OPTIMIST-2 trial, cirrhotic patients due to HCV genotype 1 infection received combination of simeprevir and sofosbuvir for 12 wk. SVR12 was achieved in 83% of patients: 88% in treatment-naïve patients and 79% in treatment-experienced patients[15].

The main side effects of Simeprevir include headache, fatigue, nausea, photosensitivity and skin rash[16]. Latent HBV infection could be reactivated if patient is on simeprevir. Simprevir can also cause hepatic failure if used in decompensated cirrhosis of liver. Simeprevir has sulphur moiety which may cause sulphur allergy. Patients on simeprevir should not take moderate to high intensity enzyme-inducers or enzyme-inhibitors as they may decrease or increase the serum levels of simeprevir. Prior to the use of Simeprevir, it is recommended to do Q80K polymorphism screening (HCV GenoSure NS3/4A Assay) which has been found in 35% of patients infected with HCV genotype 1, and in more than 40% of patients infected with HCV genotype 1a[17]. In the Interferon free era, combination of simeprevir 150 mg/d and sofosbuvir 400 mg/d for 12 wk is recommended as an alternative regimen in the treatment of treatment-naïve genotype 1a or 1b patients without cirrhosis as per the American Association for the Study of Liver Diseases (AASLD).

Drug-drug interactions: Common medications which are not recommended to be co-administered with Simeprevir include systemic antibiotics like clarithromycin, erythromycin, systemic antifungals like fluconazole, ketoconazole, itraconazole, antimycobacterials like rifampin, rifabutin, anti-hepatitis C medication ledipasvir, anti-human immunodeficiency virus (HIV) medications like ritonavir, darunavir, efavirenz, nevirapine, etravirine, delavirdine, atazanavir, nelfinavir, saquinavir, indinavir, fosamprenavir, tipranavir, anti-arrhythmic drug like amiodarone, anticonvulsants like phenytoin, phenobarbital, carbamazepine, dexamethasone, cyclosporine, milk thistle and St. John's Wort[18].

Paritaprevir is a macrocyclic NS3/4A protease inhibitor used in combination with low dose ritonavir which is a strong CYP3A inhibitor, and thus increases

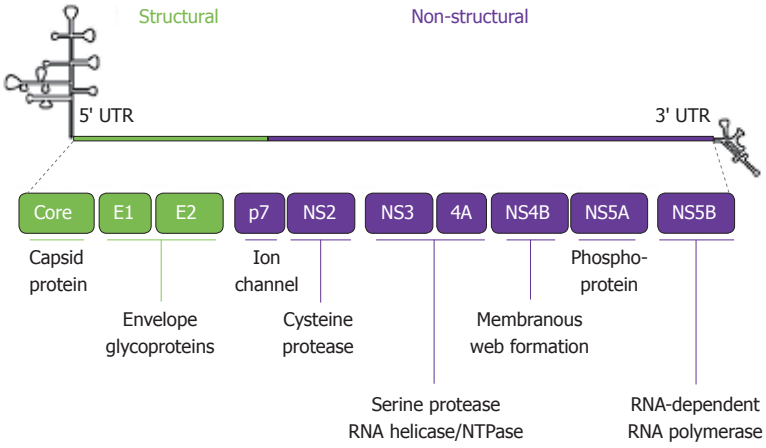

Figure 1 Hepatitis C virus RNA (genome).

peak and trough level of Paritaprevir[19]. Paritaprevir/ritonavir-ombitasvir (NS5A inhibitor) - a 3 drug fixed dose combination tablet co-packaged with dasabuvir (NS5B inhibitor) tablet in Viekira Pak, and dasabuvir, ombitasvir, paritaprevir and ritonavir - a 4 drug fixed dose combination tablet in Viekira XR with or without ribavirin are approved for the treatment of chronic hepatitis C genotype 1[20]. Paritaprevir/ritonavir-ombitasvir (in TECHNIVIE) with ribavirin is approved for the treatment hepatitis C genotype 4 without cirrhosis of liver. Viekira Pak and Viekira XR carry a higher pill burden than other regimen. They are also contraindicated in advanced cirrhosis of liver (Child-Pugh B and C). They are metabolized mainly in the liver. As a result, no dose adjustment is needed even in severe renal failure including those on dialysis[21]. If the patient has HBV/HCV co-infection and not receiving HBV antiviral therapy, HBV reactivation can occur leading to severe hepatitis, hepatic failure and death. In RUBY-1 trial, 90% of patients with HCV genotype 1 infection and end-stage renal disease (CKD 4 and CKD 5) had SVR12, *i.e.*, negative serum HCV RNA after 12 wk of therapy with dasabuvir, ombitasvir, paritaprevir and ritonavir[22]. In case of HCV genotype 1a infection - Viekira should be continued for 12 wk in non-cirrhotics and 24 wk in cirrhotics. In case of HCV genotype 1b infection - Viekira should be given for 12 wk irrespective of cirrhosis or non-cirrhosis status[23]. In liver transplant recipients with normal liver function and mild hepatic fibrosis, Viekira pak or Viekira XR with weight-based ribavirin should be given for 24 wk. The common side effects of Viekira include nausea, insomnia, itching and asthenia.

Drug-drug interaction: Common medications which are contra-indicated with Viekira include lipid lowering agent gemfibrozil, HMG co-reductase inhibitors - simvastatin, atorvastatin, lovastatin, anti-arrhythmic drug dronedarone, α-1 adrenoreceptor blocker alfuzosin, anti-anginal medication ranolazine, phosphodiesterase inhibitor sildenafil, anticonvulsants like phenytoin, phenobarbital, carbamazepine, sedative/hypnotics triazolam, midazolam, anti-gout medication colchicine, antimycobacterial medication rifampin, antipsychotic medication lurasidone and pimozide, ergot agents methylergonovine, ergotamine and dihydroergotamine, ethinyl estradiol containing medications, prokinetic agent cisapride, herbal agent St. John's Wort, immunosuppressive agents tacrolimus, sirolimus and everolimus, and anti-HIV medication efavirenz[24].

Grazoprevir is a macrocyclic NS3/4A serine protease inhibitor against HCV genotype 1 and 4. It is used in combination with Elbasvir (NS5A inhibitor) for the treatment of HCV genotypes 1 and 4. In clinical trials, combination of elbasvir and grazoprevir (Zepatier) has been shown to achieve SVR in 94% to 97% of cases of genotype 1 infection and 97% to 100% of cases of genotype 4 infection. The overall SVR for non-cirrhotics was 97% and for cirrhotics 95.7%[25]. The regimen was effective in stage 4 to 5 chronic kidney disease[26]. In case of HCV genotype 1a infection, NS5A resistance testing should be done prior to initiation of elbasvir and grazoprevir therapy. In 10% to 15% of cases, NS5A polymorphism is positive and in that case weight-based ribavirin (less than 66 kg = 800 mg/d, 66 to 80 kg = 1000 mg/d, 81 to 105 kg = 1200 mg/d, greater than 105 kg = 1400 mg/d administered in two divided doses with food) should be added and the duration should be increased from 12 to 16 wk. Otherwise HCV genotype 1a or 1b infection requires only 12 wk of Zepatier therapy irrespective of exposure to pegylated interferon and ribavirin (PEG/RIBA), and presence of cirrhosis of liver. In case of HCV genotype 4 infection, the duration of Zepatier therapy is 12 wk for treatment-naïve patients, but it should be extended to 16 wk if PEG/RIBA experienced with prior on-treatment virologic failure[27]. In C-ISLE study, Elbasvir/Grazoprevir plus Sofosbuvir was given to patients with compensated cirrhosis due to HCV genotype 3 infection for 12 wk. The SVR12 was 96% in treatment-naïve patients and 100% in PEG-interferon/Ribavirin experienced patients[28]. HBV reactivation can occur on grazoprevir/elbasvir therapy if the patient is co-infected with HBV and not receiving anti-HBV therapy. HBV serology (HBsAg and anti-HBc) and HCV resistance

associated polymorphisms as mentioned before should be tested prior to initiation of Zepatier therapy. Common side effects include headache, nausea and fatigue[29].

Common medications which are not recommended to be used with Zepatier include antibiotic Nafcillin, antifungal oral ketoconazole, anti-HIV medication etravirine, cobicistat containing medications like tenofovir, emtricitabine, cobicistat, HMG Co-A reductase inhibitors atorvastatin (dose should not exceed > 20 mg/d), rosuvastatin (dose should not exceed > 10 mg/d), simvastatin, lovastatin, fluvastatin (should be closely monitored for myopathy), nacolepsy medication modafinil, immunosuppressant tacrolimus, and endothelin antagonist bosentan[29].

Drug-drug interaction: Glecaprevir is a NS3/4A protease inhibitor coformulated with NS5A inhibitor pibrentasvir (Mavyret). Glecaprevir-pibrentasvir combination has been shown to have pangenotypic anti-HCV activity. In EXPEDITION-1 study, glecaprevir (300 mg) - pibrentasvir (120 mg) coformulation when given daily for 12 wk to treatment-naïve or treatment experienced (pegylated interferon plus ribavirin or sofosbuvir plus ribavirin) patients with HCV genotypes, infection and compensated cirrhosis, 99% of patients achieved SVR at 12 wk[30]. Another study showed glecaprevir-pibrentasvir combination when given for 8 wk to patients with HCV genotype-1 and 3 infection had SVR 12 of 99.1% and 95% respectively[31]. Patients with chronic kidney disease (CKD) have more chronic hepatitis C, particularly in patients on hemodialysis, 8.4% hemodialysis patients had chronic hepatitis C in the year of 2000. When patients with severe renal failure (stage 4 or 5 CKD or dialysis dependence) and HCV genotype 1, 2, 3, 4, 5 or 6 infection with or without compensated cirrhosis, treatment-naïve or treatment-experienced (pegylated interferon, ribavirin, sofosbuvir or a combination of these medications), were treated with glecaprevir-pibrentasvir combination for 12 wk, they had sustained SVR12 of 98%[32]. No dose adjustment was required in patients with severe renal failure or in hemodialysis patients[33].

Three tablets of fixed dose Glecaprevir 100 mg/ Pibrentasvir 40 mg (*i.e.*, 300 mg/120 mg total dose) PO once daily is given with food.

In treatment-naïve patients[33], the recommended duration is 8 wk for genotypes 1 to 6 infection without cirrhosis. But the treatment duration is 12 wk for genotypes 1 to 6 infection with compensated cirrhosis (Child-Pugh A). In treatment-experienced patients. In non-cirrhotics: Genotype 1 and NS5A inhibitor prior treatment: 16 wk. Genotype 1 and NS3/4A protease inhibitor prior treatment: 12 wk. Genotypes 1, 2, 4, 5, or 6 (prior treatment with boceprevir, or telaprevir or simeprevir with pegylated interferon and ribavirin, simeprevir and sofosbuvir): 8 wk. Genotype 3 (prior treatment with boceprevir, or telaprevir or simeprevir with pegylated interferon and ribavirin, simeprevir and sofosbuvir): 16 wk.

In treatment-experienced patients[33] with compensated cirrhotics (Child-Pugh A): Genotype 1 and NS5A inhibitor prior treatment: 16 wk. Genotype 1 and NS3/4A protease inhibitor prior treatment: 12 wk. Genotypes 1, 2, 4, 5, or 6 (prior treatment with boceprevir, or telaprevir or simeprevir with pegylated interferon and ribavirin, simeprevir and sofosbuvir): 12 wk. Genotype 3 (prior treatment with, boceprevir, or telaprevir or simeprevir with pegylated interferon and ribavirin, simeprevir and sofosbuvir): 16 wk. Common side effects of Mavyret include headache, fatigue, nausea and diarrhea.

Commonly used medications which can cause drug interaction with Mavyret include HMG Co-A reductase inhibitors atorvastatin, lovastatin, simvastatin, rosuvastatin, pravastatin, pitavastatin, fluvastatin, ethinyl estradiol containing medications, anticoagulant dabigatran, antiarrhythmic digoxin, anticonvulsant carbamazepine, antimycobacterial rifampin, anti-HIV medications efavirenz, ritonavir, lopinavir, darunavir, atazanavir, herbal medication St. John's Wort, and immunosuppressant cyclosporine[34].

Voxilaprevir is a NS3/4A protease inhibitor. It is used in the fixed dose combination of sofosbuvir and velpatasvir (NS5A inhibitor), commercially available as Vosevi. In POLARIS-1, sofosbuvir (400 mg)/velpatasvir (100 mg)/voxilaprevir (100 mg) single pill was given daily for 12 wk to NS5A inhibitor-experienced patients with HCV genotype 1-6 infection. In POLARIS-4, sofosbuvir/velpatasvir/voxilaprevir was given daily for 12 wk to DAA experienced (but not NS5A experienced) patients with HCV genotype 1-3 infection, and also to patients with HCV genotype 4 infection. Forty-six percent of all these patients had compensated cirrhosis of liver. In POLARIS-1, the SVR was 96%, and in POLARIS-4, the SVR was 98%[35]. Currently, Vosevi is approved for retreatment of: (1) HCV genotype 1-6 infection in adults who were previously treated with an NS5A inhibitor-containing regimen; and (2) HCV genotype 1a or 3 infection in adults who were previously treated with sofosbuvir-containing regimen without an NS5A inhibitor[36]. Vosevi can be given to patients without cirrhosis or with compensated cirrhosis, and no dose adjustment is required even in severe renal impairment with glomerular filtration rate (GFR) < 30 mL/min or end stage renal disease. If the patient is HBV coinfected and not receiving anti-HBV therapy, HBV reactivation can occur during or after completion of treatment with Vosevi. So, serological evidence of HBV infection (HBVsAg and anti-HBVc antibody) should be looked for before initiation of therapy with Vosevi. Common side effects of Vosevi include headache, nausea, diarrhea insomnia, asthenia and fatigue.

Drug-drug interaction: Certain medications are not recommended with Vosevi. These include anatacids like aluminum or magnesium hydroxide, H2 receptor antagonists like Famotidine, proton pump inhibitor like omeprazole, anticoagulant like dabigatran, antiarrhythmic agents like digoxin, amiodarone, HMG Co-A reductase inhibitors atorvastatin, lovastatin, fluvastatin,

simvastatin, rosuvastatin, pravastatin, pitavastatin, anticonvulsants like phenytoin, phenobarbital, carbamazepine, oxcarbazepin, anti-HIV medications efavirenz, tenofovir, lopinavir, atazanavir, tipranavir/ritonavir, anti-mycobactrial agents like rifampin, rifabutin, rifapentin, immunosuppressant cyclosporine, and herbal supplement St. John's Wort[37].

NS5A inhibitors

Inhibit hyperphosphorylation of NS5A phosphoprotein which is necessary for HCV RNA replication, and they also cause transfer of NS5A from the endoplasmic reticulum to lipid droplets in HCV replicon-containing cells leading to significant reduction of HCV RNA in cell culture[38]. Ledipasvir, ombitasvir, daclatasvir, elbasvir, velpatasvir and pibrentasvir are NS5A inhibitors. They are highly potent DAA with multigenetic coverage and intermediate barrier to resistance.

Ledipasvir is a NS5A inhibitor used as part of combination therapy with Sofosbuvir (NS5B inhibitor) for the treatment of chronic hepatitis C. The fixed dose combination called Harvoni (90 mg of Ledipasvir and 400 mg Sofosbuvir) developed by Gilead Sciences was approved by the FDA in 2014.

Effectiveness, duration and need for addition of ribavirin with this medication depend on viral load, genotype, compensated or decompensated cirrhosis, treatment naïve or treatment-experienced status, and pre or post-transplant status.

In ION-1 phase 3 clinical trial, 99% of treatment naïve patients with chronic hepatitis C genotype 1 who received Ledispavir-Sofosbuvir combination for 12 wk achieved SVR[39]. In ION-2 phase 3 clinical trial, 99% of patients with previously treated genotype-1 HCV infection who received Ledispavir-Sofosbuvir combination for 24 wk had SVR[40]. Patients with HCV genotype-1 infection and compensated cirrhosis were studied with Ledispavir-Sofosbuvir combination for 12 and 24 wk in treatment naïve and treatment experienced patients with or without ribavirin[41]. The overall SVR was 96%: 98% in treatment-naïve group *vs* 95% in treatment-experienced group, 95% in 12 wk therapy group *vs* 98% in 24 wk therapy group, 95% without ribavirin *vs* 97% with ribavirin. The only group who did not do well was the treatment-experienced group who received Ledispavir-Sofosbuvir combination for only 12 wk and without ribavirin. Their SVR was 90%[41]. As a result, this combination is recommended to be continued for 24 wk in treatment-experienced HCV genotype-1 infection with compensated cirrhosis. Patients with HCV genotype-4 with or without compensated cirrhosis were treated with Ledispavir-Sofosbuvir combination with or without ribavirin. Overall SVR12 was 95.4% in non-cirrhotics and 93.2% in cirrhotics[42]. Patients with HCV genotype 5 with or without compensated cirrhosis were treated with Ledspavir-Sofosbuvir for 12 wk in a multi-center open-label study. The SVR12 was 95% in both treatment-naïve and treatment-experienced groups; 89% of cirrhotics and 97% of non-cirrhotics achieved SVR12[43]. A small study showed when fixed dose combination of Ledispavir-Sofosbuvir was given to treatment-naïve and treatment-experienced patients with HCV genotype 6 infection for 12 wk, 96% of them achieved SVR12[44]. At the present time, Ledispavir-Sofosbuvir combination is indicated for the treatment of HCV genotype 1, 4, 5 and 6 infections with or without compensated cirrhosis. It is also used in HCV genotype 1 infection with decompensated cirrhosis in combination with ribavirin. Post liver transplant recipients who have HCV genotype 1 or 4 infection with or without compensated cirrhosis can be treated with Ledispavir-Sofosbuvir plus ribavirin for 12 wk. In one study, 96% of patients who had F-0 to F-3 fibrosis or compensated cirrhosis achieved SVR[45].

Common side effects of Harvoni include headache, fatigue, nausea and diarrhea. Harvoni like other DAAs can reactivate HBV if the patient is HBV/HCV co-infected and not receiving anti-HBV therapy. So prior to initiation of Harvoni, serological evidence of HBV infection should be tested and treated if positive.

Drug-drug interaction: Co-administration of amiodarone with Harvoni may cause serious symptomatic bradycardia. Dose adjustment or regimen change may be required for certain medications. These include antiarrhythmic drug digoxin, HMG Co-A reductase inhibitor rosuvastatin, anticoagulant warfarin, acid reducing agents proton pump inhibitors, H2 receptor antagonists, antacids, anticonvulsants phenytoin, phenobarbital, carbamazepine, oxcarbazepine, antimycobacterials rifampin, rifabutin, rifapentine, anti-HCV medication simeprevir, anti-HIV medications tenofovir DF, emtricitabine, cobicistat, elvitegravir, tipranavir/ritonavir, regimen containing tenofovor DF and an HIV protease inhibitor/ritonavir or cobicistat: darunavir/ritonavir or cobicistat + emtricitabine/tenofovir DF, atazanavir/ritonavir or cobicistat + emtricitabine/tenofovir DF, lopinavir/ritonavir + emtricitabine/tenofovir DF, and herbal supplement St. John's Wort[46].

Ombitasvir is a NS5A inhibitor. After absorption, it binds to NS5A and blocks the activity of NS5A to prevent HCV replication. It is used in combination with paritaprevir, retinovir and dasabuvir with or without ribavirin. In Viekira pak, paritaprevir, ritonavir and ombitasvir packaged in a single table, and dasabuvir is a different tablet. The daily dose of Viekira pak is 2 tablets of paritaprevir, ritonavir and ombitasvir in the morning, and one tablet of dasabuvir twice a day. In Viekira XR, each tablet contains partaprevir, ritonavir, ombitasvir and dasabuvir. 3 tablets have to be taken daily for 12 to 24 wk.

Daclatasvir is a highly selective pangenotypic NS5A inhibitor[47]. It has dual mode of action as it binds to the N-terminal of NS5A and thus prevents viral replication and viral assembly. It is the first oral medication used in combination with sofosbuvir for 12 wk for the treatment of hepatitis C genotype 3 infection. HCV genotype 3 is the most aggressive (having faster disease progression) and

most treatment resistant genotype affecting 12% of all HCV genotypes in United States. In Ally-3 trial, a 12 wk therapy of daclatasvir plus sofosbuvir achieved SVR12 in 96% of non-cirrhotic patients infected with HCV genotype 3 irrespective of prior treatment experience[48]. In Ally 3+ trial, daclatasvir plus sofosbuvir plus ribavirin were given to treatment-naïve and treatment-experienced patients with advanced fibrosis or compensated cirrhosis for 12 or 16 wk. The SVR12 was 90% in the 12 wk and 92% in the 16 wk group[49]. In Ally-2 trial, 12 wk course of daclatasvir plus sofosbuvir achieved SVR12 of 97% in patients infected with HCV genotype 1 to 4 coinfected with HIV[50]. The efficacy of daclatasvir plus sofosbuvir in cirrhotic patients infected with HCV genotype 1 to 6 is being studied in Ally-1 trial[51].

Common side effects include headache, fatigue, nausea and diarrhea. Certain drugs are contraindicated to be used with daclatasvir. These include anticonvulsants phenytoin, carbamazepine, antimycobacterial agent rifampin and herbal supplement St. John's Wort.

Drug-drug interactions: Medications which can cause significant drug interactions with daclatasvir include statins, digoxin, dabigatran, ketoconazole, itraconazole, clarithromycin, nafcillin, dexamethasone, buprenorphine, modafinil, bosentan, anti-HIV medications - protease inhibitors: atazanavir with ritonavir, indinavir, nelfinavir, saquinavir; Non-nucleoside reverse transcriptase inhibitors: Efavirenz Etravirine Nevirapine; and cobicistat-containing antiretroviral: atazanavir/cobicistat, elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate[52].

Elbasvir is a potent NS5A inhibitor. It is used in combination with grazoprevir which is a NS3/4A protease inhibitor. The combination is effective against HCV genotypes 1 and 4. Elbasvir can be less effective when there are many resistance- associated variants or substitutions (RAVs or RASs) of NS5A[53]. Elbasvir-Grazoprevir can be taken in empty stomach or with food. In C-EDGE trial, treatment-naïve HCV infected patients had a good response (SVR12) rate to 12 wk therapy of Elbasvir-Grazoprevir: 92% with genotype 1a, 99% with genotype 1b, 100% with genotype 4 and 80% with genotype 6; 97% in cirrhotics and 94% in non-cirrhotics[54]. In C-EDGE TE trial, patients with prior exposure to pegylated interferon and ribavirin, Elbasvir/Grazoprevir with or without ribavirin was highly effective in inducing an SVR12 in patients with HCV genotype 1, 4 and 6 including patients with cirrhosis[55].

Velpatasvir is a pangenotypic second generation NS5A inhibitor and is much more potent with a higher barrier to resistance than the first generation NS5A inhibitors (ledispavir and Daclatasvir). It acts as a defective substrate for NS5A and prevents HCV replication. It is used as a fixed dose co-formulation with sofosbuvir (Epclusa). In a double blind placebo-controlled study, sofosbuvir-velpatasvir combination was given daily for 12 wk to untreated and previously treated patients with HCV infection genotypes 1-6 including those with compensated cirrhosis. The SVR was 99%[56]. Another study showed that when sofosbuvir-velpatasvir combination plus ribavirin were given daily for 12 wk to decompensated cirrhotics (Child - Pugh - Turcotte class B) due to HCV infection genotype 1-6, the SVR was 94%[57].

Common side effects of Epclusa include headache, fatigue, insomnia, nausea and diarrhea. If the patient is HBV/HCV coinfected and not receiving ant-HBV therapy, administration of Epclusa may cause reactivation of HBV and fulminant hepatitis during or after treatment with Epclusa.

Drug-drug interactions: Similar to Harvoni, co-administration of Amiodarone with Epclusa may lead serious symptomatic bradycardia. Dose alteration or regimen change may be recommended for certain medications. These include acid suppressant agents proton pump inhibitors, H2 receptor blockers, antacids (aluminum and magnesium hydroxide), HMG Co-A reductase inhibitors atorvastatin, rosuvastatin, anti-arrhythmic drug digoxin, anticonvulsants phenytoin, phenobarbital, carbamazepine, oxcarbazepine, anti-mycobacterials rifampin, rifabutin, rifapentine, anti-HIV medications efavirenz, etravirine, nevirapine, tipranavir/ritonavir, Regimens containing tenofovir DF, anti-cancer medication topotecan, and herbal supplement St. John's wort[58].

Pibrentasvir is a 2^{ed} generation NS5A inhibitor coformulated with glecaprevir (Mavyret). As mentioned before glecaprevir/pibrentasvir combination is pangenotypic and can be used in severe renal failure, including hemodialysis patients.

NS5B inhibitors

They act on the catalytic site of NS5B polymerase. They cause HCV RNA chain termination after being incorporated into the RNA chain. Nucleotide NS5B inhibitors are already activated. They act on the active site of NS5B polymerase. Non-Nucleoside NS5B inhibitors need to be activated by cellular kinase 3 times to become the triphosphate which is the active form. They act on different allosteric sites (thumb, finger and palm domains) to downregulate NS5B polymerase[59]. Nucleotide NS5B inhibitors are moderately potent DAA with pangenotypic coverage and have high barrier to resistance. Non-nucleoside NS5B inhibitors are moderately potent DAA with limited genotypic coverage and low barrier to resistance.

Sofosbuvir is a nucleotide NS5B polymerase inhibitor[60]. It is coformulated with Ledipasvir for the treatment of hepatitis C genotype 1, 4, 5 and 6 infection. A meta-analysis showed no additional benefit when ribavirin was added to sofosbuvir/ledipasvir for the treatment genotype 1 infection[61]. As mentioned before, sofosbuvir/daclatasvir combination is effective for the treatment of hepatitis C genotypes 1 to 4[62]. Patients with HCV genotypes 2 and 3 infections were treated with sofosbuvir and ribavirin for 12 wk and 24 wk respectively: SVR 12 was 93% for genotype 2 infection, and 85% for genotype

Table 1 Direct acting anti-viral agents with posology

No.	Trade name	Generic name with doses
1	Sovaldi	Sofosbuvir 400 mg
2	Olysio	Simeprevir 150 mg
3	Daklinza	Daclatasvir 30 mg/60 mg/90 mg
4	Harvoni	Ledipasvir 90 mg/Sofosbuvir 400 mg
5	Viekira Pak	1 d pack contains Paritaprevir 75 mg/Ombitasvir 12.5 mg/Ritonavir 50 mg tablet × 2 and Dasabuvir 250 mg tablet × 2
6	Viekira XR	Extended release tablet contains Dasabuvir 200 mg/ombitasvir 8.33 mg/Paritaprevir 50 mg/Ritonavir 33.33 mg
7	Technivie	Ombitasvir 12.5 mg/Paritaprevir 75 mg/Ritonavir 50 mg
8	Epclusa	Sofosbuvir 400 mg/ Velpatasvir 100 mg
9	Zepatier	Elbasvir 50 mg/ Grazoprevir 100 mg
10	Mavyret	Glecaprevir 100 mg/ Pibrentasvir 40 mg
11	Vosevi	Sofosbuvir 400 mg/Velpatasvir 100 mg/Voxilaprevir 100 mg

3 infection. In non-cirrhoitc HCV genotype 3 infection, the SVR was 91% whereas in cirrhotic genotype 3 infection, the SVR was 68%[63]. Sofosbuvir has been coformulated with other NS5A inhibitor or NS3/4A protease inhibitor to make the combination more effective against HCV and also pangenotypic.

Common side effects of Sofosbuvir include headache, nausea, insomnia and fatigue. Reactivation of HBV with fulminant hepatitis can occur if the patient is HBV/HCV co-infected and not receiving anti-HBV therapy.

Drug-drug interactions: Dose alteration or change in regimen is recommended for certain medications. These include anticonvulsants phenytoin, phenobarbital, carbamazepine, oxcarbazepine, anti-HIV medications tipranavir/ritonavir, antimycobacterial agents rifampin, rifabutin, rifapentine, and herbal supplement St. John's Wort[64].

Dasabuvir is a non-nucleoside NS5B polymerase inhibitor. It is used in combination with Paritaprevir/ritonavir-ombitasvir ((in Viekira Pak and Viekira XR) with or without ribavirin for the treatment of hepatitis C genotype 1 infection with or without compensated cirrhosis (Child-Pugh A). It binds to the palm domain of NS5B, and thus prevents elongation of HCV RNA. The binding site is poorly conserved across other HCV genotypes. As a result, Dasabuvir is only effective against HCV genotype 1 infection. Every year new DAAs are being added in our clinical practice. Currently, the various DAAs available in formulation are as follows (Table 1).

Individualized treatment options

In 2018, we treat hepatitis C with combination of at least two DAAs or one DAA with ribavirin. Several factors are to be considered before planning treatment for hepatitis C. These include HCV genotype, HCV viral load, treatment-naïve or treatment-experienced (PEG/RIBA, NS3/4A protease inhibitor, NS5A inhibitor, NS5B inhibitor) patient, cirrhotic or non-cirrhotic patient, absence or presence of baseline NS5A resistance-associated substitutions (RASs), patient's current medications considering any drug-drug interactions (DDI), patient's renal function, co-infection with HIV, post-liver transplant infection, and of course, patient's insurance and financial status. Guidelines for the treatment of hepatitis C was updated by the European Association for the Study of Liver (EASL) in 2016 and the American Association for the Study of Liver Diseases (AASLD) and the Infectious Diseases Society of America (IDSA) in 2017[65,66]. Individualized treatments as per the AASLD guidelines[66] are listed in Tables 2 and 3.

Genotype 1 infection-treatment-experienced

Glecaprevir/Pibrentasvir (Mavyret): Duration of treatment depends on previous regimen and presence or absence of compensated cirrhosis. Elbasvir/Grazoprevir (Zepatier): duration depends on viral load irrespective of no cirrhosis or compensated cirrhosis as per EASL guideline. Ledipasvir/Sofosbuvir (Harvoni): Applicable for both non-cirrhotics and compensated cirrhotics. Sofosbuvir/Velpatasvir (Epclusa): treatment is same for both non-cirrhotics and compensated cirrhotics. Daclatasvir (Daklinza) plus Sofosbuvir (Sovaldi): applicable for both non-cirrhotics and compensated cirrhotics. Paritaprevir/ritonavir/ombitasvir and dasabuvir (in Viekira Pak and Viekira XR) with weight-based ribavirin (Tables 3-6).

HCV/HIV Co-infection

Twenty percent to 30% of the 34 million HIV infected individuals in the world are co-infected with HCV[67]. HCV/HIV co-infection carries increased morbidity and mortality including advanced hepatic fibrosis and cirrhosis[68]. HCV/HIV co-infected patients have higher HCV viral load, more chance of developing chronic HCV infection and rapid development of advanced liver disease than HCV mono-infected patients. The main challenge of treating these group of patients is drug-drug interactions, *i.e.*, interactions between anti-HCV DAAs and anti-retroviral medications[69]. But the SVR and side effects are similar to those of HCV mono-infected patients[70]. Ideally, HCV/HIV co-infection should be managed by or in collaboration with a HIV practitioner. No change in antiretroviral therapy should be done without consultation of the HIV practitioner. Certain guidelines given by AASLD and IDSA are as follows[66]: (1) Ledipasvir/Sofosbuvir (Harvoni): This combination has certain interactions with anti-retrovirals mentioned before. As it increases serum level of tenofovir disoproxil fumerate which may cause renal toxicity, renal function should be monitored and tenofovir disoproxil fumerare should be used with

Table 2 Genotype 1a and 1b infection - treatment-naïve (with compensated cirrhosis) and non-cirrhotic

No.	First line therapy	Alternative regimen
Genotype 1a infection - treatment-naïve and non-cirrhotic		
1	Glecaprevir/Pibrentasvir (Mavyret) - 8 wk	Paritaprevir/ritonavir/ombitasvir and dasabuvir (in Viekira Pak and Viekira XR) with weight-based ribavirin - 12 wk
2	Elbasvir/Grazoprevir (Zepatier) for patients without baseline NS5A RAVs for Elbasvir - 12 wk	Simeprevir (Olysio) plus Sofosbuvir (Sovaldi) - 12 wk
3	Ledipasvir/Sofosbuvir (Harvoni) - 12 wk < Ledipasvir/Sofosbuvir (Harvoni) - 8 wk in non-black, HIV-uninfected individuals with serum HCV RNA < 6 million units/mL	Daclatasvir(Daklinza) plus Sofosbuvir (Sovaldi) - 12 wk
4	Sofosbuvir/Velpatasvir (Epclusa) - 12 wk	Elbasvir/Grazoprevir (Zepatier) with weight-based ribavirin for patients with baseline NS5A RAVs for Elbasvir - 16 wk
Genotype 1a infection - treatment-naïve with compensated cirrhosis		
1	Glecaprevir/Pibrentasvir (Mavyret) - 12 wk	Elbasvir/Grazoprevir (Zepatier) with weight-based ribavirin for patients with baseline NS5A RASs for Elbasvir - 16 wk
2	Elbasvir/Grazoprevir (Zepatier) for patients without baseline NS5A RAVs for Elbasvir - 12 wk	
3	Ledipasvir/Sofosbuvir (Harvoni) - 12 wk	
4	Sofosbuvir/Velpatasvir (Epclusa) - 12 wk	
Genotype 1b infection - treatment-naïve and non-cirrhotic		
1	Glecaprevir/Pibrentasvir (Mavyret) - 8 wk	Paritaprevir/ritonavir/ombitasvir and dasabuvir (in Viekira Pak and Viekira XR) - 12 wk
2	Elbasvir/Grazoprevir (Zepatier) - 12 wk	Simeprevir (Olysio) plus Sofosbuvir (Sovaldi) - 12 wk
3	Ledipasvir/Sofosbuvir (Harvoni) - 12 wk < Ledipasvir/Sofosbuvir (Harvoni - 8 wk in non-black, HIV-uninfected individuals with serum HCV RNA < 6 million units/mL	Daclatasvir (Daklinza) plus Sofosbuvir (Sovaldi) - 12 wk
4	Sofosbuvir/Velpatasvir (Epclusa) - 12 wk	
Genotype 1b infection - treatment-naïve with compensated cirrhosis		
1	Glecaprevir/Pibrentasvir (Mavyret) - 12 wk	Paritaprevir/ritonavir/ombitasvir and dasabuvir (in Viekira Pak and Viekira XR) - 12 wk
2	Elbasvir/Grazoprevir (Zepatier - 12 wk	
3	Ledipasvir/Sofosbuvir (Harvoni) - 12 wk	
4	Sofosbuvir/Velpatasvir (Epclusa - 12 wk	

RAVs: Resistance- associated variants; HIV: Human immunodeficiency virus; HCV: Hepatitis C virus.

Table 3 Genotype 1 infection-treatment-experienced

Glecaprevir/Pibrentasvir (Mavyret): Duration of treatment depends on previous regimen and presence or absence of compensated cirrhosis.		
Previous regimen	No cirrhosis	Compensated cirrhosis
Pegylated IFN, ribavirin and/or Sofosbuvir but no prior exposure to NS3/4A PI or NS5A inhibitor	8 wk	12 wk
NS3/4A PI but no prior exposure to NS5A inhibitor	12 wk	12 wk
NS5A inhibitor but no prior exposure to NS3/4A PI	16 wk	16 wk
Elbasvir/Grazoprevir (Zepatier): Duration depends on viral load irrespective of no cirrhosis or compensated cirrhosis as per EASL guideline.		
Previous regimen	HCV RNA ⩽ 800000 IU/mL	HCV RNA > 800000 IU/mL
Pegylated IFN and ribavirin	12 wk without ribavirin	16 wk with ribavirin
Ledipasvir/Sofosbuvir (Harvoni): Applicable for both non-cirrhotics andcompensated cirrhotics.		
Previous regimen	With ribavirin	Without ribavirin
PEG IFN and ribavirin	12 wk	24 wk
Sofosbuvir/Velpatasvir (Epclusa): Treatment is same for both non-cirrhotics and compensated cirrhotics.		
Previous regimen	No cirrhosis	Compensated cirrhosis
PEG IFN and ribavirin	12 wk without ribavirin	12 wk without ribavirin
Daclatasvir (Daklinza) plus Sofosbuvir (Sovaldi): Applicable for both non-cirrhotics and compensated cirrhotics.		
Previous regimen	With ribavirin	Without ribavirin
PEG IFN and ribavirin	12 wk	24 wk
Paritaprevir/ritonavir/ombitasvir and dasabuvir (in Viekira Pak and Viekira XR) with weight-based ribavirin.		
Previous regimen	No cirrhosis	Compensated cirrhosis
PEG IFN and ribavirin	12 wk	24 wk

IFN: Interferon; PI; Protease inhibitors; HCV: Hepatitis C virus.

GFR > 60 mL/min. Tenofovir alafenamide could be an alternative option; (2) Paritaprevir/ritonavir/ombitasvir and dasabuvir (in Viekira Pak and Viekira XR): The anti-HIV medications which do not have substantial interaction with Vikira Pak and Viekira XR include atazanavir, dolutegravir, emtricitabine, enfuvirtide,

Table 4 Genotype 2-4 infection - treatment-naïve (with compensated cirrhosis) and non-cirrhotic

No.	First line therapy	Alternative regimen
Genotype 2 infection - treatment-naïve and non-cirrhotic		
1	Glecaprevir/Pibrentasvir (Mavyret) - 8 wk	Daclatasvir(Daklinza) plus Sofosbuvir (Sovaldi) - 12 wk
2	Sofosbuvir/Velpatasvir (Epclusa) - 12 wk	
Genotype 2 infection - treatment-naïve with compensated cirrhosis		
1	Glecaprevir/Pibrentasvir (Mavyret) - 12 wk	Daclatasvir(Daklinza) plus Sofosbuvir (Sovaldi) - 16 to 24 wk
2	Sofosbuvir/Velpatasvir (Epclusa) - 12 wk	
Genotype 3 infection - treatment-naïve and non- cirrhotic		
1	Glecaprevir/Pibrentasvir (Mavyret) - 8 wk	Daclatasvir (Daklinza) plus Sofosbuvir (Sovaldi) - 12 wk
2	Sofosbuvir/Velpatasvir (Epclusa - 12 wk	
Genotype 3 infection - treatment-naïve with compensated cirrhosis		
1	Glecaprevir/Pibrentasvir (Mavyret) - 12 wk	Vosevi - Sofosbuvir 400 mg/ Velpatasvir 100 mg/ Voxilaprevir 100 mg when Y93 is present - 12 wk
2	Sofosbuvir/Velpatasvir (Epclusa) - 12 wk	Daclatasvir (Daklinza) plus Sofosbuvir (Sovaldi) with or without weight-based ribavirin - 24 wk
Genotype 4 infection - treatment-naïve and non-cirrhotic		
1	Glecaprevir/Pibrentasvir (Mavyret) - 8 wk	Ombitasvir 25 mg/Paritaprevir 150 mg/ Ritonavir 100 mg (Technivie) with weight-based ribavirin - 12 wk
2	Sofosbuvir/Velpatasvir (Epclusa) - 12 wk	
3	Elbasvir/Grazoprevir (Zepatier) - 12 wk	
4	Ledipasvir/Sofosbuvir (Harvoni) - 12 wk	
Genotype 4 infection - treatment-naïve with compensated cirrhosis		
1	Sofosbuvir/Velpatasvir (Epclusa) - 12 wk	Ombitasvir 25 mg/Paritaprevir 150 mg/ Ritonavir 100 mg (Technivie) with weight-based ribavirin - 12 wk
2	Glecaprevir/Pibrentasvir (Mavyret) - 12 wk	
3	Elbasvir/Grazoprevir (Zepatier) - 12 wk	
4	Ledipasvir/Sofosbuvir (Harvoni) - 12 wk	

Table 5 Genotype 5 or 6 infection - treatment-naïve with and without compensated cirrhosis

No.	DAA	No cirrhosis	Compensated cirrhosis
1	Glecaprevir/Pibrentasvir (Mavyret)	8 wk	12 wk
2	Sofosbuvir/Velpatasvir (Epclusa)	12 wk	12 wk
3	Ledipasvir/Sofosbuvir (Harvoni)	12 wk	12 wk

DAA: Direct acting anti-viral agent.

lamivudine, raltegravir, and tenofovir; (3) Sofosbuvir/Velpatasvir (Epclusa): Can have drug-drug interactions with anti-retrovirals mentioned before. As velpatasvir can increase the serum level of tenofovir disoproxil fumerate, renal function should be checked and tenofovir disoproxil fumerate should not be used with a GFR of less than 60 mL/min. Tenofovir alafenamide could be used instead; (4) Elbasvir/Grazoprevir (Zepatier): Certain antiretrovirals do not have significant drug-drug interactions with elbasvir/grazoprevir combinations. These include tenofovir, lamivudine, emtricitabine, abacavir, dolutegravir, raltegravir, rilpivirine, and enfuvirtide; (5) Glecaprevir/Pibrentasvir (Mavyret): The anti-retrovirals which do not have significant drug-drug interactions with Glecaprevir/Pibrentasvir include lamivudine, tenofovir, emtricitabine, abacavir, dolutegravir, raltegravir, rilpivirine, and enfuvirtide; (6) Sofosbuvir/Velpatasvir/Voxilaprevir (Vosevi): Has some drug-drug interactions with certain anti-retrovirals mentioned before. But some of the anti-retrovirals do not have significant interactions with this combination (Vosevi). These include lamivudine, emtricitabine, dolutegravir, enfuvirtide, raltegravir and rilpivirine; (7) Simeprevir (used in combination with another DAA): Does not have significant drug-drug interactions with certain anti-retrovirals which include lamivudine, tenofovir, emtricitabine, abacavir, dolutegravir, raltegravir, rilpivirine, enfuvirtide and maraviroc; and (8) Daclatasvir (used in combination with another DAA): Has significant drug-drug interactions with certain anti-retrovirals mentioned before. Certain dose adjustment include: cobicistat/atazanavir (decrease dose to 30 mg/d), cobicistat/elvitegravir (decrease dose to 30 mg/d), ritonavir/atazanavir (decrease dose to 30 mg/d), and efavirenz or etravirine (increase dose to 90 mg/d).

HCV infection in post-liver transplant patients

Recurrence of hepatitis C infection following liver transplantation is universal in all patients with pre-transplantation viremia. The course varies from asymptomatic infection to fibrosing cholestatic hepatitis[71]. About one third of post-transplant HCV-infected patients develop allograft cirrhosis 5 to 7 years after transplantation[72]. As per AASLD and IDSA guidelines published on September 21, 2017 the various treatment options are list in Table 7[66].

There are drug-drug interactions between DAA and

Table 6 Genotype 2-6 infection - treatment-experienced

	First line therapy	Alternative regimen
Genotype 2 infection - treatment-experienced		
Pegylated IFN/ribavirin-experienced without cirrhosis	Glecaprevir/Pibrentasvir (Mavyret) - 8 wk or Sofosbuvir/Velpatasvir (Epclusa) - 12 wk	Daclatasvir (Daklinza) plus Sofosbuvir (Sovaldi) - 12 wk
Pegylated IFN/ribavirin-experienced with compensated cirrhosis	Sofosbuvir/Velpatasvir (Epclusa) - 12 wk or Glecaprevir/Pibrentasvir (Mavyret) - 12 wk	Daclatasvir (Daklinza) plus Sofosbuvir (Sovaldi) - 16 to 24 wk
Sofosbuvir plus ribavirin-experienced with or without compensated cirrhosis	Sofosbuvir/Velpatasvir (Epclusa) - 12 wk or Glecaprevir/Pibrentasvir (Mavyret) - 12 wk	
Genotype 3 infection - treatment-experienced		
Pegylated IFN/ ribavirin-experienced without cirrhosis	Sofosbuvir/Velpatasvir (Epclusa) - 12 wk	Daclatasvir (Daklinza) plus Sofosbuvir (Sovaldi) - 12 wk or Glecaprevir/Pibrentasvir (Mavyret) - 16 wk or Sofosbuvir/Velpatasvir/Voxilaprevir - 12 wk
Pegylated IFN/ ribavirin-experienced with compensated cirrhosis	Elbasvir/Grazoprevir (Zepatier) - 12 wk or Sofosbuvir/Velpatasvir/Voxilaprevir (Vosevi) -12 wk	Sofosbuvir/Velpatasvir (Epclusa) plus weight-based ribavirin - 12 wk or Glecaprevir/Pibrentasvir (Mavyret) - 16 wk
DAA-experienced including NS5A inhibitors with or without compensated cirrhosis	Sofosbuvir/Velpatasvir/Voxilaprevir (Vosevi) - 12 wk or in case of NS5A inhibitor failure and cirrhosis - Vosevi plus weight-based Ribavirin - 12 wk	
Genotype 4 infection - treatment-experienced		
Pegylated IFN/ ribavirin-experienced without cirrhosis	Sofosbuvir/Velpatasvir (Epclusa) -12 wk or Glecaprevir/Pibrentasvir (Mavyret) - 8 wk or Elbasvir/Grazoprevir (Zepatier) in virologic relapse - 12 wk or Ledipasvir/Sofosbuvir (Harvoni) - 12 wk	Ombitasvir 25 mg/Paritaprevir 150 mg/Ritonavir 100 mg plus weight based Ribavirin - 12 wk or Elbasvir/Grazoprevir (Zepatier) with weight-based Ribavirin (in case of prior on-treatment virologic failure) - 16 wk
Pegylated IFN/ ribavirin-experienced with compensated cirrhosis	Sofosbuvir/Velpatasvir (Epclusa) - 12 wk or Elbasvir/Grazoprevir (Zepatier) in virologic relapse - 12 wk or Glecaprevir/Pibrentasvir (Mavyret) - 12 wk	Ombitasvir 25 mg/Paritaprevir 150 mg/ Ritonavir 100 mg plus weight based Ribavirin - 12 wk or Elbasvir/Grazoprevir (Zepatier) with weight-based Ribavirin (in case of prior on-treatment virologic failure - 16 wk or Ledipasvir/Sofosbuvir (Harvoni) plus weight-based Ribavirin) - 12 wk
DAA-experienced including NS5A inhibitors with or without compensated cirrhosis	Sofosbuvir/Velpatasvir/Voxilaprevir (Vosevi) - 12 wk	
Genotype 5 or 6 infection - treatment-experienced (recommended regimen)		
Pegylated IFN/ ribavirin-experienced with or without compensated cirrhosis	Glecaprevir/Pibrentasvir (Mavyret) - 8 wk for patients without cirrhosis and 12 wk for patients with compensated cirrhosis or Ledipasvir/Sofosbuvir (Harvoni) plus weight-based Ribavirin - 12 wk or Sofosbuvir/ Velpatasvir (Epclusa) - 12 wk	
DAA-experienced including NS5A inhibitors with or without compensated cirrhosis	Sofosbuvir/Velpatasvir/ Voxilaprevir (Vosevi) - 12 wk	

IFN: Interferon; DAA: Direct acting anti-viral agent.

calcineurin inhibitors: cyclosporine A and Tacrolimus. When Sofosbuvir is used with cyclosporine A, serum level of sofosbuvir is increased by 4.5 fold but GS -331007 metabolite remains unchanged. So no dose adjustment is required[73]. Sofosbuvir has no drug interaction with Tacrolimus[74]. When Paritaprevir/ritonavir/ombitasvir and dasabuvir- (PrOD) in Viekira Pak and Viekira XR are used with cyclosporine A, there is 5.8 fold increased serum level of cyclosporine A suggesting use of 1/5th of dose of cyclosporine A and requirement of monitoring of serum level of cyclosporine A. When PrOD are used with tacrolimus, there is 57 fold increase in tacrolimus level in the blood, suggesting use of 0.5 mg of tacrolimus once a week, and monitoring of tacrolimus level in the blood[75]. When elbasvir/grazoprevir combination is used with cyclosporine A, there is 15 fold increases in grazoprevir level in the blood. As a result, this combination is not recommended to be used. When elbasvir/grazoprevir are used with tacrolimus, there is 43% increase in tacrolimus level, and although no prior dose adjustment is required, frequent monitoring of tacrolimus level, tacrolimus-associated side effects and renal function should be done[76]. When glecaprevir/pibrentasvir is used with higher dose (400 mg) of cyclosporine A, there is 5 fold increase in glecaprevir level in the blood. This combination is not recommended in patients requiring more than 100 mg of cyclosporine A[77]. When glecaprevir/pibrentasvir combination is used with tacrolimus, there is 1.45 fold increase in tacrolimus level suggesting no prior dose adjustment but tacrolimus level should be monitored. When sofosbuvir/velpatasvir/voxilaprevir combination is used with cyclosporine A, there is 9.4 fold increase in voxilaprevir. This combination is not recommended[78].

Table 7 Recommended and alternative therapy

Genotype	Treatment-naïve and - experienced patients with HCV infection in the allograft without cirrhosis	Treatment-naïve and - experienced patients with HCV infection in the allograft with compensated cirrhosis	Treatment-naïve and - experienced patients with HCV infection in the allograft with decompensated cirrhosis
Recommended therapy			
1, 4, 5 or 6	Glecaprevir/Pibrentasvir (Mavyret) - 12 wk or Ledipasvir/Sofosbuvir (Harvoni) for 12 wk	Ledipasvir/Sofosbuvir (Harvoni) with weight-based ribavirin - 12 wk	Ledipasvir/Sofosbuvir (Harvoni) with initial low dose of ribavirin (600 mg), increase the dose as tolerated - 12 wk
2 or 3	Glecaprevir/Pibrentasvir (Mavyret) - 12 wk or Daclatasvir (Daklinza) plus Sofosbuvir (Sovaldi) with initial low dose of ribavirin (600 mg), increase the dose as tolerated for 12 wk	Daclatasvir (Daklinza) plus Sofosbuvir (Sovaldi) with initial low dose of ribavirin (600 mg), increase the dose as tolerated - 12 wk	Daclatasvir (Daklinza) plus Sofosbuvir (Sovaldi) with initial low dose of ribavirin (600 mg), increase the dose as tolerated for 12 wk or Sofosbuvir/Velpatasvir (Epclusa) with weight-based ribavirin - 12 wk
Alternative therapy			
1, 4, 5 or 6	Daclatasvir (Daklinza) plus Sofosbuvir (Sovaldi) with initial low dose of ribavirin (600 mg), increase the dose as tolerated for 12 wk or HCV genotype 1 or 4 infection only: Simeprevir (Olysio) plus Sofosbuvir (Sovaldi) with or without weight-based ribavirin	Daclatasvir (Daklinza) plus Sofosbuvir (Sovaldi) with initial low dose of ribavirin (600 mg), increase the dose as tolerated for 12 wk or HCV genotype 1 or 4 infection only: Simeprevir (Olysio) plus Sofosbuvir (Sovaldi) with or without weight-based ribavirin	
2 or 3		Glecaprevir/Pibrentasvir (Mavyret) for 12 wk or Sofosbuvir/Velpatasvir (Epclusa) with weight-based ribavirin for 12 wk	

HCV: Hepatitis C virus.

DAA and hepatocellular carcinoma

Concern emerged after the study done by Reig *et al*[79] in 2016 showing higher rate of hepatocellular carcinoma (HCC) recurrence after DAA treatment in patients with prior HCC. Subsequently few studies were done to evaluate this finding. Large VA cohort study did not find any increased risk of developing HCC after DAA therapy[80]. Fortunately, the concern has lost its importance. But all cirrhotic patients should be under surveillance for HCC after DAA therapy.

DAA failure

DAA failure or relapse has occurred in less than 10% of HCV infected patients in clinical trials. In a multicenter real life study, the overall failure rate was found to be 3.6%[81]. As mentioned before, HCV exists as a quasispecies with production of various genetically distinct variants due to replication errors. Due to this high mutation rate of HCV genome, changes in the critical coding regions may occur making the virus less susceptible to anti-HCV therapy. Viral variants containing polymorphisms or substitutions resistant to DAA are also called baseline resistant-associated substitutions (RASs) and they exist in a small percentage of patients with chronic HCV infection prior to anti-HCV therapy. Sometimes RASs develop after initiation of DAA therapy when they are called treatment-emergent or treatment-selected RASs. Subtherapeutic DAA level may predispose to the development of RASs. NS5A and NS3 RASs are frequently seen in cases of failure of DAA containing NS5A or NS3 inhibitor[82]. On the other hand, there is rare development of NS5B RASs even after exposure to a failed DAA therapy containing. NS5B inhibitor, possibly due to binding of NS5B inhibitor to the highly conserved catalytic site of the viral genome making generation of RASs extremely difficult[83].

AASLD and IDSA recommend testing for NS5A RASs if DAAs containing NS5A inhibitors fail[82]. Testing for baseline NS5A RASs is recommended in patients infected with HCV genotype 1a prior to initiation of elbasvir and grazoprevir therapy[84]. Baseline NS5A RASs testing should also be considered in patients with cirrhosis of liver due to HCV genotype 1a infection prior to sofosbuvir plus daclatasvir therapy[85]. NS5A RASs testing should be considered in certain situations: (1) treatment-naïve HCV genotype 3 infection with compensated cirrhosis of liver prior to treatment with either Sofosbuvir/Velpatasvir (Epclusa) for 12 wk or Daclatasvir (Daklinza) plus Sofosbuvir (Sovaldi) for 24 wk; (2) treatment-experienced HCV genotype 3 infection without cirrhosis prior to treatment with Daclatasvir(Daklinza) plus Sofosbuvir (Sovaldi) for 12 wk; and (3) treatment-experienced HCV genotype 3 infection with or without cirrhosis prior to treatment with Sofosbuvir/Velpatasvir (Epclusa) for 12 wk. If Y93H is found, weight-based ribavirin should be added[82]. DAA failure has been associated with certain predisposing factors like presence of RASs, sofosbuvir/ribavirin treatment, HCV genotype 3, and cirrhosis of liver[81,86].

CONCLUSION

DAAs have changed the treatment plan and outcome of chronic hepatitis C infection in this pegylated interferion free era. There are various DAAs available in the market and these include second generation NS3/4A protease inhibitors, NS5A and NS5B inhibitors. AASLD, IDSA and EASL recommend combination of DAAs with or without ribavirin. As the success rate is high, side effects are low, drug-drug interactions are less, and duration of

therapy is relatively short, more and more patients are getting treatment for the cure of hepatitis C infection. Some DAAs require preliminary testing to find out the effectiveness of that particular DAA, for example Q80K polymorphism in case of Simeprevir, and NS5A resistance testing in case of elabasvir. HBV co-infection testing should be done prior to initiation of any DAA therapy to prevent fulminant hepatitis and liver failure. Drug-drug interaction, and specific conditions like HCV/HIV co-infection and HCV infection in post-liver transplant patients should considered before initiating any treatment plan. Although DAA can eradicate HCV infection, it does not decrease the chance of developing HCC in cirrhotic patients. So they should be under regular HCC surveillance. The next challenge will be the development of HCV vaccine to reduce the incidence of HCV infection in the world.

REFERENCES

1 **Petruzziello A**, Marigliano S, Loquercio G, Cozzolino A, Cacciapuoti C. Global epidemiology of hepatitis C virus infection: An up-date of the distribution and circulation of hepatitis C virus genotypes. *World J Gastroenterol* 2016; **22**: 7824-7840 [PMID: 27678366 DOI: 10.3748/wjg.v22.i34.7824]

2 **Pawlotsky JM**. NS5A inhibitors in the treatment of hepatitis C. *J Hepatol* 2013; **59**: 375-382 [PMID: 23567084 DOI: 10.1016/j.jhep.2013.03.030]

3 **Ogata N**, Alter HJ, Miller RH, Purcell RH. Nucleotide sequence and mutation rate of the H strain of hepatitis C virus. *Proc Natl Acad Sci USA* 1991; **88**: 3392-3396 [PMID: 1849654 DOI: 10.1073/pnas.88.8.3392]

4 **Pawlotsky JM**. Hepatitis C virus population dynamics during infection. *Curr Top Microbiol Immunol* 2006; **299**: 261-284 [PMID: 16568902 DOI: 10.1007/3-540-26397-7_9]

5 **Myers RP**, Regimbeau C, Thevenot T, Leroy V, Mathurin P, Opolon P, Zarski JP, Poynard T. Interferon for interferon naive patients with chronic hepatitis C. *Cochrane Database Syst Rev* 2002; **(2)**: CD000370 [PMID: 12076394 DOI: 10.1002/14651858.CD000370]

6 **Palumbo E**. Pegylated interferon and ribavirin treatment for hepatitis C virus infection. *Ther Adv Chronic Dis* 2011; **2**: 39-45 [PMID: 23251740 DOI: 10.1177/2040622310384308]

7 **De Meyer S**, Dierynck I, Ghys A, Beumont M, Daems B, Van Baelen B, Sullivan JC, Bartels DJ, Kieffer TL, Zeuzem S, Picchio G. Characterization of telaprevir treatment outcomes and resistance in patients with prior treatment failure: results from the REALIZE trial. *Hepatology* 2012; **56**: 2106-2115 [PMID: 22806681 DOI: 10.1002/hep.25962]

8 **Chatel-Chaix L**, Baril M, Lamarre D. Hepatitis C Virus NS3/4A Protease Inhibitors: A Light at the End of the Tunnel. *Viruses* 2010; **2**: 1752-1765 [PMID: 21994705 DOI: 10.3390/v2081752]

9 **Salam KA**, Akimitsu N. Hepatitis C virus NS3 inhibitors: current and future perspectives. *Biomed Res Int* 2013; **2013**: 467869 [PMID: 24282816 DOI: 10.1155/2013/467869]

10 **Jacobson IM**, Dore GJ, Foster GR, Fried MW, Radu M, Rafalsky VV, Moroz L, Craxi A, Peeters M, Lenz O, Ouwerkerk-Mahadevan S, De La Rosa G, Kalmeijer R, Scott J, Sinha R, Beumont-Mauviel M. Simeprevir with pegylated interferon alfa 2a plus ribavirin in treatment-naive patients with chronic hepatitis C virus genotype 1 infection (QUEST-1): a phase 3, randomised, double-blind, placebo-controlled trial. *Lancet* 2014; **384**: 403-413 [PMID: 24907225 DOI: 10.1016/S0140-6736(14)60494-3]

11 **Manns M**, Marcellin P, Poordad F, de Araujo ES, Buti M, Horsmans Y, Janczewska E, Villamil F, Scott J, Peeters M, Lenz O, Ouwerkerk-Mahadevan S, De La Rosa G, Kalmeijer R, Sinha R, Beumont-Mauviel M. Simeprevir with pegylated interferon alfa 2a or 2b plus ribavirin in treatment-naive patients with chronic hepatitis C virus genotype 1 infection (QUEST-2): a randomised, double-blind, placebo-controlled phase 3 trial. *Lancet* 2014; **384**: 414-426 [PMID: 24907224 DOI: 10.1016/S0140-6736(14)60538-9]

12 **Forns X**, Lawitz E, Zeuzem S, Gane E, Bronowicki JP, Andreone P, Horban A, Brown A, Peeters M, Lenz O, Ouwerkerk-Mahadevan S, Scott J, De La Rosa G, Kalmeijer R, Sinha R, Beumont-Mauviel M. Simeprevir with peginterferon and ribavirin leads to high rates of SVR in patients with HCV genotype 1 who relapsed after previous therapy: a phase 3 trial. *Gastroenterology* 2014; **146**: 1669-79.e3 [PMID: 24602923 DOI: 10.1053/j.gastro.2014.02.051]

13 **Lawitz E**, Sulkowski MS, Ghalib R, Rodriguez-Torres M, Younossi ZM, Corregidor A, DeJesus E, Pearlman B, Rabinovitz M, Gitlin N, Lim JK, Pockros PJ, Scott JD, Fevery B, Lambrecht T, Ouwerkerk-Mahadevan S, Callewaert K, Symonds WT, Picchio G, Lindsay KL, Beumont M, Jacobson IM. Simeprevir plus sofosbuvir, with or without ribavirin, to treat chronic infection with hepatitis C virus genotype 1 in non-responders to pegylated interferon and ribavirin and treatment-naive patients: the COSMOS randomised study. *Lancet* 2014; **384**: 1756-1765 [PMID: 25078309 DOI: 10.1016/S0140-6736(14)61036-9]

14 **Kwo P**, Gitlin N, Nahass R, Bernstein D, Etzkorn K, Rojter S, Schiff E, Davis M, Ruane P, Younes Z, Kalmeijer R, Sinha R, Peeters M, Lenz O, Fevery B, De La Rosa G, Scott J, Witek J. Simeprevir plus sofosbuvir (12 and 8 weeks) in hepatitis C virus genotype 1-infected patients without cirrhosis: OPTIMIST-1, a phase 3, randomized study. *Hepatology* 2016; **64**: 370-380 [PMID: 26799692 DOI: 10.1002/hep.28467]

15 **Lawitz E**, Matusow G, DeJesus E, Yoshida EM, Felizarta F, Ghalib R, Godofsky E, Herring RW, Poleynard G, Sheikh A, Tobias H, Kugelmas M, Kalmeijer R, Peeters M, Lenz O, Fevery B, De La Rosa G, Scott J, Sinha R, Witek J. Simeprevir plus sofosbuvir in patients with chronic hepatitis C virus genotype 1 infection and cirrhosis: A phase 3 study (OPTIMIST-2). *Hepatology* 2016; **64**: 360-369 [PMID: 26704148 DOI: 10.1002/hep.28422]

16 **Cunha JP**. Olysio Side Effects Center. Last reviewed on May 25, 2017. Available from: URL: https://www.rxlist.com/olysio-side-effects-drug-center.htm

17 **Vidal LL**, Soares MA, Santos AF. NS3 protease polymorphisms and genetic barrier to drug resistance of distinct hepatitis C virus genotypes from worldwide treatment-naïve subjects. *J Viral Hepat* 2016; **23**: 840-849 [PMID: 26775769 DOI: 10.1111/jvh.12503]

18 **OLYSIO**. Potentially significant drug interactions—drug-drug interactions (DDIs) [Internet]. Available from: URL: https://www.olysio.com/hcp/safety-profile/drug-interactions

19 **Hussaini T**. Paritaprevir/ritonavir-ombitasvir and dasabuvir, the 3D regimen for the treatment of chronic hepatitis C virus infection: a concise review. *Hepat Med* 2016; **8**: 61-68 [PMID: 27274322 DOI: 10.2147/HMER.S72429]

20 **Viekira XR**. An individualized approach for patients like Michael—with renal impairment [Internet]. Available from: URL: https://www.viekira.com/hcp/individualized-approaches/michael-renal-impairment

21 Viekira Pak and Viekira XR [Internet]. Available from: URL: http://hepatitiscmedications.hcvadvocate.org/viekira-pak/

22 **Pockros PJ**, Reddy KR, Mantry PS, Cohen E, Bennett M, Sulkowski MS, Bernstein DE, Cohen DE, Shulman NS, Wang D, Khatri A, Abunimeh M, Podsadecki T, Lawitz E. Efficacy of Direct-Acting Antiviral Combination for Patients With Hepatitis C Virus Genotype 1 Infection and Severe Renal Impairment or End-Stage Renal Disease. *Gastroenterology* 2016; **150**: 1590-1598 [PMID: 26976799 DOI: 10.1053/j.gastro.2016.02.078]

23 **Raedler LA**. Viekira Pak (Ombitasvir, Paritaprevir, and Ritonavir Tablets; Dasabuvir Tablets): All-Oral Fixed Combination Approved for Genotype 1 Chronic Hepatitis C Infection. *Am Health Drug Benefits* 2015; **8**: 142-147 [PMID: 26629280]

24 Viekira PakTM and Drug Interaction Information for Patients. Available from: URL: https://www.hepatitis.va.gov/pdf/viekira-drug-interactions.pdf

25 **Ahmed H**, Abushouk AI, Menshawy A, Attia A, Mohamed A,

Negida A, Abdel-Daim MM. Meta-Analysis of Grazoprevir plus Elbasvir for Treatment of Hepatitis C Virus Genotype 1 Infection. *Ann Hepatol* 2018; **17**: 18-32 [PMID: 29311409 DOI: 10.5604/01.3001.0010.7532]

26 **Roth D**, Nelson DR, Bruchfeld A, Liapakis A, Silva M, Monsour H Jr, Martin P, Pol S, Londoño MC, Hassanein T, Zamor PJ, Zuckerman E, Wan S, Jackson B, Nguyen BY, Robertson M, Barr E, Wahl J, Greaves W. Grazoprevir plus elbasvir in treatment-naive and treatment-experienced patients with hepatitis C virus genotype 1 infection and stage 4-5 chronic kidney disease (the C-SURFER study): a combination phase 3 study. *Lancet* 2015; **386**: 1537-1545 [PMID: 26456905 DOI: 10.1016/S0140-6736(15)00349-9]

27 **Al-Salama ZT**, Deeks ED. Elbasvir/Grazoprevir: A Review in Chronic HCV Genotypes 1 and 4. *Drugs* 2017; **77**: 911-921 [PMID: 28417245 DOI: 10.1007/s40265-017-0739-8]

28 **Foster GR**, Agarwal K, Cramp ME, Moreea S, Barclay S, Collier J, Brown AS, Ryder SD, Ustianowski A, Forton DM, Fox R, Gordon F, Rosenberg WM, Mutimer DJ, Du J, Gilbert CL, Asante-Appiah E, Wahl J, Robertson MN, Barr E, Haber B. Elbasvir/grazoprevir and sofosbuvir for hepatitis C virus genotype 3 infection with compensated cirrhosis: A randomized trial. *Hepatology* 2018; **67**: 2113-2126 [PMID: 29473975 DOI: 10.1002/hep.29852]

29 Drugs without clinically significant interactions with zepatier. Available from: URL: https://www.merckconnect.com/zepatier/drug-interactions.html?hcpUser=yes

30 **Forns X**, Lee SS, Valdes J, Lens S, Ghalib R, Aguilar H, Felizarta F, Hassanein T, Hinrichsen H, Rincon D, Morillas R, Zeuzem S, Horsmans Y, Nelson DR, Yu Y, Krishnan P, Lin CW, Kort JJ, Mensa FJ. Glecaprevir plus pibrentasvir for chronic hepatitis C virus genotype 1, 2, 4, 5, or 6 infection in adults with compensated cirrhosis (EXPEDITION-1): a single-arm, open-label, multicentre phase 3 trial. *Lancet Infect Dis* 2017; **17**: 1062-1068 [PMID: 28818546 DOI: 10.1016/S1473-3099(17)30496-6]

31 **Zeuzem S**, Foster GR, Wang S, Asatryan A, Gane E, Feld JJ, Asselah T, Bourlière M, Ruane PJ, Wedemeyer H, Pol S, Flisiak R, Poordad F, Chuang WL, Stedman CA, Flamm S, Kwo P, Dore GJ, Sepulveda-Arzola G, Roberts SK, Soto-Malave R, Kaita K, Puoti M, Vierling J, Tam E, Vargas HE, Bruck R, Fuster F, Paik SW, Felizarta F, Kort J, Fu B, Liu R, Ng TI, Pilot-Matias T, Lin CW, Trinh R, Mensa FJ. Glecaprevir-Pibrentasvir for 8 or 12 Weeks in HCV Genotype 1 or 3 Infection. *N Engl J Med* 2018; **378**: 354-369 [PMID: 29365309 DOI: 10.1056/NEJMoa1702417]

32 **Gane E**, Lawitz E, Pugatch D, Papatheodoridis G, Bräu N, Brown A, Pol S, Leroy V, Persico M, Moreno C, Colombo M, Yoshida EM, Nelson DR, Collins C, Lei Y, Kosloski M, Mensa FJ. Glecaprevir and Pibrentasvir in Patients with HCV and Severe Renal Impairment. *N Engl J Med* 2017; **377**: 1448-1455 [PMID: 29020583 DOI: 10.1056/NEJMoa1704053]

33 Warning: Risk of hepatitis B virus reactivation in patients coinfected with HCV and HBV. Highlights of prescribing information. Available from: URL: https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/209394s000lbl.pdf

34 Mavyret (glecaprevir and pibrentasvir). Available from: URL: https://www.rxlist.com/mavyret-drug.htm

35 **Bourlière M**, Gordon SC, Flamm SL, Cooper CL, Ramji A, Tong M, Ravendhran N, Vierling JM, Tran TT, Pianko S, Bansal MB, de Lédinghen V, Hyland RH, Stamm LM, Dvory-Sobol H, Svarovskaia E, Zhang J, Huang KC, Subramanian GM, Brainard DM, McHutchison JG, Verna EC, Buggisch P, Landis CS, Younes ZH, Curry MP, Strasser SI, Schiff ER, Reddy KR, Manns MP, Kowdley KV, Zeuzem S; POLARIS-1 and POLARIS-4 Investigators. Sofosbuvir, Velpatasvir, and Voxilaprevir for Previously Treated HCV Infection. *N Engl J Med* 2017; **376**: 2134-2146 [PMID: 28564569 DOI: 10.1056/NEJMoa1613512]

36 **Chahine EB**, Kelley D, Childs-Kean LM. Sofosbuvir/Velpatasvir/Voxilaprevir: A Pan-Genotypic Direct-Acting Antiviral Combination for Hepatitis C. *Ann Pharmacother* 2018; **52**: 352-363 [PMID: 29115151 DOI: 10.1177/1060028017741508]

37 **GILEAD**. Available from: URL: http://www.gilead.com/~/media/Files/pdfs/medicines/liver-disease/vosevi/vosevi_pi.pdf

38 **Targett-Adams P**, Graham EJ, Middleton J, Palmer A, Shaw SM, Lavender H, Brain P, Tran TD, Jones LH, Wakenhut F, Stammen B, Pryde D, Pickford C, Westby M. Small molecules targeting hepatitis C virus-encoded NS5A cause subcellular redistribution of their target: insights into compound modes of action. *J Virol* 2011; **85**: 6353-6368 [PMID: 21507963 DOI: 10.1128/JVI.00215-11]

39 **Afdhal N**, Zeuzem S, Kwo P, Chojkier M, Gitlin N, Puoti M, Romero-Gomez M, Zarski JP, Agarwal K, Buggisch P, Foster GR, Bräu N, Buti M, Jacobson IM, Subramanian GM, Ding X, Mo H, Yang JC, Pang PS, Symonds WT, McHutchison JG, Muir AJ, Mangia A, Marcellin P; ION-1 Investigators. Ledipasvir and sofosbuvir for untreated HCV genotype 1 infection. *N Engl J Med* 2014; **370**: 1889-1898 [PMID: 24725239 DOI: 10.1056/NEJMoa1402454]

40 **Afdhal N**, Reddy KR, Nelson DR, Lawitz E, Gordon SC, Schiff E, Nahass R, Ghalib R, Gitlin N, Herring R, Lalezari J, Younes ZH, Pockros PJ, Di Bisceglie AM, Arora S, Subramanian GM, Zhu Y, Dvory-Sobol H, Yang JC, Pang PS, Symonds WT, McHutchison JG, Muir AJ, Sulkowski M, Kwo P; ION-2 Investigators. Ledipasvir and sofosbuvir for previously treated HCV genotype 1 infection. *N Engl J Med* 2014; **370**: 1483-1493 [PMID: 24725238 DOI: 10.1056/NEJMoa1316366]

41 **Reddy KR**, Bourlière M, Sulkowski M, Omata M, Zeuzem S, Feld JJ, Lawitz E, Marcellin P, Welzel TM, Hyland R, Ding X, Yang J, Knox S, Pang P, Dvory-Sobol H, Subramanian GM, Symonds W, McHutchison JG, Mangia A, Gane E, Mizokami M, Pol S, Afdhal N. Ledipasvir and sofosbuvir in patients with genotype 1 hepatitis C virus infection and compensated cirrhosis: An integrated safety and efficacy analysis. *Hepatology* 2015; **62**: 79-86 [PMID: 25846144 DOI: 10.1002/hep.27826]

42 **Crespo J**, Calleja JL, Fernández I, Sacristan B, Ruiz-Antorán B, Ampuero J, Hernández-Conde M, García-Samaniego J, Gea F, Buti M, Cabezas J, Lens S, Morillas RM, Salcines JR, Pascasio JM, Turnes J, Sáez-Royuela F, Arenas J, Rincón D, Prieto M, Jorquera F, Sanchez Ruano JJ, Navascués CA, Molina E, Moya AG, Moreno-Planas JM; Spanish Group for the Study of the Use of Direct-acting Drugs Hepatitis C Collaborating Group. Real-World Effectiveness and Safety of Oral Combination Antiviral Therapy for Hepatitis C Virus Genotype 4 Infection. *Clin Gastroenterol Hepatol* 2017; **15**: 945-949.e1 [PMID: 28238958 DOI: 10.1016/j.cgh.2017.02.020]

43 **Abergel A**, Asselah T, Metivier S, Kersey K, Jiang D, Mo H, Pang PS, Samuel D, Loustaud-Ratti V. Ledipasvir-sofosbuvir in patients with hepatitis C virus genotype 5 infection: an open-label, multicentre, single-arm, phase 2 study. *Lancet Infect Dis* 2016; **16**: 459-464 [PMID: 26803446 DOI: 10.1016/S1473-3099(15)00529-0]

44 **Gane EJ**, Hyland RH, An D, Svarovskaia E, Pang PS, Brainard D, Stedman CA. Efficacy of ledipasvir and sofosbuvir, with or without ribavirin, for 12 weeks in patients with HCV genotype 3 or 6 infection. *Gastroenterology* 2015; **149**: 1454-1461.e1 [PMID: 26261007 DOI: 10.1053/j.gastro.2015.07.063]

45 **Charlton M**, Everson GT, Flamm SL, Kumar P, Landis C, Brown RS Jr, Fried MW, Terrault NA, O'Leary JG, Vargas HE, Kuo A, Schiff E, Sulkowski MS, Gilroy R, Watt KD, Brown K, Kwo P, Pungpapong S, Korenblat KM, Muir AJ, Teperman L, Fontana RJ, Denning J, Arterburn S, Dvory-Sobol H, Brandt-Sarif T, Pang PS, McHutchison JG, Reddy KR, Afdhal N; SOLAR-1 Investigators. Ledipasvir and Sofosbuvir Plus Ribavirin for Treatment of HCV Infection in Patients With Advanced Liver Disease. *Gastroenterology* 2015; **149**: 649-659 [PMID: 25985734 DOI: 10.1053/j.gastro.2015.05.010]

46 **GILEAD**. Available from: URL: https://www.gilead.com/~/media/Files/pdfs/medicines/liver-disease/harvoni/harvoni_pi.pdf

47 **Gandhi Y**, Eley T, Fura A, Li W, Bertz RJ, Garimella T. Daclatasvir: A Review of Preclinical and Clinical Pharmacokinetics. *Clin Pharmacokinet* 2018; **57**: 911-928 [PMID: 29353349 DOI: 10.1007/s40262-017-0624-3]

48 **Nelson DR**, Cooper JN, Lalezari JP, Lawitz E, Pockros PJ, Gitlin N, Freilich BF, Younes ZH, Harlan W, Ghalib R, Oguchi G, Thuluvath PJ, Ortiz-Lasanta G, Rabinovitz M, Bernstein D, Bennett M, Hawkins T, Ravendhran N, Sheikh AM, Varunok P, Kowdley KV, Hennicken D, McPhee F, Rana K, Hughes EA; ALLY-3 Study

Team. All-oral 12-week treatment with daclatasvir plus sofosbuvir in patients with hepatitis C virus genotype 3 infection: ALLY-3 phase III study. *Hepatology* 2015; **61**: 1127-1135 [PMID: 25614962 DOI: 10.1002/hep.27726]

49 **Leroy V**, Angus P, Bronowicki JP, Dore GJ, Hezode C, Pianko S, Pol S, Stuart K, Tse E, McPhee F, Bhore R, Jimenez-Exposito MJ, Thompson AJ. Daclatasvir, sofosbuvir, and ribavirin for hepatitis C virus genotype 3 and advanced liver disease: A randomized phase III study (ALLY-3+). *Hepatology* 2016; **63**: 1430-1441 [PMID: 26822022 DOI: 10.1002/hep.28473]

50 **Wyles DL**, Ruane PJ, Sulkowski MS, Dieterich D, Luetkemeyer A, Morgan TR, Sherman KE, Dretler R, Fishbein D, Gathe JC Jr, Henn S, Hinestrosa F, Huynh C, McDonald C, Mills A, Overton ET, Ramgopal M, Rashbaum B, Ray G, Scarsella A, Yozviak J, McPhee F, Liu Z, Hughes E, Yin PD, Noviello S, Ackerman P; ALLY-2 Investigators. Daclatasvir plus Sofosbuvir for HCV in Patients Coinfected with HIV-1. *N Engl J Med* 2015; **373**: 714-725 [PMID: 26196502 DOI: 10.1056/NEJMoa1503153]

51 **Pol S**, Corouge M, Vallet-Pichard A. Daclatasvir-sofosbuvir combination therapy with or without ribavirin for hepatitis C virus infection: from the clinical trials to real life. *Hepat Med* 2016; **8**: 21-26 [PMID: 27019602 DOI: 10.2147/HMER.S62014]

52 **Garimella T**, You X, Wang R, Huang SP, Kandoussi H, Bifano M, Bertz R, Eley T. A Review of Daclatasvir Drug-Drug Interactions. *Adv Ther* 2016; **33**: 1867-1884 [PMID: 27664109 DOI: 10.1007/s12325-016-0407-5]

53 **Cada DJ**, Kim AP, Baker DE. Elbasvir/Grazoprevir. *Hosp Pharm* 2016; **51**: 665-686 [PMID: 27698508 DOI: 10.1310/hpj5108-665]

54 **Zeuzem S**, Ghalib R, Reddy KR, Pockros PJ, Ben Ari Z, Zhao Y, Brown DD, Wan S, DiNubile MJ, Nguyen BY, Robertson MN, Wahl J, Barr E, Butterton JR. Grazoprevir-Elbasvir Combination Therapy for Treatment-Naive Cirrhotic and Noncirrhotic Patients With Chronic Hepatitis C Virus Genotype 1, 4, or 6 Infection: A Randomized Trial. *Ann Intern Med* 2015; **163**: 1-13 [PMID: 25909356 DOI: 10.7326/M15-0785]

55 **Kwo P**, Gane EJ, Peng CY, Pearlman B, Vierling JM, Serfaty L, Buti M, Shafran S, Stryszak P, Lin L, Gress J, Black S, Dutko FJ, Robertson M, Wahl J, Lupinacci L, Barr E, Haber B. Effectiveness of Elbasvir and Grazoprevir Combination, With or Without Ribavirin, for Treatment-Experienced Patients With Chronic Hepatitis C Infection. *Gastroenterology* 2017; **152**: 164-175.e4 [PMID: 27720838 DOI: 10.1053/j.gastro.2016.09.045]

56 **Feld JJ**, Jacobson IM, Hézode C, Asselah T, Ruane PJ, Gruener N, Abergel A, Mangia A, Lai CL, Chan HL, Mazzotta F, Moreno C, Yoshida E, Shafran SD, Towner WJ, Tran TT, McNally J, Osinusi A, Svarovskaia E, Zhu Y, Brainard DM, McHutchison JG, Agarwal K, Zeuzem S; ASTRAL-1 Investigators. Sofosbuvir and Velpatasvir for HCV Genotype 1, 2, 4, 5, and 6 Infection. *N Engl J Med* 2015; **373**: 2599-2607 [PMID: 26571066 DOI: 10.1056/NEJMoa1512610]

57 **Curry MP**, O'Leary JG, Bzowej N, Muir AJ, Korenblat KM, Fenkel JM, Reddy KR, Lawitz E, Flamm SL, Schiano T, Teperman L, Fontana R, Schiff E, Fried M, Doehle B, An D, McNally J, Osinusi A, Brainard DM, McHutchison JG, Brown RS Jr, Charlton M; ASTRAL-4 Investigators. Sofosbuvir and Velpatasvir for HCV in Patients with Decompensated Cirrhosis. *N Engl J Med* 2015; **373**: 2618-2628 [PMID: 26569658 DOI: 10.1056/NEJMoa1512614]

58 **GILEAD**. Available from: URL: https://www.gilead.com/~/media/files/pdfs/medicines/liver-disease/epclusa/epclusa_pi.pdf

59 **Vachon ML**, Dieterich DT. The era of direct-acting antivirals has begun: the beginning of the end for HCV? *Semin Liver Dis* 2011; **31**: 399-409 [PMID: 22189979 DOI: 10.1055/s-0031-1297928]

60 **Bhatia HK**, Singh H, Grewal N, Natt NK. Sofosbuvir: A novel treatment option for chronic hepatitis C infection. *J Pharmacol Pharmacother* 2014; **5**: 278-284 [PMID: 25422576 DOI: 10.4103/0976-500X.142464]

61 **Ferreira VL**, Assis Jarek NA, Tonin FS, Borba HH, Wiens A, Muzzillo DA, Pontarolo R. Ledipasvir/sofosbuvir with or without ribavirin for the treatment of chronic hepatitis C genotype 1: A pairwise meta-analysis. *J Gastroenterol Hepatol* 2017; **32**: 749-755 [PMID: 27785825 DOI: 10.1111/jgh.13620]

62 **El-Khayat H**, Fouad Y, Mohamed HI, El-Amin H, Kamal EM, Maher M, Risk A. Sofosbuvir plus daclatasvir with or without ribavirin in 551 patients with hepatitis C-related cirrhosis, genotype 4. *Aliment Pharmacol Ther* 2018; **47**: 674-679 [PMID: 29314146 DOI: 10.1111/apt.14482]

63 **Zeuzem S**, Dusheiko GM, Salupere R, Mangia A, Flisiak R, Hyland RH, Illeperuma A, Svarovskaia E, Brainard DM, Symonds WT, Subramanian GM, McHutchison JG, Weiland O, Reesink HW, Ferenci P, Hézode C, Esteban R; VALENCE Investigators. Sofosbuvir and ribavirin in HCV genotypes 2 and 3. *N Engl J Med* 2014; **370**: 1993-2001 [PMID: 24795201 DOI: 10.1056/NEJMoa1316145]

64 Sovaldi. Available from: URL: https://www.rxlist.com/sovaldi-drug.htm#indications_dosage

65 **European Association for the Study of the Liver**. Electronic address: easloffice@easloffice.eu. EASL Recommendations on Treatment of Hepatitis C 2016. *J Hepatol* 2017; **66**: 153-194 [PMID: 27667367 DOI: 10.1016/j.jhep.2016.09.001]

66 **AASLD-IDSA**. Recommendations for testing, managing, and treating hepatitis C. 2017. Available from: URL: http://hcvguidelines.org/

67 **Hernandez MD**, Sherman KE. HIV/hepatitis C coinfection natural history and disease progression. *Curr Opin HIV AIDS* 2011; **6**: 478-482 [PMID: 22001892 DOI: 10.1097/COH.0b013e32834bd365]

68 **Thein HH**, Yi Q, Dore GJ, Krahn MD. Natural history of hepatitis C virus infection in HIV-infected individuals and the impact of HIV in the era of highly active antiretroviral therapy: a meta-analysis. *AIDS* 2008; **22**: 1979-1991 [PMID: 18784461 DOI: 10.1097/QAD.0b013e32830e6d51]

69 **AIDSinfo**. Guidelines for the Use of Antiretroviral Agents in Adults and Adolescents Living with HIV. Available from: URL: https://aidsinfo.nih.gov/guidelines/html/1/adult-and-adolescent-arv/26/hcv-hiv

70 **Bhattacharya D**, Belperio PS, Shahoumian TA, Loomis TP, Goetz MB, Mole LA, Backus LI. Effectiveness of All-Oral Antiviral Regimens in 996 Human Immunodeficiency Virus/Hepatitis C Virus Genotype 1-Coinfected Patients Treated in Routine Practice. *Clin Infect Dis* 2017; **64**: 1711-1720 [PMID: 28199525 DOI: 10.1093/cid/cix111]

71 **Mitchell O**, Gurakar A. Management of Hepatitis C Post-liver Transplantation: a Comprehensive Review. *J Clin Transl Hepatol* 2015; **3**: 140-148 [PMID: 26357641 DOI: 10.14218/JCTH.2015.00005]

72 **Vinaixa C**, Rubín A, Aguilera V, Berenguer M. Recurrence of hepatitis C after liver transplantation. *Ann Gastroenterol* 2013; **26**: 304-313 [PMID: 24714603]

73 **Talavera Pons S**, Boyer A, Lamblin G, Chennell P, Châtenet FT, Nicolas C, Sautou V, Abergel A. Managing drug-drug interactions with new direct-acting antiviral agents in chronic hepatitis C. *Br J Clin Pharmacol* 2017; **83**: 269-293 [PMID: 27530469 DOI: 10.1111/bcp.13095]

74 Drug interactions with Sofosbuvir. Available from: URL: http://www.hcvdruginfo.ca/downloads/Hepatitis%20C-sofosbuvir%20int.pdf

75 Drug interactions with ABBVIE'S 3D regimen. Available from: URL: http://hcvdruginfo.ca/downloads/Hepatitis%20C-int_Abbvie%203D%20regimen.pdf

76 **Faragon JJ**. Elbasvir/Grazoprevir (Zepatier™) Drug Interactions A Quick Guide for Clinicians. Available from: URL: https://aidsetc.org/sites/default/files/resources_files/ELBGRZ%20HIV_and_HCV_Drug_Interaction_Quick_Guide%20February%202018.pdf

77 **Faragon JJ**. Glecaprevir/Pibrentasvir (Mavyret™) Drug Interactions A Quick Guide for Clinicians. Available from: URL: https://aidsetc.org/sites/default/files/resources_files/PBRGCPHIV_and_HCV_Drug_Interaction_Quick_Guide%20February%202018.pdf

78 **FDA**. Available from: URL: https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/209195s000lbl.pdf

79 **Reig M**, Mariño Z, Perelló C, Iñarrairaegui M, Ribeiro A, Lens S, Díaz A, Vilana R, Darnell A, Varela M, Sangro B, Calleja JL, Forns X, Bruix J. Unexpected high rate of early tumor recurrence in patients with HCV-related HCC undergoing interferon-free therapy. *J Hepatol* 2016; **65**: 719-726 [PMID: 27084592 DOI: 10.1016/j.jhep.2016.04.008]

80 **Kanwal F**, Kramer J, Asch SM, Chayanupatkul M, Cao Y, El-Serag HB. Risk of Hepatocellular Cancer in HCV Patients Treated With Direct-Acting Antiviral Agents. *Gastroenterology* 2017; **153**: 996-1005.e1 [PMID: 28642197 DOI: 10.1053/j.gastro.2017.06.012]

81 **Kondili LA**, Gaeta GB, Brunetto MR, Di Leo A, Iannone A, Santantonio TA, Giammario A, Raimondo G, Filomia R, Coppola C, Amoruso DC, Blanc P, Del Pin B, Chemello L, Cavalletto L, Morisco F, Donnarumma L, Rumi MG, Gasbarrini A, Siciliano M, Massari M, Corsini R, Coco B, Madonia S, Cannizzaro M, Zignego AL, Monti M, Russo FP, Zanetto A, Persico M, Masarone M, Villa E, Bernabucci V, Taliani G, Biliotti E, Chessa L, Pasetto MC, Andreone P, Margotti M, Brancaccio G, Ieluzzi D, Borgia G, Zappulo E, Calvaruso V, Petta S, Falzano L, Quaranta MG, Weimer LE, Rosato S, Vella S, Giannini EG. Incidence of DAA failure and the clinical impact of retreatment in real-life patients treated in the advanced stage of liver disease: Interim evaluations from the PITER network. *PLoS One* 2017; **12**: e0185728 [PMID: 28977040 DOI: 10.1371/journal.pone.0185728]

82 **American Association for the Study of Liver Diseases (AASLD) and Infectious Diseases Society of America (IDSA)**. Recommendations for testing, managing, and treating hepatitis C. Available from: URL: http://www.hcvguidelines.org/full-report-view.

83 **Svarovskaia ES**, Dvory-Sobol H, Parkin N, Hebner C, Gontcharova V, Martin R, Ouyang W, Han B, Xu S, Ku K, Chiu S, Gane E, Jacobson IM, Nelson DR, Lawitz E, Wyles DL, Bekele N, Brainard D, Symonds WT, McHutchison JG, Miller MD, Mo H. Infrequent development of resistance in genotype 1-6 hepatitis C virus-infected subjects treated with sofosbuvir in phase 2 and 3 clinical trials. *Clin Infect Dis* 2014; **59**: 1666-1674 [PMID: 25266287 DOI: 10.1093/cid/ciu697]

84 Zepatier™ [package insert]. Whitehouse Station, NJ: Merck Co Inc, 2016

85 Daklinza™ [package insert]. Princeton, NJ: Bristol-Myers Squibb Co, 2016

86 **Boesecke C**, Ingiliz P, Berger F, Lutz T, Schewe K, Schulze Zur Wiesch J, Baumgarten A, Christensen S, Rockstroh JK, Mauss S; GECCO Study Group. Liver Cirrhosis as a Risk Factor for Direct-Acting Antiviral Therapy Failure in Real-Life Hepatitis C Virus/Human Immunodeficiency Virus Coinfection. *Open Forum Infect Dis* 2017; **4**: ofx158 [PMID: 28948181 DOI: 10.1093/ofid/ofx158]

P- Reviewer: Blackadar C, Santos-Lopez G **S- Editor**: Ji FF
L- Editor: A **E- Editor**: Bian YN

World Journal of Hepatology

Submit a Manuscript: http://www.f6publishing.com

World J Hepatol 2018 October 27; 10(10): 685-694

DOI: 10.4254/wjh.v10.i10.685

ISSN 1948-5182 (online)

MINIREVIEWS

Nutritional support in chronic liver disease and cirrhotics

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Author contributions: Shergill R drafted the initial manuscript, conceived of the idea, and conducted research; Syed W, Rizvi SA and Singh I assisted with data analysis and editing of the final manuscript.

Conflict-of-interest statement: No conflict of interest.

Manuscript source: Unsolicited manuscript

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Received: June 27, 2018
Peer-review started: June 30, 2018
First decision: July 19, 2018
Revised: August 4, 2018
Accepted: August 6, 2018
Article in press: August 6, 2018
Published online: October 27, 2018

Abstract

The liver is a major organ and an essential component in maintaining an appropriate nutritional status in healthy individuals through metabolism of protein, carbohydrates, and fat. In individuals with chronic liver disease (CLD), along with a number of other essential functions that the liver serves, its role in nutrition maintenance is severely impaired. Common causes of CLD include hepatitis C, alcoholic liver disease, and non-alcoholic liver disease. Amongst this population, the most common manifestation of impaired nutritional maintenance is protein-calorie malnutrition. Aside from inherent abnormalities in metabolism, such as malabsorption and maldigestion, CLD can be associated with anorexia as well as increased metabolic requirements, all of which contribute to a state of malnutrition. Given the systemic implications and impact on prognosis of malnutrition, proper nutritional assessment is essential and can be achieved through a thorough history and physical, as well as biochemical investigations and anthropometry as needed. Following an appropriate assessment of a patient's nutritional status, an approach to management can be decided upon and is based on the extent of malnutrition which directly reflects the severity of disease. Management options can be grossly separated into enteral and parenteral nutrition. The former is usually sufficient in the form of oral supplements in less severe cases of malnutrition, but as the CLD worsens, parenteral nutrition becomes necessary. With appropriate assessment and early intervention, many of the complications of CLD can be avoided, and ultimately better outcomes can be achieved.

Key words: Chronic liver disease; Cirrhosis; Energy requirements; Nutrition; Malnutrition; Anthropometry; Liver

Core tip: This paper highlights the most recent evidence in the clinical approach to dealing with nutrition in patients with chronic liver disease and cirrhotics.

We will review the pathophysiology of liver disease, etiology, and management of nutrition.

Shergill R, Syed W, Rizvi SA, Singh I. Nutritional support in chronic liver disease and cirrhotics. *World J Hepatol* 2018; 10(10): 685-694 Available from: URL: http://www.wjgnet.com/1948-5182/full/v10/i10/685.htm DOI: http://dx.doi.org/10.4254/wjh.v10.i10.685

INTRODUCTION

The liver is a major organ involved in maintaining appropriate nutritional status. Its role includes metabolism of protein, carbohydrates and fat, which are essential sources of nutrition for the body. Accordingly, patients who suffer from chronic liver disease (CLD) often experience debilitating and severe malnutrition that both reflects the severity of the disease, and thereby disease prognosis, but is also an independent predictor of mortality[1,2]. Malnutrition in CLD is a function of multiple factors including, but not limited to, impaired absorption and/or digestion, increased metabolic requirements, as well as anorexia and overall decreased oral intake. The permanent functional deficits in cirrhosis result in nutritional deficiencies with systemic impacts, and coupled with the several mechanisms by which these nutritional deficiencies are realized, approaches to management and support are increasingly complicated[3]. Given the prognostic implications of nutritional status in patients with CLD, further insight into the assessment and therapy of these patients is essential to appropriate management. This literature review aims to summarize a general approach to nutritional support in patients with CLD, including exploring etiology, clinical assessment and key investigations, as well as management.

ETIOLOGY AND PREVALENCE

CLD involves a process of continuous inflammation and regeneration that eventually results in permanent fibrosis and cirrhosis. The most common cause of this condition is hepatitis C virus infection[4]. Other common causes include alcoholic liver disease, non-alcoholic fatty liver disease (NAFLD) and hepatitis B virus infection. Although it is difficult to assess, experts estimate that over 844 million people worldwide have CLD, and this associated with a mortality rate of approximately 2 million deaths per year. Of those affected by CLD, approximately 20% with compensated cirrhosis and 65%-95% with decompensated cirrhosis have protein-calorie malnutrition (PCM)[5]. PCM is a condition involving cachexia due to nutritional deficiencies in calories and protein. This decline in essential macronutrients can lead to extensive body wasting as well as other related sequelae. Micronutrient deficiencies are also present in patients with CLD, albeit with a more subtle presentation than PCM. In the context of cirrhosis, patients with associated malnutrition have higher rates of hepatic encephalopathy, infection, ascites and variceal bleeding[6]. Multiple studies have documented increased complications and overall length of hospital stay in malnourished patients[7]. It is no question that malnutrition complicates patient management significantly, leading to increased rates of morbidity and mortality in CLD patients.

MALNUTRITION

Dietary intake

The underlying cause of malnutrition in patients with CLD and cirrhosis is multifactorial, and is typically related to a loss of appetite, malabsorption and increased metabolic requirements. Low dietary intake is commonly found among cirrhotic patients. Davidson *et al*[8] describes the hepatic role in appetite regulation through clearance of chemical mediators like cholecystokinin, which contributes to feelings of satiety. The liver also contributes to splanchnic production of cytokines that reduce the hypothalamic-mediated drive for appetite. Furthermore, considering that ascites is a common complication of CLD, the mechanical compression may possibly lead to a premature feeling of fullness. In addition to a low overall dietary intake, there is an alteration in the pattern of fuel consumption in the body. There is a higher rate of fat oxidation in the fasting state of CLD patients. During an overnight fast, a research study showed that 58% of energy came from fat oxidation in cirrhotics, whereas healthy controls derived 55% of their energy from carbohydrates[9]. This may reflect the low hepatic glycogen stores found in patients with cirrhosis. Additionally, in a study by Nielsen *et al*[10], they demonstrated that body size in cirrhotic patients increased during refeeding therapy. This indicates that the volume of dietary intake was in fact a contributing factor to the decline in body mass and overall malnutrition in CLD patients.

Malabsorption

Malabsorption is also an important consideration in patients who present with malnutrition in the setting of CLD. Liver disease that leads to a decline in the bile-salt pool can contribute to fat malabsorption[11]. This is commonly found in patients who suffer from comorbid biliary and pancreatic disease, as there is a decrease in fat and fat-soluble vitamin absorption. The extent of malabsorption in CLD patients without related cholestatic disease is currently a topic of controversy. Some studies claim that regardless of etiology, CLD patients experience bacterial overgrowth

from small bowel hypomotility and portal hypertension that can contribute to malabsorption[12]. While others have found that neither fat nor protein are noticeably malabsorbed unless there is co-existing biliary or pancreatic disease.

Energy requirements

Whether or not increased metabolic requirements contribute to malnutrition in CLD is considered uncertain, with varying levels of evidence to support this claim. Müller *et al*[13] found that the average resting energy expenditure (REE) in a study of 473 cirrhotic patients was found to be normal. However, 34% of patients had an increased REE of over 120% of the expected value[13]. Various causes for hypermetabolism in CLD have been proposed, including infection, ascites and portal hypertension. The link between energy expenditure and malnutrition in cirrhotics remains unclear and further research on this topic is required. In addition to metabolic changes, the overall protein requirement is also increased in patients with CLD. Druml *et al*[14] credits this to a decrease in the production of protein, and an increase in the rate of protein degradation. Low glycogen reserves in the liver trigger increased rates of gluconeogenesis from amino acids, which are derived from protein breakdown. These elevated protein requirements in cirrhotic patients can contribute to a state of malnourishment.

NUTRITIONAL ASSESSMENT (PHYSICAL EXAM AND SERUM MARKERS)

Considering that PCM and micronutrient deficiencies have major prognostic implications in patients with CLD, it is essential to effectively and regularly assess nutritional status. Clinicians should consider and analyze multiple factors in order to make a comprehensive nutritional assessment. This typically includes a medical history, extensive physical examination, laboratory data and more.

History

A thorough medical history can offer significant insight as a preliminary assessment of nutritional status. Discussing the patient's eating behaviors and dietary intake through 24-h recall can help the clinician identify possible sources of malnutrition. Recent weight loss is also important to review, as it can point towards the severity of nutritional deficit. This can be complicated in cases of decompensated cirrhosis, as water retention and ascites can lead to inappropriate increases in weight. It is also imperative to identify comorbidities, as they can indirectly impact nutritional status. For example, underlying nausea, vomiting, or anorexia can decrease dietary intake and contribute to malnourishment irrespective of the underlying CLD. Additionally, the severity of liver disease should be assessed through various clinical tools like the Child-Pugh score or Model for End-Stage Liver Disease (MELD) score[15]. The clinical suspicion and onset of malnourishment is directly related to the extent of liver disease in patients.

Physical examination

An appropriate physical examination is a critical component of an effective nutritional status assessment. Macronutrient and micronutrient deficiencies can have a variety of unique physical manifestations. Common examples include pallor in iron deficiency, dermatitis in vitamin A deficiency, bruising in vitamin K or C deficiency and many more[16]. Of particular importance is being able to recognize and assess sarcopenia, which is the generative loss of skeletal muscle mass. Sarcopenia is the most common complication of cirrhosis and so will often be the initial or only presentation of someone with malnutrition secondary to CLD[17]. One of most notable and effective measures of nutritional assessment in clinical practice is known as the subjective global assessment (SGA). The SGA is a standardized array of questions and physical exam findings that are collectively scored to provide a comprehensive rating of nutritional status[18]. In the medical history component, patients are asked a variety of questions about weight changes, dietary intake, associated symptoms, and functional capability. The physical examination includes an assessment of muscle wasting, peripheral edema, ascites, and fat loss. They are then graded with a letter score: Grade A - well nourished, Grade B - Moderately malnourished, Grade C - Severely malnourished. The SGA has been found to serve as a good prognostic indicator in post-liver transplant and chronic dialysis patients[19]. Unfortunately, the SGA is a subjective assessment and it has been found to underestimate the severity of malnutrition in cirrhotic patients compared to the handgrip strength (HG) tool[20]. However, despite being a subjective assessment, there is an 80% inter-rater reproducibility of SGA results[19]. A variation of the SGA includes Royal Free Hospital Global Assessment (RFHGA) tool. This assessment tool adds anthropometric measurements and incorporates gender differences into its final rating of nutritional status[3].

Serum markers

In addition to an appropriate history and physical, there are various laboratory markers that help evaluate the nutritional status of a patient. Currently, various plasma proteins, vitamin levels, and creatinine are considered useful for nutritional assessment. Albumin, pre-albumin, and occasionally transferrin are major plasma proteins that are included in biochemical investigations. Prealbumin, also known as

transthyretin, is a hepatic protein that has been found to correlate well with the body protein status. With a half-life of approximately 2 d, its serum concentration closely reflects recent dietary intake. Production is measurably decreased roughly 14 d following insufficient dietary protein intake, which is less than 60% of the required amount[21]. Devoto *et al*[22] found that prealbumin levels correlated well with the Detailed Nutritional Assessment (DNA) tool, which was used as a reference standard for detecting PCM. They concluded that prealbumin is a good screening tool for protein malnutrition. Furthermore, low prealbumin levels as a nutritional marker have been shown to correlate with higher rates of complications and mortality[21]. In an independent study of dialysis patients, prealbumin was found to be the best nutritional predictor of survival in comparison to serum albumin, cholesterol and creatinine[23]. A serum level less than 15 mg/dL denotes concern for malnutrition[21]. Limitations to the use of prealbumin include inappropriate fluctuations after an alcoholic binge, prednisone use, or active infection and inflammation[24].

In addition to prealbumin, serum albumin was historically considered a good nutritional marker for protein status. However, recent studies have placed significant doubt on this claim and reported no significant link between albumin levels and nutritional status[25]. In malnourished patients, albumin levels were effectively maintained despite the clinical presence of severe PCM. Furthermore, albumin has a half-life of 20 d, making it a particularly slow tool for use in tracking clinical improvement or decline[25]. The serum concentration is also heavily impacted by active inflammation, where hepatic protein synthesis is reduced in order to prioritize the production of acute phase reactants[26]. Several studies have therefore recommended against the use of albumin for nutritional assessment, claiming that it does not serve as a marker for PCM. Similar to albumin, transferrin is also major hepatic protein that was historically measured to assess nutritional status. Transferrin functions as a transport protein for iron. Its use as a marker for protein status is limited because of the large number of factors that influence serum levels[27].

Finally, another major serum marker that has historically been used as a measure of protein status includes creatinine. Researchers hypothesized that urinary creatinine can be used as a serum indicator because it is almost entirely derived from processes in muscle tissue. A creatinine-height index (CHI) was developed as a method of assessing protein status[28]. However, this tool requires 24-h urine measurements and this can serve as a limitation to clinical application. Nevertheless, studies have shown that CHI as a nutritional marker serves as a better prognostic indicator than total body protein and serum albumin.

In terms of micronutrient malnutrition, serum measurements of various vitamins and minerals can serve as a rough measure of total body stores. The specific micronutrients that warrant clinical attention can depend on the etiology of cirrhosis. For example, alcoholic liver cirrhosis is often associated with a thiamine deficiency and cholestatic liver disease can include deficiencies in fat-soluble vitamins.

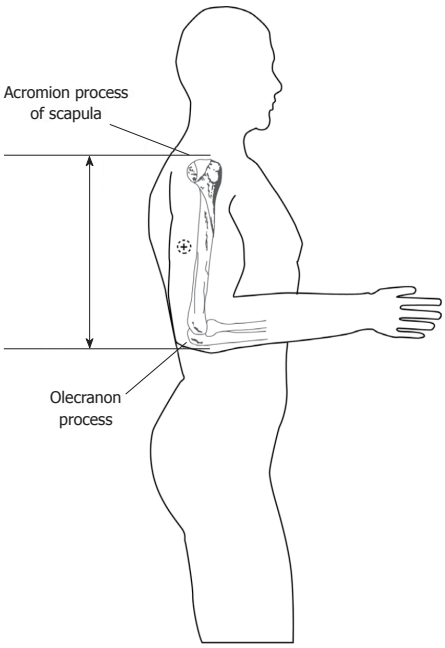

Figure 1 Position for mid-arm muscle circumference measurement[49].

Anthropometry

The mainstay of nutritional assessment almost always involves a history, physical and biochemical investigation. However, patients with a more complicated picture of malnutrition can undergo anthropometric ancillary tests to better characterize their nutritional status. One of the simplest methods of anthropometric assessment includes the body mass index (BMI). BMI evaluates patient height and weight to determine if they are underweight, normal, or overweight. However, the use of BMI in liver disease is very limited as patients often have volume overload complications, which can lead to an overestimation of nutritional status[29]. An anthropometric test that is less affected by fluid status is known as mid-arm muscle circumference (MAMC). MAMC can be used as a measurement of lean tissue levels and muscle bulk (Figure 1). Multiple studies have demonstrated that MAMC correlates well with various other markers in estimating muscle mass[18]. Specifically, it has been shown to correlate well with body cell mass (BCM) measurement, which is a proven marker for PCM in CLD patients. There is also prognostic value to MAMC measurements, as it is associated with mortality risk in CLD patients[1]. In addition to MAMC, triceps skinfold

Figure 2 Position and technique for triceps skinfold thickness measurement[49].

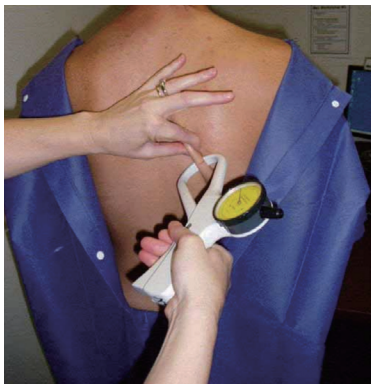

Figure 3 Position and technique for subscapular skinfold thickness measurement[49].

thickness (ST) is another anthropometric test that uses a caliper to measure fat reserve (Figure 2). ST was found to correlate with DEXA scan as a marker of body fat stores. Both ST and MAMC compare results on a percentile score, with values below the 5th percentile indicating severe malnutrition[20]. Anthropometric measurements are widely available, inexpensive and easy to complete. This makes them quite invaluable as bedside tools for nutritional status assessment. However, a major limitation to the use of anthropometric measurements as nutritional markers includes poor inter-rater reproducibility of results[30] (Figure 3) .

Functional measurements have also gained popularity as tools to assess nutritional status in CLD patients. In particular, HG has been shown to be a strong predictor of malnutrition. In a study by Alvares-da-Silva *et al*[20], HG was found to be more sensitive than SGA in predicting malnutrition and the incidence of major complications at 1-year in cirrhotic patients. This functional assessment is completed with a dynamometer and is useful for tracking clinical change in patients. Refer to Table 1 for a summary of these anthropometric measurements and their unique limitations.

Miscellaneous

Less commonly used tests for nutritional status assessment include a DEXA scan, bioelectrical impedance analysis, and *in vivo* neutron activation analysis (IVNAA). These tests are rarely used, primarily due to their limited availability and high cost[30]. DEXA scans can accurately assess the fat mass in CLD patients. Sarcopenia is the most common complication of cirrhosis and DEXA scans can also be used to assess skeletal muscle mass[31]. Additionally, CT and MRI scans can be used to measure the extent of sarcopenia as they both can determine muscle cross-sectional area. These two tests are not used as commonly as DXA scans would be in assessment of sarcopenia however because CT scans are expensive and expose the patient to significant radiation, while MRI scans are also expensive and less available[17]. Bioelectrical impedance analysis uses electrodes to accurately estimate fat content. IVNAA is used to measure total body protein and can serve as a good indicator of PCM[30]. Nevertheless, these are not typical tools used in a standard nutritional assessment.

MANAGEMENT - ENTERAL AND PARENTERAL

Therapeutic interventions to maintain adequate nutritional status in CLD patients can be divided into enteral or parenteral forms. The indications and contraindications for each mode of therapy vary widely depending on the patient and the extent of disease.

Generally speaking, guidelines suggest that the required energy intake for cirrhotic patients is 35-40 kcal/kg-BW per day and a protein intake of 1.2-1.5 g/kg-BW per day[32,33]. The dietary plan for an average 70 kg adult does not significantly differ from a practically "normal" diet, as long as the above caloric and protein intake guidelines are met (usually through protein supplementation). In fact, the diet of CLD patients is largely based on a standard diet with added supplements as needed[31]. It is however important to discuss variations in a patient's condition, as they may concomitantly have hepatic encephalopathy or hepatic-renal syndrome, and these must be addressed as well. With regards to hepatic encephalopathy, the general recommendation currently is to simply continue usual diet, while maximizing appropriate treatment with lactulose rifaximin, and so on[33]. In hepato-renal syndrome, there do not appear to be any recommendations as per the current literature to adjust nutrition, and the current approach remains to address the underlying mechanism causing HRS (*e.g.*, correcting hypovolemia with albumin infusions)[34].

Note that CLD can arise through different pathologies, including NAFLD. This is of particular importance because the pathophysiology through which NAFLD arises is metabolic syndrome and diet/

Table 1 Anthropometric techniques: Benefits and limitations

Technique	Benefits	Limitations
BMI	Weight (kg)/height (m^2) Indicator of choice for chronic undernutrition in adults Probability of misclassifying nutritional status on basis of BMI considered to be very small	Confounded in cirrhotics with ascites and peripheral edema
Mid-arm muscle circumference	Measured in centimeters using flexible measuring tape (halfway between olecranon and acromion process) Less influenced by patient fluid status (upper limbs less commonly edematous) Recognize malnutrition earlier relative to BMI Better at recognizing mild-moderate malnutrition	Possibly significant inter-observer variability Poorly recognizes patients with severe malnutrition
Skinfold thickness (triceps, biceps, subscapular, suprailiac)	Measured in millimeters using skinfold caliper Less influenced by patient fluid status Recognize malnutrition earlier relative to BMI Better at recognizing mild-moderate malnutrition	Possibly significant inter-observer variability Poorly recognizes patients with severe malnutrition
Handgrip strength	Measured in kilogram force, using hydraulic dynamometer adjusted to patient hand size Highly sensitive indicator of functional impairment, reflective of protein-calorie malnutrition Correlates with severity of clinical outcome in different disease states	Requires certain equipment to measure which may not be widely available

BMI: Body mass index.

lifestyle is largely implicated[35]. Given this, addressing diet in patients with CLD from NAFLD is of utmost importance. General recommendations are to reduce total fat, saturated fats, trans fats, and fracture, while simultaneously increasing intake of polyunsaturated fats, and monosaturated fats[35]. These changes will likely benefit any patient with CLD but are especially important in those with NAFLD.

As prognosis is closely linked to nutrition in patients with chronic liver pathology, the goal of any therapeutic measure is to achieve the recommended intake amount.

Enteral

Enteral means of nutritional administration implies that food is absorbed primarily through the digestive processes of the gastrointestinal tract. The intake of nutritional substances can be done orally, directly into the stomach, or from the rectum. In the setting of comorbidities or inability to consume food orally, a nasogastric (NG) or percutaneous endoscopic gastrostomy (PEG) tube can be inserted for direct gastric administration.

Oral

The content and distribution of diet must be regulated to ensure adequate nutritional intake in CLD and cirrhotic patients. The general diet recommendation is to have multiple (5-6) small meals that are rich in complex carbohydrates, while lipids may compose 20%-30% of the overall caloric intake[32]. Initially, it was thought that protein restriction was essential as it was shown to decrease the incidence of encephalopathy in end-stage liver disease. However, recent insight into the value of protein restriction has shown that there is minimal impact on the onset of encephalopathy and rather that overall protein intake should be increased since requirements are higher[36]. A diet low in protein should therefore be avoided.

One of the largest topics of study in patients with liver disease includes the possible benefits of oral supplement use. More specifically, there have been multiple research studies on the use of branched chain amino acids (BCAAs) for nutritional support. BCAAs include leucine, isoleucine, and valine, which cannot be synthesized in the body and must be obtained through diet[37]. Patients with CLD or cirrhosis are known to have low levels of BCAAs, which impacts a variety of bodily functions including ammonia detoxification. Furthermore, inadequate levels have been shown to worsen hepatic encephalopathy and ultimately contribute to a worsened clinical outcome[36]. Multiple research studies have demonstrated benefits of using BCAA supplementation for patients with severe liver disease. A randomized clinical trial showed that long-term supplementation with oral BCAAs helped prevent progressive hepatic failure[37]. They have also been noted to improve cases with pre-existent hepatic encephalopathy. In addition to BCAAs, usage of a controlled diet with nutritional supplements like casein-based protein mixtures were associated with lower bilirubin levels, improved prothrombin time and an overall reduction in infection[37]. A 2012 systematic review by Koretz *et al*[32] identified that the use nutritional supplements in oral feeding for patients with liver disease were associated lower rates of ascites, infection and hepatic encephalopathy. Finally, multivitamins are also recommended but there is

limited research on the benefits in CLD patients.

Increasing numbers of patients with CLD are being found to have coexisting celiac disease and this introduces an additional barrier with regards to tailoring feeds to achieve appropriate nutrition[38-40]. Given that general recommendations for diet include meals that are rich in complex carbohydrates, it is essential that patients with concomitant celiac disease ensure the carbohydrates that they intake are gluten-free. Many BCAA supplements do not contain gluten, and further supplementation with protein mixtures can be achieved by ensuring the protein mixture acquired is gluten-free[41]. Although celiac disease does in fact introduce an additional barrier, much of it can be overcome with a fastidious approach to which foods are consumed. These principles apply to other methods of nutritional intake explored below as well.

Tube feeding

If it is evident that nutritional requirements cannot be met through oral feeding, the next line of therapy is tube feeding. In regards to the use of a NG or PEG tube, clinicians are often concerned about the possibility of an NG tube causing gastrointestinal bleeding. However, literature has found that the risk of GI bleeding in NG tube placement is quite low and should not deter clinicians away from this form of tube feeding[42]. The European Society for Parenteral and Enteral Nutrition (ESPEN) guidelines originally indicated that tube feeding could be used in patients who were not able to maintain adequate nutritional status through oral intake, even in the presence esophageal varices. Though, this was modified shortly after the results of a small-randomized trial identified the possibility of tube feeding causing recurrence of bleeding[42]. The guidelines were then changed to state that tube feeding in patients with esophageal varices is dangerous, and that patients should be closely monitored should this therapy be initiated[42].

When NG tubes are needed for extended periods of time, smaller diameter NG tubes are recommended to avoid irritation of the nasal mucosa when in use for extended periods of time. However, in cases where long term enteral tube feeding is needed, there is typically discussion on the merits of using a PEG tube. Major problems with using PEG tubes in this patient population are that there are various instances in liver disease where PEG tubes are contraindicated; specifically in the setting of ascites, bleeding varices, coagulopathy or other sequela of decompensated cirrhosis[43]. Due to this, clinicians are rarely inclined to use a PEG tube for CLD or cirrhotic patients.

Research comparing oral diets to enteral-tube feeding has shown variable results in determining which mode of nutrition provides better outcomes. Studies by Kearns *et al*[44] and Cabre *et al*[45] have shown significant clinical improvements in serum albumin, Child's score, and a reduction in mortality with patients who were on enteral-tube feeding compared to oral diet controls[46]. Accordingly, a recent multicenter trial in 99 cirrhotic patients randomly placed half on enteral tube-feeding for 4 wk, followed by oral supplements for 8 wk while the other half was kept on a strict oral diet[47]. Using short-term enteral tube feeding allowed investigators to achieve the recommended caloric intake in 70% of patients, but there was no difference in 1-year survival, or other liver parameters between the 2 groups[47]. Considering the multitude of studies on the topic of oral nutrition *vs* tube feeding, Hasse *et al*[48] performed an extensive review of enteral nutrition in the setting of liver disease. This review indicated that evidence for starting a patient on oral or tube feeding is currently inconclusive, as the majority of data show highly variable results[48]. However, the discussion of when to use oral or tube feeding is typically not a matter of preference, but more so what a patient is able to tolerate. Currently, this review of enteral nutrition outlines that patients should maximize oral intake with a targeted diet and supplements initially. At this stage, individuals should be closely monitored to see if oral feeding is able to achieve the desired caloric and nutrient intake. If nutritional status continues to decline, it is recommended that patients begin tube feeding within 1 wk of inadequate oral intake[42,48]. Delaying the onset of tube feeding is associated with worse outcomes and delayed improvement in nutritional status. NG tubes are recommended, since PEG tubes are often contraindicated in CLD patients.

There are various formulas that are suitable for tube-feeding depending on the individual patient disease. They may typically be a standard formula that is protein rich, or nutrient-dense in those who are on fluid restrictions. These can also be hydrolyzed for those who have impaired digestion, include more BCAAs or offer immune complexes for patients that are immunocompromised. These formulas are highly variable and can be altered for individual patient needs.

Parenteral

Parenteral nutrition therapy is an intervention in which feeding is done entirely through an intravenous line. In patients with liver disease, parenteral nutrition is often recommended when caloric and nutritional intake is insufficient through either oral or enteral means. It can also be considered in short-term situations where patients must undergo prolonged fasts for procedural considerations. It is also a consideration in patients with compromised airways, encephalopathy or impaired swallowing reflexes that would make oral and sometimes tube feeding difficult.

Cirrhotic patients require a caloric intake that is

approximately 1.2-1.3 times the REE[43]. Nutrient intake is divided into carbohydrates, lipids, proteins and micronutrients. Through parenteral nutrition, carbohydrates are provided in the form of glucose to make up for approximately 50%-60% of non-protein energy requirements. Lipids are provided as emulsions of unsaturated fatty acids that make up approximately 40%-50% of non-protein energy requirements. Protein requirements are met through an infusion of amino acids that ranges from 1.2-1.5 g/kg per day depending on the patient's current disease severity[43]. These amino acid solutions typically contain a larger proportion of BCAAs and a lower fraction of aromatic amino acids[16]. The inclusion of micronutrients into parenteral formulas is a topic of controversy as many studies have failed to show actual therapeutic benefits. Nevertheless, various water and fat-soluble vitamins, and electrolytes are included in the overall infusion by clinicians. This is primarily because CLD is often associated with significant micronutrient deficiencies, specifically those that have an alcohol-related etiology[44]. Once again, the recommendations for beginning parenteral nutrition are when oral and enteral means have been tried and failed.

There are a variety of complications involved with parenteral nutrition. Since it often requires chronic vascular access, there is risk of catheter infection and clotting. Furthermore, there is risk of total parenteral nutrition (TPN) induced liver disease, particularly due to interactions between linoleic acid (major lipid source of calories) and the liver parenchyma[43]. Furthermore, patients may experience severe hunger pains because TPN use completely bypasses the GI system. Refeeding syndrome is also a consideration in patients with severe cirrhosis or CLD that have been experiencing severe nutrient deficiencies for a prolonged period of time[9].

CONCLUSION

In summary, given the essential role of the liver in maintaining appropriate nutritional status, the importance of prompt recognition of nutritional deficiency in patients with CLD cannot be understated given the immense implications it has on overall morbidity and mortality. The etiology of this malnutrition is multifactorial, including decreased intake, increased metabolic requirements, and malabsorption/maldigestion. Recognizing and assessing nutritional deficiencies can be challenging in patients with CLD. Although a history and physical examination can prove helpful, often they are insufficient and further investigations in the form of serum markers and anthropometry are required for proper assessment. Ultimately, management is in the form of enteral or parenteral nutrition, with the appropriate choice being reflective of disease severity. Although enteral nutrition in oral form with appropriately selected supplements can be sufficient in managing the majority of patients with CLD and malnutrition, often with worsening disease and malabsorption/maldigestion, a step-wise approach moving from oral, to tube feeding, and if necessary, parenteral nutrition is required. Early recognition and intervention is beneficial as the systemic implications of malnutrition greatly impact the prognosis and can limit management options as the disease progresses. With an appropriate approach to malnutrition in CLD, many of the associated complications can be avoided and overall improve the outcomes in this patient population.

REFERENCES

1 **Alberino F**, Gatta A, Amodio P, Merkel C, Di Pascoli L, Boffo G, Caregaro L. Nutrition and survival in patients with liver cirrhosis. *Nutrition* 2001; **17**: 445-450 [PMID: 11399401 DOI: 10.1016/S0899-9007(01)00521-4]

2 **Merli M**, Riggio O, Dally L. Does malnutrition affect survival in cirrhosis? PINC (Policentrica Italiana Nutrizione Cirrosi). *Hepatology* 1996; **23**: 1041-1046 [PMID: 8621131 DOI: 10.1002/hep.510230516]

3 **Gunsar F**, Raimondo ML, Jones S, Terreni N, Wong C, Patch D, Sabin C, Burroughs AK. Nutritional status and prognosis in cirrhotic patients. *Aliment Pharmacol Ther* 2006; **24**: 563-572 [PMID: 16827812 DOI: 10.1111/j.1365-2036.2006.03003.x]

4 **Heidelbaugh JJ**, Bruderly M. Cirrhosis and chronic liver failure: part I. Diagnosis and evaluation. *Am Fam Physician* 2006; **74**: 756-762 [PMID: 16970019]

5 **Cheung K**, Lee SS, Raman M. Prevalence and mechanisms of malnutrition in patients with advanced liver disease, and nutrition management strategies. *Clin Gastroenterol Hepatol* 2012; **10**: 117-125 [PMID: 21893127 DOI: 10.1016/j.cgh.2011.08.016]

6 **Kalaitzakis E**, Simrén M, Olsson R, Henfridsson P, Hugosson I, Bengtsson M, Björnsson E. Gastrointestinal symptoms in patients with liver cirrhosis: associations with nutritional status and health-related quality of life. *Scand J Gastroenterol* 2006; **41**: 1464-1472 [PMID: 17101578 DOI: 10.1080/00365520600825117]

7 **Lin SJ**, Hwang SJ, Liu CY, Lin HR. The relationship between nutritional status and physical function, admission frequency, length of hospital stay, and mortality in old people living in long-term care facilities. *J Nurs Res* 2012; **20**: 110-121 [PMID: 22592106 DOI: 10.1097/jnr.0b013e318254eac9]

8 **Davidson HI**, Richardson R, Sutherland D, Garden OJ. Macronutrient preference, dietary intake, and substrate oxidation among stable cirrhotic patients. *Hepatology* 1999; **29**: 1380-1386 [PMID: 10216119 DOI: 10.1002/hep.510290531]

9 **Henkel AS**, Buchman AL. Nutritional support in patients with chronic liver disease. *Nat Clin Pract Gastroenterol Hepatol* 2006; **3**: 202-209 [PMID: 16582962 DOI: 10.1038/ncpgasthep0443]

10 **Nielsen K**, Kondrup J, Martinsen L, Døssing H, Larsson B, Stilling B, Jensen MG. Long-term oral refeeding of patients with cirrhosis of the liver. *Br J Nutr* 1995; **74**: 557-567 [PMID: 7577893 DOI: 10.1079/BJN19950158]

11 **Shapiro H**, Tehilla M, Attal-Singer J, Bruck R, Luzzatti R, Singer P. The therapeutic potential of long-chain omega-3 fatty acids in nonalcoholic fatty liver disease. *Clin Nutr* 2011; **30**: 6-19 [PMID: 20619513 DOI: 10.1016/j.clnu.2010.06.001]

12 **Mueller KJ**, Crosby LO, Oberlander JL, Mullen JL. Estimation of fecal nitrogen in patients with liver disease. *JPEN J Parenter Enteral Nutr* 1983; **7**: 266-269 [PMID: 6683334 DOI: 10.1177/0148607183007003266]

13 **Müller MJ**, Böttcher J, Selberg O, Weselmann S, Böker KH, Schwarze M, von zur Mühlen A, Manns MP. Hypermetabolism in

clinically stable patients with liver cirrhosis. *Am J Clin Nutr* 1999; **69**: 1194-1201 [PMID: 10357739 DOI: 10.1093/ajcn/69.6.1194]
14 **Druml W**. Global quality assurance in parenteral nutrition. *Clin Nutr* 1996; **15**: 39; author reply 39-39; author reply 40 [PMID: 16843996 DOI: 10.1016/S0261-5614(96)80188-9]
15 **Ebell MH**. Predicting prognosis in patients with end-stage liver disease. *Am Fam Physician* 2006; **74**: 1762-1763 [PMID: 17137008]
16 **Nishikawa H**, Osaki Y. Liver Cirrhosis: Evaluation, Nutritional Status, and Prognosis. *Mediators Inflamm* 2015; **2015**: 872152 [PMID: 26494949 DOI: 10.1155/2015/872152]
17 **Kim TN**, Choi KM. Sarcopenia: definition, epidemiology, and pathophysiology. *J Bone Metab* 2013; **20**: 1-10 [PMID: 24524049 DOI: 10.11005/jbm.2013.20.1.1]
18 **Figueiredo FA**, Perez RM, Freitas MM, Kondo M. Comparison of three methods of nutritional assessment in liver cirrhosis: subjective global assessment, traditional nutritional parameters, and body composition analysis. *J Gastroenterol* 2006; **41**: 476-482 [PMID: 16799890 DOI: 10.1007/s00535-006-1794-1]
19 **Stephenson GR**, Moretti EW, El-Moalem H, Clavien PA, Tuttle-Newhall JE. Malnutrition in liver transplant patients: preoperative subjective global assessment is predictive of outcome after liver transplantation. *Transplantation* 2001; **72**: 666-670 [PMID: 11544428 DOI: 10.1097/00007890-200108270-00018]
20 **Alvares-da-Silva MR**, Reverbel da Silveira T. Comparison between handgrip strength, subjective global assessment, and prognostic nutritional index in assessing malnutrition and predicting clinical outcome in cirrhotic outpatients. *Nutrition* 2005; **21**: 113-117 [PMID: 15723736 DOI: 10.1016/j.nut.2004.02.002]
21 **Beck FK**, Rosenthal TC. Prealbumin: a marker for nutritional evaluation. *Am Fam Physician* 2002; **65**: 1575-1578 [PMID: 11989633]
22 **Devoto G**, Gallo F, Marchello C, Racchi O, Garbarini R, Bonassi S, Albalustri G, Haupt E. Prealbumin serum concentrations as a useful tool in the assessment of malnutrition in hospitalized patients. *Clin Chem* 2006; **52**: 2281-2285 [PMID: 17068165 DOI: 10.1373/clinchem.2006.080366]
23 **Sreedhara R**, Avram MM, Blanco M, Batish R, Avram MM, Mittman N. Prealbumin is the best nutritional predictor of survival in hemodialysis and peritoneal dialysis. *Am J Kidney Dis* 1996; **28**: 937-942 [PMID: 8957050 DOI: 10.1016/S0272-6386(96)90398-4]
24 **Shenkin A**. Serum prealbumin: Is it a marker of nutritional status or of risk of malnutrition? *Clin Chem* 2006; **52**: 2177-2179 [PMID: 17138848 DOI: 10.1373/clinchem.2006.077412]
25 **Ikizler TA**. The use and misuse of serum albumin as a nutritional marker in kidney disease. *Clin J Am Soc Nephrol* 2012; **7**: 1375-1377 [PMID: 22904120 DOI: 10.2215/CJN.07580712]
26 **Kuzuya M**, Izawa S, Enoki H, Okada K, Iguchi A. Is serum albumin a good marker for malnutrition in the physically impaired elderly? *Clin Nutr* 2007; **26**: 84-90 [PMID: 16996659 DOI: 10.1016/j.clnu.2006.07.009]
27 **Guerra LT**, Rosa AR, Romani RF, Gurski RR, Schirmer CC, Kruel CD. Serum transferrin and serum prealbumin as markers of response to nutritional support in patients with esophageal cancer. *Nutr Hosp* 2009; **24**: 241-242 [PMID: 19593499]
28 **Datta D**, Foley R, Wu R, Grady J, Scalise P. Can Creatinine Height Index Predict Weaning and Survival Outcomes in Patients on Prolonged Mechanical Ventilation After Critical Illness? *J Intensive Care Med* 2018; **33**: 104-110 [PMID: 27179057 DOI: 10.1177/0885066616648133]
29 **Prijatmoko D**, Strauss BJ, Lambert JR, Sievert W, Stroud DB, Wahlqvist ML, Katz B, Colman J, Jones P, Korman MG. Early detection of protein depletion in alcoholic cirrhosis: role of body composition analysis. *Gastroenterology* 1993; **105**: 1839-1845 [PMID: 8253360 DOI: 10.1016/0016-5085(93)91083-T]
30 **Patton HM**. Nutritional assessment of patients with chronic liver disease. *Gastroenterol Hepatol* (N Y) 2012; **8**: 687-690 [PMID: 24683378]
31 **Silva M**, Gomes S, Peixoto A, Torres-Ramalho P, Cardoso H, Azevedo R, Cunha C, Macedo G. Nutrition in Chronic Liver Disease. *GE Port J Gastroenterol* 2015; **22**: 268-276 [PMID: 28868418 DOI: 10.1016/j.jpge.2015.06.004]
32 **Koretz RL**, Avenell A, Lipman TO. Nutritional support for liver disease. *Cochrane Database Syst Rev* 2012; **(5)**: CD008344 [PMID: 22592729 DOI: 10.1002/14651858.CD008344.pub2]
33 **O'Brien A**, Williams R. Nutrition in end-stage liver disease: principles and practice. *Gastroenterology* 2008; **134**: 1729-1740 [PMID: 18471550 DOI: 10.1053/j.gastro.2008.02.001]
34 **Lenz K**, Buder R, Kapun L, Voglmayr M. Treatment and management of ascites and hepatorenal syndrome: an update. *Therap Adv Gastroenterol* 2015; **8**: 83-100 [PMID: 25729433 DOI: 10.1177/1756283X14564673]
35 **Dongiovanni P**, Lanti C, Riso P, Valenti L. Nutritional therapy for nonalcoholic fatty liver disease. *J Nutr Biochem* 2016; **29**: 1-11 [PMID: 26895659 DOI: 10.1016/j.jnutbio.2015.08.024]
36 **Bémeur C**, Desjardins P, Butterworth RF. Role of nutrition in the management of hepatic encephalopathy in end-stage liver failure. *J Nutr Metab* 2010; **2010**: 489823 [PMID: 21234351 DOI: 10.1155/2010/489823]
37 **Bianchi G**, Marzocchi R, Agostini F, Marchesini G. Update on branched-chain amino acid supplementation in liver diseases. *Curr Opin Gastroenterol* 2005; **21**: 197-200 [PMID: 15711213 DOI: 10.1097/01.mog.0000153353.45738.bf]
38 **Singh P**, Agnihotri A, Jindal G, Sharma PK, Sharma M, Das P, Gupta D, Makharia GK. Celiac disease and chronic liver disease: is there a relationship? *Indian J Gastroenterol* 2013; **32**: 404-408 [PMID: 23918040 DOI: 10.1007/s12664-013-0352-z]
39 **Zali MR**, Rostami Nejad M, Rostami K, Alavian SM. Liver complications in celiac disease. *Hepat Mon* 2011; **11**: 333-341 [PMID: 22087157]
40 **Rubio-Tapia A**, Murray JA. Liver involvement in celiac disease. *Minerva Med* 2008; **99**: 595-604 [PMID: 19034257]
41 **van Hees NJ**, Giltay EJ, Tielemans SM, Geleijnse JM, Puvill T, Janssen N, van der Does W. Essential amino acids in the gluten-free diet and serum in relation to depression in patients with celiac disease. *PLoS One* 2015; **10**: e0122619 [PMID: 25884227 DOI: 10.1371/journal.pone.0122619]
42 **Plauth M**, Cabré E, Riggio O, Assis-Camilo M, Pirlich M, Kondrup J; DGEM (German Society for Nutritional Medicine), Ferenci P, Holm E, Vom Dahl S, Müller MJ, Nolte W; ESPEN (European Society for Parenteral and Enteral Nutrition). ESPEN Guidelines on Enteral Nutrition: Liver disease. *Clin Nutr* 2006; **25**: 285-294 [PMID: 16707194 DOI: 10.1016/j.clnu.2006.01.018]
43 **Plauth M**, Cabré E, Campillo B, Kondrup J, Marchesini G, Schütz T, Shenkin A, Wendon J; ESPEN. ESPEN Guidelines on Parenteral Nutrition: hepatology. *Clin Nutr* 2009; **28**: 436-444 [PMID: 19520466 DOI: 10.1016/j.clnu.2009.04.019]
44 **Kearns PJ**, Young H, Garcia G, Blaschke T, O'Hanlon G, Rinki M, Sucher K, Gregory P. Accelerated improvement of alcoholic liver disease with enteral nutrition. *Gastroenterology* 1992; **102**: 200-205 [PMID: 1727754 DOI: 10.1016/0016-5085(92)91801-A]
45 **Cabre E**, Gonzalez-Huix F, Abad-Lacruz A, Esteve M, Acero D, Fernandez-Bañares F, Xiol X, Gassull MA. Effect of total enteral nutrition on the short-term outcome of severely malnourished cirrhotics. A randomized controlled trial. *Gastroenterology* 1990; **98**: 715-720 [PMID: 2105256 DOI: 10.1016/0016-5085(90)90293-A]
46 **Cabré E**, Gassull MA. Nutritional and metabolic issues in cirrhosis and liver transplantation. *Curr Opin Clin Nutr Metab Care* 2000; **3**: 345-354 [PMID: 11151078 DOI: 10.1097/00075197-200009000-00004]
47 **Dupont B**, Dao T, Joubert C, Dupont-Lucas C, Gloro R, Nguyen-Khac E, Beaujard E, Mathurin P, Vastel E, Musikas M, Ollivier I, Piquet MA. Randomised clinical trial: enteral nutrition does not improve the long-term outcome of alcoholic cirrhotic patients with jaundice. *Aliment Pharmacol Ther* 2012; **35**: 1166-1174 [PMID:

22452620 DOI: 10.1111/j.1365-2036.2012.05075.x]
48 **Hasse JM**, DiCecco SR. Enteral Nutrition in Chronic Liver Disease: Translating Evidence Into Practice. *Nutr Clin Pract* 2015; **30**: 474-487 [PMID: 26113562 DOI: 10.1177/0884533615591058]
49 **National Health And Nutrition Examination Survey (NHANES)**. Anthropometry Procedures Manual. 2007. Available from: URL: https://www.cdc.gov/nchs/data/nhanes/nhanes_07_08/manual_an.pdf

P- Reviewer: Borzio M, Ferraioli G, Kapoor S, Namisaki T, Qi X
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World J Hepatol 2018 October 27; 10(10): 695-701

DOI: 10.4254/wjh.v10.i10.695

ISSN 1948-5182 (online)

MINIREVIEWS

Anthropometric indicators of visceral adiposity as predictors of non-alcoholic fatty liver disease: A review

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Author contributions: All authors equally contributed to this paper with conception, literature review, drafting and critical revision, editing, and approval of the final version.

Conflict-of-interest statement: No potential conflicts of interest. No financial support.

Manuscript source: Invited manuscript

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Received: March 30, 2018
Peer-review started: March 30, 2018
First decision: April 26, 2018
Revised: June 1, 2018
Accepted: June 8, 2018
Article in press: June 9, 2018
Published online: October 27, 2018

Abstract

The objective was to critically analyze studies that evaluated the predictive capacity of indicators of visceral adiposity in non-alcoholic fatty liver disease (NAFLD). The bibliographic research was carried out using the electronic database PubMed, LILACS and SciELO, references of selected articles. Although we found few studies, they have already used several indicators of visceral adiposity as waist circumference, waist-to-hip ratio, waist-to-height ratio, Lipid accumulation product, Body Shape Index, Body Roundness Index and most them were good predictors of NAFLD. Thus, the anthropometric indicators may contribute for the diagnosis of NAFLD in a simple, low-cost and non-invasive way, allowing early therapeutic measures to prevent the evolution to non-alcoholic steatohepatitis.

Key words: Anthropometry; Adiposity; Non-alcoholic fatty liver disease; Abdominal fat; Pediatrics; Predictive value

Core tip: Non-alcoholic fatty liver disease (NAFLD) is one of the most common liver diseases worldwide and presents evolutional potential for more severe forms of the disease such as cirrhosis and hepatocellular carcinoma. The most effective treatment is based on changes in lifestyle, diet and exercise; however, this presents the challenge of having to be performed for

a long time. The diagnosis, especially of non-alcoholic steatohepatitis (NASH), requires invasive examination such as liver biopsy. The anthropometric clinical indicators of visceral obesity, of easy applicability and low cost, have been very promising in the prediction of NAFLD. Thus, future studies could be conducted to use them in the prediction of NASH, besides assisting in the therapeutic and preventive conduction NASH.

Almeida NS, Rocha R, Cotrim HP, Daltro C. Anthropometric indicators of visceral adiposity as predictors of non-alcoholic fatty liver disease: A review. *World J Hepatol* 2018; 10(10): 695-701 Available from: URL: http://www.wjgnet.com/1948-5182/full/v10/i10/695.htm DOI: http://dx.doi.org/10.4254/wjh.v10.i10.695

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a relevant metabolic disorder with a high evolutional potential, including from isolated hepatic steatosis to non-alcoholic steatohepatitis (NASH), and if left untreated, can progress to fibrosis, cirrhosis and even liver failure[1].

Excess weight, mainly the accumulation of visceral fat, is considered the main risk factor for NAFLD, being closely related to the severity of the disease[2-4]. Currently, clinical anthropometric indicators such as waist-to-height ratio (WHtR) and lipid accumulation product (LAP) are described in the literature as the most sensitive and specific for discriminating visceral fat compared to the classic parameters as waist circumference (WC) and body mass index (BMI)[5].

Some formulas recommended in the literature for NAFLD prediction already use anthropometric indicators such as BMI and WC[6,7]. However, there are few studies that have evaluated predictive capacity as well as the cutoff points of these new indicators in individuals with NAFLD[8-11]. This review aimed to critically analyze studies that assessed the ability of anthropometric indicators of adiposity to predict NAFLD.

LITERATURE SEARCH

To perform the search and retrieval of scientific articles, we used the PubMed (National Library of Medicine), Scielo (Scientific Eletronic Library Online) and LILACS (Literatura Latino-Americana e do Caribe em Ciências da Saúde) databases. The "OR" and "AND" connectors were used to combine the descriptors: ("non-alcoholic fatty liver disease" or "non-alcoholic steatohepatitis" or "fatty liver" or "NAFLD") and ("adult") and ("waist circumference" or "body mass index" or "waist-to-height ratio" or "conicity index" or "lipid accumulation product") and ("discriminatory performance" or "optimal cutoff points"). The articles of interest listed in the references have also been identified and reviewed.

Inclusion criteria were: the availability of the full text in the database; publication in the last ten years (Feb 2008-Feb 2018); written in Portuguese, English or Spanish. Exclusion criteria were: review studies, repeated studies in more than one database, research outside the previously determined context.

After analyzing the titles and abstracts, according to the eligibility criteria, the studies were selected for reading in full. One thousand eight hundred and sixty-three studies were found in the databases. After completing the selection of the studies based on the reading of the titles and abstracts, 1858 studies were excluded: 1845 did not meet the eligibility criteria; 1 duplicated, 1 Chinese, 1 cohort study; 1 evaluated individuals with hepatitis B and C virus, and 2 studies were included after the screening of the references.

Thus, eleven articles were included in the review. The main information of the studies as authorship and year of publication, where the study was conducted, the study population characteristics (number of individuals and age), anthropometric indicators evaluated BMI, WC, WHtR, LAP, Waist-To-Hip Ratio (WHR), Body Shape Index (ABSI), Body Roundness Index (BRI) and diagnosis criteria of NAFLD (Table 1).

CLINICAL ANTHROPOMETRIC INDICATORS OF VISCERAL ADIPOSITY

Classical anthropometric indicators, such as BMI and WC, are disseminated in clinical practice to assess nutritional status, since they are relatively simple and inexpensive tools[12,13]. Visceral obesity is the most important risk factor for NAFLD[2-4] and recent studies have been investigating the relevance of WC and other new clinical indicators of visceral adiposity (WHtR and LAP) in NAFLD diagnosis (Table 2).

In the studies selected here it is observed that different methods were used to identify hepatic steatosis: liver biopsy[9], computed tomography[14], proton magnetic resonance spectroscopy[8,15] being that the great majority realized abdominal ultrasonography[10,11,16-20].

Most of these methods such as ultrasonography, computed tomography and magnetic resonance imaging are important in the diagnosis of NAFLD, however these techniques cannot distinguish benign steatosis from steatohepatitis, severity of inflammation and grade and degree of fibrosis or stage of disease[21]. The liver biopsy, although not used in routine clinical practice remains the only available procedure to grade and to stage NAFLD and to exclude other causes of liver disease. It has been considered a gold standard method[22].

WAIST CIRCUMFERENCE

The WC is an anthropometric indicator widely used

Table 1 Studies evaluating the anthropometric indicators as predictors of non-alcoholic fatty liver disease

Ref.	Country	Population (*n*)	Age (yr)	Anthropometric indicator	NAFLD diagnosis
Yoo *et al*[14]	South Korea	NAFLD (77) Non-NAFLD (379)	20-88	WC and WHtR	Tomography
Zheng *et al*[9]	China	NAFLD (250) Non-NAFLD (240)	36.6 ± 11.1 37.3 ± 10.2	WHR, BMI, WC and WHtR	Liver biopsy
Ju *et al*[10]	South Korea	NAFLD (2553) Non-NAFLD (6606)	42.5 ± 5.1 41.6 ± 4.9	BMI and WC	Ultrasonography
Cuthbertson *et al*[8]	England and Germany	NAFLD (168) Non-NAFLD (168)	50.3 ± 11.9 48.6 ± 10.9	LAP	Proton magnetic resonance spectroscopy
Monteiro *et al*[16]	Brazil	Obese with NAFLD (45) Obese without NAFLD (100)	11-17	WC	Ultrasonography
Zhang *et al*[17]	China	NAFLD (362) Non-NAFLD (6867)	7-18	WHtR, BMI and WC	Ultrasonography
Motamed *et al*[11]	Iran	NAFLD (2048) Non-NAFLD (2824)	48.6 ± 12.7 39.0 ± 15.4	WHtR, WHR, ABSI, BRI	Ultrasonography
Özhan *et al*[18]	Turkey	Obese without NAFLD (130) Obese with NAFLD (202)	11.6 ± 2.7 12.1 ± 2.6	WHtR	Ultrasonography
Lin *et al*[19]	Taiwan	NAFLD (167) Non-NAFLD (1043)	18.8 ± 1.9 15.1 ± 2.8	WHtR, WHR	Ultrasonography
Lee *et al*[15]	United States	Black (94) and White (58) overweight and obese adolescents	14.7 ± 1.8 14.5 ± 1.5	WC	Proton magnetic resonance spectroscopy
Dai *et al*[20]	China	NAFLD (12150) Non-NAFLD (28309)	18-94	LAP	Ultrasonography

NAFLD: Non-alcoholic fatty liver disease; WHR: Waist-to-hip ratio; BMI: Body mass index; WC: Waist circumference; WHtR: Waist-to-height ratio; ABSI: Body shape index; BRI: Body roundness index; LAP: Lipid accumulation product.

in population studies and in clinical practice to assess central obesity, better reflecting the content of visceral fat, in addition to having a good relationship with total body fat[14,23]. However, it presents important limitations when used alone as a predictor of visceral fat[24].

WC and BMI indicators are widely used in the evaluation of individuals with NAFLD[2,8,25]. However, it is known BMI limitations in the evaluation of the composition and distribution of body fat, considering that this indicator only evaluates total body mass[26]. In most studies, the WC was assessed by WHO[26] (midpoint between the iliac crest and the last rib)[9,14,17]. Only Ju *et al*[10] performed the measurement of WC in the umbilical line. Yoo *et al*[14], in an observational cohort study, investigated the WC in 456 adults and elderly Koreans, with BMI of 26.5 ± 2.5 kg/m^2 in men NAFLD group and 27.3 ± 3.3 kg/m^2 in women NAFLD group and found a cutoff point of 89.0 cm for men and 84.0 cm for women, with sensitivity above 74%, in the prediction of NAFLD. Ju *et al*[10] investigated the WC in 9159 adults Koreans in a cross-sectional study with BMI of 22.4 ± 2.5 kg/m^2 in Non-NAFLD group and 25.6 ± 2.61 kg/m^2 in NAFLD group and identified with better levels of sensitivity and specificity, the WC cutoff of 84.9 cm for men and 80.4 cm for women. They observed that WC and BMI showed similar predictive capacity in both sexes, showing that there is still controversy about the best anthropometric parameter to predict NAFLD. In the study conducted by Zheng *et al*[9] in a cross-sectional study with 490 patients Chinese, between 15-56 years, which included 96 patients with liver disease, 86 hepatitis, 19 hepatic hemangioma, and 39 autoimmune liver disease, with BMI 24.8 ± 10.1 kg/m^2 in Non-NAFLD group and 36.8 ± 10.1 kg/m^2 in NAFLD group, BMI and WC had AUC above 0.84 in the total sample.

In children, the WC AUC was 0.94, with the cutoff point at the 80th percentile for age, in the study by Zhang *et al*[17], a cross sectional study with 7229 students in China. Lee *et al*[15] evaluated white and black obese adolescents in the United States and found an optimal WC cutoff point for predicting NAFLD of 101.5 cm, AUC: 0.847, 93% sensitivity and 80% specificity in white obese women, and with no statistical difference between the values found between the blacks. The cross-sectional study by Monteiro *et al*[16], which evaluated 145 obese children and adolescents in Brazil, found higher WC (AUC: 0.720) when compared to trunk fat mass (AUC: 0.661), estimated by Dual-energy X-ray absorptiometry, however lower than the intra-abdominal adipose tissue (AUC: 0.741), measured by ultrasound examination. It should be noted that the authors did not present the cut-off points of the indicators. It is observed that most of these studies were developed in Asian populations[9,14,17], considering that WC cutoff points may differing between racial and ethnic groups more studies are needed for this identification.

WAIST-TO-HIP RATIO

The Waist-To-Hip Ratio (WHR) is determined by dividing

Table 2 Cut-offs and areas under the ROC curve, sensitivity and specificity of anthropometric indicators to determine non-alcoholic fatty liver disease

Ref.	Indicator	Total				Women				Men			
		AUC (95%CI)	Cut-offs point	Sens (%)	Spec (%)	AUC (95%CI)	Cut-offs point	Sens (%)	Spec (%)	AUC (95%CI)	Cut-offs point	Sens (%)	Spec (%)
Yoo *et al*[14]	WHtR					0.80 (0.74-0.86)	0.53	90	63	0.72 (0.63-0.81)	0.52	71	65
	WC					0.79 (0.73-0.86)	84	84	62	0.74 (0.65-0.83)	89	75	64
Zheng *et al*[9]	WHR	0.916 (0.86-0.97)	0.89	99	66								
	BMI	0.854 (0.78-0.93)	24.22	96	64								
	WC	0.876 (0.81-0.94)	82.5	95	68								
	WHtR	0.878 (0.82-0.94)	0.49	96	64								
Ju *et al*[10]	WC					0.821 (0.801-0.840)	80.395			0.759 (0.746-0.773)	84.945		
	BMI					0.83 (0.811-0.850)	22.715			0.76 (0.747-0.773)	24.465		
Cuthbertson *et al*[8]	LAP	0.78 (0.73-0.83)				0.77 (0.69-0.85)				0.74 (0.66-0.82)			
Monteiro *et al*[16]	WC	0.72 (0.636-0.804)		66.7	64			0.733	0.704			0.567	0.6
Zhang *et al*[17]	WHtR	0.95 (0.94-0.96)	0.47	85.2	92.5								
	WC	0.94 (0.92-0.95)	80[1]	86.2	87.4								
	BMI	0.93 (0.91-0.94)	80[1]	86.2	87.6								
Motamed *et al*[11]	WHtR					0.8566 (0.8419-0.8714)	0.58	83.3	71.7	0.8457 (0.8320-0.8593)	0.533	82.7	70.8
	BRI					0.8566 (0.8419-0.8714)	5	83.3	71.7	0.8457 (0.8320-0.8593)	4	82.7	70.8
	WHR					0.7673 (0.7487-0.7860)				0.8018 (0.7862-0.8173)			
	ABSI					0.6598 (0.6382-0.6814)				0.6539 (0.6351-0.6727)			
Özhan *et al*[18]	WHtR		0.62	48.4	73.8								
Lin *et al*[19]	WHtR	0.80 (0.76-0.83)	0.469	70.1	76.9								
	WHR	0.755 (0.714-0.795)											
[1]Lee *et al*[15]	WC	0.847	101.5	93	80								
Dai *et al*[20]	LAP					0.887 (0.882-0.892)	23	82	79	0.843 (0.837-0.849)	30.5	77	75

[1]Data shown for white obese adolescents only. AUC: Area under ROC curve; Sens: Sensitivity; Spec: Specificity; WHtR: Waist-to-height ratio; WC: Waist circumference; WHR: Waist-to-hip ratio; BMI: Body mass index; LAP: Lipid accumulation product; BRI: Body roundness index; ABSI: Body shape index.

the waist circumference by the circumference of the hip. This indicator is used to characterize how body fat is distributed, if it is concentrated in the central region or in body extremities. Sometimes the gain or loss of weight causes similar alterations in waist and hip circumferences, without altering the final ratio, so in these cases, WHR is not so useful to evaluate body mass changes[27].

Few studies have evaluated the WHR in predicting NAFLD. However, the WHR was the indicator with the highest predictive capacity (AUC: 0.91) for NAFLD in the study by Zheng *et al*[9], as a cutoff point of 0.89 for the total sample. Motamed *et al*[11], in a cross-sectional study with Iranian adults, reported an AUC above 0.70 of the WHR in both sexes to predict NAFLD, but cut points were not presented. Despite the limited amount of studies found in the literature that addressed the predictive capacity of WHR in NAFLD, the results found in both studies show that this indicator can be considered as a good predictor of NAFLD.

WAIST-TO-HEIGHT RATIO

The WHtR is an indicator of abdominal obesity that has been used in several studies to evaluate metabolic disorders[28-30]. The analysis of the WHtR suggests that a person WC should not exceed half the height value, presenting a better sensitivity in the evaluation of the health risk when compared to the measurement of isolated WC in different populations[31].

Yoo *et al*[14], Zheng *et al*[9] and Motamed *et al*[11] reported an AUC above 0.71 of the WHtR to detect NAFLD. Yoo *et al*[14] identified cutoff points of 0.53 (sens: sensitivity 90%, spec: specificity 63%) for women and 0.52 (sens: 71%, spec: 65%) for men. The cutoff points found in the study conducted by Motamed *et al*[11] with Iranians were similar for men 0.533 (sens: 82.7%, spec: 70.8%), but not for women which was 0.580 (sens: 83.3%, spec: 71.7%). Already Zheng *et al*[9] did not stratify the results by sex.

In children, the WHtR was the indicator that presented the highest AUC 0.95 and with cutoff points of 0.47 (sens: 95%, spec: 96%) in Zhang *et al*[17]. Lin *et al*[19] evaluated 1210 children aged 10-19 years in Taiwan and identified that WHtR had AUC higher than that of WHR. The cutoff point of WHtR to predict NAFLD in the study population was 0.499 (sens: 70.1%, spec: 76.9%). Özhan *et al*[18], evaluated 332 obese children with and without NAFLD, and the WHtR presented an optimal cutoff point for the prediction of NAFLD of 0.62, but with low sensitivity (48.5%) and high specificity (73.8%). In all this study WC was measured according to WHO[26].

In adults, the advantage of this indicator is related to the ability to neutralize the differences between the heights, allowing to individualize the interpretation of the fat concentration for different ages, since only a change in the value of the indicator occurs if the modification comes from the WC. In addition to be a low-cost indicator, it uses traditional and simple body measurements, and is a non-invasive method, being easily interpreted and reproducible[29,30].

LIPID ACCUMULATION PRODUCT

LAP is a proposed indicator for estimating lipid concentration in adults. Its formula includes data from WC and the serum concentration of triglycerides in the fasted state. The indicator proposes to investigate if the concentration of lipids exerts effect in the evaluation of the cardiovascular risks, better than the BMI[32].

Only two studies used LAP in the prediction of NAFLD[8,20]. Cuthbertson *et al*[8] evaluated 4 cohorts in Germany and England and although it has demonstrated an AUC of 0.78, the authors did not calculate cutoff points for NAFLD. Already a large cross-sectional study[20] that evaluated 40459 Chinese, the LAP showed high accuracy for diagnosing NAFLD in both men and women (AUC 0.843 and 0.887, respectively), with cutoff points of 30.5 (sens: 77%, spec: 75%) and 23.0 (sens: 82%, spec: 79%), respectively, and especially in the younger population between 18 and 44 years old.

Therefore, further studies are needed to confirm its usefulness in predicting this condition in other population groups since it is an excellent predictor of NAFLD in the Asian population. In addition, it is important to highlight a limitation in the clinical practice of this indicator, which requires application of complex formula and serum triglyceride values[33].

OTHER INDICATORS

Two new indicators of obesity, Ody Shape Index (ABSI) e Body Roundness Index (BRI) were described in literature. The ABSI was proposed by Krakauer *et al*[34] and it is calculated with the values of WC, BMI and height. Their analysis suggests that the ABSI allows to evaluate the excess risk of high WC. And Thomas *et al*[35] developed BRI to predict body fat and percentage of visceral adipose tissue, calculated with WC and height data.

Motamed *et al*[11] evaluated the ability of two new obesity indicators to predict NAFLD. In this study BRI and WHtR presented the same discriminatory power (AUC above 0.84) for NAFLD. However, ABSI was not a good indicator to predict NAFLD. Because BRI is an indicator of obesity that is still recent and little studied, the use in clinical practice to assess the discriminatory capacity of NAFLD deserves caution.

Of all the articles studied only one study, Ju *et al*[10], stratified the population in NAFLD and NASH. However, this study did not evaluate the predictive capacity of these indicators in NASH, only in NAFLD, besides NASH classification was evaluated by the presence of steatosis associated with elevation of alanine aminotransferase. Therefore, these studies that investigated the anthropometric indicators of visceral adiposity can only predict NAFLD. In addition, we can highlight in these studies the predictive capacity of the anthropometric clinical indicators for NAFLD that the main limitations were the different diagnostic methods used to detect NAFLD, the different ethnicities and different age groups, such as the inclusion of the elderly in some samples.

CONCLUSION

There are still few studies that evaluated anthropometric indicators of visceral obesity in the prediction of NAFLD, and these already have promising results. The identification of these indicators, with specific cutoff points considering the ethnic and racial groups, for use in the prediction of NAFLD, and especially NASH, may help in the early diagnosis, allowing a therapeutic and preventive approach to this population.

REFERENCES

1 **Gastaldelli A**. Fatty liver disease: the hepatic manifestation of metabolic syndrome. *Hypertens Res* 2010; **33**: 546-547 [PMID: 20463681 DOI: 10.1038/hr.2010.60]

2 **Rocha R**, Cotrim HP, Carvalho FM, Siqueira AC, Braga H, Freitas LA. Body mass index and waist circumference in non-alcoholic fatty liver disease. *J Hum Nutr Diet* 2005; **18**: 365-370 [PMID:

16150132 DOI: 10.1111/j.1365-277X.2005.00634.x]
3 **Eguchi Y**, Eguchi T, Mizuta T, Ide Y, Yasutake T, Iwakiri R, Hisatomi A, Ozaki I, Yamamoto K, Kitajima Y, Kawaguchi Y, Kuroki S, Ono N. Visceral fat accumulation and insulin resistance are important factors in nonalcoholic fatty liver disease. *J Gastroenterol* 2006; **41**: 462-469 [PMID: 16799888 DOI: 10.1007/s00535-006-1790-5]
4 **van der Poorten D**, Milner KL, Hui J, Hodge A, Trenell MI, Kench JG, London R, Peduto T, Chisholm DJ, George J. Visceral fat: a key mediator of steatohepatitis in metabolic liver disease. *Hepatology* 2008; **48**: 449-457 [PMID: 18627003 DOI: 10.1002/hep.22350]
5 **Roriz AK**, Passos LC, de Oliveira CC, Eickemberg M, Moreira Pde A, Sampaio LR. Evaluation of the accuracy of anthropometric clinical indicators of visceral fat in adults and elderly. *PLoS One* 2014; **9**: e103499 [PMID: 25078454 DOI: 10.1371/journal.pone.0103499]
6 **Bedogni G**, Bellentani S, Miglioli L, Masutti F, Passalacqua M, Castiglione A, Tiribelli C. The Fatty Liver Index: a simple and accurate predictor of hepatic steatosis in the general population. *BMC Gastroenterol* 2006; **6**: 33 [PMID: 17081293 DOI: 10.1186/1471-230X-6-33]
7 **Wang J**, Xu C, Xun Y, Lu Z, Shi J, Yu C, Li Y. ZJU index: a novel model for predicting nonalcoholic fatty liver disease in a Chinese population. *Sci Rep* 2015; **5**: 16494 [PMID: 26568423 DOI: 10.1038/srep16494]
8 **Cuthbertson DJ**, Weickert MO, Lythgoe D, Sprung VS, Dobson R, Shoajee-Moradie F, Umpleby M, Pfeiffer AF, Thomas EL, Bell JD, Jones H, Kemp GJ. External validation of the fatty liver index and lipid accumulation product indices, using 1H-magnetic resonance spectroscopy, to identify hepatic steatosis in healthy controls and obese, insulin-resistant individuals. *Eur J Endocrinol* 2014; **171**: 561-569 [PMID: 25298375 DOI: 10.1530/EJE-14-0112]
9 **Zheng RD**, Chen ZR, Chen JN, Lu YH, Chen J. Role of Body Mass Index, Waist-to-Height and Waist-to-Hip Ratio in Prediction of Nonalcoholic Fatty Liver Disease. *Gastroenterol Res Pract* 2012; **2012**: 362147 [PMID: 22701476 DOI: 10.1155/2012/362147]
10 **Ju DY**, Choe YG, Cho YK, Shin DS, Yoo SH, Yim SH, Lee JY, Park JH, Kim HJ, Park DI, Sohn CI, Jeon WK, Kim BI. The influence of waist circumference on insulin resistance and nonalcoholic fatty liver disease in apparently healthy Korean adults. *Clin Mol Hepatol* 2013; **19**: 140-147 [PMID: 23837138 DOI: 10.3350/cmh.2013.19.2.140]
11 **Motamed N**, Rabiee B, Hemasi GR, Ajdarkosh H, Khonsari MR, Maadi M, Keyvani H, Zamani F. Body Roundness Index and Waist-to-Height Ratio are Strongly Associated With Non-Alcoholic Fatty Liver Disease: A Population-Based Study. *Hepat Mon* 2016; **16**: e39575 [PMID: 27822266 DOI: 10.5812/hepatmon.39575]
12 **Tonding SF**, Silva FM, Antonio JP, Azevedo MJ, Canani LH, Almeida JC. Adiposity markers and risk of coronary heart disease in patients with type 2 diabetes mellitus. *Nutr J* 2014; **13**: 124 [PMID: 25539716 DOI: 10.1186/1475-2891-13-124]
13 **Veena J**, Muragundla A, Sidgiddi S, Subramaniam S. Non-alcoholic fatty liver disease: need for a balanced nutritional source. *Br J Nutr* 2014; **112**: 1858-1872 [PMID: 25274101 DOI: 10.1017/S0007114514002591]
14 **Yoo HJ**, Park MS, Lee CH, Yang SJ, Kim TN, Lim KI, Kang HJ, Song W, Yeon JE, Baik SH, Choi DS, Choi KM. Cutoff points of abdominal obesity indices in screening for non-alcoholic fatty liver disease in Asians. *Liver Int* 2010; **30**: 1189-1196 [PMID: 20602679 DOI: 10.1111/j.1478-3231.2010.02300.x]
15 **Lee S**, Kuk JL, Boesch C, Arslanian S. Waist circumference is associated with liver fat in black and white adolescents. *Appl Physiol Nutr Metab* 2017; **42**: 829-833 [PMID: 28334548 DOI: 10.1139/apnm-2016-0410]
16 **Monteiro PA**, Antunes Bde M, Silveira LS, Christofaro DG, Fernandes RA, Freitas Junior IF. Body composition variables as predictors of NAFLD by ultrasound in obese children and adolescents. *BMC Pediatr* 2014; **14**: 25 [PMID: 24476029 DOI: 10.1186/1471-2431-14-25]
17 **Zhang X**, Wan Y, Zhang S, Lu L, Chen Z, Liu H, Jiang X, Luo K, Cai W. Nonalcoholic fatty liver disease prevalence in urban school-aged children and adolescents from the Yangtze River delta region: a cross-sectional study. *Asia Pac J Clin Nutr* 2015; **24**: 281-288 [PMID: 26078245 DOI: 10.6133/apjcn.2015.24.2.13]
18 **Özhan B**, Ersoy B, Özkol M, Kiremitci S, Ergin A. Waist to height ratio: a simple screening tool for nonalcoholic fatty liver disease in obese children. *Turk J Pediatr* 2016; **58**: 518-523 [PMID: 28621093 DOI: 10.24953/turkjped.2016.05.009]
19 **Lin MS**, Lin TH, Guo SE, Tsai MH, Chiang MS, Huang TJ, Chen MY. Waist-to-height ratio is a useful index for nonalcoholic fatty liver disease in children and adolescents: a secondary data analysis. *BMC Public Health* 2017; **17**: 851 [PMID: 29084519 DOI: 10.1186/s12889-017-4868-5]
20 **Dai H**, Wang W, Chen R, Chen Z, Lu Y, Yuan H. Lipid accumulation product is a powerful tool to predict non-alcoholic fatty liver disease in Chinese adults. *Nutr Metab* (Lond) 2017; **14**: 49 [PMID: 28775758 DOI: 10.1186/s12986-017-0206-2]
21 **Parekh S**, Anania FA. Abnormal lipid and glucose metabolism in obesity: implications for nonalcoholic fatty liver disease. *Gastroenterology* 2007; **132**: 2191-2207 [PMID: 17498512 DOI: 10.1053/j.gastro.2007.03.055]
22 **Gaidos JK**, Hillner BE, Sanyal AJ. A decision analysis study of the value of a liver biopsy in nonalcoholic steatohepatitis. *Liver Int* 2008; **28**: 650-658 [PMID: 18331241 DOI: 10.1111/j.1478-3231.2008.01693.x]
23 **Klein S**, Allison DB, Heymsfield SB, Kelley DE, Leibel RL, Nonas C, Kahn R; Association for Weight Management and Obesity Prevention; NAASO; Obesity Society; American Society for Nutrition; American Diabetes Association. Waist circumference and cardiometabolic risk: a consensus statement from shaping America's health: Association for Weight Management and Obesity Prevention; NAASO, the Obesity Society; the American Society for Nutrition; and the American Diabetes Association. *Diabetes Care* 2007; **30**: 1647-1652 [PMID: 17360974 DOI: 10.2337/dc07-9921]
24 **Chan DC**, Watts GF, Barrett PH, Burke V. Waist circumference, waist-to-hip ratio and body mass index as predictors of adipose tissue compartments in men. *QJM* 2003; **96**: 441-447 [PMID: 12788963 DOI: 10.1093/qjmed/hcg069]
25 **Aller R**, Izaola O, Ruiz-Rebollo L, Pacheco D, de Luis DA. Predictive factors of non-alcoholic steatohepatitis: relationship with metabolic syndrome. *Nutr Hosp* 2015; **31**: 2496-2502 [PMID: 26040357 DOI: 10.3305/nh.2015.31.6.8908]
26 **World Health Organization**. Obesity: Preventing and Managing the Global Epidemic. Geneva, World Health Organization, 2000. Available from: URL:http://www.who.int/nutrition/publications/obesity/WHO_TRS_894/en
27 **Vasques ACJ**, Priore ES, Rosado LEFPL, Francesch SCC. The use of anthropometric measures to assess visceral fat accumulation. *Rev Nutr* 2010; **23**: 107-118 [DOI: 10.1590/S1415-52732010000100012]
28 **Pitanga FJ**, Lessa I. Sensitivity and specificity of the conicity index as a coronary risk predictor among adults in Salvador. *Brazil Rev Bras Epidemiol* 2004; **7**: 259-269 [DOI: 10.1590/S1415-790X2004000300004]
29 **Haun DR**, Pitanga FJ, Lessa I. Waist-height ratio compared to other anthropometric indicators of obesity as predictors of high coronary risk. *Rev Assoc Med Bras* (1992) 2009; **55**: 705-711 [PMID: 20191225]
30 **Ashwell M**, Gunn P, Gibson S. Waist-to-height ratio is a better screening tool than waist circumference and BMI for adult cardiometabolic risk factors: systematic review and meta-analysis. *Obes Rev* 2012; **13**: 275-286 [PMID: 22106927 DOI: 10.1111/j.1467-789X.2011.00952.x]
31 **Ashwell M**, Hsieh SD. Six reasons why the waist-to-height ratio is a rapid and effective global indicator for health risks of obesity and how its use could simplify the international public health

message on obesity. *Int J Food Sci Nutr* 2005; **56**: 303-307 [PMID: 16236591 DOI: 10.1080/09637480500195066]

32 **Kahn HS**. The "lipid accumulation product" performs better than the body mass index for recognizing cardiovascular risk: a population-based comparison. *BMC Cardiovasc Disord* 2005; **5**: 26 [PMID: 16150143 DOI: 10.1186/1471-2261-5-26]

33 **de Oliveira CC**, Roriz AK, Ramos LB, Gomes Neto M. Indicators of Adiposity Predictors of Metabolic Syndrome in the Elderly. *Ann Nutr Metab* 2017; **70**: 9-15 [PMID: 28103600 DOI: 10.1159/000455333]

34 **Krakauer NY**, Krakauer JC. A new body shape index predicts mortality hazard independently of body mass index. *PLoS One* 2012; **7**: e39504 [PMID: 22815707 DOI: 10.1371/journal.pone.0039504]

35 **Thomas DM**, Bredlau C, Bosy-Westphal A, Mueller M, Shen W, Gallagher D, Maeda Y, McDougall A, Peterson CM, Ravussin E, Heymsfield SB. Relationships between body roundness with body fat and visceral adipose tissue emerging from a new geometrical model. *Obesity* (Silver Spring) 2013; **21**: 2264-2271 [PMID: 23519954 DOI: 10.1002/oby.20408]

P- Reviewer: Marzuillo P, Streba LLAM, Tzamaloukas AH, Tzamaloukas AHH **S- Editor**: Ji FF **L- Editor**: A **E- Editor**: Song H

World Journal of Hepatology

Submit a Manuscript: http://www.f6publishing.com

World J Hepatol 2018 October 27; 10(10): 702-707

DOI: 10.4254/wjh.v10.i10.702

ISSN 1948-5182 (online)

MINIREVIEWS

Dental pulp cell bank as a possible future source of individual hepatocytes

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Author contributions: Ohkoshi S wrote the paper; Hirono H, Nakahara T and Ishikawa H had critical discussions regarding the study and the manuscript with Ohkoshi S.

Supported by Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science, No. 17K08966 (to Ohkoshi S).

Conflict-of-interest statement: The authors do not have any commercial affiliation or consultancy that could be construed as a conflict of interest.

Manuscript source: Invited manuscript

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Received: March 29, 2018
Peer-review started: March 29, 2018
First decision: May 9, 2018
Revised: May 30, 2018
Accepted: July 10, 2018
Article in press: July 10, 2018
Published online: October 27, 2018

Abstract

Mesenchymal stem cells (MSCs) as a source for regenerative medicine are now the subject of much clinical attention. There are high expectations due to their safety, low tumorigenic risk, and low ethical concerns. MSC therapy has been approved for acute graft-versus host diseases since 2015. Tooth-derived MSCs are known to have a great potential in their proliferation and differentiation capacities, even when compared with bone-marrow-derived MSCs. In particular, stem cells from human exfoliated deciduous teeth (SHEDs) are the best candidates for personal cell banking (dental pulp cell bank), because they can be obtained less invasively in the natural process of individual growth. SHEDs are known to differentiate into hepatocytes. There have been several studies showing the effectiveness of SHEDs on the treatment of liver failure in animal models. They may exert their effects either by repopulation of cells in injured liver or by paracrine mechanisms due to their immune-regulatory functions. Moreover, it may be possible to use each individuals' dental pulp cells as a future source of tailor-made differentiated hepatocytes in the context of a bioartificial liver or liver-on-a-chip to screen for drug toxicity.

Key words: Mesenchymal stem cells; Stem cells from

human exfoliating teeth; Hepatocytes; Dental pulp cell bank; Liver diseases

Core tip: Dental pulp-origin mesenchymal stem cells have a remarkable potential for regenerative medicine in both differentiation and proliferation capacity. Dental pulp cell banks are currently under operation in several institutions in Japan, as they can be obtained easily and less invasively in the personal growth process. Recent findings that they can differentiate into hepatocytes suggest that they can be applied to refractory liver diseases as either auto or allogenic cell therapies. These hepatocytes can be used as tailor-made components for a bioartificial liver or liver-on-a-chip to screen for drug toxicities in preparation for future use.

Ohkoshi S, Hirono H, Nakahara T, Ishikawa H. Dental pulp cell bank as a possible future source of individual hepatocytes. *World J Hepatol* 2018; 10(10): 702-707 Available from: URL: http://www.wjgnet.com/1948-5182/full/v10/i10/702.htm DOI: http://dx.doi.org/10.4254/wjh.v10.i10.702

INTRODUCTION

Mesenchymal stem cells (MSCs), which reside in a variety of tissues, are able to differentiate into many cell types. They have a low risk of tumorigenesis because they do not need the introduction of foreign genes to differentiate, unlike induced pluripotent stem (iPS) cells. They also have a low risk of immune rejection. They can be obtained in a minimally invasive manner such as umbilical cord blood, providing a promising cell source for regenerative medicine. Application of MSCs in the treatment of refractory liver diseases is currently under great clinical scrutiny[1-3].

It was first reported in 2000 that MSCs were present in dental pulp tissues within the teeth[4]. Dental pulp-derived MSCs (DP-MSCs) are known to differentiate into many cell types like other MSCs, such as osteoblasts, adipocytes and neural cells[5]. DP-MSCs also have good potential for proliferation and differentiation similarly to other types of MSC. In particular, MSCs derived from exfoliated deciduous teeth (SHED) in childhood have been reported to have a pronounced potential of proliferation[6,7]. Because these are normally discarded in the process of personal growth, they are perfectly suited for cell banking in a manner similar to umbilical cord blood[8]. The dental pulp cell bank is a best fit for future tailor-made medicine, where people deposit their own tooth-derived MSCs, preparing for their future medical needs. In this review, we concisely review the current and future status of DP-MSCs, including SHED-based regenerative medicines, particularly focusing on their application for liver diseases and for the construction of bio-assay systems that are suitable for drug side-effect testing, with the aim of achieving tailor-made medicine.

Cells from teeth for regenerative medicine

Recent progress in regenerative medicine has been outstanding; it is now possible to remove one's own cells or tissues, differentiate them into many cell types, and use these to repair dysfunctional organs. The development of iPS cells has contributed greatly to this movement. In Japan, a clinical study for age-related macular degeneration using iPS cells started in 2014[9]. However, there remain some clinical concerns regarding iPS cell-based regenerative medicine. For instance, because autologous transplantation of self-iPS cells is costly, heterologous cells must be used in practical situations. Establishment of cell panels to cover all HLA types remains costly and laborious. In addition, because they are prepared with transfection by foreign genes, the risk of tumorigenesis cannot be ignored[10].

On the other hand, because MSCs do not need transfection of genes, they may have a lower risk of tumorigenesis. In addition, they induce immune tolerance in general, so rejection of cells is unlikely. MSCs might also increase the acceptance of regenerative medicine because they do not undergo any gene manipulation.

In the dental field, starting with their acquisition from wisdom and deciduous teeth, MSCs from dental pulp, periodontal ligament, apical papilla, and dental follicle have been reported[11-13]. These dental stem cells have variety of differentiation and active proliferation capacities. These are obtained in a less-invasive manner, and the concept of "waste material re-utilization" is the main rationale to promote a system of dental pulp cell banking.

Gronthos *et al*[4] first reported that dental pulp-derived cells from adults were clonogenic, rapidly-progressive and produced dentin/pulp-like complex under specific conditions. This study opened the way for the application of DP-MSCs to regenerative medicine. Subsequently, it was shown that DP-MSCs could differentiate into cells that were irrelevant to teeth, such as adipocytes or neural cells[5] and are known to be osteogenic, odontogenic, dentinogenic, cementogenic, adipogenic, chondrogenic and neurogenic[11,14]. Miura *et al*[6] showed that SHEDs had a higher potential of proliferation and differentiation, and would therefore be a hopeful source for regenerative medicine. This may be the beginning of the concept of dental bank reusing the exfoliated juvenile teeth that would be discarded otherwise. MSCs from an early age may be expected to be more capable of regeneration and differentiation, as was shown by study finding that SHEDs were more proliferative than other DP-MSCs[7]. There was also a report showing a superior differentiation capacity for SHED when compared with stem cells from adult dental pulp[15].

It is known that stem cells from bone marrow or blood are able to differentiate into cells like hepatocytes[16-18]. Ishkitiev *et al*[19] first reported that DP-MSCs

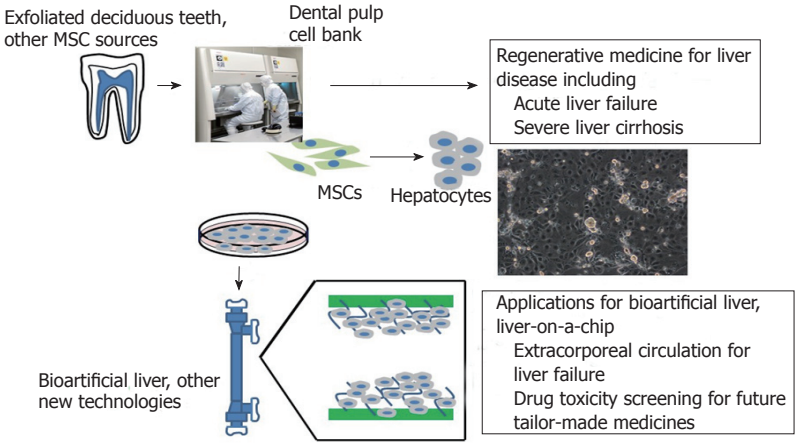

Figure 1 Schematic representation of processes that utilize dental pulp cell bank for future use as hepatocytes. They may be used as cellular sources for cytotherapies to treat refractory liver diseases or as a component of bioartificial liver aiming at tailor-made applications such as future drug-toxicity screening. A hepatocyte-like cell induced from dental pulp-derived-mesenchymal stem cells in our laboratory is shown (unpublished data).

could differentiate into hepatocytes. They cultured cells from deciduous teeth in medium containing HGF, Insulin-Transferrin-Selenium-X, and oncostatin M, and found that they differentiated into cells with an appearance of hepatocytes and produced albumin. These hepatocytes were able to metabolize ammonia to urea, suggesting the presence of a urea cycle. Purification of the cell fractions positive for CD117 enabled efficient induction of hepatocytes[20]. The level of hepatic differentiation in SHED when compared with bone marrow-derived MSC (BM-MSC)s was the same or higher[21]. A recent report also showed a higher expression of hepatocyte-markers in DP-MSCs than in BM-MSCs at both the genetic and protein levels[22]. We also succeeded in differentiating DP-MSC into cells with hepatocyte-morphology, by culturing them first under the presence of activin A, Na-butylate and fibroblast growth factor, and then insulin, dexamethasone, and hepatocyte growth factor (unpublished results, Figure 1).

CELL TRANSPLANTATION THERAPY WITH SHEDS FOR LIVER DISEASES

The effects of MSC-based therapy consist of two major mechanisms. The first is that MSCs transdifferentiate into the cells of damaged-tissues and compensate for organ dysfunction. The second is that, responding to cytokines from the inflamed tissues, MSCs exert paracrine functions including immunomodulation and tissue repair[23]. MSCs produce a variety of cytokines, chemokines and growth factors. The immunomodulatory effects may be one of the main mechanisms of MSC treatment for acute graft versus host disease that has been approved[24].

To date, about half of the papers describing cytotherapies with MSCs were those using bone-marrow-derived MSCs, followed by umbilical cord blood and adipose tissues, and very few were on DP-MSCs[23], despite their promising capabilities. This may partly be due to difficulties of collaboration between dental and medical departments.

Cytotherapies with MSCs have been applied for refractory liver diseases with severe dysfunctions and fibrosis[3]. Transdifferentiation of MSCs into hepatocytes and paracrine mechanisms have been considered to be the main effects. Shi *et al*[25] reported that 13/15 pigs with acute liver failure that were administered bone-marrow derived MSCs survived, while none of the controls did. They showed that 4.5% of cells in surviving liver were repopulated by MSC-derived hepatocytes, concluding that MSC paracrine mechanisms as well as repopulation of hepatocytes by transdifferentiated MSCs contributed to the effects of MSC treatment.

Paracrine mechanisms, including immunomodulation, have attracted the most clinical attention[26]. As the immune effects of MSCs are most likely caused by soluble factors, restriction by HLA in donor selection can be ignored[27]. Moreover, DP-MSCs might induce stronger immune tolerance than bone-marrow derived MSCs[28].

There have been several experimental reports that showed the application of DP-MSCs for liver diseases. Ishikiev *et al*[29] reported that transplantation of hepatocytes induced by SHEDs into the spleen of rats with acute and chronic liver failure improved hepatic functions *via* transdifferentiation and repopulation of the cells. Yamaza *et al*[30] also reported that trans-spleen administration of SHEDs into CCL4-induced cirrhotic mice significantly improved liver function, inflammation, and fibrosis. Both studies attributed the effects to the direct implantation of cells through their differentiation into hepatocytes. Ito *et al*[31] reported that only conditioned medium (CM) from SHEDs resulted in significant survival effects in rats with acute liver failure due to D-galactosamine. They reported that the survival effect of CM on liver failure was induced by anti-inflammatory M2 macrophages that suppressed hepatocyte apoptosis, and promoted hepatocyte proliferation. It is important

Table 1 Comparison of benefits and disadvantages among 3 types of cell sources, mesenchymal stem cells, induced pluripotent stem and embryogenic stem cells

	MSC	iPS cells	ES cells
Proliferation	Low	High	High
Differentiation	Limited	Pluripotent	Pluripotent
Gene transfer	No	Yes	No
Cancer risk	Low	Not neglected	Not neglected
Immune rejection	Low	Possible	High
Paracrine mechanism	Yes	Unknown	Unknown
Banking	Easy	Easy	Possible
Ethical hurdle	Low	Low	High

MSC: Mesenchymal stem cells; iPS: Induced pluripotent stem; ES: Embryogenic stem.

to know that only soluble factors, not the use of cells, induce significant clinical outcomes. Moreover, exosomes secreted by MSCs have been reported to be effective in the improvement of liver function and fibrosis[32,33]. Future studies should verify the effects of no-cell-therapy with conditioned medium or intracellular vesicles on liver diseases.

ESTABLISHMENT AND OPERATION OF DENTAL PULP CELL BANK

Three cellular resources, embryonic stem (ES) cells, iPS cells and MSCs, are currently the major candidates for the clinical application of regenerative medicine. A comparison of the benefits and disadvantages among these cellular resources is shown in Table 1. MSCs do not have higher potential of proliferation or differentiation than ES cells or iPS cells, some consider them to be a primary source for regenerative medicine because of the low possibility of tumorigenesis and the lack of ethical concerns.

SHEDs are an ideal resource in regenerative medicine because of their high capacity, low ethical concerns and cost, and re-use concept[8]. In addition, dental pulp is viable 5 d after extraction[34]. Not only could they be used as a low immunogenic source for allogenic transplantation therapy, but they can also be applied as a tailor-made self-source preparing for future needs[35].

Aiming at the future progress of regenerative medicine from ethical and technical aspects, new legislation was introduced in Japan in 2014. Regenerative medicine using tissue stem cells including MSCs is classified as medium risk, while those using iPS or ES cells are classified as high risk.

The dental pulp cell bank should be officially approved under investigation by the regenerative medicine committee, on the premise of acquisition of informed consent and act of protection of personal information. It must fulfill the requirement of Pharmaceuticals and Medical Devices Agency (PMDA). In Japan, two dental banks are currently under operation, including the Dental Cell Bank™ of The Nippon Dental University which started in 2016 after obtaining permission to operate as a cell processing facility (CPF) from the Japanese Government. Extracted teeth from registered dental clinics are stored in preservation solution and are sent to the Dental Cell Bank™. Dental pulp cells are propagated in culture and stored.

The merits of using dental pulp cells for regenerative medicines, in addition to the general benefits of MSC (Table 1), are follows: the stock cells are obtained when in good health and in a minimally-invasive manner, low cost, and low external radiation exposure because of their confinement in the enamel.

Although some difficulties remain to be overcome in order to achieve successful dental cell bank operations including cost barriers, restrictions imposed by current preservation technology, and the limitation of operation method, the promising capabilities of SHEDs and other tooth-derived sources are supporting the development of the dental pulp cell banking system.

APPLICATION OF HEPATOCYTES FROM DENTAL PULP CELL BANK TO TAILOR-MADE MEDICINE TO MEET FUTURE NEEDS

Fulminant hepatic failure is an aggressive disease that has an extremely poor prognosis. Liver transplantation may be the only medical method to rescue most patients. Because the keys of the success of liver transplantation depend on the acquisition of donor liver, medical bridging therapies while waiting for the appearance of donor liver are critical for life-saving. Extra-corporeal circulation using bioartificial livers that have hepatocytes in the column to reduce toxic substances such as ammonia that can affect consciousness levels have been developed[36]. Although primary hepatocytes or highly differentiated hepatoma cell lines were used for the column, significant survival elongation using bioartificial livers have not yet been confirmed. Recently, development of artificial livers using iPS cells has been reported. Takebe *et al*[37] cultured iPS cells with vascular endothelial cells and macrophages, and succeeded in the creation of an organ bud or mini-liver. Because DP-MSC-derived hepatocytes had high proliferation activity, express hepatocyte nuclear factor 4a (HNF-4a), and metabolize ammonia to urea (unpublished observation), they are expected to bear the function of bioartificial livers.

On the other hand, the liver is an organ involved in drug metabolism. In the era of new medicine development, there will certainly be a need to predict the adverse effects of drugs in a tailor-made manner. Because drug metabolism varies from individual to individual, it is necessary to use self-hepatocytes to screen for drug toxicity. Hepatocytes derived from dental pulp cell bank may suit this purpose. Cells lose differentiation levels in two dimension or spheroid cultures where diffusion of materials is the only way to feed the cells. Recently, microenvironments of the cells in tissues have been

simulated in the organ-on-a-chip system that reproduces the dynamic environments of real tissues[38]. Nakao *et al*[39] reported liver-on-a-chip that reproduced the cord-like structures of hepatocytes with bile -duct canalicular formations. Vernetti *et al*[40], succeeded in drug toxicity screening with construction of a culture system that had hepatocytes, vascular endothelial cells, immune and stellate cells. Hepatocytes derived from a dental pulp cell bank may be a good cellular source of such a three-dimensional culture system and may enable people who deposit their teeth to meet the future use of hepatocytes, such as in drug screening, while providing an allo-auto cellular source to cure liver diseases (Figure 1).

REFERENCES

1 **Lee CW**, Chen YF, Wu HH, Lee OK. Historical Perspectives and Advances in Mesenchymal Stem Cell Research for the Treatment of Liver Diseases. *Gastroenterology* 2018; **154**: 46-56 [PMID: 29107021 DOI: 10.1053/j.gastro.2017.09.049]

2 **Shiota G**, Itaba N. Progress in stem cell-based therapy for liver disease. *Hepatol Res* 2017; **47**: 127-141 [PMID: 27188253 DOI: 10.1111/hepr.12747]

3 **Terai S**, Tsuchiya A. Status of and candidates for cell therapy in liver cirrhosis: overcoming the "point of no return" in advanced liver cirrhosis. *J Gastroenterol* 2017; **52**: 129-140 [PMID: 27631592 DOI: 10.1007/s00535-016-1258-1]

4 **Gronthos S**, Mankani M, Brahim J, Robey PG, Shi S. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. *Proc Natl Acad Sci U S A* 2000; **97**: 13625-13630 [PMID: 11087820 DOI: 10.1073/pnas.240309797]

5 **Gronthos S**, Brahim J, Li W, Fisher LW, Cherman N, Boyde A, DenBesten P, Robey PG, Shi S. Stem cell properties of human dental pulp stem cells. *J Dent Res* 2002; **81**: 531-535 [PMID: 12147742 DOI: 10.1177/154405910208100806]

6 **Miura M**, Gronthos S, Zhao M, Lu B, Fisher LW, Robey PG, Shi S. SHED: stem cells from human exfoliated deciduous teeth. *Proc Natl Acad Sci U S A* 2003; **100**: 5807-5812 [PMID: 12716973 DOI: 10.1073/pnas.0937635100]

7 **Nakamura S**, Yamada Y, Katagiri W, Sugito T, Ito K, Ueda M. Stem cell proliferation pathways comparison between human exfoliated deciduous teeth and dental pulp stem cells by gene expression profile from promising dental pulp. *J Endod* 2009; **35**: 1536-1542 [PMID: 19840643 DOI: 10.1016/j.joen.2009.07.024]

8 **Arora V**, Arora P, Munshi AK. Banking stem cells from human exfoliated deciduous teeth (SHED): saving for the future. *J Clin Pediatr Dent* 2009; **33**: 289-294 [PMID: 19725233 DOI: 10.17796/jcpd.33.4.y887672r0j703654]

9 **Mandai M**, Watanabe A, Kurimoto Y, Hirami Y, Morinaga C, Daimon T, Fujihara M, Akimaru H, Sakai N, Shibata Y, Terada M, Nomiya Y, Tanishima S, Nakamura M, Kamao H, Sugita S, Onishi A, Ito T, Fujita K, Kawamata S, Go MJ, Shinohara C, Hata KI, Sawada M, Yamamoto M, Ohta S, Ohara Y, Yoshida K, Kuwahara J, Kitano Y, Amano N, Umekage M, Kitaoka F, Tanaka A, Okada C, Takasu N, Ogawa S, Yamanaka S, Takahashi M. Autologous Induced Stem-Cell-Derived Retinal Cells for Macular Degeneration. *N Engl J Med* 2017; **376**: 1038-1046 [PMID: 28296613 DOI: 10.1056/NEJMoa1608368]

10 **Aoi T**. 10th anniversary of iPS cells: the challenges that lie ahead. *J Biochem* 2016; **160**: 121-129 [PMID: 27387749 DOI: 10.1093/jb/mvw044]

11 **Huang GT**, Gronthos S, Shi S. Mesenchymal stem cells derived from dental tissues vs. those from other sources: their biology and role in regenerative medicine. *J Dent Res* 2009; **88**: 792-806 [PMID: 19767575 DOI: 10.1177/0022034509340867]

12 **Odontology prize 2017**. *Odontology* 2017; **105**: 391 [PMID: 28988286 DOI: 10.1007/s10266-017-0325-2]

13 **Tamaki Y**, Nakahara T, Ishikawa H, Sato S. In vitro analysis of mesenchymal stem cells derived from human teeth and bone marrow. *Odontology* 2013; **101**: 121-132 [PMID: 22772774 DOI: 10.1007/s10266-012-0075-0]

14 **Liu H**, Gronthos S, Shi S. Dental pulp stem cells. *Methods Enzymol* 2006; **419**: 99-113 [PMID: 17141053 DOI: 10.1016/s0076-6879(06)19005-9]

15 **Feng X**, Xing J, Feng G, Sang A, Shen B, Xu Y, Jiang J, Liu S, Tan W, Gu Z, Li L. Age-dependent impaired neurogenic differentiation capacity of dental stem cell is associated with Wnt/β-catenin signaling. *Cell Mol Neurobiol* 2013; **33**: 1023-1031 [PMID: 24043508 DOI: 10.1007/s10571-013-9965-0]

16 **Krause DS**, Theise ND, Collector MI, Henegariu O, Hwang S, Gardner R, Neutzel S, Sharkis SJ. Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. *Cell* 2001; **105**: 369-377 [PMID: 11348593 DOI: 10.1016/S0092-8674(01)00328-2]

17 **Lagasse E**, Connors H, Al-Dhalimy M, Reitsma M, Dohse M, Osborne L, Wang X, Finegold M, Weissman IL, Grompe M. Purified hematopoietic stem cells can differentiate into hepatocytes in vivo. *Nat Med* 2000; **6**: 1229-1234 [PMID: 11062533 DOI: 10.1038/81326]

18 **Schwartz RE**, Reyes M, Koodie L, Jiang Y, Blackstad M, Lund T, Lenvik T, Johnson S, Hu WS, Verfaillie CM. Multipotent adult progenitor cells from bone marrow differentiate into functional hepatocyte-like cells. *J Clin Invest* 2002; **109**: 1291-1302 [PMID: 12021244 DOI: 10.1172/jci15182]

19 **Ishkitiev N**, Yaegaki K, Calenic B, Nakahara T, Ishikawa H, Mitiev V, Haapasalo M. Deciduous and permanent dental pulp mesenchymal cells acquire hepatic morphologic and functional features in vitro. *J Endod* 2010; **36**: 469-474 [PMID: 20171365 DOI: 10.1016/j.joen.2009.12.022]

20 **Ishkitiev N**, Yaegaki K, Imai T, Tanaka T, Nakahara T, Ishikawa H, Mitev V, Haapasalo M. High-purity hepatic lineage differentiated from dental pulp stem cells in serum-free medium. *J Endod* 2012; **38**: 475-480 [PMID: 22414832 DOI: 10.1016/j.joen.2011.12.011]

21 **Okada M**, Ishkitiev N, Yaegaki K, Imai T, Tanaka T, Fukuda M, Ono S, Haapasalo M. Hydrogen sulphide increases hepatic differentiation of human tooth pulp stem cells compared with human bone marrow stem cells. *Int Endod J* 2014; **47**: 1142-1150 [PMID: 24517624 DOI: 10.1111/iej.12262]

22 **Kumar A**, Kumar V, Rattan V, Jha V, Pal A, Bhattacharyya S. Molecular spectrum of secretome regulates the relative hepatogenic potential of mesenchymal stem cells from bone marrow and dental tissue. *Sci Rep* 2017; **7**: 15015 [PMID: 29118330 DOI: 10.1038/s41598-017-14358-0]

23 **Tsuchiya A**, Kojima Y, Ikarashi S, Seino S, Watanabe Y, Kawata Y, Terai S. Clinical trials using mesenchymal stem cells in liver diseases and inflammatory bowel diseases. *Inflamm Regen* 2017; **37**: 16 [PMID: 29259715 DOI: 10.1186/s41232-017-0045-6]

24 **Ball LM**, Bernardo ME, Roelofs H, van Tol MJ, Contoli B, Zwaginga JJ, Avanzini MA, Conforti A, Bertaina A, Giorgiani G, Jol-van der Zijde CM, Zecca M, Le Blanc K, Frassoni F, Egeler RM, Fibbe WE, Lankester AC, Locatelli F. Multiple infusions of mesenchymal stromal cells induce sustained remission in children with steroid-refractory, grade III-IV acute graft-versus-host disease. *Br J Haematol* 2013; **163**: 501-509 [PMID: 23992039 DOI: 10.1111/bjh.12545]

25 **Shi D**, Zhang J, Zhou Q, Xin J, Jiang J, Jiang L, Wu T, Li J, Ding W, Li J, Sun S, Li J, Zhou N, Zhang L, Jin L, Hao S, Chen P, Cao H, Li M, Li L, Chen X, Li J. Quantitative evaluation of human bone mesenchymal stem cells rescuing fulminant hepatic failure in pigs. *Gut* 2017; **66**: 955-964 [PMID: 26884426 DOI: 10.1136/gutjnl-2015-311146]

26 **Wang Y**, Chen X, Cao W, Shi Y. Plasticity of mesenchymal stem cells in immunomodulation: pathological and therapeutic implications. *Nat Immunol* 2014; **15**: 1009-1016 [PMID: 25329189 DOI: 10.1038/ni.3002]

27 **Le Blanc K**, Frassoni F, Ball L, Locatelli F, Roelofs H, Lewis I, Lanino E, Sundberg B, Bernardo ME, Remberger M, Dini G, Egeler

RM, Bacigalupo A, Fibbe W, Ringdén O; Developmental Committee of the European Group for Blood and Marrow Transplantation. Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: a phase II study. *Lancet* 2008; **371**: 1579-1586 [PMID: 18468541 DOI: 10.1016/S0140-6736(08)60690-X]

28 **Pierdomenico L**, Bonsi L, Calvitti M, Rondelli D, Arpinati M, Chirumbolo G, Becchetti E, Marchionni C, Alviano F, Fossati V, Staffolani N, Franchina M, Grossi A, Bagnara GP. Multipotent mesenchymal stem cells with immunosuppressive activity can be easily isolated from dental pulp. *Transplantation* 2005; **80**: 836-842 [PMID: 16210973 DOI: 10.1097/01.tp.0000173794.72151.88]

29 **Ishkitiev N**, Yaegaki K, Imai T, Tanaka T, Fushimi N, Mitev V, Okada M, Tominaga N, Ono S, Ishikawa H. Novel management of acute or secondary biliary liver conditions using hepatically differentiated human dental pulp cells. *Tissue Eng Part A* 2015; **21**: 586-593 [PMID: 25234861 DOI: 10.1089/ten.TEA.2014.0162]

30 **Yamaza T**, Alatas FS, Yuniartha R, Yamaza H, Fujiyoshi JK, Yanagi Y, Yoshimaru K, Hayashida M, Matsuura T, Aijima R, Ihara K, Ohga S, Shi S, Nonaka K, Taguchi T. In vivo hepatogenic capacity and therapeutic potential of stem cells from human exfoliated deciduous teeth in liver fibrosis in mice. *Stem Cell Res Ther* 2015; **6**: 171 [PMID: 26358689 DOI: 10.1186/s13287-015-0154-6]

31 **Ito T**, Ishigami M, Matsushita Y, Hirata M, Matsubara K, Ishikawa T, Hibi H, Ueda M, Hirooka Y, Goto H, Yamamoto A. Secreted Ectodomain of SIGLEC-9 and MCP-1 Synergistically Improve Acute Liver Failure in Rats by Altering Macrophage Polarity. *Sci Rep* 2017; **7**: 44043 [PMID: 28272428 DOI: 10.1038/srep44043]

32 **Tan CY**, Lai RC, Wong W, Dan YY, Lim SK, Ho HK. Mesenchymal stem cell-derived exosomes promote hepatic regeneration in drug-induced liver injury models. *Stem Cell Res Ther* 2014; **5**: 76 [PMID: 24915963 DOI: 10.1186/scrt465]

33 **Li T**, Yan Y, Wang B, Qian H, Zhang X, Shen L, Wang M, Zhou Y, Zhu W, Li W, Xu W. Exosomes derived from human umbilical cord mesenchymal stem cells alleviate liver fibrosis. *Stem Cells Dev* 2013; **22**: 845-854 [PMID: 23002959 DOI: 10.1089/scd.2012.0395]

34 **Perry BC**, Zhou D, Wu X, Yang FC, Byers MA, Chu TM, Hockema JJ, Woods EJ, Goebel WS. Collection, cryopreservation, and characterization of human dental pulp-derived mesenchymal stem cells for banking and clinical use. *Tissue Eng Part C Methods* 2008; **14**: 149-156 [PMID: 18489245 DOI: 10.1089/ten.tec.2008.0031]

35 **Collart-Dutilleul PY**, Chaubron F, De Vos J, Cuisinier FJ. Allogenic banking of dental pulp stem cells for innovative therapeutics. *World J Stem Cells* 2015; **7**: 1010-1021 [PMID: 26328017 DOI: 10.4252/wjsc.v7.i7.1010]

36 **Bañares R**, Catalina MV, Vaquero J. Liver support systems: will they ever reach prime time? *Curr Gastroenterol Rep* 2013; **15**: 312 [PMID: 23392862 DOI: 10.1007/s11894-013-0312-x]

37 **Takebe T**, Sekine K, Enomura M, Koike H, Kimura M, Ogaeri T, Zhang RR, Ueno Y, Zheng YW, Koike N, Aoyama S, Adachi Y, Taniguchi H. Vascularized and functional human liver from an iPSC-derived organ bud transplant. *Nature* 2013; **499**: 481-484 [PMID: 23823721 DOI: 10.1038/nature12271]

38 **Zhang J**, Zhao X, Liang L, Li J, Demirci U, Wang S. A decade of progress in liver regenerative medicine. *Biomaterials* 2018; **157**: 161-176 [PMID: 29274550 DOI: 10.1016/j.biomaterials.2017.11.027]

39 **Nakao Y**, Kimura H, Sakai Y, Fujii T. Bile canaliculi formation by aligning rat primary hepatocytes in a microfluidic device. *Biomicrofluidics* 2011; **5**: 22212 [PMID: 21799718 DOI: 10.1063/1.3580753]

40 **Vernetti LA**, Senutovitch N, Boltz R, DeBiasio R, Shun TY, Gough A, Taylor DL. A human liver microphysiology platform for investigating physiology, drug safety, and disease models. *Exp Biol Med* (Maywood) 2016; **241**: 101-114 [PMID: 26202373 DOI: 10.1177/1535370215592121]

P- Reviewer: Du Z, McMillin MA, Shi YJ, Tomizawa M
S- Editor: Ma YJ **L- Editor**: A **E- Editor**: Wu YXJ

World Journal of Hepatology

Submit a Manuscript: http://www.f6publishing.com

World J Hepatol 2018 October 27; 10(10): 708-718

DOI: 10.4254/wjh.v10.i10.708

ISSN 1948-5182 (online)

MINIREVIEWS

Adiponectin as a novel biomarker for liver fibrosis

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Author contributions: Udomsinprasert W, Honsawek S and Poovorawan Y contributed to the work, conceived and designed this review; Udomsinprasert W generated the figure and wrote the manuscript, Udomsinprasert W, Honsawek S and Poovorawan Y reviewed and edited the manuscript; all authors reviewed and approved the final manuscript.

Supported by The Research Chair Grant from NSTDA, No. P-15-50004; the Center of Excellence in Clinical Virology, No. GCE 59-00930-005; and Department of Pediatrics, Faculty of Medicine, Chulalongkorn University and Hospital.

Conflict-of-interest statement: The authors declare that they have no competing interests regarding the publication of this paper.

Manuscript source: Invited manuscript

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Received: July 2, 2018
Peer-review started: July 2, 2018
First decision: July 19, 2018
Revised: August 2, 2018
Accepted: August 6, 2018
Article in press: August 7, 2018
Published online: October 27, 2018

Abstract

Adiponectin is known to play primary roles in the regulation of systemic glucose homeostasis and lipid metabolism. Interestingly, emerging evidence indicates beneficial effects of adiponectin on liver fibrosis; however, the exact mechanisms of this action remain unclear. Herein, we aimed to summarize the recent findings regarding the role of adiponectin in liver fibrogenesis and update the current comprehensive knowledge regarding usefulness of adiponectin-based treatments in liver fibrosis. Adiponectin has been demonstrated to have an anti-fibrotic action in the liver by blocking the activation of hepatic stellate cell-mediated adenosine monophosphate-activated protein kinase and peroxisome proliferator-activated receptor-alpha pathways, which in turn diminish the expression of pro-fibrotic genes. In addition, hyperadiponectinemia was noted in patients with various chronic liver diseases (CLDs)-related liver fibrosis. An increase in circulating adiponectin levels was also found to be associated with the development of liver fibrosis, indicating a role of adiponectin as a non-invasive biomarker for predicting the progression of liver fibrosis. It is therefore reasonable to speculate that adiponectin may be developed as a new therapeutic candidate for the treatment of liver fibrosis. Nonetheless, future observations are still necessary to fully elucidate the extent of the effects of adiponectin on

liver fibrotic outcomes, in order to modify adiponectin as an anti-fibrotic therapy that would speed up fibrosis reversal in patients with CLD.

Key words: Adiponectin; Hyperadiponectinemia; Liver fibrosis; Chronic liver disease; Biomarker

Core tip: Adiponectin plays a protective role against the development of liver fibrosis *via* inhibition of hepatic stellate cell activation, induced by specific signal transduction pathways. Among patients with chronic liver diseases (CLDs), hyperadiponectinemia is associated with the degree of liver fibrosis. The potential link between adiponectin and the limited progression of liver fibrosis has accelerated attraction in seeking adiponectin as a target for diagnostic detection tools and novel treatment methods. Nonetheless, additional current therapeutic and clinical trials of adiponectin in liver fibrosis are needed. In this context, we reviewed additional potential therapeutic applications of adiponectin in patients with various CLDs in the context of liver fibrosis.

Udomsinprasert W, Honsawek S, Poovorawan Y. Adiponectin as a novel biomarker for liver fibrosis. *World J Hepatol* 2018; 10(10): 708-718 Available from: URL: http://www.wjgnet.com/1948-5182/full/v10/i10/708.htm DOI: http://dx.doi.org/10.4254/wjh.v10.i10.708

INTRODUCTION

Liver fibrosis is a leading cause of morbidity and mortality associated with end-stage liver complications in patients with chronic liver disease (CLD). It is presently recognized a reversible wound-healing reaction to chronic hepatic damage. The morphological characteristics of liver fibrosis include the excessive accumulation of extracellular matrix (ECM) components, mainly fibrillar collagens[1]. The complexity of liver fibrosis has hindered attempts to understand its pathology, which remains unclear. However, there is a wide spectrum of stimuli known to influence liver fibrogenesis, such as drugs, alcohol abuse, toxins, metabolic disorders, viral infections, and cholestasis[2]. If the underlying cause of hepatic fibrosis cannot be ameliorated, the majority of CLD patients with severe hepatic fibrosis will develop cirrhosis, hepatic failure, and hepatocellular carcinoma (HCC) and ultimately require hepatic transplantation. This has driven researchers toward the search and development of effective anti-fibrotic approaches focused on constraining the growth of fibrogenic cells and/or prohibiting the synthesis of ECM molecules, which would be helpful in improving the clinical outcomes of patients with CLD. Hepatic stellate cells (HSCs) are classically recognized as primary fibrogenic cells in the liver. Given that HSCs are the primary source of activated myofibroblasts and portal fibroblasts, HSCs unsurprisingly take part in enhancing the synthesis of ECM components and modulating matrix degradation in the injured liver. Generally, quiescent HSCs are dormant and their activity is to deposit retinoids. In response to liver injury, HSCs undergo an activation process, and become myofibroblast-like cells that secrete various cytokines/growth factors and produce ECM proteins[3]. Consequently, elucidating the molecular mechanisms of liver fibrosis and their relevance to HSCs is of paramount importance for the discovery of new therapeutic targets.

Adiponectin, a 28 kDa protein adipocytokine, is mainly produced and secreted into the circulation by white adipose tissue. The primary function of adiponectin is the regulation of carbohydrate and lipid metabolism. However, the full extent of its biological action remains to be elucidated, with a variety of effects on different cell and tissue types, including its immune modulatory, anti-inflammatory[4], and anti-fibrotic properties[5,6]. Regarding its protective effects, an experimental study demonstrated the extensive development of liver fibrosis in adiponectin-knockout mice[6]. Acting *via* transmembrane receptors, adiponectin regulates HSC proliferation, as well as migration, and induces their apoptosis through the activation of adenosine monophosphate-activated protein kinase (AMPK)[7]. Moreover, adiponectin can attenuate HSC activation and suppress the expression of pro-fibrogenic genes, including collagen Ⅰ, transforming growth factor-beta 1 (TGF-β1), and alpha-smooth muscle actin (α-SMA)[8], leading to the inhibition of liver fibrogenesis. With such potent effects on HSCs against liver fibrosis, adiponectin may be developed as a novel therapeutic agent in liver fibrosis. As adiponectin can be detected in the circulation and exerts its effects on various cells, it may have prognostic and diagnostic value for several human diseases. Interestingly, hyperadiponectinemia has been documented as highly prevalent in patients with CLD and liver fibrosis[9], thereby establishing the possible influence of adiponectin levels in the development and progression of liver fibrosis. Nevertheless, the underlying mechanisms of association between hyperadiponectinemia and liver fibrosis have yet to be completely elucidated. Therefore, the purpose of this minireview is to summarize an update on experimental and clinical studies that have been focused on the promising beneficial impacts of adiponectin on the treatment of liver fibrosis associated with many aspects of CLDs.

The articles published between 2000-2018 were searched manually from the PubMed and Scopus using the following keywords or combination of keywords: "Adiponectin", "Adiponectin levels", "Liver disease", and "Liver fibrosis". Initially, titles and abstracts related to the keywords were screened, and further full articles were evaluated for inclusion. Human clinical studies of any design providing circulating adiponectin levels associated with the severity of liver fibrosis in patients with various

CLDs were eligible for this review. Articles not written in English-language, letters to the editor, case reports/series, and editorials were excluded from this review. No restrictions on gender, ethnic background, number of study subjects, or publishing year were applied.

ADIPONECTIN BIOLOGY

Protein structure of adiponectin

Human adiponectin encoded by the *Adipo Q* gene spanning 17 kb on chromosome locus 3q27 is a multimeric protein hormone and exerts diverse biological functions. The encoded protein comprises an N-terminal signal sequence being a collagenous domain and a C-terminal globular domain maintaining biological properties after cleavage[10]. Pre-secretion, post-translational mechanisms (*e.g.*, hydroxylation and glycosylation) occurring in the collagenous domain of adiponectin at the four lysines have been shown to improve the activity of sub-physiological levels of insulin, which leads to the inhibition of gluconeogenesis in liver cells[11]. The globular domains of adiponectin form three major complexes including trimers, hexamers, and high-molecular-weight (HMW) multimers, classically existing in the circulation[12]. It seems clear that accurate adiponectin folding and assembly are an important step in regulating its complex distribution in the circulation. Although the different oligomeric complexes distributed in the circulation have distinct downstream biological effects on specific target tissues, HMW adiponectin, the dominant form in the circulation, is considered a marker for disease-associated adipocyte dysfunctions[13]. Among adipocytokines, circulating levels of adiponectin have been observed at high levels in healthy individuals, with approximately 0.01% of the total circulating protein ranging from 5 to 30 μg/mL[14]. On the other hand, hypoadiponectinemia has been previously associated with metabolic alterations, including insulin resistance, dyslipidemia, and atherosclerosis[15-17]. It is noteworthy that a physiological level of circulating adiponectin is important for defense against metabolic disorders and may be related to other chronic diseases including chronic obstructive pulmonary disease[18], chronic kidney disease[19], and knee osteoarthritis[20]. Notwithstanding, the precise mechanisms regulating adiponectin levels in the human body remain poorly understood. It has been suggested that there are multiple factors with important roles in regulating adiponectin levels in the human body, including genetics, mechanisms affecting its clearance, and post-translational modifications associated with controlling adiponectin gene expression[21-23]. Moreover, the regulation of adiponectin receptors is thought to be important for facilitating essential physiological functions of adiponectin.

Adiponectin receptors

The adiponectin receptors, through which adiponectin acts, include seven-transmembrane domains, consisting of two predominant isoforms: adiponectin receptor type 1 (adipo R1) and adiponectin receptor type 2 (adipo R2). These 2 major receptors have been identified in numerous tissues. In human tissues, both of them are observed in the brain and peripheral tissues. However, adipo R1 is ubiquitously detected, most abundantly in the skeletal muscle. The major expression of adipo R2 is in the liver[24]. The adiponectin receptors bind globular and full-length adiponectin with different affinities. The adipoR1 has a greater affinity for globular adiponectin, whereas adipo R2 has an intermediate affinity for both isoforms. Even though the adiponectin receptors comprise seven-transmembrane domains, their structure and functions are distinct from those of G protein-coupled receptors. Physiologically, the engagement of adiponectin with adipo R1 stimulates the phosphorylation of AMPK, which results in a concomitant suppression of energy-consuming biosynthetic pathways, including lipid synthesis and gluconeogenesis. A second common pathway activated by the adiponectin-adipo R2 axis is the peroxisome proliferator-activated receptor-alpha (PPAR-α) signaling pathway, which regulates fatty acid beta-oxidation[25]. Collectively, the aforementioned pathways boost catabolic processes to renew cellular energy under conditions of energy stress. From a classical perspective, adiponectin takes part in controlling glucose and lipid metabolism, through which it exerts a multitude of beneficial effects such as controlling insulin sensitivity. It is conceivable that adiponectin interacting with its distinct receptors influences the stimulation of an appropriate signaling pathway that becomes different in liver pathology.

MULTIFACETED ROLES OF ADIPONECTIN IN THE LIVER

It is commonly recognized that adiponectin regulates the metabolism of both glucose and lipid in the liver, and has been implicated in inhibiting gluconeogenesis, as well as activating fatty acid oxidation and glycolytic pathways. These metabolic impacts of adiponectin are activated by the stimulation of adipo R1 and adipo R2. Following the binding of adiponectin to its receptors, the downstream signaling effects of these receptors are associated with the activation of AMPK and the PPAR-α cascade. As a crucial downstream effector of Adipo R1, AMPK is essentially an energy-sensing gauge that is stimulated by AMP and inhibited by ATP. Activation of AMPK inhibits the transcriptional activity of glucose-6-phosphatase (G-6-Pase) and phosphoenolpyruvate carboxykinase (PEPCK), which in turn decreases gluconeogenesis. Through the phosphorylation of acetyl-CoA carboxylase (ACC) converted into an inactive form and malonyl-CoA catalysis, AMPK also induces fatty acid degradation. Malonyl-CoA, the product of the carboxylase reaction, plays an essential role in inhibiting carnitine palmitoyl transferase-1 (CPT-1), which prevents the transport of long-chain fatty acyl-CoA into the mitochondria, re-

sulting in reduced fatty acid biosynthesis. In addition to its effects mediated by the inactivation of PEPCK, G-6-Pase, and ACC signaling, AMPK phosphorylation can limit the activity of sterol regulatory element binding protein-1c (SREBP-1c), which is a transcription factor regulating lipid combustion in the liver[26,27]. This action can cause a reduction in hepatic triglyceride content. It is comprehensible that adiponectin-AMPK signaling can promote fatty acid catabolism and inhibit gluconeogenesis and triglyceride production in the hepatocytes[28]. In parallel with its stimulation of AMPK, adiponectin-adipo R2 axis activates the PPAR-α signaling cascade that increases fatty acid combustion and energy consumption. This mechanism leads to reduced triglyceride accumulation in the liver and thus, increased insulin sensitivity[29].

Although the triggering of adipo R1 and adipo R2 reportedly controls glucose and lipid metabolism by the stimulation of AMPK and PPAR-α signaling cascades, there are additional signaling molecules related to a wide range of beneficial systemic effects of adiponectin in the liver. The pleiotropic actions of adiponectin have been reportedly associated with the activation of its cognate receptor-mediated stimulation of ceramidase activity[30]. Ceramidase is known to take a primary part in the control of ceramide and sphingosine metabolism, which results in the breakdown of ceramide to produce sphingosine for phosphorylation to sphingosine-1-phosphate. Ceramide and sphingosine comprise an important class of bioactive lipids associated with changes in insulin sensitivity, inflammation, and survival[31]. In 2017, Holland *et al*[32] also demonstrated the significant involvement of adiponectin in declining intracellular ceramide levels, which was accompanied by increased ceramidase activity. This peculiar effect of adiponectin-induced ceramidase signaling was supported in transgenic mice exhibiting an overexpression of adiponectin receptors. Overexpression of adiponectin receptors ameliorated ceramidase activity and induced metabolic improvements in glucose and lipid homeostasis, in addition to insulin sensitivity[32]. These observations provide further evidence regarding the beneficial effects of adiponectin, especially its insulin-sensitizing action, in which adiponectin is activated by its own receptor-mediated stimulation of ceramidase activity.

Besides its primary roles in regulating insulin sensitivity and fatty acid metabolism, adiponectin has been shown to possess anti-inflammatory effects. In particular, adiponectin expands its hepatoprotective effects by decreasing inflammation through blocking the activation of nuclear factor-kappa B (NF-κB)[33] and suppressing the release of tumor necrosis factor-alpha (TNF-α), which is an inflammatory cytokine[4]. The TNF-α that is predominantly secreted by HSCs and Kupffer cells in the liver has a critical effect on hepatic damage, given its capability to promote inflammation and apoptosis in liver cells under the appropriate circumstances of oxidative stress[34]. Adiponectin also limits the production of interleukin-1β, which is a pro-inflammatory cytokine that is responsible for liver injury and initiates the secretion of interleukin-10[35]. The anti-inflammatory actions of adiponectin have also been implicated in macrophage dysfunction[36], through which it suppresses the growth and movement of vascular smooth muscle cells[37] and modulates lymphopoiesis[38]. The pleiotropic biological actions of adiponectin in the liver are illustrated in Figure 1.

Anti-fibrotic action of adiponectin in the liver

One of the critical mechanisms by which adiponectin exerts an anti-fibrogenic effect on the liver is characterized by the induction of the activated phenotype of HSCs. It has been well established that activated HSCs constitutively express both types of adiponectin receptors[39,40], suggesting a potential physiologic action for adiponectin receptor-mediated liver fibrosis in HSCs. As described above, the alteration of HSCs into myofibroblasts is the keystone event of the pathogenesis of liver fibrosis during liver injury. Adiponectin was shown to repress the growth and movement of mouse HSCs stimulated by platelet-derived growth factor (PDGF)[7], which is considered a marker for activated HSCs. An experimental study further demonstrated that adiponectin diminished the effect of TGF-β1-induced expression of connective tissue growth factor (CTGF, also known as fibrogenic gene) on HSCs *via* suppression of the nuclear translocation of mothers against decapentaplegic homolog 2 (SMAD2)[8]. This is important evidence that supports the hypothesis that adiponectin may have anti-fibrogenic effects that are independent of metabolic actions. In this context, overexpression of adipoR2 in mice was found to have a protective role against the progression of liver fibrosis *via* the enhancement of PPAR-α signaling with reduced expression of TGF-β1[41]. Moreover, in cultured HSCs, the enhancement of adiponectin expression remarkably worsened HSC proliferation, as well as the expression of α-SMA, and prevented liver fibrosis through the modulation of caspase-mediated HSC apoptosis[8]. Adiponectin expression is considerably downregulated in activated HSCs, but it is abundantly present in the quiescent phenotype, suggesting a permissive role of adiponectin that favors the quiescent state of HSCs in the physiology of liver fibrosis. In this regard, adiponectin diminishes the development of liver fibrosis by modulating the activity of inhibitor of cytokine signaling-3 (SOCS-3) mediated by the long-form of the leptin receptor (Ob-Rb) and inducing the expression and activation of protein tyrosine phosphatase 1B (PTP1B). As negative regulators of Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signaling, SOCS-3 and PTP1B inhibit JAK-2/STAT-3, which in turn regulate the formation of extracellular components, including tissue inhibitor of metalloproteinases-1 (TIMP-1) and matrix metalloproteinase-1 (MMP-1)[42].

ANTI-FIBROTIC ROLE OF ADIPONECTIN IN CLINICAL PRACTICE

Indeed, adiponectin protects liver injury and reverses

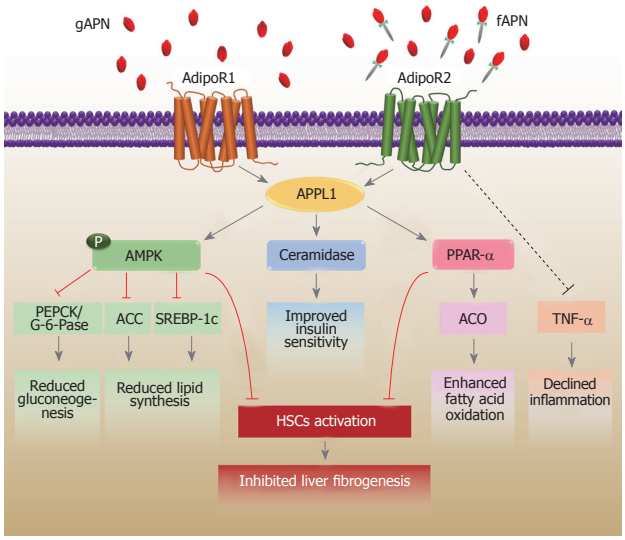

Figure 1 The biological effects of adiponectin on the liver. Adiponectin interacts with adiponectin receptors to prompt a number of signaling pathways. AdipoR1 and R2 dependent signaling is mediated *via* adaptor protein phosphotyrosine interaction (APPL) 1. The signaling activates AMP-activated protein kinase (AMPK), ceramidase activity, and peroxisome proliferator-activated receptor-alpha (PPAR-α) to suppress the accumulation of lipids and regulate glucose homeostasis. The adiponectin-adipoR2 axis can reduce inflammation by inhibiting tumor necrosis factor-alpha (TNF-α) activity. Importantly, activated adiponectin also limits the activation of hepatic stellate cells (HSCs) *via* both AMPK and PPAR-α activation, leading to the inhibition of liver fibrogenesis. ACC: Acetyl-CoA carboxylase; ACO: Acyl-CoA oxidase; AdipoR: Adiponectin receptor; fAPN: Full-length adiponectin; gAPN: Globular adiponectin; PEPCK: Phosphoenolpyruvate carboxykinase; SREBP-1c: Sterol regulatory element binding protein-1c.

the activation of HSCs in animal models. These effects support the possibility of its role against liver damage and fibrogenesis in humans. In pursuit of developing the clinical advantage of adiponectin as a potential predictor for the progression of liver fibrosis, a number of studies have assessed circulating adiponectin levels in a wide range of CLDs.

Adiponectin as a biological marker for liver fibrosis

Generally, liver fibrosis is a reversible physiologic and pathologic event in response to chronic liver injury that leads to liver cirrhosis and eventually end-stage liver disease. The reversal and prevention of these conditions have come to be critical endpoints in clinical trials with novel anti-fibrotic therapies. A liver biopsy has long remained a definitive diagnostic approach for the staging of fibrosis; however, highly efficient and specific non-invasive tools to assess liver fibrosis will be necessary for monitoring the development and progression of disease. Taking into account the risks for complications with respect to sampling error from a needle liver biopsy, there has been enormous interest in the search and development of noninvasive biological markers. Physical characteristics of adiponectin, *e.g.*, high stability in the circulation, little diurnal variation, and great abundance in the human body have made it a promising biomarker in medical contexts for clinical investigation, prognosis, and therapy of liver fibrosis in patients with CLD.

Given that adiponectin has been shown to have predominantly hepatoprotective and anti-fibrogenic effects in conditions of liver injury, whether circulating adiponectin levels are associated with an increased risk of development of liver fibrosis in CLD remains to be determined. Several studies have been focused on investigating the possible associations between adiponectin concentration and the stage of liver fibrosis in various CLDs, including, liver cirrhosis, biliary atresia (BA), hepatitis C viral infection (HCV), hepatitis B viral infection (HBV), non-alcoholic fatty liver disease (NAFLD), and non-alcoholic steatohepatitis (NASH), as summarized in Table 1. First, in a cross-sectional study of 232 fasting patients with CLD, serum adiponectin levels were significantly elevated in patients with cirrhosis, as compared to those patients with other liver diseases. Serum adiponectin concentrations were also positively associated with surrogate biomarkers of liver fibrogenesis, including transient elastography, fasting serum bile acids, and hyaluronate in patients with CLD[9]. This study supports a recent report in which adiponectin levels were significantly elevated in patients with cirrhosis compared to controls and also associated with the severity of hepatic dysfunction in those patients[43]. The possible relevance of adiponectin in CLD has been attested by our previous studies, which evaluated circulating adiponectin concentrations in patients with cholestatic BA. A case-control study of 106 patients with BA and 40 healthy controls by Udomsinprasert *et al*[44] demonstrated that patients with BA exhibited considerably greater levels of serum adiponectin than healthy controls. Notably, serum adiponectin levels were positively correlated with the degree of liver fibrosis in patients with BA[44]. When assessing the relationship between circulating adiponectin levels and clinical parameters in patients with BA - particularly liver stiffness scores, serum adiponectin concentrations were also observed to be remarkably higher in patients with significant liver fibrosis than those with insignificant fibrosis, and a direct correlation of serum adiponectin and the severity of liver fibrogenesis was reported[45].

In addition to its significant involvement in the clinical outcome of BA with respect to the degree of liver fibrosis, a number of studies have reported the relationships between hyperadiponectinemia and the clinical parameters of liver fibrosis in cohorts with hepatitis viral infection. A more recent study by Carvalho *et al*[46] found that patients with HCV infection had significantly greater adiponectin levels than healthy controls. In 2013, Korah *et al*[47] examined serum adiponectin levels in patients with chronic HCV genotype 4 associated with steatosis and fibrosis. They also found that 45 men with chronic HCV genotype 4 and advanced fibrosis had significantly increased adiponectin levels, whereas these levels were remarkably reduced in patients with steatosis. Sumie *et al*[48] added another piece of supporting data, revealing that serum adiponectin levels were predictors of liver fibrosis in patients with HCC, in response to chronic HCV infection. Likewise, when Corbetta *et al*[49] analyzed

Table 1 Summary of studies on the association between circulating adiponectin levels and liver fibrosis in various types of chronic liver diseases

Reference	Yr	Diagnosis	Study design	Subjects	Significant results
Hyperadiponectinemia					
Balmer *et al*[9]	2010	CLD	Cross-sectional study	232 fasting patients with CLD, 64 with NAFLD, 71 patients with viral hepatitis, 18 patients with autoimmune disease, 3 patients with alcohol-induced liver disease, 31 patients with elevated liver enzyme of unknown origin, and 45 patients with cirrhosis	Adiponectin levels were substantially increased in cases of cirrhosis Adiponectin levels were positively correlated with surrogate markers of hepatic fibrosis, including transient elastography, fasting serum bile acids, and hyaluronate
da Silva *et al*[43]	2018	Cirrhosis	Case-control study	122 patients with cirrhosis and 30 healthy controls	Patients with CLD had higher adiponectin levels than controls Adiponectin levels were also associated with the severity of liver dysfunction and worse prognosis in those patients
Udomsinprasert *et al*[44]	2012	BA	Case-control study	106 patients with BA and 40 healthy controls	Serum adiponectin levels were significantly higher in BA patients than in healthy controls Adiponectin levels were associated with the severity of fibrosis in BA patients.
Honsawek *et al*[45]	2011	BA	Case-control study	60 patients with BA and 20 healthy controls	BA patients with significant liver fibrosis exhibited remarkably greater serum adiponectin than insignificant fibrosis Serum adiponectin was positively correlated with the degree of fibrosis.
Carvalho *et al*[46]	2018	HCV	Case-control study	33 patients with untreated HCV infection and 30 healthy controls	Patients with HCV infection had higher adiponectin levels, especially those with women
Korah *et al*[47]	2013	HCV	Case-control study	45 untreated men with chronic HCV genotype 4, and 15 healthy men	Serum adiponectin levels were significantly elevated in hepatic fibrosis, but decreased in steatosis
Sumie *et al*[48]	2011	HCV	Case-control study	97 patients with HCC and chronic HCV infection, and 97 patients (controls) with underlying disease	Serum total and HMW adiponectin levels were predictors of liver fibrosis in HCC patients, in response to chronic HCV infection
Corbetta *et al*[49]	2011	HCV	Case-control study	54 patients with chronic HCV hepatitis and healthy controls	Serum adiponectin levels were higher in patients with chronic HCV hepatitis Adiponectin levels were significantly related to the severity of fibrosis in patients with chronic HCV hepatitis
Derbala *et al*[50]	2009	HCV	Case-control study	92 patients with chronic HCV genotype 4 and 66 healthy controls	Adiponectin levels were associated with hepatic fibrosis and inflammation
Hsu *et al*[51]	2015	HBV	Case-control study	187 patients with chronic HBV infection and 187 without chronic HBV infection	Serum adiponectin levels were remarkably correlated with advanced liver fibrosis in elder male HBeAg-negative patients
Hui *et al*[52]	2007	HBV	Cross-sectional study	100 patients with HBV	Patients with fibrosis reduction had a marked decline in serum adiponectin levels after antiviral therapy Adiponectin levels were significantly correlated with fibrosis stage
Hypoadiponectinemia					
Lucero *et al*[54]	2017	NAFLD	Cross-sectional study	36 patients with NAFLD associated with metabolic syndrome and 24 metabolic syndrome patients without NAFLD	Adiponectin levels were significantly lower in NAFLD patients with metabolic syndrome than those patients without metabolic syndrome Adiponectin levels were associated with metabolic parameters and the degree of liver fibrosis
Nazal *et al*[55]	2010	NAFLD	Case-control study	70 patients with NAFLD and 69 normal controls	NAFLD patients had significantly lower serum adiponectin levels than controls Adiponectin levels were independently associated with liver fibrosis
Savvidou *et al*[56]	2009	NAFLD	Cross-sectional study	42 patients with NAFLD	Adiponectin levels were negatively associated with higher stages of fibrosis Adiponectin levels were independent predictors of advanced fibrosis
Yoneda *et al*[57]	2007	NAFLD	Cross-sectional study	248 patients with NAFLD and type 2 diabetes	A reduction in levels of serum adiponectin was independently associated with the severity of hepatic fibrosis in NAFLD patients with type 2 diabetes
Musso *et al*[58]	2005	NASH	Case-control study	20 patients with biopsy-proven NASH and 45 healthy controls	Serum adiponectin levels were significantly reduced in the NASH group, as compared to control groups Adiponectin levels correlated with the severity of hepatic steatosis and fibrosis

CLD: Chronic liver diseases; BA: Biliary atresia; HCV: Hepatitis C virus; HBV: Hepatitis B virus; NAFLD: Non-alcoholic fatty liver disease; NASH: Non-alcoholic steatohepatitis.

circulating adiponectin levels in 54 patients with chronic HCV, they reported increased adiponectin levels. The investigators also reported that serum adiponectin levels were further correlated with the severity of liver fibrosis in those patients. The aforementioned findings suggest that monitoring adiponectin levels may be employed to improve care for liver fibrosis in patients with HCV infection. In support of this hypothesis, a study of Derbala *et al*[50] also examined circulating adiponectin levels in patients with chronic HCV. They observed that adiponectin concentration was directly associated with hepatic fibrosis and inflammation in the chronic HCV subjects. In addition, strong evidence for a possible correlation between adiponectin levels and progressive liver fibrosis in patients with chronic HBV was recently described by Hsu *et al*[51]. The authors showed that serum adiponectin levels were independently associated with the development of liver fibrosis in patients with HBV. This information supports the observations of Hui and coworkers, which showed that serum adiponectin levels were significantly reduced in patients with reduced fibrosis after antiviral therapy. In particular, adiponectin levels were also positively associated with the stage of fibrosis[52]. All of these findings support the notion that adiponectin has non-classical metabolic actions, including a potential capacity to suppress fibrosis and therefore, may link its role to the development of hepatic fibrogenesis.

Even though former clinical studies have demonstrated a direct relationship of adiponectin and liver fibrogenesis in patients with various CLDs, the exact mechanism responsible for an increase in circulating adiponectin in liver fibrosis remains uncertain. The possible explanation for these findings might be attributed to a reduction in its clearance. In CLD patients with liver fibrosis, declined adiponectin clearance could result from reduced uptake of adiponectin by liver sinusoidal endothelial cells (LSECs), which may lead to elevated adiponectin levels in the circulation. It is widely known that dysfunction of LSECs is one of pathologic events in liver fibrogenesis. In the healthy liver, LSECs generally promote HSCs quiescence. During the process of liver fibrosis, LSECs undergo phenotypic changes with the loss of several receptors and LSECs fenestration, leading to the capillarization of liver sinusoids and the abnormality of various substances uptake[3]. It has been shown that adiponectin levels and adipoR2 expression are decreased in the LSECs response to liver injury[53]. These phenomena may help explain why hyperadiponectinemia has been observed in CLDs patients with liver fibrosis.

In apparent contrast to the aforementioned studies, circulating adiponectin levels have been shown to be reduced in patients with steatosis and steatohepatitis, such as those with NAFLD and NASH. Most recently, Lucero *et al*[54] investigated systemic adiponectin levels in 36 patients with NAFLD related to metabolic syndrome and 24 metabolic syndrome patients without NAFLD. They reported that adiponectin levels were significantly elevated in NAFLD patients with metabolic syndrome when compared to those patients without metabolic syndrome. Besides, circulating adiponectin levels were correlated with metabolic parameters and the degree of liver fibrosis in NAFLD patients. Furthermore, a case-control study of 70 patients with NAFLD and 69 healthy controls conducted by Nazal *et al*[55], explored a possible correlation between adiponectin levels and NAFLD pathology. The authors reported that plasma adiponectin levels were markedly lower in patients with NAFLD than in controls, and they were inversely related to the presence of liver fibrosis. The findings of Savvidou *et al*[56] provide support to a negative association between adiponectin concentrations and higher stages of fibrosis in patients with NAFLD, suggesting that adiponectin levels can be used as predictors of advanced fibrosis. Supporting this finding, a large cohort study of 248 patients with NAFLD and type 2 diabetes found that reduced adiponectin levels were independently associated with the degree of liver fibrosis[57]. Finally, in a case-control study of 60 patients with NAFLD and 60 healthy controls, patients with NAFLD had markedly reduced plasma adiponectin levels, when compared to controls. Notably, a decline in adiponectin levels was found to be closely associated with the degree of liver fibrosis. Similarly, when Musso *et al*[58] determined adiponectin levels in 20 patients with biopsy-proven NASH and 45 healthy controls, they observed that low adiponectin levels were associated with the severity of hepatic steatosis and fibrosis in patients with NASH. Based on these findings, hypoadiponectinemia has been proposed as a contributory factor to the development of metabolic dysfunction in NAFLD and NASH. The reasons for these conflicting findings remain unexplained. These are likely due to differences in populations, disease advancement, or measurements applied, or to incomplete control of confounding variables.

The contrasting results regarding hypoadiponectinemia in patients with NAFLD and those with NASH compared to those with other CLDs could be partly attributed to the differences in pathophysiology of the diseases. Indeed, hypoadiponectinemia has been suggested to play a pathogenic role in the pancreatic-cell dysfunction observed in both NAFLD and NASH[59], and accumulated visceral fat can cause a decline in levels of circulating adiponectin[60]. Regardless of the role of environmental and genetic factors, adiponectin appears to be strongly associated with the hepatic phenotype, which is a major cause of morbidity in NAFLD. It has also been discovered that the adiponectin promoter polymorphism rs266729 was associated with the susceptibility of NAFLD, and the subjects with the GG genotype of rs266729 exhibited substantially lesser adiponectin values than those subjects with the GC or CC genotypes[61]. It is tempting to speculate that genetic variation of adiponecin and lifestyles choices causing visceral fat deposition/obesity may lead to reduced circulating adiponetin levels in patients with NAFLD and NASH. From these reports regarding its significant association with liver fibrosis, it is apparent that adiponectin levels in the circulation may be

a prognosticator for the development of liver fibrosis in various CLDs.

ADIPONECTIN-TARGETED TREATMENT FOR LIVER FIBROSIS

Given that accumulating data correlate the hepatoprotective functions of adiponectin with restricting HSC proliferation and myofibroblast restoration, which are both pivotal mechanisms of liver fibrogenesis, increases in adiponectin levels and its agonists could be an alternative treatment option for the protection and therapy of liver fibrosis. Currently, there are two prime approaches to increase adiponectin concentrations in the body.

Firstly, recombinant adiponectin, or drugs that bypass adiponectin by directly stimulating AMPK, have been extensively utilized in preclinical models. For example, trials utilizing ADP355, which is an adiponectin-like small synthetic peptide agonist, show promise in inhibiting tumorigenesis in a mouse xenograft breast cancer model[62]. Regarding its protective effects on liver fibrosis, an adiponectin-mimetic peptide analog (ADP355) that docks into the adiponectin receptors binding pocket, possesses potency in modulating multiple anti-fibrogenic mechanisms, in particular, AMPK phosphorylation, and has been shown to eliminate hepatic fibrosis in mice with carbon tetrachloride (CCl_4)-induced liver fibrosis. These mechanisms are mediated by the diminishing expression of pro-fibrogenic genes, including *α-SMA*, desmin, *CTGF*, *TGF-β1*, and *TIMP-1*, but enhanced expression of matrix metalloproteinase-13 (*MMP-13*) as a marker for restructuring of the collagen matrix[63]. In the light of these considerations, ADP355 may represent an alternative anti-fibrogenic agent in the treatment of liver fibrosis. However, due to the lack of clinical data concerning liver toxicity, further studies assessing the toxic and off-target effects of this agent will be necessary to determine the practicability and clinical efficiency of adiponectin for practical applications

In contrast, one additional method would be to characterize the classes of substances that could bring about an increase in the secretion or expression of adiponectin. Recently, a few studies have suggested that activation of PPAR-γ can enhance the expression and circulating concentrations of adiponectin *via* regulation of gene transcription[64]. The PPAR-γ cascade is therefore interesting, as it may be associated with direct targets that can modify adiponectin for potential clinical applications. Among the drugs presently in clinical use that are PPAR-γ agonists, the thiazolidinediones (TZDs) exert beneficial anti-proliferative and anti-inflammatory effects *via* PPAR-γ activation[65], and have been found to induce adipose tissue to release adiponectin into the circulation[66]. As TZDs have been broadly applied to improve insulin sensitivity in patients with diabetes, we cannot discern the specific conditions under which adiponectin has defensive effects against liver fibrosis. Accordingly, the rigorous identification of new drugs that target adiponectin in the treatment of liver fibrosis is presently even more important. However, further research efforts will be needed to validate the safety and efficacy of adiponectin before a standardized treatment regimen can be established.

CONCLUSION

The close relationships between the pathology of liver fibrogenesis and clinical appearance of the disease affirm the significance of extended basic and translational studies into the pathogenesis of liver fibrosis. To date, the development of anti-fibrotic therapies in fibrosis and/or cirrhosis has been largely unsuccessful. Considerable research conducted over the past several years has demonstrated the multifaceted and potentially contradicting effects of adiponectin as a novel regulator of liver fibrogenesis. The possible relevance of adiponectin has emerged from studies in cell cultures and animal models, in addition to clinical investigations. Indeed, experimental studies document the multiple effects of adiponectin on limiting HSC proliferation and suppressing the expression of pro-fibrogenic genes through specific signal transduction pathways. The possible effect of adiponectin against liver fibrosis is supported by clinical studies that link hyperadiponectinemia to the severity of liver fibrosis in many liver diseases, including BA, HCV, HBV, and liver cirrhosis; whereas reduced adiponectin levels have been reported to be a key factor in the development of metabolic disorders contributing to NAFLD and NASH. Based on these observations, adiponectin could be a plausible noninvasive biochemical marker identifying the severity of liver fibrosis in patients with CLDs. However, additional research is warranted to better comprehend the precise aspect of adiponectin in the pathogenesis of liver fibrosis, which will help to develop adiponectin as circulating indicator for distinct CLD patients who are at risk of developing liver fibrosis.

ACKNOWLEDGMENTS

The authors would like to thank Assistant Professor Jiraphun Jittikoon for kindly reviewing and proofreading the manuscript.

REFERENCES

1 **Bataller R**, Brenner DA. Liver fibrosis. *J Clin Invest* 2005; **115**: 209-218 [PMID: 15690074 DOI: 10.1172/JCI24282]
2 **Rowe IA**. Lessons from Epidemiology: The Burden of Liver Disease. *Dig Dis* 2017; **35**: 304-309 [PMID: 28468017 DOI: 10.1159/000456580]
3 **DeLeve LD**. Liver sinusoidal endothelial cells in hepatic fibrosis. *Hepatology* 2015; **61**: 1740-1746 [PMID: 25131509 DOI: 10.1002/hep.27376]
4 **Masaki T**, Chiba S, Tatsukawa H, Yasuda T, Noguchi H, Seike M, Yoshimatsu H. Adiponectin protects LPS-induced liver injury through modulation of TNF-alpha in KK-Ay obese mice. *Hepatology* 2004; **40**: 177-184 [PMID: 15239101 DOI: 10.1002/hep.20282]
5 **Handy JA**, Saxena NK, Fu P, Lin S, Mells JE, Gupta NA, Anania

FA. Adiponectin activation of AMPK disrupts leptin-mediated hepatic fibrosis via suppressors of cytokine signaling (SOCS-3). *J Cell Biochem* 2010; **110**: 1195-1207 [PMID: 20564215 DOI: 10.1002/jcb.22634]

6 **Kamada Y**, Tamura S, Kiso S, Matsumoto H, Saji Y, Yoshida Y, Fukui K, Maeda N, Nishizawa H, Nagaretani H, Okamoto Y, Kihara S, Miyagawa J, Shinomura Y, Funahashi T, Matsuzawa Y. Enhanced carbon tetrachloride-induced liver fibrosis in mice lacking adiponectin. *Gastroenterology* 2003; **125**: 1796-1807 [PMID: 14724832 DOI: 10.1053/j.gastro.2003.08.029]

7 **Adachi M**, Brenner DA. High molecular weight adiponectin inhibits proliferation of hepatic stellate cells via activation of adenosine monophosphate-activated protein kinase. *Hepatology* 2008; **47**: 677-685 [PMID: 18220291 DOI: 10.1002/hep.21991]

8 **Ding X**, Saxena NK, Lin S, Xu A, Srinivasan S, Anania FA. The roles of leptin and adiponectin: a novel paradigm in adipocytokine regulation of liver fibrosis and stellate cell biology. *Am J Pathol* 2005; **166**: 1655-1669 [PMID: 15920151 DOI: 10.1016/S0002-9440(10)62476-5]

9 **Balmer ML**, Joneli J, Schoepfer A, Stickel F, Thormann W, Dufour JF. Significance of serum adiponectin levels in patients with chronic liver disease. *Clin Sci* (Lond) 2010; **119**: 431-436 [PMID: 20524936 DOI: 10.1042/CS20100008]

10 **Zhao L**, Fu Z, Liu Z. Adiponectin and insulin cross talk: the microvascular connection. *Trends Cardiovasc Med* 2014; **24**: 319-324 [PMID: 25220977 DOI: 10.1016/j.tcm.2014.08.001]

11 **Wang Y**, Lam KS, Yau MH, Xu A. Post-translational modifications of adiponectin: mechanisms and functional implications. *Biochem J* 2008; **409**: 623-633 [PMID: 18177270 DOI: 10.1042/BJ20071492]

12 **Waki H**, Yamauchi T, Kamon J, Ito Y, Uchida S, Kita S, Hara K, Hada Y, Vasseur F, Froguel P, Kimura S, Nagai R, Kadowaki T. Impaired multimerization of human adiponectin mutants associated with diabetes. Molecular structure and multimer formation of adiponectin. *J Biol Chem* 2003; **278**: 40352-40363 [PMID: 12878598 DOI: 10.1074/jbc.M300365200]

13 **Pajvani UB**, Du X, Combs TP, Berg AH, Rajala MW, Schulthess T, Engel J, Brownlee M, Scherer PE. Structure-function studies of the adipocyte-secreted hormone Acrp30/adiponectin. Implications fpr metabolic regulation and bioactivity. *J Biol Chem* 2003; **278**: 9073-9085 [PMID: 12496257 DOI: 10.1074/jbc.M207198200]

14 **Scherer PE**, Williams S, Fogliano M, Baldini G, Lodish HF. A novel serum protein similar to C1q, produced exclusively in adipocytes. *J Biol Chem* 1995; **270**: 26746-26749 [PMID: 7592907 DOI: 10.1074/jbc.270.45.26746]

15 **Matsuda M**, Shimomura I, Sata M, Arita Y, Nishida M, Maeda N, Kumada M, Okamoto Y, Nagaretani H, Nishizawa H, Kishida K, Komuro R, Ouchi N, Kihara S, Nagai R, Funahashi T, Matsuzawa Y. Role of adiponectin in preventing vascular stenosis. The missing link of adipo-vascular axis. *J Biol Chem* 2002; **277**: 37487-37491 [PMID: 12138120 DOI: 10.1074/jbc.M206083200]

16 **Okamoto Y**, Kihara S, Funahashi T, Matsuzawa Y, Libby P. Adiponectin: a key adipocytokine in metabolic syndrome. *Clin Sci* (Lond) 2006; **110**: 267-278 [PMID: 16464169 DOI: 10.1042/CS20050182]

17 **Chen MC**, Lee CJ, Yang CF, Chen YC, Wang JH, Hsu BG. Low serum adiponectin level is associated with metabolic syndrome and is an independent marker of peripheral arterial stiffness in hypertensive patients. *Diabetol Metab Syndr* 2017; **9**: 49 [PMID: 28670347 DOI: 10.1186/s13098-017-0247-8]

18 **Bianco A**, Mazzarella G, Turchiarelli V, Nigro E, Corbi G, Scudiero O, Sofia M, Daniele A. Adiponectin: an attractive marker for metabolic disorders in Chronic Obstructive Pulmonary Disease (COPD). *Nutrients* 2013; **5**: 4115-4125 [PMID: 24128974 DOI: 10.3390/nu5104115]

19 **Lim CC**, Teo BW, Tai ES, Lim SC, Chan CM, Sethi S, Wong TY, Sabanayagam C. Elevated serum leptin, adiponectin and leptin to adiponectin ratio is associated with chronic kidney disease in Asian adults. *PLoS One* 2015; **10**: e0122009 [PMID: 25793395 DOI: 10.1371/journal.pone.0122009]

20 **Honsawek S**, Chayanupatkul M. Correlation of plasma and synovial fluid adiponectin with knee osteoarthritis severity. *Arch Med Res* 2010; **41**: 593-598 [PMID: 21199727 DOI: 10.1016/j.arcmed.2010.11.007]

21 **Kedenko L**, Lamina C, Kiesslich T, Kapur K, Bergmann S, Waterworth D, Heid IM, Wichmann HE, Kedenko I, Kronenberg F, Paulweber B. Genetic polymorphisms of the main transcription factors for adiponectin gene promoter in regulation of adiponectin levels: association analysis in three European cohorts. *PLoS One* 2012; **7**: e52497 [PMID: 23285067 DOI: 10.1371/journal.pone.0052497]

22 **Jin D**, Sun J, Huang J, Yu X, Yu A, He Y, Li Q, Yang Z. Peroxisome proliferator-activated receptor γ enhances adiponectin secretion via up-regulating DsbA-L expression. *Mol Cell Endocrinol* 2015; **411**: 97-104 [PMID: 25917454 DOI: 10.1016/j.mce.2015.04.015]

23 **Shehzad A**, Iqbal W, Shehzad O, Lee YS. Adiponectin: regulation of its production and its role in human diseases. *Hormones* (Athens) 2012; **11**: 8-20 [PMID: 22450341 DOI: 10.1007/BF03401534]

24 **Yamauchi T**, Kamon J, Ito Y, Tsuchida A, Yokomizo T, Kita S, Sugiyama T, Miyagishi M, Hara K, Tsunoda M, Murakami K, Ohteki T, Uchida S, Takekawa S, Waki H, Tsuno NH, Shibata Y, Terauchi Y, Froguel P, Tobe K, Koyasu S, Taira K, Kitamura T, Shimizu T, Nagai R, Kadowaki T. Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. *Nature* 2003; **423**: 762-769 [PMID: 12802337 DOI: 10.1038/nature01705]

25 **Yamauchi T**, Kadowaki T. Adiponectin receptor as a key player in healthy longevity and obesity-related diseases. *Cell Metab* 2013; **17**: 185-196 [PMID: 23352188 DOI: 10.1016/j.cmet.2013.01.001]

26 **Li Y**, Xu S, Mihaylova MM, Zheng B, Hou X, Jiang B, Park O, Luo Z, Lefai E, Shyy JY, Gao B, Wierzbicki M, Verbeuren TJ, Shaw RJ, Cohen RA, Zang M. AMPK phosphorylates and inhibits SREBP activity to attenuate hepatic steatosis and atherosclerosis in diet-induced insulin-resistant mice. *Cell Metab* 2011; **13**: 376-388 [PMID: 21459323 DOI: 10.1016/j.cmet.2011.03.009]

27 **Shklyaev S**, Aslanidi G, Tennant M, Prima V, Kohlbrenner E, Kroutov V, Campbell-Thompson M, Crawford J, Shek EW, Scarpace PJ, Zolotukhin S. Sustained peripheral expression of transgene adiponectin offsets the development of diet-induced obesity in rats. *Proc Natl Acad Sci USA* 2003; **100**: 14217-14222 [PMID: 14617771 DOI: 10.1073/pnas.2333912100]

28 **Misra P**. AMP activated protein kinase: a next generation target for total metabolic control. *Expert Opin Ther Targets* 2008; **12**: 91-100 [PMID: 18076373 DOI: 10.1517/14728222.12.1.91]

29 **Yamauchi T**, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K, Mori Y, Ide T, Murakami K, Tsuboyama-Kasaoka N, Ezaki O, Akanuma Y, Gavrilova O, Vinson C, Reitman ML, Kagechika H, Shudo K, Yoda M, Nakano Y, Tobe K, Nagai R, Kimura S, Tomita M, Froguel P, Kadowaki T. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nat Med* 2001; **7**: 941-946 [PMID: 11479627 DOI: 10.1038/90984]

30 **Holland WL**, Miller RA, Wang ZV, Sun K, Barth BM, Bui HH, Davis KE, Bikman BT, Halberg N, Rutkowski JM, Wade MR, Tenorio VM, Kuo MS, Brozinick JT, Zhang BB, Birnbaum MJ, Summers SA, Scherer PE. Receptor-mediated activation of ceramidase activity initiates the pleiotropic actions of adiponectin. *Nat Med* 2011; **17**: 55-63 [PMID: 21186369 DOI: 10.1038/nm.2277]

31 **Yang G**, Badeanlou L, Bielawski J, Roberts AJ, Hannun YA, Samad F. Central role of ceramide biosynthesis in body weight regulation, energy metabolism, and the metabolic syndrome. *Am J Physiol Endocrinol Metab* 2009; **297**: E211-E224 [PMID: 19435851 DOI: 10.1152/ajpendo.91014.2008]

32 **Holland WL**, Xia JY, Johnson JA, Sun K, Pearson MJ, Sharma AX, Quittner-Strom E, Tippetts TS, Gordillo R, Scherer PE. Inducible overexpression of adiponectin receptors highlight the roles of adiponectin-induced ceramidase signaling in lipid and glucose homeostasis. *Mol Metab* 2017; **6**: 267-275 [PMID: 28271033 DOI: 10.1016/j.molmet.2017.01.002]

33 **Lira FS**, Rosa JC, Pimentel GD, Seelaender M, Damaso AR, Oyama LM, do Nascimento CO. Both adiponectin and interleukin-10 inhibit LPS-induced activation of the NF-κB pathway in 3T3-L1 adipocytes. *Cytokine* 2012; **57**: 98-106 [PMID: 22047972 DOI: 10.1016/j.cyto.2011.10.001]

34 **Liu C**, Tao Q, Sun M, Wu JZ, Yang W, Jian P, Peng J, Hu Y, Liu

C, Liu P. Kupffer cells are associated with apoptosis, inflammation and fibrotic effects in hepatic fibrosis in rats. *Lab Invest* 2010; **90**: 1805-1816 [PMID: 20921949 DOI: 10.1038/labinvest.2010.123]

35 **Wolf AM**, Wolf D, Rumpold H, Enrich B, Tilg H. Adiponectin induces the anti-inflammatory cytokines IL-10 and IL-1RA in human leukocytes. *Biochem Biophys Res Commun* 2004; **323**: 630-635 [PMID: 15369797 DOI: 10.1016/j.bbrc.2004.08.145]

36 **Tsatsanis C**, Zacharioudaki V, Androulidaki A, Dermitzaki E, Charalampopoulos I, Minas V, Gravanis A, Margioris AN. Adiponectin induces TNF-alpha and IL-6 in macrophages and promotes tolerance to itself and other pro-inflammatory stimuli. *Biochem Biophys Res Commun* 2005; **335**: 1254-1263 [PMID: 16115611 DOI: 10.1016/j.bbrc.2005.07.197]

37 **Arita Y**, Kihara S, Ouchi N, Maeda K, Kuriyama H, Okamoto Y, Kumada M, Hotta K, Nishida M, Takahashi M, Nakamura T, Shimomura I, Muraguchi M, Ohmoto Y, Funahashi T, Matsuzawa Y. Adipocyte-derived plasma protein adiponectin acts as a platelet-derived growth factor-BB-binding protein and regulates growth factor-induced common postreceptor signal in vascular smooth muscle cell. *Circulation* 2002; **105**: 2893-2898 [PMID: 12070119 DOI: 10.1161/01.CIR.0000018622.84402.FF]

38 **Yokota T**, Meka CS, Kouro T, Medina KL, Igarashi H, Takahashi M, Oritani K, Funahashi T, Tomiyama Y, Matsuzawa Y, Kincade PW. Adiponectin, a fat cell product, influences the earliest lymphocyte precursors in bone marrow cultures by activation of the cyclooxygenase-prostaglandin pathway in stromal cells. *J Immunol* 2003; **171**: 5091-5099 [PMID: 14607907 DOI: 10.4049/jimmunol.171.10.5091]

39 **Bertolani C**, Marra F. Role of adipocytokines in hepatic fibrosis. *Curr Pharm Des* 2010; **16**: 1929-1940 [PMID: 20370673 DOI: 10.2174/138161210791208857]

40 **Caligiuri A**, Bertolani C, Guerra CT, Aleffi S, Galastri S, Trappoliere M, Vizzutti F, Gelmini S, Laffi G, Pinzani M, Marra F. Adenosine monophosphate-activated protein kinase modulates the activated phenotype of hepatic stellate cells. *Hepatology* 2008; **47**: 668-676 [PMID: 18098312 DOI: 10.1002/hep.21995]

41 **Tomita K**, Oike Y, Teratani T, Taguchi T, Noguchi M, Suzuki T, Mizutani A, Yokoyama H, Irie R, Sumimoto H, Takayanagi A, Miyashita K, Akao M, Tabata M, Tamiya G, Ohkura T, Hibi T. Hepatic AdipoR2 signaling plays a protective role against progression of nonalcoholic steatohepatitis in mice. *Hepatology* 2008; **48**: 458-473 [PMID: 18666257 DOI: 10.1002/hep.22365]

42 **Handy JA**, Fu PP, Kumar P, Mells JE, Sharma S, Saxena NK, Anania FA. Adiponectin inhibits leptin signalling via multiple mechanisms to exert protective effects against hepatic fibrosis. *Biochem J* 2011; **440**: 385-395 [PMID: 21846328 DOI: 10.1042/BJ20102148]

43 **da Silva TE**, Costa-Silva M, Correa CG, Denardin G, Alencar MLA, Coelho MSPH, Muraro-Wildner L, Luiza-Bazzo M, González-Chica DA, Dantas-Correa EB, Narciso-Schiavon JL, Schiavon LL. Clinical Significance of Serum Adiponectin and Resistin Levels in Liver Cirrhosis. *Ann Hepatol* 2018; **17**: 286-299 [PMID: 29469045 DOI: 10.5604/01.3001.0010.8659]

44 **Udomsinprasert W,** Honsawek S, Anomasiri W, Chongsrisawat V, Vejchapipat P, Poovorawan Y. Elevated adiponectin is associated with poor outcome in children with biliary atresia. *Asian Biomedicine* 2012; **6**: 369-376

45 **Honsawek S**, Chayanupatkul M, Chongsrisawat V, Theamboonlers A, Praianantathavorn K, Udomsinprasert W, Vejchapipat P, Poovorawan Y. Serum adiponectin and transient elastography as non-invasive markers for postoperative biliary atresia. *BMC Gastroenterol* 2011; **11**: 16 [PMID: 21356120 DOI: 10.1186/1471-230X-11-16]

46 **Carvalho RF**, Atta AM, de Oliveira IS, Santos TPS, Santos JPA, Schinoni MI, de Sousa-Atta MLB. Adiponectin levels and insulin resistance among patients with chronic hepatitis C. *Acta Trop* 2018; **178**: 258-263 [PMID: 29217381 DOI: 10.1016/j.actatropica.2017.12.004]

47 **Korah TE**, El-Sayed S, Elshafie MK, Hammoda GE, Safan MA. Significance of serum leptin and adiponectin levels in Egyptian patients with chronic hepatitis C virus associated hepatic steatosis and fibrosis. *World J Hepatol* 2013; **5**: 74-81 [PMID: 23646232 DOI: 10.4254/wjh.v5.i2.74]

48 **Sumie S**, Kawaguchi T, Kuromatsu R, Takata A, Nakano M, Satani M, Yamada S, Niizeki T, Torimura T, Sata M. Total and high molecular weight adiponectin and hepatocellular carcinoma with HCV infection. *PLoS One* 2011; **6**: e26840 [PMID: 22110596 DOI: 10.1371/journal.pone.0026840]

49 **Corbetta S**, Redaelli A, Pozzi M, Bovo G, Ratti L, Redaelli E, Pellegrini C, Beck-Peccoz P, Spada A. Fibrosis is associated with adiponectin resistance in chronic hepatitis C virus infection. *Eur J Clin Invest* 2011; **41**: 898-905 [PMID: 21539538 DOI: 10.1111/j.1365-2362.2011.02498.x]

50 **Derbala M**, Rizk N, Al-Kaabi S, Amer A, Shebl F, Al Marri A, Aigha I, Alyaesi D, Mohamed H, Aman H, Basem N. Adiponectin changes in HCV-Genotype 4: relation to liver histology and response to treatment. *J Viral Hepat* 2009; **16**: 689-696 [PMID: 19486470 DOI: 10.1111/j.1365-2893.2009.01096.x]

51 **Hsu CS**, Liu WL, Chao YC, Lin HH, Tseng TC, Wang CC, Chen DS, Kao JH. Adipocytokines and liver fibrosis stages in patients with chronic hepatitis B virus infection. *Hepatol Int* 2015; **9**: 231-242 [PMID: 25788201 DOI: 10.1007/s12072-015-9616-2]

52 **Hui CK**, Zhang HY, Lee NP, Chan W, Yueng YH, Leung KW, Lu L, Leung N, Lo CM, Fan ST, Luk JM, Xu A, Lam KS, Kwong YL, Lau GK; Hong Kong Liver Fibrosis Study Group. Serum adiponectin is increased in advancing liver fibrosis and declines with reduction in fibrosis in chronic hepatitis B. *J Hepatol* 2007; **47**: 191-202 [PMID: 17462782 DOI: 10.1016/j.jhep.2007.02.023]

53 **Sun LJ**, Yu JW, Shi YG, Zhang XY, Shu MN, Chen MY. Hepatitis C virus core protein induces dysfunction of liver sinusoidal endothelial cell by down-regulation of silent information regulator 1. *J Med Virol* 2018; **90**: 926-935 [PMID: 29350417 DOI: 10.1002/jmv.25034]

54 **Lucero D**, Miksztowicz V, Gualano G, Longo C, Landeira G, Álvarez E, Zago V, Brites F, Berg G, Fassio E, Schreier L. Nonalcoholic fatty liver disease associated with metabolic syndrome: Influence of liver fibrosis stages on characteristics of very low-density lipoproteins. *Clin Chim Acta* 2017; **473**: 1-8 [PMID: 28802640 DOI: 10.1016/j.cca.2017.08.006]

55 **Nazal L**, Riquelme A, Solís N, Pizarro M, Escalona A, Burotto M, Méndez JI, Saint-Jean C, Concha MJ, Giovanni S, Awruch D, Morales A, Baudrand R, Carrasco G, Domínguez MA, Padilla O, Espinoza M, Miquel JF, Nervi F, Arrese M. Hypoadiponectinemia and its association with liver fibrosis in morbidly obese patients. *Obes Surg* 2010; **20**: 1400-1407 [PMID: 20066503 DOI: 10.1007/s11695-009-0051-0]

56 **Savvidou S**, Hytiroglou P, Orfanou-Koumerkeridou H, Panderis A, Frantzoulis P, Goulis J. Low serum adiponectin levels are predictive of advanced hepatic fibrosis in patients with NAFLD. *J Clin Gastroenterol* 2009; **43**: 765-772 [PMID: 19525862 DOI: 10.1097/MCG.0b013e31819e9048]

57 **Yoneda M**, Iwasaki T, Fujita K, Kirikoshi H, Inamori M, Nozaki Y, Maeyama S, Wada K, Saito S, Terauchi Y, Nakajima A. Hypoadiponectinemia plays a crucial role in the development of nonalcoholic fatty liver disease in patients with type 2 diabetes mellitus independent of visceral adipose tissue. *Alcohol Clin Exp Res* 2007; **31**: S15-S21 [PMID: 17331160 DOI: 10.1111/j.1530-0277.2006.00281.x]

58 **Musso G**, Gambino R, Biroli G, Carello M, Fagà E, Pacini G, De Michieli F, Cassader M, Durazzo M, Rizzetto M, Pagano G. Hypoadiponectinemia predicts the severity of hepatic fibrosis and pancreatic Beta-cell dysfunction in nondiabetic nonobese patients with nonalcoholic steatohepatitis. *Am J Gastroenterol* 2005; **100**: 2438-2446 [PMID: 16279898 DOI: 10.1111/j.1572-0241.2005.00297.x]

59 **Maeda N**, Shimomura I, Kishida K, Nishizawa H, Matsuda M, Nagaretani H, Furuyama N, Kondo H, Takahashi M, Arita Y, Komuro R, Ouchi N, Kihara S, Tochino Y, Okutomi K, Horie M, Takeda S, Aoyama T, Funahashi T, Matsuzawa Y. Diet-induced insulin resistance in mice lacking adiponectin/ACRP30. *Nat Med* 2002; **8**: 731-737 [PMID: 12068289 DOI: 10.1038/nm724]

60 **Burgert TS**, Taksali SE, Dziura J, Goodman TR, Yeckel CW, Papademetris X, Constable RT, Weiss R, Tamborlane WV, Savoye M, Seyal AA, Caprio S. Alanine aminotransferase levels and fatty liver in

childhood obesity: associations with insulin resistance, adiponectin, and visceral fat. *J Clin Endocrinol Metab* 2006; **91**: 4287-4294 [PMID: 16912127 DOI: 10.1210/jc.2006-1010]

61 **Hsieh CJ**, Wang PW, Hu TH. Association of adiponectin gene polymorphism with nonalcoholic fatty liver disease in Taiwanese patients with type 2 diabetes. *PLoS One* 2015; **10**: e0127521 [PMID: 26042596 DOI: 10.1371/journal.pone.0127521]

62 **Otvos L Jr**, Haspinger E, La Russa F, Maspero F, Graziano P, Kovalszky I, Lovas S, Nama K, Hoffmann R, Knappe D, Cassone M, Wade J, Surmacz E. Design and development of a peptide-based adiponectin receptor agonist for cancer treatment. *BMC Biotechnol* 2011; **11**: 90 [PMID: 21974986 DOI: 10.1186/1472-6750-11-90]

63 **Kumar P**, Smith T, Rahman K, Thorn NE, Anania FA. Adiponectin agonist ADP355 attenuates CCl4-induced liver fibrosis in mice. *PLoS One* 2014; **9**: e110405 [PMID: 25310107 DOI: 10.1371/journal.pone.0110405]

64 **Maeda N**, Takahashi M, Funahashi T, Kihara S, Nishizawa H, Kishida K, Nagaretani H, Matsuda M, Komuro R, Ouchi N, Kuriyama H, Hotta K, Nakamura T, Shimomura I, Matsuzawa Y. PPARgamma ligands increase expression and plasma concentrations of adiponectin, an adipose-derived protein. *Diabetes* 2001; **50**: 2094-2099 [PMID: 11522676 DOI: 10.2337/diabetes.50.9.2094]

65 **Yki-Järvinen H**. Thiazolidinediones. *N Engl J Med* 2004; **351**: 1106-1118 [PMID: 15356308 DOI: 10.1056/NEJMra041001]

66 **Pajvani UB**, Hawkins M, Combs TP, Rajala MW, Doebber T, Berger JP, Wagner JA, Wu M, Knopps A, Xiang AH, Utzschneider KM, Kahn SE, Olefsky JM, Buchanan TA, Scherer PE. Complex distribution, not absolute amount of adiponectin, correlates with thiazolidinedione-mediated improvement in insulin sensitivity. *J Biol Chem* 2004; **279**: 12152-12162 [PMID: 14699128 DOI: 10.1074/jbc.M311113200]

P- Reviewer: Citores MJ, Pallav K, Reichert MCC **S- Editor**: Ji FF
L- Editor: A **E- Editor**: Yin SY

World Journal of Hepatology

Submit a Manuscript: http://www.f6publishing.com

World J Hepatol 2018 October 27; 10(10): 719-730

DOI: 10.4254/wjh.v10.i10.719

ISSN 1948-5182 (online)

ORIGINAL ARTICLE

Basic Study

Experimental bio-artificial liver: Importance of the architectural design on ammonia detoxification performance

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Author contributions: Pizarro MD performed the majority of experiments, analyzed data, and wrote the manuscript; Mamprin ME and Mediavilla MG designed the research, contributed new reagents, analyzed data, and wrote the manuscript; Daurelio LD designed and supervised the statistical analysis; Rodriguez JV developed the cylindrical bioreactor and metabolite mass balance equations, performed a critical revision, and contributed to the research and the redaction of this article; all the authors were involved in reviewing the literature for latest contributions in the field, writing, and editing the manuscript; Mamprin ME and Mediavilla MG have equally contributed to this work.

Supported by Universidad Nacional de Rosario (UNR), BIO 272, Resol. C.S., No. 677/2013; Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT), PICT-03-14492, BID 1728 OC/AR (Argentina); and a grant from Regione Autonoma Friuli-Venezia Giulia, Italy.

Institutional review board statement: The study was reviewed and approved by the National University of Rosario Institutional Review Board (Resol. C.S. No. 677/2013).

Institutional animal care and use committee statement: All procedures involving animals were reviewed and approved by the Institutional Animal Care and Use Committee of the Faculty of Biochemical and Pharmaceutical Sciences-UNR (Resol. No. 139/2011).

Conflict-of-interest statement: No potential conflicts of interest relevant to this article were reported.

Manuscript source: Invited manuscript

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Received: May 22, 2018
Peer-review started: May 23, 2018
First decision: June 14, 2018
Revised: July 12, 2018
Accepted: August 6, 2018

Article in press: August 7, 2018
Published online: October 27, 2018

Abstract

AIM

To determine the influence of the construction design over the biological component's performance in an experimental bio-artificial liver (BAL) device.

METHODS

Two BAL models for liver microorgans (LMOs) were constructed. First, we constructed a cylindrical BAL and tested it without the biological component to establish its correct functioning. Samples of blood and biological compartment (BC) fluid were taken after 0, 60, and 120 min of perfusion. Osmolality, hematocrit, ammonia and glucose concentrations, lactate dehydrogenase (LDH) release (as a LMO viability parameter), and oxygen consumption and ammonia metabolizing capacity (as LMO functionality parameters) were determined. CPSI and OTC gene expression and function were measured. The second BAL, a "flat bottom" model, was constructed using a 25 cm^2 culture flask while maintaining all other components between the models. The BC of both BALs had the same capacity (approximately 50 cm^3) and both were manipulated with the same perfusion system. The performances of the two BALs were compared to show the influence of architecture.

RESULTS

The cylindrical BAL showed a good exchange of fluids and metabolites between blood and the BC, reflected by the matching of osmolalities, and glucose and ammonia concentration ratios after 120 min of perfusion. No hemoconcentration was detected, the hematocrit levels remained stable during the whole study, and the minimal percentage of hemolysis (0.65% ± 0.10%) observed was due to the action of the peristaltic pump. When LMOs were used as biological component of this BAL they showed similar values to the ones obtained in a Normothermic Reoxygenation System (NRS) for almost all the parameters assayed. After 120 min, the results obtained were: LDH release (%): 14.7 ± 3.1 in the BAL and 15.5 ± 3.2 in the NRS (n = 6); oxygen consumption (μmol/min·g wet tissue): 1.16 ± 0.21 in the BAL and 0.84 ± 0.15 in the NRS (n = 6); relative expression of *Cps1* and *Otc*: 0.63 ± 0.12 and 0.67 ± 0.20, respectively, in the BAL, and 0.86 ± 0.10 and 0.82 ± 0.07, respectively, in the NRS (n = 3); enzymatic activity of CPSI and OTC (U/g wet tissue): 3.03 ± 0.86 and 222.0 ± 23.5, respectively, in the BAL, and 3.12 ± 0.73 and 228.8 ± 32.8, respectively, in the NRS (n = 3). In spite of these similarities, LMOs as a biological component of the cylindrical BAL were not able to detoxify ammonia at a significant level (not detected *vs* 35.1% ± 7.0% of the initial 1 mM NH_4^+ dose in NRS, n = 6). Therefore, we built a second BAL with an entirely different design that offers a flat base BC. When LMOs were placed in this "flat bottom" device they were able to detoxify 49.3% ± 8.8% of the initial ammonia overload after 120 min of perfusion (n = 6), with a detoxification capacity of 13.2 ± 2.2 μmol/g wet tissue.

CONCLUSION

In this work, we demonstrate the importance of adapting the BAL architecture to the biological component characteristics to obtain an adequate BAL performance.

Key words: Bio-artificial liver; Ammonia detoxification; Device design; Ornithine Transcarbamylase; Rat liver microorgans; Carbamyl Phosphate Synthetase I

Core tip: This work describes the adaptation of a simplified bio-artificial liver (BAL) prototype to make it suitable to house rat liver microorgans (LMOs) as a biological component, and the evaluation of the performance in this new model. We demonstrate that the modification in the design of the artificial parts employed allows a good performance of LMOs, thus showing the importance of architecture and model configuration on the design of these devices. Besides its application as BAL, this mini bioreactor could serve as a suitable laboratory tool to evaluate the behavior and functionality of LMOs subjected to different incubation conditions due to its simple design and the utilization of standard materials.

Pizarro MD, Mamprin ME, Daurelio LD, Rodriguez JV, Mediavilla MG. Experimental bio-artificial liver: Importance of the architectural design on ammonia detoxification performance. *World J Hepatol* 2018; 10(10): 719-730 Available from: URL: http://www.wjgnet.com/1948-5182/full/v10/i10/719.htm DOI: http://dx.doi.org/10.4254/wjh.v10.i10.719

INTRODUCTION

Bio-artificial liver (BAL) devices are extracorporeal systems that contain functional hepatic tissue or cells (the biological component) seeded into a man-made bioreactor (the artificial component) and separated from blood flow by semipermeable membranes[1]. The aim of these devices is to fulfill the necessary hepatic functions to keep patients with hepatic failure stabilized until the regeneration of their own livers or until the appearance of a compatible donor[1,2].

Though the majority of liver functions are performed by hepatocytes, the other hepatic cellular types (Kupffer, Pit, stellate or Ito, and sinusoidal epithelial cells) are also involved and exert some influence over the different functions *in vivo*[2]. Therefore, we became interested in studying a biological component possessing all these different liver constituent cells in search of a better performance of our BAL prototype with respect to a

previous one designed in our laboratory to house isolated hepatocytes[3]. In this sense, liver microorgans (LMOs) are an appropriate choice mostly because they are thin slices of tissue that keep the liver structure and its physiological characteristics and allow a good exchange of substances with the surrounding liquid and gaseous environment[4,5].

Ammonia elimination from the blood of patients with liver failure is a key metabolic reaction that any biological component of an efficient BAL should accomplish in light of the correlation existing between this metabolite accumulation and the progression of hepatic failure[3,6]. Nonetheless, etiology of hyperammonemia can also be from non-hepatic situations, including sepsis[7,8], urea cycle inborn disorders[9], complications in a hepatic transplantation setting[10], complications from cirrhosis[11,12], and urinary tract infections[13]. Hyperammonemia should be quickly treated to avoid brain damage or even death, and simple hemofiltration is sometimes not sufficient to cope with it[10,11]. In this context, a device capable of efficiently detoxifying ammonia could be useful to ameliorate the condition of the patient. Also, it could be utilized in the non-hepatic situations until the origin of the complication can be determined and controlled[6].

When studying ammonia detoxification function displayed by LMOs, we found that these tissue slices had excellent performance when incubated in suspension in a shaker. This simple system was called the Normothermic Reoxygenation System (NRS)[14]. Surprisingly, they completely lost this detoxifying capacity in the BAL device when the blood was overloaded with ammonia.

In our laboratory we had previously designed a BAL consisting of a cylindrical shaped device to house isolated rat hepatocytes as the biological component[4]. This system showed a good performance and allowed the maintenance of adequate viability and functionality of fresh hepatocyte suspensions, and successfully fulfilling different *in vitro* tests that constitute the first step in the development of any BAL system[4]. In this work, we present the results obtained when we enlarged this original prototype to allow accommodation to the bulkier LMOs, hypothesizing that this was a straightforward path in the design. However, we showed that the LMOs were unable to detoxify NH_4^+, and therefore had to redesign the artificial component receptacle to make it suitable to this biological component. To the best of our knowledge, this is the first report that evaluates such an essential issue and is valuable information for scientists studying this field of research.

MATERIALS AND METHODS

Experimental design

Figure 1 summarizes the path followed to develop a BAL prototype suitable for using LMOs as the biological component. Step 1: Evaluation of LMO inherent performance, mainly regarding ammonia detoxification capacity in the NRS[5] (Figure 2A); Step 2: Adaptation of the BAL, originally designed for hepatocytes[3], to house LMOs on its biological compartment (BC) (Figure 2B). Testing of this prototype without any biological component establishes its correct functioning. Testing of this prototype with LMOs as biological component to establish its performance as BAL, especially in relation to ammonia detoxification. Comparison with the results obtained in the NRS; Step 3: Due to the failure of LMOs to detoxify ammonia in the cylindrical BAL (in comparison with NRS): development of a "flat bottom" BAL (Figure 2C). Testing with LMOs as a biological component to determine the influence of the BAL configuration and design over the LMO ammonia detoxification capacity. Other parameters remained similar to the values encountered for the cylindrical BAL and were already published[15].

LMO obtainment

LMOs were obtained from livers of 60-day-old male Wistar rats with a weight of 250-300 g. Animals were given a fourteen day adaptation period to get used to the experimental laboratory environment, during which they could access standard rodent laboratory food and water *ad libitum*. Rats received care according to the principles and recommendations given by the National Academy of Sciences (Argentina). The School of Biochemical and Pharmaceutical Sciences Institutional Animal Care and Use Committee (Universidad Nacional de Rosario, Res No. 139/2011) approved all experimental procedures.

Rats were anesthetized (chloral hydrate, 500 mg/kg body weight, i.p.) and the liver was surgically removed. The right medial lobe was cut into blocks and LMOs were manually obtained from these blocks as thin slices (thickness: 338 ± 27 μm, n = 25), using a disposable microtome blade. We did all the manipulations over an ice-cooled cutting device to reduce liver deterioration, and on top of a piece of filter paper to avoid tissue slippage and ensure the accurate cutting of LMOs[14]. After slicing, LMOs were placed in KH-Base (KHB) solution (KHB, Table 1) and were pre-incubated for 15 min before loading into the NRS or the BAL prototype. The different KH solution compositions are shown in Table 1. KHB media was used for the LMO obtaining procedure and during the pre-incubation period.

LMOs have been characterized regarding histology, water content, and viability (published results[5,14]). They were weighted for normalization of results and their weights were 0.070 ± 0.020 g (n = 25), showing size consistency.

NRS

To evaluate the biological component inherent performance before introducing it into our BAL, we

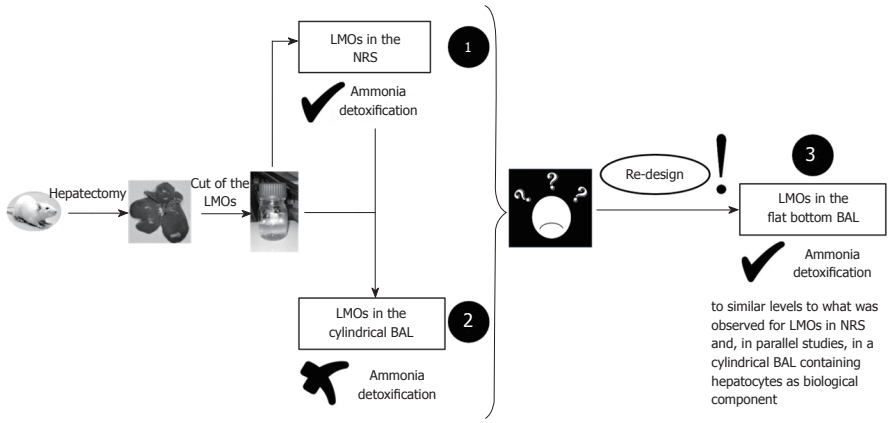

Figure 1 Schematic representation of the steps followed to arrive to the design of a "flat -bottom" bio-artificial liver device suitable to support the appropriate performance of liver microorgans as the biological component. Liver microorgans (LMOs) were obtained from Wistar rat livers: (1) Evaluation of LMO performance parameters in Normothermic Reoxygenation System; (2) Evaluation of LMO performance in cylindrical bio-artificial liver (BAL) and finding that they were unable to detoxify an ammonia overload in the test blood; (3) Evaluation of LMO performance in the newly designed "flat -bottom" BAL that proved suitable to support ammonia detoxification function. LMO: Liver microorgan; NRS: Normothermic Reoxygenation System; BAL: Bio-artificial liver.

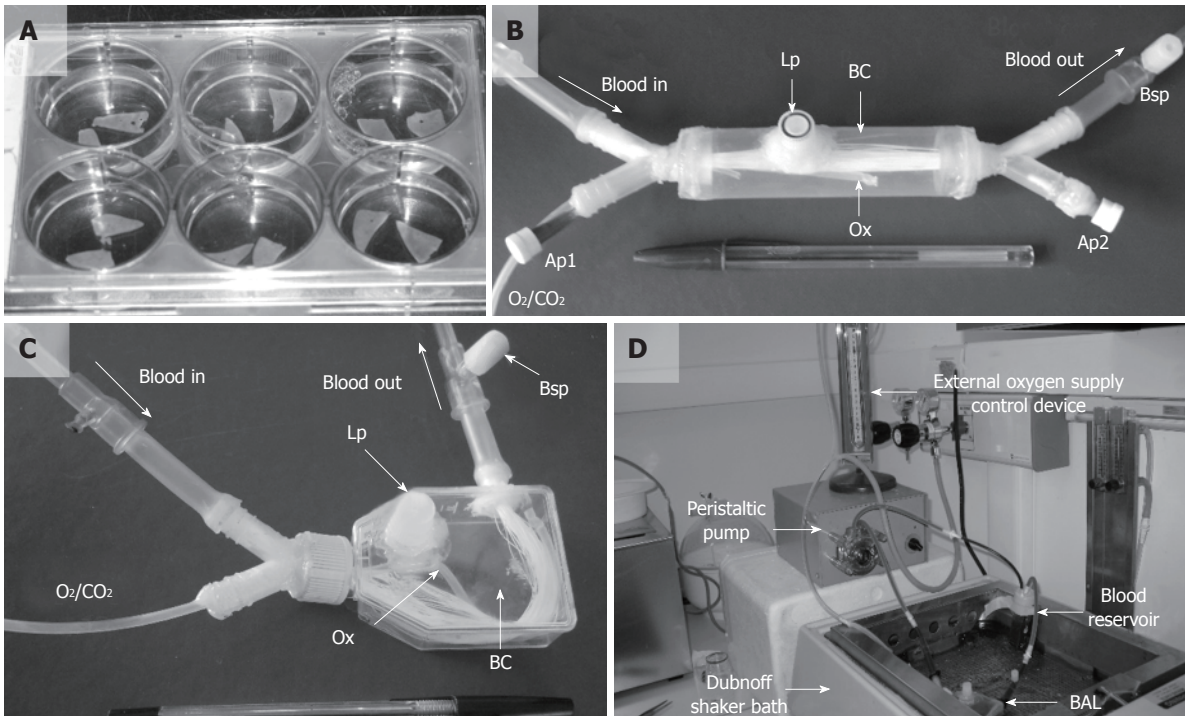

Figure 2 Devices used to analyze liver microorgan performance. A: Liver microorgan (LMO) disposition in the Normothermic Reoxygenation System (NRS).; B: Cylindrical shaped bio-artificial liver (BAL).; C: "Flat bottom" shaped BAL.; D: Perfusion system components. BC: Biological compartment; Lp: LMO loading port; Ap 1 and 2: Biological compartment access ports; Bsp: Blood sampling port; O_2/CO_2: Carbogen supply; Ox: Silicone oxygenating tube.

tested LMOs in a NRS (Figure 2A). In the NRS, they were maintained in Krebs-Henseleit (KH) solution at 37 ℃ under carbogen atmosphere (95% O_2: 5% CO_2) using a six-well culture plate for 120 min[5]. To determine LMO NH_4^+ metabolizing capability, we added an ammonia overload (1 mmol/L approximate NH_4^+ final concentration[16]) to KH-ammonia solution (KHA, Table 1) from a concentrated ammonium chloride solution (approximate concentration: 350 mmol/L). This ammonia overload is far in excess of the level of this metabolite encountered in plasma of patients with liver failure (approximately 0.2 mmol/L). We chose this condition of work to challenge LMOs because they are supposed to deal with continuously infused plasma when applied to animal models of hepatic failure or patients in the future[16–18]. The exact amount of ammonia in KHA solution was then determined, as explained later in this section.

LMO disposition in the NRS is shown in Figure 2A. Two LMOs were placed on each well with 5 mL

Table 1 Composition of the different Krebs-Henseleit solution used

Components	KHB solution	KHA solution
NaCl	114 mmol/L	114 mmol/L
KH_2PO_4	1.2 mmol/L	1.2 mmol/L
KCl	4.8 mmol/L	4.8 mmol/L
$MgSO_4$	1.2 mmol/L	1.2 mmol/L
$CaCl_2$	1.5 mmol/L	1.5 mmol/L
HEPES	10 mmol/L	10 mmol/L
$NaHCO_3$	25 mmol/L	25 mmol/L
Glucose	25 mmol/L	25 mmol/L
Allopurinol	1 mmol/L	1 mmol/L
Fructose	5 mmol/L	5 mmol/L
Glycine	-	3 mmol/L
Adenosine	-	10 μmol/L
Ornithine	-	6 mmol/L
Sodium lactate	-	10 mmol/L
pH	7.4	7.4
mOsm/kg H_2O	293 ± 6 (n = 10)	328 ± 5 (n = 10)

HEPES: 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; mOsm/kg H_2O: Osmolality; KHB: KH-Base solution; KHA: KH-ammonia solution.

of KHA solution plus the ammonia overload, and the plate was then introduced in a Dubnoff metabolic shaker maintained at 37 ℃ and stirred at 60 cycles/min. Samples of tissue and the bathing solution were taken after 0, 60, and 120 min to evaluate lactate dehydrogenase (LDH) release, oxygen consumption, and ammonia metabolism[5]. This system is intended to evaluate the biological component performance and condition *per se* in a simpler and less stressing manner than in the BAL device.

The cylindrical shaped BAL designed to house LMOs as biological component

The device designed for hepatocytes[4] was modified to use LMOs as biological component (Figure 2B). It was constructed with a cylindrical plastic cartridge that contained 120 to 140 hollow fibers (Polyamix™, GAMBRO®) assembled to the ends of the cartridge by two Y-connectors (Nalgene, # 6152–0375) connected to S/P silicone tubes (6.4 mm i.d., 11.2 mm o.d., and 2.4 mm wall). Two Teflon large catheters (14 gauge, 2 mm i.d.) and an oxygenator (Ox) made of oxygen permeable tube (silicone tubing, 0.078 in. i.d., 0.125 in. o.d.; cat. No. T5715-9, Baxter Healthcare Corp., Deerfield, IL, United States) were assembled through the Y-connectors. LMOs were seeded through an access port (Lp, Figure 2B) in the space outside the fibers (BC capacity: approximately 50 cm^3), while ram blood (obtained from animals in the animal house facility of our school) circulated along the inner space of the fibers (the blood compartment).

The "flat bottom" BAL to house LMOs as biological component

We constructed a device with a flat base BC using a standard 25 cm^2 culture flask[15] as shown in Figure 2C. The culture flask was adapted introducing a Y-shape connector (Nalgene, # 6152–0375) onto its tap and a plain connector (S/P silicone tube, 6.4 mm i.d., 11.2 mm o.d., and 2.4 mm wall) on one side, to assemble the 140 hollow fibers (Polyamix™, GAMBRO®). The loading port (Lp) was introduced on its top surface and the oxygenating tube (Ox) (silicone tubing, 0.078 in. i.d., 0.125 in. o.d.; cat. no. T5715-9, Baxter Healthcare Corp., Deerfield, IL, United States) access the BC through the free branch of the Y-connector. Both prototypes of BALs were tested using the perfusion system (Figure 2D). The peristaltic pump ensured ram blood recirculation from its reservoir passing through a clot filter/bubble trap before entering the hollow fibers. The perfusion system was kept at 37 ℃ by the thermostatic bath.

System operation protocol

First of all, a sample of ram blood (basal blood) was taken before adding the ammonia overload and filling the blood perfusion system by the aid of a peristaltic pump (blood volume: approximately 35 mL). The BC was then filled with KHA alone (to test the bioreactor performance) or with KHA plus 1 g of LMO. Samples of blood and BC fluid were taken after 0, 60, and 120 min of perfusion to assay the different parameters mentioned below. Blood samples were centrifuged at 12000 × g for 3 min in a mini spin centrifuge.

During the experiments (BAL runs), the device was operated in horizontal position, immersed in the Dubnoff shaker bath at 37 ℃ stirred at 60 cycles/min. Carbogen gas pressure was kept at 85 mm Hg to introduce oxygen in the BC media *via* the silicone tube oxygenator, and in this way pH was maintained at 7.40 ± 0.10, which was controlled throughout the run. The blood maintained proper oxygenation, and its recirculation flow rate was maintained at 9 mL/min.

All the devices designed were subjected to two different types of studies: (1) without any biological component, to establish the adequate functioning of the device and the perfusion system[4]; and (2) with biological component, to determine if the chosen biological component could accomplish ammonia detoxification.

Study of system performance

In experiments without LMOs, we first checked the correct system functioning by evaluating the following parameters: (1) hematocrit of blood samples after centrifugation (10000 × g for 3 min, Rolco CH24 centrifuge), as the percentage of volume that red cells represent from the total (blood cells + plasma). It was used to evaluate fluid exchange and the occurrence of hemolysis during perfusion; (2) osmolality of plasma and extra-fiber fluid was determined with a freezing point osmometer (Osmomat 030 Gonotec, GmbH, Berlin, Germany) to check the proper passage of fluids through the fiber walls; and (3) glucose and ammonia concentrations were assessed as described below

Table 2 Sequences of the primers employed

Primers	Fragment size	Genbank access number
Cps1 5′-ATCTGAGGAAGGAGCTGTCT-3′ (sense) *Cps1* 5′-AAAACCACTTGTCAATGGAT-3′ (anti-sense)	120 bp	NM_017072
Otc 5′-ATGACAGATGCAGTGTTAGC-3′ (sense) *Otc* 5′-CAGGATCTGGATAGGATGAT-3′ (anti-sense)	120 bp	NM_013078
Actb 5′-CAACCTTCTTGCCAGCTCCTC-3′ (sense) *Actb* 5′-GACGAGCGCAGCGATATC-3′ (anti-sense)	79 bp	NM_031144.2
Rn18S 5′-TAACCCGTTGAACCCCATT-3′ (sense) *Rn18S* 5′-CCATCCAATCGGTAGTAGCG-3′ (anti-sense)	150 bp	X01117
Gapdh 5′-CCATCACCATCTTCCAGGAG-3′ (sense) *Gapdh* 5′-CCTGCTTCACCACCTTCTTG-3′ (anti-sense)	576 bp	NM_017008.4

and their mass balance was calculated to monitor the adequate exchange of metabolites between blood and the BC in the device.

Hemolysis assessment

The quantity of hemoglobin in plasma samples obtained after 0, 60, and 120 min of perfusion was assessed using the oxyhemoglobin method as previously described[19]. The equation proposed by Arnaud *et al*[20] was then used to calculate hemolysis percentage as follows:

$$\text{Hemolysis (\%)} = 100 \times [(Hb_S \times (1 - Ht))/Hb_T]$$

where Hb_S represents the sample hemoglobin contents, in g/100 mL; Hb_T is the total hemoglobin content determined in whole blood, and Ht represents the respective hematocrit value.

Glucose level determination

This parameter was assessed with a commercially available kit ("Glicemia Enzimática AA", Wiener Laboratories, Rosario, Argentina), as instructed in the information leaflet.

LDH release quantification

We used LDH release as a LMO viability parameter. This enzyme activity was determined in the KH incubation solution and in the liver slices as previously described[21]. Results are shown as the percentage of the total enzyme activity released to the bathing media.

Ammonia level assessment

Samples were stored in liquid nitrogen until ammonia quantification was done using the enzymatic method described by van Anken[22]. The reaction media (0.8 mL) was composed of 66.7 mmol/L phosphate buffer, pH = 8.30, 0.14 mmol/L NADPH, 6.5 mmol/L sodium α-ketoglutarate, 2.5 mmol/L ADP, and 120 UI/mL glutamate dehydrogenase (cat. #G2626, Sigma Aldrich, St. Louis, MO, United States).

In the BAL, ammonia mass balance was calculated using the equations described next:

$$Q_{B,t} = ([A]_{B,t} \times V_{B,t}) - ([A]_{B,Bas} \times V_{B,t})$$

$$Q_{BC,t} = ([A]_{BC,t} \times V_{BC,t}) - ([A]_{BC,Bas} \times V_{BC,t})$$

$$Q_{T,t} = Q_{B,t} + Q_{BC,t}$$

where: $Q_{B,t}$ and $Q_{BC,t}$ are the ammonia mass in blood and the BC solution at time t, respectively; $[A]_{B,t}$ and $[A]_{BC,t}$ represent respective ammonia concentrations in blood and BC fluid; $V_{B,t}$ and $V_{BC,t}$ are, respectively, the volumes of blood and the BC fluid at each time, and $Q_{T,t}$ is the total ammonia mass at the different assayed times.

Then, we calculated the percentage of ammonia initial dose metabolized at each time with the subsequent equation:

$$\text{Initial Dose Detoxified (\%)} = 100 - [Q_{T,t} \times 100/ Q_{T,0}]$$

Also, we estimated the μmol of ammonia detoxified per gram of LMO as follows:

$$\text{Ammonia Detoxification (μmol/g wet tissue)} = (Q_{T,0} - Q_{T,t})/ P_T$$

where P_T represents the mass of LMOs in grams.

In the case of the NRS, ammonia concentration was determined in the LMO bathing solution and the equations applied were:

$$\text{Initial Dose Detoxified (\%)} = 100 - [(Q_t \times 100)/ Q_i]$$

$$\text{Ammonia Detoxification (μmol/g wet tissue)} = (Q_i - Q_t)/ P_T$$

where Q_i and Q_t represent the amount of ammonia measured at the beginning of the experiments and after t minutes, respectively, and P_T is the total weight of LMOs in grams.

Cps1 and Otc expression analysis

As part of the LMO ammonia metabolism study, we determined the mRNA and activity levels of Carbamyl Phosphate Synthetase Ⅰ (CPSI) and Ornithine Transcarbamylase (OTC) that catalyze the first and second steps of the urea cycle, respectively.

Total RNA extractions were performed using TriReagent™ (Sigma Chem. Co., St. Louis, United State) and following the instructions provided by the manufacturer. We carried out reverse transcription and semi-quantitative PCR as previously described[23]. In Table 2, the base sequences of the primers used for the study of each gene expression are listed. We applied the $2^{-\Delta\Delta CT}$ method to obtain relative expression, using Glyceraldehyde Phosphate Dehydrogenase (*Gapdh*), β-Actin (*Actb*) and rRNA 18S (*Rn18S*) as reporter mRNAs to normalize the values. Each sample was analyzed in triplicate, and its template cDNA initial

quantity was expressed relative to a reference sample, considered 1X. This reference sample was taken at time 0.

CPSI and OTC enzymatic activity determination

To determine CPSI activity we performed the Pierson's colorimetric test[24] that involves the reaction of carbamyl phosphate and hydroxylamine to obtain hydroxyurea. An enhanced colorimetric test for ureido compounds allows the quantification of the hydroxyurea produced due to the chromophore absorbance measurement at 458 nm. CPSI activity is expressed as U/g of wet tissue, being U the μmoles of carbamyl phosphate produced per minute at 37 ℃.

OTC activity was measured with the method described by Ceriotti[25] as the rate of citrulline formation from ornithine and carbamyl phosphate is catalyzed by OTC. The quantity of citrulline produced was determined by the diacetyl monoxime-antipyrine reaction and OTC activity was also expressed as U/g of wet tissue, where U represents the μmoles of citrulline synthesized per minute at 37 ℃.

Oxygen consumption

Samples of LMOs were taken at different times (0, 60, and 120 min) from the NRS or the BAL prototype and were put into a thermostized oxygen electrode chamber constructed in our laboratory, filled with respiration media (KH plus 10 mmol/L HEPES and 2 mmol/Lm pyruvate, pH = 7.40 at 36 ℃)[14]. The oxygen levels in the incubation media were measured using a Clark-type oxygen electrode (YSI 5300, Yellow Spring, OH, United States). After a 2 min stabilization period, the endogenous respiration rate was recorded and calculated over a 5 min period. Results are expressed as μmol O_2/min/g wet tissue[14].

Statistical analysis

Results are expressed as mean ± SD. Data was analyzed using one-way or multifactor analysis of variance with Scheffe's multiple range tests as post-test. Differences between means were considered statistically significant when $P \leqslant 0.05$. Dr. Lucas D Daurelio (Estadística, Facultad de Cs. Bioquímicas y Farmacéuticas, UNR) has designed and supervised the statistical analysis performed in this work.

RESULTS

Cylindrical shaped BAL for LMOs: Performance without biological component

As was formerly explained, the first BAL constructed in our laboratory was designed to house hepatocyte suspensions as the biological component and showed a good performance[4]. In order to evaluate the use of LMOs as alternative biocomponent of our BAL prototype, we first studied their viability and functionality in a simpler model, the NRS, as shown in Figure 2A[5]. Once was established, their ability to metabolize ammonia (detoxification of 35.1% ± 7.0 %, or 14.3 ± 3.6 μmol/g wet tissue, after 120 min incubation, $n = 6$), we modified the cylindrical BAL, originally designed to contain hepatocytes, in order to accommodate LMOs on its BC.

The cylindrical shaped BAL for LMOs is shown in Figure 2B. The hollow fiber cartridge configuration is maintained: blood circulates inside the fibers and LMOs are placed in the compartment delimited between the plastic receptacle and the fiber outer surfaces, but this BC has a larger capacity (50 cm^3 *vs* 9 cm^3 in the previous model). Also, a bigger loading port (Lp in Figure 2B) was assembled to the plastic cartridge. Both, Lp and BC capacity are larger than in the original BAL[4] to allow easy LMO loading and retrieval, and to accommodate the biological material, respectively. In both designs, approximately 140 hollow fibers are aligned inside the cartridge connected to two Y-shaped connectors.

We studied the functioning of the device and the perfusion system in experiments without a biological component. The values obtained for the different parameters assayed can be seen in Table 3 ($n = 6$ independent runs). Hematocrit values and osmolality ratios remained stable during the 120 min of perfusion and, though there was an increment in the percentage of hemolysis, it was minimal. Ammonia and glucose could readily cross the fiber walls because their concentrations in both compartments evened out after 120 min. Total ammonia mass (Q_{NH4+}) did not change during experiments.

Cylindrical shaped BAL: performance with LMOs as a biological component

After determining that the BAL was functioning correctly without a biological component, the modified device was tested with LMOs to establish its capacity to keep these tissue slices functional and viable for 120 min of perfusion.

As a viability parameter, we assayed the release of the cytosolic enzyme LDH. The values measured for LMOs in the BAL prototype are shown in Table 4 ($n = 6$ independent LMO preparations in separate runs), compared to the values obtained in the NRS[5] ($n = 6$ different LMO preparations). In our BAL, the amount of LDH release increased with perfusion time. A similar pattern was observed in the NRS and no difference was detected when both systems were compared, reaching almost the same percentages after 120 min (14.7% ± 3.1% for the BAL prototype and 15.5% ± 3.2% for the NRS).

Respiratory activity determination constitutes a very specific metabolic test to evaluate tissue functionality and notice the existence of hidden damages[26]. LMOs were taken out at different times and put into an oxygen chamber to test their oxygen consumption capacity (Table 4, $n = 6$ LMOs taken at each time,

Table 3 Functional parameters of the cylindrical shaped bio-artificial liver[1] (*n* = 6 independent runs)

Perfusion time	Osm_B/Osm_{BC}	Ht (%)	Hemolysis (%)	$[Glucose]_B/[Glucose]_{BC}$	$[NH_4^+]_B/[NH_4^+]_{BC}$	Q_{NH4+} (μmol)
0 min	0.95 ± 0.05	43 ± 3	0.12 ± 0.06	0.12 ± 0.01	23.2 ± 1.2	31.1 ± 3.9
60 min	0.99 ± 0.01	42 ± 6	0.30 ± 0.08	0.81 ± 0.03	1.4 ± 0.4	31.1 ± 4.2
120 min	1.00 ± 0.01	38 ± 2	0.65 ± 0.10	0.94 ± 0.02	1.2 ± 0.2	31.3 ± 3.8

[1]The cylindrical bio-artificial liver designed for liver microorgans functioning without any biological component. B: Blood; BC: Biological compartment; Ht: Hematocrit.

Table 4 Viability and functional parameters evaluated for liver microorgans in the cylindrical shaped bio-artificial liver and the Normothermic Reoxygenation System (*n* = 6 different liver microorgan preparations in independent bio-artificial liver runs/Normothermic Reoxygenation System incubations)

Evaluated parameters		LDH release (%)			Oxygen Consumption (μmol/min·g wet tissue)		
Time (min)		0	60	120	0	60	120
Model	Cylindrical BAL	1.9 ± 0.9	9.4 ± 3.0[a]	14.7 ± 3.1[a]	1.21 ± 0.24	1.15 ± 0.20	1.16 ± 0.21
	NRS	2.6 ± 0.1	8.3 ± 1.2[a]	15.5 ± 3.2[a]	1.13 ± 0.11	0.85 ± 0.15[c]	0.84 ± 0.15[c]

[a]$P < 0.05$, different from other times; [c]$P < 0.05$, different from time 0. BAL: Bio-artificial liver; NRS: Normothermic Reoxygenation System; LDH: Lactate dehydrogenase.

Table 5 Relative gene expression of *Cps1* and *Otc* for liver microorgans in the cylindrical shaped bio-artificial liver and the Normothermic Reoxygenation System (*n* = 3 different liver microorgan preparations in independent bio-artificial liver runs/Normothermic Reoxygenation System incubations)

Relative gene expression		*Cps1*			*Otc*		
Time (min)		0	60	120	0	60	120
Model	Cylindrical BAL	1.05 ± 0.17	0.84 ± 0.09	0.63 ± 0.12	1.02 ± 0.26	0.87 ± 0.21	0.67 ± 0.20
	NRS	1.00 ± 0.24	0.96 ± 0.12	0.86 ± 0.10	1.00 ± 0.20	0.87 ± 0.16	0.82 ± 0.07

BAL: Bio-artificial liver; NRS: Normothermic Reoxygenation System; *Cps1*: Carbamyl phosphate synthetase Ⅰ; *Otc*: Ornithine transcarbamylase.

Table 6 Carbamyl phosphate synthetase Ⅰ and ornithine transcarbamylase enzymatic activity in the cylindrical shaped bio-artificial liver and the Normothermic Reoxygenation System (*n* = 3 different liver microorgan preparations in independent bio-artificial liver runs/Normothermic Reoxygenation System incubations)

Enzymatic activity		CPSI (U/g wet tissue)			OTC (U/g wet tissue)		
Time (min)		0	60	120	0	60	120
Model	Cylindrical BAL	2.48 ± 0.62	2.72 ± 0.61	3.03 ± 0.86	241.7 ± 12.6	235.1 ± 11.9	222.0 ± 23.5
	NRS	2.47 ± 0.60	2.82 ± 0.76	3.12 ± 0.73	209.7 ± 33.2	251.8 ± 29.4	228.8 ± 32.8

CPSI: Carbamyl phosphate synthetase Ⅰ; OTC: Ornithine transcarbamylase; BAL: Bio-artificial liver; NRS: Normothermic Reoxygenation System.

coming from different preparations in independent BAL-runs/NRS-incubations). This parameter remained stable during the 120 min of perfusion in the BAL and again no difference was observed compared to the NRS, even though in this system there was a significant decrease at 60 min and 120 min compared to the initial value.

Any biological component employed in a BAL must face and deal with the high levels of ammonia in the blood of the patients to be treated because the accumulation of this metabolite is associated with the progression of hepatic failure[3]. Therefore, we analyzed different parameters related to ammonia metabolism in LMOs. Relative gene expression and activity of CPSI and OTC, two key enzymes of the urea cycle, were studied and the results obtained are shown in Tables 5 and 6 (*n* = 3 different LMO preparations in independent BAL-runs/NRS-incubations). The levels of mRNA and activity for both enzymes did not significantly change after 120 min of perfusion in the BAL prototype. In addition, there were no significant differences in the levels measured for LMOs in the NRS[5], indicating proper enzyme quantities to perform ammonia detoxification. However, despite all these similarities, when the LMOs were used as the biological component of the cylindrical shaped BAL they were unable to metabolize ammonia (Table 7), while in the NRS they detoxified 22.2% ± 5.5% and 35.1% ± 7.0% of the initial dose after 60 min and 120 min, respectively (*n* = 6 different LMO preparations).

Table 7 Ammonia detoxification capacity (μmol/g wet tissue) for fresh liver microorgans and hepatocyte suspensions used as biological component of the "flat bottom" and cylindrical bio-artificial livers (*n* = 6 different liver microorgan/hepatocyte preparations in independent runs)

Time (min)	Ammonia detoxification capacity (μmol/g wet tissue)		
	Flat bottom BAL	Cylindrical BAL	
	LMOs	LMOs	Hepatocytes
60	8.1 ± 1.2[a]	ND	12.5 ± 1.8
120	13.2 ± 2.2	ND	18.6 ± 4.9

[a]$P < 0.05$, different from hepatocytes at time 60 min. 128×10^6 hepatocytes = 1 g of liver. ND: Not detectable, means that the total ammonia mass remained equivalent to the 100% of initial dose at the indicated times; LMO: Liver microorgan; BAL: Bio-artificial liver.

Cylindrical vs "flat bottom" shaped BAL prototypes: The importance of architecture

We hypothesized that the LMOs were unable to metabolize ammonia in the cylindrical shaped device was not related to the biological component because all the other functionality and viability parameters were similar to the NRS. Therefore, the problem could be due to the artificial part of the device, specifically the BC. To test this hypothesis, we decided to change the BC architecture of our BAL and mimic the configuration and disposition of the LMOs in the NRS (Figure 2A). The result was the design of a BAL prototype with a completely different shaped BC: The "flat bottom" BAL displayed in Figure 2C. Instead of using a cylindrical plastic cartridge, we utilized a 25 cm^2 culture flask that offers a flat base BC where LMOs adopt a similar distribution as the one in the NRS. All the other components and materials utilized were the same in both devices, including the perfusion system. This "flat bottom" BAL performed well in experiments without a biological component[15].

Ammonia detoxification was the key task that LMOs could not perform in the cylindrical shape BAL. When they were evaluated in the "flat bottom" BAL, fresh LMOs were able to metabolize 32.1% ± 2.2% and 49.3% ± 8.8% of the initial NH_4^+ dose at 60 min and 120 min, respectively (n = 6 different LMO preparations in independent runs)[15], results that support the importance of architecture.

According to the results, the devices designed in our laboratory could be successfully incorporated into BAL systems depending on the chosen biological component. In Table 7, we compare both ammonia detoxification capacities between LMOs in the "flat bottom" and cylindrical BAL prototypes as well as with hepatocyte suspensions in our previous cylindrical BAL (n = 6 different LMO/hepatocyte preparations in independent runs). For the purpose of comparison between the different biological components (LMOs *vs* hepatocytes), we performed calculations using the following equivalence: 128×10^6 hepatocytes = 1 g of liver[27-29]. After 60 min of perfusion, LMOs in the "flat bottom" BAL showed a significantly decreased level of ammonia detoxification compared to hepatocytes in the cylindrical model (Table 7). However, after 120 min both models had similar metabolizing capacities. Rat hepatocyte isolation and incubation in the cylindrical BAL were performed as already reported by our group[4] (routine protocol for hepatocyte isolation is shown in Figure S1; and cylindrical BAL operation in Figure S2).

DISCUSSION

The first step before using LMOs in our BAL device was the adaptation of the prototype designed for hepatocytes, and testing it without any biological components. The results obtained (Table 3) demonstrated a proper exchange of fluids between the blood and the BC since the relationship between osmolality and the hematocrit values remained stable during the 120 min of perfusion. Also, it can be appreciated that solute transport functioned properly in both directions: ammonia diffused from blood to the extra-fiber fluid, while glucose flowed the opposite way, almost matching their concentrations during the initial hour (Table 3). We did not detect unspecific loss of ammonia or interaction with any part of the device (Q_{NH4+} remained constant) and the small percentage of hemolysis measured can be attributed to the action of the peristaltic pump. Taken together, the results showed that the cylindrical shaped device made in our laboratory, with its simple design, could be easily adapted and scaled up, which is a very important issue in the construction of a BAL intended for clinical application.

After corroborating the proper functioning of the device, we performed several experiments with LMOs as its biological component. The results of these tests were unexpected: LMOs showed satisfactory viability levels (assayed by LDH release), oxygen consumption capacity did not change during the perfusion, and conserved levels of transcripts and adequate enzymatic activities of CPSI and OTC, but they were not able to metabolize ammonia in a significant amount. Even more astonishing was the fact that all the assayed parameters showed similar values to the ones determined in the NRS[5], where LMOs could detoxify 35.1% ± 7.0% of the initial 1 mmol/L NH_4^+ dose after 120 min.

When adapting the cylindrical BAL model to house LMOs as a biological component, we found that, in spite of the shaking applied, LMOs piled up over each other at the bottom of the cartridge in an array that could prevent the correct exchange of nutrients, oxygen, and metabolites. Furthermore, the experiments in the NRS were accomplished in flat wells in 6-well multi dishes. Then, we came up with the idea of mimicking the NRS configuration, and developed a BAL prototype with a flat surface BC. We reasoned that this geometrical disposition would allow LMOs to adopt a looser, not

stacked distribution that would benefit the exchange mechanisms between LMOs and the bathing media. In this way, provided that the volume of the BC is equivalent to that of the previous prototype (50 cm^3), this flat bottomed device offers a surface that allows an enhanced bathing and shaking of plane LMOs due to their better distribution.

When we tested fresh LMO ammonia detoxification efficiency in this new "flat bottom" BAL, the results obtained supported our hypothesis. They were able to detoxify 49.3% ± 8.8% of the initial ammonia overload after 120 min of perfusion[15], contrary to the LMOs applied to the cylindrical BAL (Table 7). This observation demonstrates the importance of adjusting the architecture and design of the artificial compartment to a given biological component in order to obtain an optimal BAL performance. Although we cannot rule out other factors influencing LMO detoxification activities that remained unnoticed, the only change between the cylindrical and flat bottom BALs was the shape of the BC. The BC volume, shaking speed, blood flow velocity, oxygen tension, and the brand of fibers used in their construction were identical.

Both systems published, *i.e.*, the cylindrical one operating with isolated hepatocytes[4] and the "flat bottom" one operating with LMOs[15], function satisfactorily in relation to ammonia detoxification (Table 7) and, according to the needs of the patient, could be optionally selected in the future. The device could also be used as a suitable mini-bioreactor to analyze drug toxicity or other parameters of interest. We are performing further studies to establish the synthetic capacity of the biological components in our prototypes.

At present no BAL is in use to clinically treat liver failure. Some clinical trials have been conducted (for an updated review consult the work of Sakiyama *et al*[30] and references therein) with some success. All of these devices use isolated liver-derived cells. Four of them use porcine primary hepatocytes either fresh (named LSS, Excorp Medical BLSS and AMC-BAL) or cryopreserved and microcarrier attached (HepatAssit), one uses human primary hepatocytes (MELS), and one uses HepG2/C3A cell line (Vitagen ELAD). These devices have not become mainstream in clinical settings yet because of the complexity of isolating hepatocytes and the difficulties for maintaining the viability of these cells viable for prolonged periods of time. This limits the assembly and transport of the devices and confines its use to the centers where they were developed. An exception to this could be the ELAD system that uses a cell line, but the costs of producing the required amount of cellular material makes it expensive. It is also limited to places with the facilities and expertise to conduct cell culture at a large scale. In this sense our BAL prototype, although it needs further testing, requires hand-cut LMOs, which are obtained by a low-cost technique that is easily learned. Also, the required biological material could be obtained from donor livers not acceptable for transplantation but meeting the criteria to obtain adequate pieces to feed this kind of BAL and possibly used in multiple treatments. Therefore, pre-assembled BALs could be filled with LMOs obtained in the same center where they will be immediately applied. The prototype we are presenting in this work is constructed with standard laboratory and medical supplies, and we envisage that they could be constructed at reasonable costs and, consequently, commercialized at reasonable prices.

After publishing our previous results[15], we became aware that it is difficult to predict the conditions needed for good performance of the devices based on rational design and, instead, much of the experience we have accumulated over the years mostly originated by trial and error. Especially in this field of BAL research, every laboratory seems to apply different strategies to achieve the objective of extracorporeal treatment of liver failure. Although some basic principles and features are followed by all of us, there seems to really exist one different BAL for each group of work[31,32]. As far as we know, this is the first time that a comparison of the same biological component applied to different configurations of BALs is reported. In the literature, we have always found comparisons of different BALs only in review articles that use data from different research groups, which renders the analysis incomplete and inadequate. However, we have made comparisons in the same set of experiments using cells and tissues from the same brood of experimental animals, same batches of solutions, same laboratory instruments, *etc*. These conditions render our results coherent and valid, and strongly demonstrate that architecture of BALs can determine the success of this kind of device.

Further investigations must be done in order to bring these types of devices to the clinic. Very few clinical trials have been performed, and they are typically carried out on patients with advanced deterioration[32-35]. In conclusion, we demonstrate the importance of adapting the artificial component architecture to the biological component characteristics to obtain an adequate BAL performance regarding blood ammonia detoxification.

ARTICLE HIGHLIGHTS

Research background

Liver failure is a condition that usually requires liver transplantation, but in some cases acute liver failure resolves spontaneously due to the viable hepatic mass remaining after the cause of the damage has disappeared. If the amount of this functional tissue has the sufficient capacity to handle the detoxification of harmful metabolites produced by the insult and to provide the needed essential hepatic molecules and factors, then the regeneration capacity of the organ allows the recovery. This is why many attempts have been pursued to help the patient´s liver to pass through this acute failure and either recover or extend the time frame for a liver transplantation. Artificial livers, either dialysis based or incorporating hepatic cells and tissues (these later referred to as bio-

artificial livers or BALs), are extracorporeal devices intended to aid the failing livers to overcome failure or at least to permit the patient to improve to undergo transplantation. In this sense, BALs are considered the choice to accomplish this job but until now they have been applied only by medical care teams that are able to obtain the biological component and to assemble the device at the same location making the practice limited to very few centers in the world.

Research motivation

The BAL research field is several decades old but still no successful device has been developed. Several prototypes have been submitted to clinical trials but none are routinely used in clinical settings or commercially available. These prototypes use isolated cells of hepatic origin (isolated primary human or pig hepatocytes and HepG2/C3A cell line), which is a biological material that requires expertise and money to be obtained and/or maintained. Additionally, cryopreservation of primary hepatocytes is not a very successful technique and recovery after thawing is poor. Among other researchers in the field, we propose and are testing the use of liver microorgans (LMOs) as the biological component for BAL devices, which are promising in terms of bearing all hepatic cellular types and microarchitecture and involve a simple method to obtain. These characteristics are appealing because they bring the possibility of using procured organs not suitable for transplantation to get the material necessary to feed pre-assembled cartridges at the same centers were the BAL would be needed. Our experience in the field has taught us that changes in the artificial part of these devices can have an impact on the biological component function. The finding that these changes in design, that can be minor or significant, have an influence on performance with effects ranging from subtle to massive, address the importance of finely tuning the interplay between the components of the device to optimize BAL operation.

Research objectives

LMOs failed to detoxify ammonia in a scaled-up BAL configuration that was previously successful. We set out to solve this problem and analyze the possible reasons of the phenomenon we were observing.

Research methods

The methodology used is the standard in our laboratory and has been previously published. The novelty is to perform and report the comparison of different BAL designs using the same biological component. This is the first report making such a comparison in the same set of experiments using cells and tissues from the same brood of experimental animals, same batches of solutions, same laboratory instruments, same materials to construct the devices, and so forth.

Research results

The main result we achieved in this work is that LMOs were totally incompetent to detoxify ammonia when placed in a cylindrical shaped BAL while they were fully able to detoxify ammonia inside a flat bottomed BAL.

Research conclusions

The accumulation of high levels of ammonia in the blood is an important issue in patients presenting liver failure, and is of the highest interest when studying this kind of device. In the literature, we have always found comparisons of different BALs only in review articles that use data from different research groups, which renders the comparative analysis incomplete. Our experimental design makes our comparison coherent and valid, and strongly demonstrates that the architecture of BALs can determine the success of this kind of device.

Research perspectives

We consider that this is an especially important finding, particularly in the light of the results presented, compelling future research to put an effort to finely tune the interplay between the artificial and biological components of BALs in order to achieve optimal performance and finally reach the clinical setting.

ACKNOWLEDGMENTS

We would like to thank GAMBRO, especially to Mr. Jorge Auerbuch, for kindly providing the dialysis cartridge from which we obtained Polyamix™ fibers.

REFERENCES

1 **Rahman TM**, Hodgson HJ. Review article: liver support systems in acute hepatic failure. *Aliment Pharmacol Ther* 1999; **13**: 1255-1272 [PMID: 10540040 DOI: 10.1046/j.1365-2036.1999.00597.x]
2 **Selden C**. Bioartificial liver. In: Nedović V, Willaert R, editors. Applications of Cell Immobilisation Biotechnology. 1st ed. Berlin/Heidelberg: Springer-Verlag, 2005: 69-83 [DOI: 10.1007/1-4020-3363-X_4]
3 **Bernal W**, Hall C, Karvellas CJ, Auzinger G, Sizer E, Wendon J. Arterial ammonia and clinical risk factors for encephalopathy and intracranial hypertension in acute liver failure. *Hepatology* 2007; **46**: 1844-1852 [PMID: 17685471 DOI: 10.1002/hep.21838]
4 **Rodriguez JV**, Pizarro MD, Scandizzi AL, Guibert EE, Almada LL, Mamprin ME. Construction and performance of a minibioreactor suitable as experimental bioartificial liver. *Artif Organs* 2008; **32**: 323-328 [PMID: 18370948 DOI: 10.1111/j.1525-1594.2007.00435.x]
5 **Pizarro MD**, Mediavilla MG, Berardi F, Tiribelli C, Rodríguez JV, Mamprin ME. Cold storage of liver microorgans in ViaSpan and BG35 solutions: study of ammonia metabolism during normothermic reoxygenation. *Ann Hepatol* 2014; **13**: 256-264 [PMID: 24552868]
6 **Matoori S**, Leroux JC. Recent advances in the treatment of hyperammonemia. *Adv Drug Deliv Rev* 2015; **90**: 55-68 [PMID: 25895618 DOI: 10.1016/j.addr.2015.04.009]
7 **McEwan P**, Simpson D, Kirk JM, Barr DG, McKenzie KJ. Short report: Hyperammonaemia in critically ill septic infants. *Arch Dis Child* 2001; **84**: 512-513 [PMID: 11369572 DOI: 10.1136/ADC.84.6.512]
8 **Bharat A**, Cunningham SA, Scott Budinger GR, Kreisel D, DeWet CJ, Gelman AE, Waites K, Crabb D, Xiao L, Bhorade S, Ambalavanan N, Dilling DF, Lowery EM, Astor T, Hachem R, Krupnick AS, DeCamp MM, Ison MG, Patel R. Disseminated Ureaplasma infection as a cause of fatal hyperammonemia in humans. *Sci Transl Med* 2015; **7**: 284re3 [PMID: 25904745 DOI: 10.1126/scitranslmed.aaa8419]
9 **Häberle J**, Boddaert N, Burlina A, Chakrapani A, Dixon M, Huemer M, Karall D, Martinelli D, Crespo PS, Santer R, Servais A, Valayannopoulos V, Lindner M, Rubio V, Dionisi-Vici C. Suggested guidelines for the diagnosis and management of urea cycle disorders. *Orphanet J Rare Dis* 2012; **7**: 32 [PMID: 22642880 DOI: 10.1186/1750-1172-7-32]
10 **Mouat S**, Bishop J, Glamuzina E, Chin S, Best EJ, Evans HM. Fatal hyperammonemia associated with disseminated Serratia marcescens infection in a pediatric liver transplant recipient. *Pediatr Transplant* 2018; **22**: e13180 [PMID: 29624817 DOI: 10.1111/petr.13180]
11 **Vilstrup H**, Amodio P, Bajaj J, Cordoba J, Ferenci P, Mullen KD, Weissenborn K, Wong P. Hepatic encephalopathy in chronic liver disease: 2014 Practice Guideline by the American Association for the Study of Liver Diseases and the European Association for the Study of the Liver. *Hepatology* 2014; **60**: 715-735 [PMID: 25042402 DOI: 10.1002/hep.27210]
12 **Olde Damink SW**, Jalan R, Deutz NE, Redhead DN, Dejong CH, Hynd P, Jalan RA, Hayes PC, Soeters PB. The kidney plays a major role in the hyperammonemia seen after simulated or actual GI bleeding in patients with cirrhosis. *Hepatology* 2003; **37**: 1277-1285 [PMID: 12774005 DOI: 10.1053/jhep.2003.50221]
13 **De Jonghe B**, Janier V, Abderrahim N, Hillion D, Lacherade JC, Outin H. Urinary tract infection and coma. *Lancet* 2002; **360**: 996 [PMID: 12383670 DOI: 10.1016/S0140-6736(02)11084-1]
14 **Mandolino C**, Pizarro MD, Quintana AB, Rodríguez JV, Mamprin ME. Hypothermic preservation of rat liver microorgans (LMOs) in bes-gluconate solution. Protective effects of polyethyleneglycol (PEG) on total water content and functional viability. *Ann Hepatol*

2011; **10**: 196-206 [PMID: 21502682]
15 **Pizarro MD**, Mediavilla MG, Quintana AB, Scandizzi ÁL, Rodriguez JV, Mamprin ME. Performance of cold-preserved rat liver Microorgans as the biological component of a simplified prototype model of bioartificial liver. *World J Hepatol* 2016; **8**: 1442-1451 [PMID: 27957242 DOI: 10.4254/wjh.v8.i33.1442]
16 **Calligaris SD**, Almada LL, Guibert EE, Tiribelli C, Rodriguez JV. Ammonium detoxifying activity is maintained after 72 hours of cold preservation of rat hepatocytes in University of Wisconsin (UW) solution. *Cryo Letters* 2002; **23**: 245-254 [PMID: 12391485]
17 **Proelss HF**, Wright BW. Rapid determination of ammonia in a perchloric acid supernate from blood, by use of an ammonia-specific electrode. *Clin Chem* 1973; **19**: 1162-1169 [PMID: 4355040]
18 **Blei AT**. Diagnosis and treatment of hepatic encephalopathy. *Baillieres Best Pract Res Clin Gastroenterol* 2000; **14**: 959-974 [PMID: 11139349 DOI: 10.1053/bega.2000.0141]
19 **Kalinov A**. El Laboratorio y Su Interpretación Semiológica. 1st ed. Buenos Aires: Lopez Libreros Editores, 1975
20 **Arnaud FG**, Khirabadi BS, Fahy GM. Normothermic blood perfusion of isolated rabbit kidneys. Ⅲ. In vitro physiology of kidneys after perfusion with Euro-Collins solution or 7.5 M cryoprotectant (VS4). *Transpl Int* 2002; **15**: 278-289 [PMID: 12072898 DOI: 10.1007/s00147-002-0395-z]
21 **Olinga P**, Merema MT, Hof IH, De Jager MH, De Jong KP, Slooff MJ, Meijer DK, Groothuis GM. Effect of cold and warm ischaemia on drug metabolism in isolated hepatocytes and slices from human and monkey liver. *Xenobiotica* 1998; **28**: 349-360 [PMID: 9604299 DOI: 10.1080/004982598239461]
22 **van Anken HC**, Schiphorst ME. A kinetic determination of ammonia in plasma. *Clin Chim Acta* 1974; **56**: 151-157 [DOI: 10.1016/0009-8981(74)90223-X]
23 **Miszczuk G**, Mediavilla MG, Pizarro MD, Tiribelli C, Rodríguez J, Mamprin ME. Expression and distribution of aquaporin 8 in rat hepatocytes cold stored 72 hours in modified University of Wisconsin and bes-gluconate-sucrose solutions. Study of their correlation with water content. *Cryo Letters* 2012; **33**: 75-85 [PMID: 22434125]
24 **Pierson DL**. A rapid colorimetric assay for carbamyl phosphate synthetase I. *J Biochem Biophys Methods* 1980; **3**: 31-37 [DOI: 10.1016/0165-022X(80)90004-4]
25 **Ceriotti G**. Ornithine Carbamoyltransferase. *Methods Enzym Anal vol III* 1983; **2**: 319-325
26 **Bilzer M**, Gerbes AL. Preservation injury of the liver: mechanisms and novel therapeutic strategies. *J Hepatol* 2000; **32**: 508-515 [PMID: 10735623 DOI: 10.1016/S0168-8278(00)80404-3]
27 **Bayliss MK**, Bell JA, Jenner WN, Park GR, Wilson K. Utility of hepatocytes to model species differences in the metabolism of loxtidine and to predict pharmacokinetic parameters in rat, dog and man. *Xenobiotica* 1999; **29**: 253-268 [PMID: 10219966 DOI: 10.1080/004982599238650]
28 **Pang KS**, Kong P, Terrell JA, Billings RE. Metabolism of acetaminophen and phenacetin by isolated rat hepatocytes. A system in which the spatial organization inherent in the liver is disrupted. *Drug Metab Dispos* 1985; **13**: 42-50 [PMID: 2858375]
29 **Sohlenius-Sternbeck AK**. Determination of the hepatocellularity number for human, dog, rabbit, rat and mouse livers from protein concentration measurements. *Toxicol In Vitro* 2006; **20**: 1582-1586 [PMID: 16930941 DOI: 10.1016/j.tiv.2006.06.003]
30 **Sakiyama R**, Blau BJ, Miki T. Clinical translation of bioartificial liver support systems with human pluripotent stem cell-derived hepatic cells. *World J Gastroenterol* 2017; **23**: 1974-1979 [PMID: 28373763 DOI: 10.3748/wjg.v23.i11.1974]
31 **Bader A**, Knop E, Frühauf N, Crome O, Böker K, Christians U, Oldhafer K, Ringe B, Pichlmayr R, Sewing KF. Reconstruction of liver tissue in vitro: geometry of characteristic flat bed, hollow fiber, and spouted bed bioreactors with reference to the in vivo liver. *Artif Organs* 1995; **19**: 941-950 [PMID: 8687303 DOI: 10.1111/j.1525-1594.1995.tb02456.x]
32 **Carpentier B**, Gautier A, Legallais C. Artificial and bioartificial liver devices: present and future. *Gut* 2009; **58**: 1690-1702 [PMID: 19923348 DOI: 10.1136/gut.2008.175380]
33 **Krisper P**, Stadlbauer V, Stauber RE. Clearing of toxic substances: are there differences between the available liver support devices? *Liver Int* 2011; **31** Suppl 3: 5-8 [PMID: 21824275 DOI: 10.1111/j.1478-3231.2011.02588.x]
34 **Sussman NL**, McGuire BM, Kelly JH. Hepatic assist devices: will they ever be successful? *Curr Gastroenterol Rep* 2009; **11**: 64-68 [PMID: 19166661 DOI: 10.1007/s11894-009-0010-x]
35 **Sussman NL**, Kelly JH. Artificial liver. *Clin Gastroenterol Hepatol* 2014; **12**: 1439-1442 [PMID: 24909908 DOI: 10.1016/j.cgh.2014.06.002]

P- Reviewer: Kao JT, Xiao J **S- Editor**: Ji FF
L- Editor: Filipodia **E- Editor**: Song H

World Journal of Hepatology

Submit a Manuscript: http://www.f6publishing.com

World J Hepatol 2018 October 27; 10(10): 731-742

DOI: 10.4254/wjh.v10.i10.731

ISSN 1948-5182 (online)

ORIGINAL ARTICLE

Retrospective Cohort Study

Spleen stiffness mirrors changes in portal hypertension after successful interferon-free therapy in chronic-hepatitis C virus patients

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Author contributions: Ravaioli F, Dajti E, Marasco G and Alemanni V collected data, analysed data, wrote the manuscript, approved the final manuscript; Tamè M, Azzaroli F, Brillanti S and Mazzella G analysed data and contributed to the drafting and final approval of the manuscript; Colecchia A, Mazzella G and Festi D provided overall oversight of the study, analysed data and contributed to the drafting and final approval of the manuscript.

Institutional review board statement: This study was approved by the National Institutional Review Board of the Italian Medicines Agency Committee. Local IRB [Institutional Ethics Committee of Sant'Orsola-Malpighi University Hospital (Bologna, Italy)] approval was authorized.

Informed consent statement: Patients were not required to give informed consent to the study because the analysis used anonymous data that were obtained after each patient agreed to treatment by written consent.

Conflict of interest statement: The authors disclose no conflicts.

STROBE statement: The guidelines of the STROBE statement have been adopted and a fulfilled version of the checklist has been attached with the submission of the manuscript.

Manuscript source: Invited manuscript

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Received: July 3, 2018
Peer-review started: July 3, 2018
First decision: July 24, 2018
Revised: July 27, 2018
Accepted: August 12, 2018
Article in press: August 13, 2018
Published online: October 27, 2018

Abstract

AIM

To investigate changes in spleen stiffness measurements (SSMs) and other non-invasive tests (NITs) after treatment with direct-acting antivirals (DAAs) and identify predictors of SSM change after sustained

virological response (SVR).

METHODS

We retrospectively analysed 146 advanced-chronic liver disease (ACLD) patients treated with DAA with available paired SSM at baseline and SVR24. Liver stiffness (LSM), spleen diameter (SD), platelet count (PLT) and liver stiffness-spleen diameter to platelet ratio score(LSPS) were also investigated. LSM $\geqslant$ 21 kPa was used as a cut-off to rule-in clinically significant portal hypertension (CSPH). SSM reduction > 20% from baseline was defined as significant.

RESULTS

SSM significantly decreased at SVR24, in both patients with and without CSPH; in 44.8% of cases, SSM reduction was > 20%. LSPS significantly improved in the entire cohort at SVR24; SD and PLT changed significantly only in patients without CSPH. LSM significantly decreased in 65.7% of patients and also in 2/3 patients in whom SSM did not decrease. The independent predictor of decreased SSM was median relative change of LSM. CSPH persisted in 54.4% patients after SVR. Delta LSM and baseline SSM were independent factors associated with CSPH persistence.

CONCLUSION

SSM and other NITs significantly decrease after SVR, although differently according to the patient's clinical condition. SSM faithfully reflects changes in portal hypertension and could represent a useful NIT for the follow-up of these patients.

Key words: Clinically significant portal hypertension; Spleen stiffness measurement; Advanced chronic liver disease; Direct-acting antivirals; Portal hypertension; Hepatitis C; Non-invasive test

Core tip: Liver stiffness measurement (LSM) and spleen stiffness measurement (SSM) are widely validated surrogates of portal hypertension (PH) and its complications. Their role in the assessment of therapy response, such as treatment with direct-acting antivirals (DAAs) of hepatitis C virus patients, is still under investigation. We demonstrated in a large cohort that not only LSM, but also SSM, is reduced six months after successful DAA therapy. As opposed to LSM, SSM directly reflects PH and is less influenced by the immediate reduction of liver necro-inflammation. We believe that SSM could represent a helpful tool for the clinician in the follow-up of these patients.

Ravaioli F, Colecchia A, Dajti E, Marasco G, Alemanni LV, Tamè M, Azzaroli F, Brillanti S, Mazzella G, Festi D. Spleen stiffness mirrors changes in portal hypertension after successful interferon-free therapy in chronic-hepatitis C virus patients. *World J Hepatol* 2018; 10(10): 731-742 Available from: URL: http://www.wjgnet.com/1948-5182/full/v10/i10/731.htm DOI: http://dx.doi.org/10.4254/wjh.v10.i10.731

INTRODUCTION

Chronic hepatitis C virus (HCV) infection represents one of the major causes of liver disease and is a leading cause of liver transplantation[1,2]. Recently, the introduction of the highly effective interferon-free direct-acting antivirals (DAAs) has enormously increased the number of patients who have achieved sustained virological response (SVR), even in patients with liver cirrhosis[3–5].

Studies, mostly from the interferon era, have shown that achieving SVR improves liver function[6,7], liver histology[8] and overall clinical outcomes[9]. However, the real impact of SVR in the DAA era, in terms of changes in portal hypertension (PH) and risk of decompensation on immediate follow-up, is not completely known, especially in patients with advanced chronic liver diseases (ACLD). PH is a progressive condition that represents a key point in the natural history of liver diseases[10]; therefore, its assessment by the hepatic venous pressure gradient (HVPG) measurement is fundamental in ACLD patients[11–14]. Indeed, the development of clinically significant portal hypertension (CSPH) in patients with compensated ACLD (cACLD)[11] is highly associated with the risk of clinical decompensation events (ascites, variceal bleeding, jaundice and hepatic encephalopathy)[10].

To date, several studies have demonstrated a significant reduction in HVPG (> 10%-20%) after achieving SVR, both after interferon-based[15–17] and DAA-based regimens[18–21]. Although the HVPG measurement is the gold standard to assess PH[11], it remains an invasive method[22] and its use is still limited only to highly specialized centres[12]; thus, its repeated measurements during the follow-up would hardly be applicable.

Consequently, many non-invasive tests (NITs) in the last decade, including liver and spleen stiffness measurements (LSM and SSM) as well as liver stiffness-spleen diameter to platelet ratio score (LSPS), have been developed and validated to accurately assess PH degree and its complications[11,22–29]. In fact, the Baveno Ⅵ Consensus recently recommended that LSM values of 10 kPa should rule out cACLD patients, and values of 20-25 kPa should accurately identify CSPH in patients with viral hepatitis[11]. However, to date, few studies have evaluated the role of NITs in the PH assessment of SVR patients after DAA treatment, and their role in the follow-up. Even if most studies agree on the fact that LSM rapidly decreases after virus eradication[18,19,30–32], controversial data have emerged regarding the changes of SSM after SVR[30–32].

MATERIALS AND METHODS

Aims of the study

We aimed to: (1) investigate the possible effect of HCV-

DAA treatment on PH, evaluated by spleen stiffness changes as a mirror of PH; (2) as well as those of other NITs, after HCV-DAA treatment; moreover, we aimed to (3) identify the presence of predictors of the SSM changes in SVR patients after DAA therapy.

Study design and population

This is a retrospective analysis of prospectively collected data of HCV-related cACLD patients treated with DAAs between January 2015 and September 2017 at our department, with valid measurements of LSM and SSM by transient elastography (TE) at baseline (BL) and at six mo after the end of DAA treatment (SVR24).

According to the Baveno Ⅵ Criteria[11], values of LSM > 10 kPa at TE were considered suggestive of having cACLD; LSM cut-off ⩾ 21 kPa was used to rule-in CSPH, as previously described[33,34]. At baseline, laboratory values, Model for end-stage liver disease (MELD) and Child-Turcotte Pugh (CTP) scores were also reported for each patient.

We excluded patients who (1) had incomplete response to surgical resection or loco-regional ablation of previous HCC; (2) developed HCC during antiviral treatment; (3) developed variceal bleeding and/or endoscopic banding legation (EBL) during the study period; and (4) initiated or changed the dosage of non-selective beta-blockers (NSBB) or had portal vein thrombosis, transjugular intrahepatic portosystemic shunt (TIPS) and non-cirrhotic PH. A subgroup of the patients who did not achieve SVR were separately investigated.

Antiviral treatment

Eligibility for treatment of HCV patients with DAAs was assessed following the priority criteria established in the protocol approved by the Italian Medicines Agency committee. The choice of DAA and treatment duration (12 or 24 wk) was based on viral genotype and stage of disease, according to the guidelines available at the time of enrollment[35]. SVR was defined as undetectable HCV-RNA using real-time PCR, with a detection limit of 15 IU/mL at the 12-wk post-treatment follow-up visit.

NITs for PH assessments

LSM values were assessed by expert operators using the FibroScan® apparatus with "M" probe (Echosens®, Paris, France) after overnight fasting and after a complete abdominal US examination. LSM values were obtained as previously reported[16], and the reliability criteria considered were according to the last EFSUMB Guidelines and Recommendations on the Clinical Use of Ultrasound Elastography[36]. SSM was assessed on the same day as LSM assessment, with the same probe utilized to perform LSM using the FibroScan® apparatus, as previously described[24]. Since no specific literature is present, we translated data from HVPG experience[11] and defined significant SSM as a reduction > 20% from BL. LSPS was calculated as liver stiffness × (spleen diameter (SD)/platelet count)[37]. SD was considered to be the bipolar diameter of the spleen as assessed by ultrasound.

Statistical analysis

Categorical data are expressed as numbers (percentages) and continuous variables as medians (IQR or range). For group comparison, the Mann-Whitney *U* test was used for continuous variables and the chi^2 test for categorical variables. Group comparisons among NITs at BL and SVR24 were evaluated with Friedman's non-parametric test, and Bonferroni-corrected alphas were used for post hoc pairwise comparison. Demographic, clinical, functional and elastometric variables were evaluated with univariate and multivariate Logistic Regression models in order to assess the predictive factors associated with PH improvement as assessed by SSM. After evaluation of multicollinearity, variables with a *P*-value < 0.10 upon univariate analysis were included in several multivariate Logistic Regression models with stepwise backward procedures. Prevalence of esophageal varices (EV) was not included in the multivariate analysis due to the limited number of patients with available EGD data (within 6 mo from TE assessment). The estimated odds ratios with their 95% confidence intervals, LR chi^2 and Area under ROC Curve were presented. For each multivariable logistic regression, the model discrimination and calibration were reported together with Akaike information criterion and Bayesian information criterion measures for comparing maximum likelihood models. Only *P*-values < 0.05 were considered statistically significant. The statistical analysis was conducted using Stata/SE (Version 14.0; Stata Corp, Texas, United States).

Ethics

The DAAs treatment protocol was approved by the National Institutional Review Board (IRB) of the Italian Medicines Agency committee. Local IRB [Institutional Ethics Committee of Sant'Orsola-Malpighi University Hospital (Bologna, Italy)] approval was authorized.

RESULTS

Patients characteristics

One hundred-ninety-seven cACLD patients treated with DAAs and with available valid baseline LS and SS measurements were evaluated. The following patients were excluded: two (1%) had HCC occurrence and three (1.5%) presented with active HCC, one (0.5%) underwent EBL during the study period, four (2%) had previous EBL, two (1%) patients presented with complete portal vein thrombosis, one (0.5%) required an increase in NSBB dosage and one (0.5%) had previous TIPS placement. An additional 37 (18.8%) patients were excluded: 22 (out of 197, 11.2%) due to lack of follow-up and 15 (out of 197, 7.6%) due to unfeasible SSM at follow-up. Accordingly, a total of 134

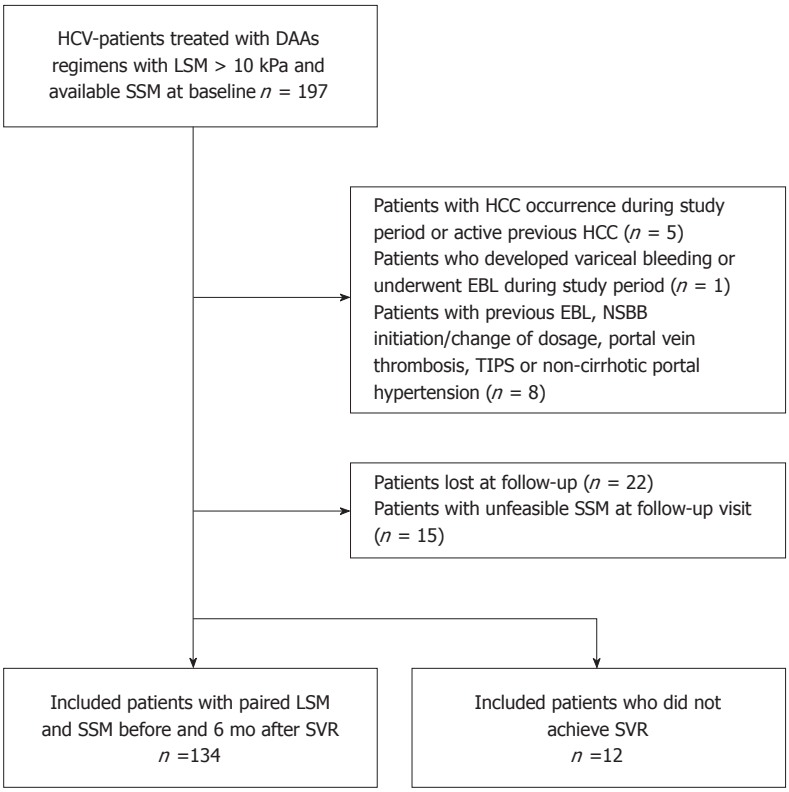


Figure 1 Flowchart of study design. DAA: Direct-acting antiviral; EBL: Endoscopic band ligation; HCC: Hepatocellular carcinoma; HCV: Hepatitis C virus; LSM: Liver stiffness measurement; NSBB: Non-selective beta-blocker; SSM: Spleen stiffness measurement; SVR: Sustained virological response; TIPS: Transjugular intrahepatic portosystemic shunt.

patients with paired LSM and SSM at BL and SVR24 were included in the final analysis; 12 (6%) patients who did not achieve SVR were analysed separately (Figure 1).

Table 1 depicts the baseline characteristics of the study cohort. Regarding main NITs, the median values at BL were LSM 19.3 kPa (14.1-27 kPa) and SSM 58.8 kPa (42.2-75 kPa). In a sub-analysis, patients with CSPH (LSM $\geqslant$ 21 kPa) differed significantly for MELD score, platelet count, total serum bilirubin, INR, SSM, LSM, SSM and LSPS.

Changes in SSM and LSM after SVR

In the patients who achieved SVR, the median SSM significantly decreased from 58.8 kPa to 38.2 kPa (P = 0.001), with a median delta change in SSM of − 12.3%. The decrease in SSM was statistically significant in both groups, CSPH and not (Figure 2A); the median delta SSM was higher in patients without CSPH at baseline when compared to patients with CSPH (-20.4% *vs* -4.7%), although this difference did not reach statistical significance. A decrease in SSM values was found in 92 (68.7%) patients, of whom 73 (54.5%) had a decrease > 10% and 60 (44.8%) > 20% (Table 2 and Figure 3A). LSM values also decreased after SVR, with respective median values of 19.3 kPa and 13.8 kPa at baseline and SVR24 (P < 0.0001). The median delta LSM was -30% with similar changes in both groups; LSM decreased in 114 (85.1%) patients, of whom 88 (65.7%) had a decrease of > 20% (Table 2 and Figure 3A).

A LSM decrease was found in almost all patients in whom SSM decreased (95.3%). On the other hand, LSM significantly decreased (P = 0.022) in 2/3 of the patients in whom SSM did not decrease, with a median delta LSM of -28.3%. (Figure 3B).

Changes in other NITs after SVR

The median spleen diameter (SD) at baseline and SVR24 were 14 cm and 13.2 cm, respectively. Although the reduction was not statistically significant in the overall population, it reached significance in the subgroup of patients without CSPH. The increase of PLT (from 110 10^9/L to 130 10^9/L) did not reach statistical significance in the entire cohort either, but only in patients without CSPH (Figure 2B). Moreover, median LSPS differed significantly between baseline (2.78) and SVR (1.34), and in both subgroups as well.

Non-SVR patients

Twelve patients did not achieve SVR in our cohort. Baseline characteristics did not statistically differ from the patients included in the final analysis. In particular, in non-SVR patients, an LSM decrease (23.2 kPa at BL *vs* 21.6 kPa at FU24), an SSM increase (45.6 kPa at BL *vs* 57.8 kPa at FU24) and a PLT decrease (128 ×

Table 1 Baseline characteristics of included patients

Variable	Overall (n = 134)	CSPH (LSM ⩾ 21 kPa) (n = 60)	No CSPH (LSM < 21 kPa) (n = 74)
Age (yr)	60 (51-69)	57 (50.5-65)	61.5 (51-70)
Male	92 (68.7)	42 (70)	50 (67.6)
HCV-genotype			
1	95 (70.9)	41 (68.3)	54 (72.5)
2	12 (8.9)	4 (6.7)	8 (10.8)
3	20 (14.9)	11 (18.3)	9 (12.2)
4	7 (5.3)	4 (6.7)	3 (4.5)
Treatment regimen			
SOF/RBV	33 (24.6)	10 (16.7)	23 (31.1)
SOF/SMV	29 (21.6)	15 (25)	14 (18.9)
SOF/DCV	38 (28.4)	19 (31.6)	19 (25.6)
SOF/LDV	16 (12)	7 (11.7)	9 (12.2)
Other	18 (13.4)	9 (15)	9 (12.2)
Child Pugh Score			
A	115 (85.8)	52 (86.7)	63 (85.1)
B	19 (14.2)	8 (13.3)	11 (14.9)
MELD Score	8 (7-10)	9 (8-10)	8 (7-10)
Spleen Diameter (cm)	14 (12.3-15.5)	14.7 (12.8-15.8)	13.9 (12.1-15)
Laboratory results			
Platelets (cells × 10^9/L)	110 (79-150)	102 (74-132)	134 (92-159)
ALT (U/L)	58 (39-95)	55 (39-84)	60 (38-105)
Bilirubin (mg/dL)	0.9 (0.67-1.29)	1 (0.84-1.52)	0.8 (0.6-1.1)
Albumin (g/dL)	3.8 (3.6-4.1)	3.8 (3.5-4.1)	3.8 (3.6-4.1)
Creatinine (mg/dL)	0.8 (0.7-0.98)	0.8 (0.70-0.96)	0.85 (0.71-1.08)
INR	1.1 (1.06-1.2)	1.17 (1.1-1.21)	1.08 (1.04-1.13)
NITs			
SSM (kPa)	58.8 (42.2-75)	69.9 (55.7-75)	46.2 (31.6-63.9)
LSM (kPa)	19.3 (14.1-27)	29.1 (23.9-39.7)	14.6 (12-17)
LSPS	2.78 (1.4-4.94)	5.1 (3.05-7.48)	1.58 (1.09-2.79)

Qualitative data were expressed as number and percentage (%); quantitative data were expressed as median (25%-75% quantiles). ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; CSPH: Clinically significant portal hypertension; DCV: Daclatasvir; HRV: High risk varices; INR: International normalized ratio; LDV: Ledipasvir; LSM: Liver stiffness measurement; LSPS: Liver stiffness to spleen/platelet score; MELD: Model for end-stage liver disease; NITs: Non-invasive tests; RBV: Ribavirin; SMV: Simeprevir; SOF: Sofosbuvir; SVR: Sustained virological response; SSM: Spleen stiffness measurement.

10^9/L at BL *vs* 100 × 10^9/L at FU24) were observed; none of these changes reached statistical significance (Supplementary Table 1).

Predictors of significant SSM Decrease (> 20%)

Table 3 shows the differences observed between patients who had an SSM decrease > 20% and those who did not. In the entire cohort, patients with significant SSM reduction differed in the prevalence of EV, MELD score, albumin levels, as well as baseline SSM, LSPS values and LSM-related variables. In multivariate analysis, relative LSM changes remained as the only independent predictor of an SSM decrease > 20%. Furthermore, predictors of an SSM decrease > 20% (Supplementary Table 2) were investigated among patients with CSPH at baseline. Once again, a higher prevalence of EV, higher creatinine levels, lower LSM values at SVR24 and higher delta LSM were observed among patients with an SSM decrease > 20%. In multivariate analysis, higher serum creatinine levels and delta LSM > 20% were the predictors of a significant SSM decrease.

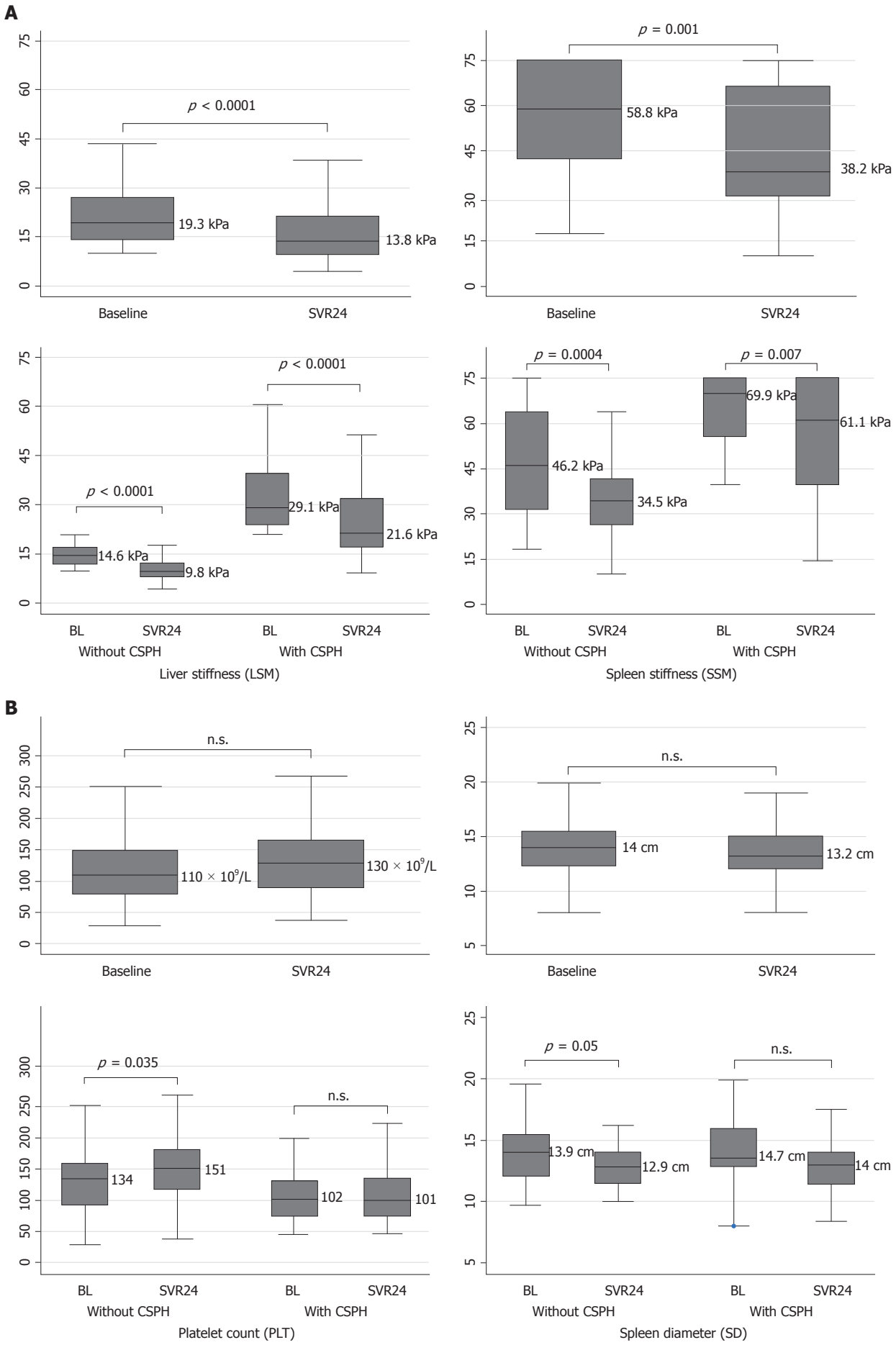
Changes of CSPH state after SVR

Figure 4 shows that 60 (44.8%) patients presented with CSPH at baseline, defined as an LSM ⩾ 21 kPa. After a 6 mo follow-up, none of the 74 patients without CSPH at baseline progressed to CSPH. In patients with CSPH, 46.7% of them had reduced LSM under the CSPH threshold after treatment. Supplementary Table 3 shows the predictors of CSPH persistence after DAA treatment.

DISCUSSION

The main aim of our study was to evaluate PH changes assessed by non-invasive methods after successful viral eradication in patients treated with DAAs. Our data show that SSM and LSM significantly decrease after SVR, according to the baseline clinical patient condition.

The IFN-free regimens are highly effective, allowing to treat and achieve SVR in patients who also have ACLD[4,38]. However, the individual clinical benefit in these patients is still under debate, especially in terms of changes in PH and CSPH-driven complications[39–41]. While results from the interferon era might not necessarily be translatable to DAA regimens[21], recent studies have also unanimously demonstrated that HVPG significantly decreases after SVR[18–21]. Although many


A
p < 0.0001
19.3 kPa
13.8 kPa
Baseline
SVR24
p = 0.001
58.8 kPa
38.2 kPa
Baseline
SVR24
p < 0.0001
p < 0.0001
14.6 kPa
9.8 kPa
29.1 kPa
21.6 kPa
BL
SVR24
BL
SVR24
Without CSPH
With CSPH
Liver stiffness (LSM)
p = 0.0004
p = 0.007
46.2 kPa
34.5 kPa
69.9 kPa
61.1 kPa
BL
SVR24
BL
SVR24
Without CSPH
With CSPH
Spleen stiffness (SSM)
B
n.s.
110 × 10⁹/L
130 × 10⁹/L
Baseline
SVR24
n.s.
14 cm
13.2 cm
Baseline
SVR24
p = 0.035
n.s.
134
151
102
101
BL
SVR24
BL
SVR24
Without CSPH
With CSPH
Platelet count (PLT)
p = 0.05
n.s.
13.9 cm
12.9 cm
14.7 cm
14 cm
BL
SVR24
BL
SVR24
Without CSPH
With CSPH
Spleen diameter (SD)

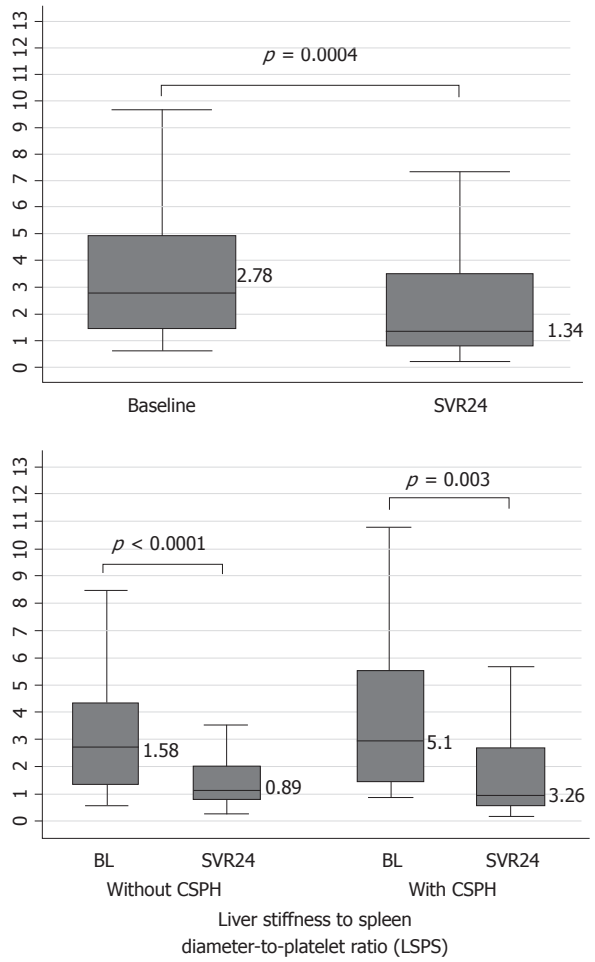


Figure 2 Non-invasive tests changes after sustained viral response by clinically significant portal hypertension presence. A: LSM and SSM changes; B: PLT, SD, LSPS changes. CSPH: Clinically significant portal hypertension; LSM: Liver stiffness measurement; SSM: Spleen stiffness measurement; PLT: Platelet count; SD: Spleen diameter; LSPS: Liver stiffness-to-spleen diameter-to-platelet count ratio score.

studies have shown that LSM rapidly decreases after DAA treatment[42,43], not much is known about the changes of PH surrogate NITs, such as SSM and LSPS, after viral eradication. In fact, NITs have yet to be validated in SVR patients, and their role in the clinical follow-up is still to be determined.

The main finding of this study is that SSM significantly changes after 24 wk of SVR in patients with cACLD, with a median relative change of -12.3% (Table 2). To our knowledge, only two complete papers[30,32] and one letter to the editor[39] have investigated the changes in SSM after SVR, with opposing results. In fact, only in the study by Pons *et al*[32] SSM was found to rapidly decrease at only 4 wk after therapy initiation in 41 patients, with no ulterior significant changes until 48 wk of follow-up; the other studies concluded that SSM did not significantly decrease at SVR24[30,32].

In our study that analyzed a large cohort of cACLD patients, we demonstrated that SSM significantly decreased after DAA treatment. These results confirm previous studies in which PH was assessed by paired HVPG measurements[18-21]. Moreover, our study is the first to assess and demonstrate the improvement of LSPS, another accurate surrogate of PH, after SVR24. Moreover, in the eight patients who did not achieve SVR, SSM and other NITs did not significantly differ during follow-up measurements (Supplementary Table 1).

We classified patients with and without CSPH according to a LSM cut-off of 21 kPa[33,34]. Interestingly, the relative changes in SSM and LSM performed differently in patients with and without CSPH. In fact, while the median delta LSM in patients with and without CSPH was very similar (-28.3% *vs* -30.8%), the reduction of SSM was much more evident in patients without CSPH (-20.4% *vs* -4.7%). This last result is consistent with the relative HVPG changes described by Mandorfer *et al*[18]. Moreover, the other surrogates of PH, including the platelet and spleen diameter**,** significantly changed only when split by CSPH presence. Regarding the different changes of NITs in patients with and without CSPH, we could speculate that this behaviour can reflect the different stages of underlying PH pathogenic mechanisms. Indeed, determinants of portal pressure affecting SSM, such as intrahepatic resistance and liver necro-inflammation[44], improve in both subgroups. However, in CSPH, other major actors of PH, such as

Table 2 Liver and Spleen stiffness measurement decreases after sustained viral response

Variable	Overall (n = 134)	CSPH (LSM ⩾ 21 kPa) (n = 60)	No CSPH (LSM < 21 kPa) (n = 74)
Relative SSM decrease (%)	12.3 (0-36.3)	4.7 (0-32.5)	20.4 (0-39.7)
Overall SSM decrease	92 (68.7)	40 (66.7)	52 (70.3)
> 10%	73 (54.5)	31 (51.7)	42 (56.8)
> 20%	60 (44.8)	23 (38.3)	37 (50)
Relative LSM decrease (%)	30 (13.5-42.4)	28.3 (11.4-41.9)	30.8 (13.9-42.4)
Overall LSM decrease	114 (85.1)	51 (85)	63 (85.1)
> 10%	108 (80.6)	48 (80)	60 (81.1)
> 20%	88 (65.7)	40 (66.7)	48 (64.9)
PLT Increase (%)	12.4 (-10.1 to 29.6)	5.5 (-15.6 to 25.9)	17.4 (-0.67 to 35.6)

CSPH: Clinically significant portal hypertension; LSM: Liver stiffness measurement; PLT: Platelet count; SSM: Spleen stiffness measurement.

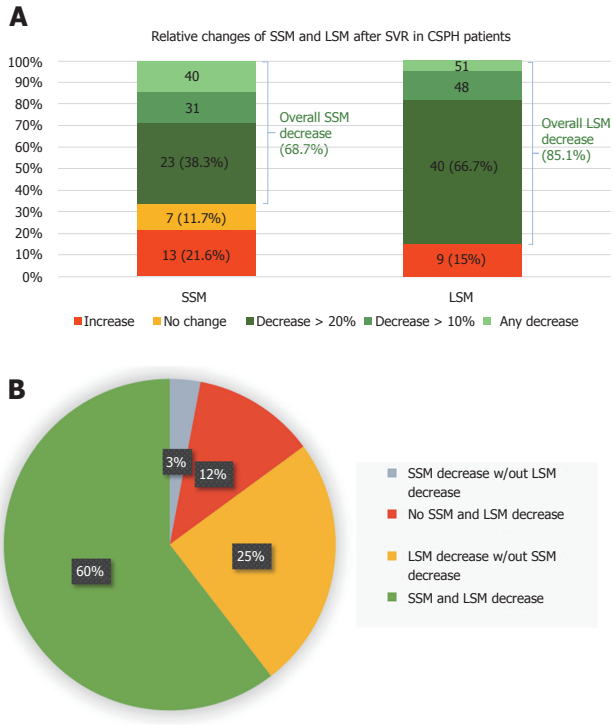


Figure 3 Spleen and liver stiffness measurement decreases after sustained viral response (A), and liver stiffness measurement decreases in patients without spleen stiffness measurement improvements (B). BL: Baseline; CSPH: Clinically significant portal hypertension; LSM: Liver stiffness measurement; SSM: Spleen stiffness measurement; SVR: Sustained virological response.

extra-hepatic hemodynamic factors[34] and spleen structural changes[45], might not ameliorate in the short-term follow-up (6 mo after SVR). This hypothesis could explain why we found a less prominent SSM decrease (-4.7% *vs* -20.4%), even when liver necro-inflammation reduction as assessed by delta LSM (-28.3% *vs* -30.8%) was the same.

SSM reduction was present in 68.7% of patients after 6 mo of follow-up. We found that the only independent predictor of a significant PH improvement, as reflected by a SSM decrease > 20%, was the relative change in LSM (Table 3), confirming previous studies with HVPG[18,21]. However, when we assessed PH improvement as reflected by SSM in our study, as PH surrogate, and by HVPG in the study by Lens *et al*[21], we noticed similar proportions of patients with a significant response (> 20%) when comparing SSM and HVPG (38.3% *vs* 39.8%, respectively), but not LSM and HVPG (66.7% *vs* 39.8%, respectively) (Figure 3A). Even if a correlation between HVPG and SSM changes after DAA treatment has not been demonstrated to date, our data may suggest that an SSM reduction > 20% could be a more accurate non-invasive predictor of a significant HVPG reduction[11].

A statement in the Baveno VI consensus was that the main therapeutic goal in patients with mild PH (6-9 mmHg) is to prevent CSPH development[11]. In our cohort, none of the patients who achieved SVR progressed to CSPH. More challenging, however, is the concept of assessment of CSPH presence/absence after SVR due to its clinical implications, since there is not sufficient evidence showing that the cut-offs after DAAs are the same as the ones used in the pre-treatment phase[23,46]. However, promising data documented that a LSM of 20-25 kPa could be an accurate cut-off to rule-in CSPH after DAA therapy[21]. Accordingly, we also investigated CSPH persistence after SVR (Figure 4). Using these cut-offs, we found that 53% of the patients with CSPH at baseline presented CSPH at SVR24. In multivariate analysis, higher baseline values of SSM (indicating a more severe PH) and lower LSM relative changes were found to be predictors of CSPH persistence (Supplementary Table 3). These results are in line with another study[21] in which higher BL HVPG and relative LSM changes were predictors of CSPH persistence after DAA treatment.

All of the above results seem to reflect the different dynamics in LSM and SSM changes after achieving SVR. LSM consensually decreased in almost all patients with SSM reduction (95.2%), while the opposite was not found to be true. In fact, LSM significantly decreased, with a median delta -28.3%, in 2/3 of the patients in whom no SSM reduction was found. This result emphasizes the fact that LSM is heavily influenced by the reduction of liver necro-inflammation[44] after SVR, and that changes in LSM might not be the most adequate predictors of PH changes in this context. On the other hand, a SSM decrease > 20% could identify patients who significantly clinically benefit from viral eradication.

Table 3 Univariate and multivariate analysis of factors associated with a SSM decrease > 20%

Variable	Entire Population (*n* = 134)					
	Univariate analysis				Multivariate analysis	
					LR Chi-2 = 16.48 AUROC = 0.6821	AIC = 171.8 BIC = 177.6
	SSM Decrease > 20% (*n* = 60)	No SSM Decrease > 20% (*n* = 74)	OR (95%CI)	*P* value	OR (95%CI)	*P* value
Age (yr)	62 (52-69)	56 (50-68)	1.005 (0.975-1.037)	0.727		
Sex (male)	21 (28.4)	21 (35)	1.359 (0.653-2.828)	0.412		
Presence of varices (*n* = 67) (yes)	9 (34.2)	24 (82.8)	0.110 (0.031-0.388)	0.001		
Spleen diameter (cm)	13.6 (11.65-15.15)	14.5 (13-16)	0.800 (0.660-0.970)	0.023		
Child Pugh Score	5 (5-6)	5 (5-6)	0.885 (0.601-1.303)	0.535		
Child Pugh Score B (yes)	8 (13.3)	11 (14.9)	0.881 (0.330-2.352)	0.801		
MELD score	8 (7-10)	9 (8-10)	0.786 (0.648-0.954)	0.015		
MELD > 10	12 (20)	30 (40.5)	0.367 (0.167-0.804)	0.012		
AST (U/L)	54.5 (38-85)	56 (35.5-87)	0.996 (0.987-1.004)	0.322		
ALT (U/L)	62 (37-105)	53 (40-90)	1.002 (0.995-1.008)	0.620		
ALT ⩾ 2 × ULN at BL	59.5 (37.5-101)	54 (40-91.5)	1.326 (0.597-2.944)	0.489		
INR	1.09 (1.05-1.17)	1.12 (1.09-1.21)	0.127 (0.001-0.551)	0.023		
Bilirubin (mg/dl)	0.85 (0.65-1.16)	1.02 (0.71-1.52)	0.903 (0.535-1.525)	0.703		
Albumin (g/dl)	3.8 (3.52-4.12)	3.78 (3.52-4.12)	2.096 (0.959-4.581)	0.063		
Creatinine (mg/d)	0.8 (0.7-1)	0.81 (0.69-0.93)	0.327 (0.674-1.585)	0.165		
Platelet count (10^9/L)	118 (92-154)	91 (74-137)	1.002 (0.996-1.007)	0.579		
LSM BL (kPa)	18 (14.6-25.7)	21.1 (14-38.5)	0.988 (0.962-1.015)	0.391		
LSM SVR24 (kPa)	12.4 (9.4-18)	17.5 (10.4-32.4)	0.944 (0.908-0.981)	0.004		
SSM BL (kPa)	60.4 (45.7-70.7)	53.2 (37.4-75)	1.012 (0.992-1.032)	0.225		
LSPS BL	2.17 (1.33-3.77)	4.15 (1.65-6.26)	0.817 (0.684-0.975)	0.025		
LSM decrease (Delta, %)	33 (18.1–44.6)	19.4 (0–31.3)	0.0332 (0.005-0.225)	< 0.0001	0.0332 (0.005-0.225)	< 0.0001
LSM decrease > 10% (yes)	54 (90)	54 (73)	3.333 (1.242-8.946)	0.017		
LSM decrease > 20% (yes)	47 (78.3)	41 (55.4)	2.910 (1.352-6.262)	0.006		

Qualitative data were expressed as number and percentage (%); quantitative data were expressed as median (25%-75% quantiles). AIC: Akaike information criterion; ALT: Alanine aminotransferase; AUROC: Area under curve ROC; AST: Aspartate aminotransferase; BIC: Bayesian information criterion; CSPH: Clinically significant portal hypertension; DCV: Daclatasvir; HRV: High risk varices; INR: International normalized ratio; LDV: Ledipasvir; LR: Like-hood ratio; LSM: Liver stiffness measurement; LSPS: Liver stiffness to spleen/platelet score; MELD: Model for end-stage liver disease; NITs: Non-invasive tests; RBV: Ribavirin; SMV: Simeprevir; SOF: Sofosbuvir; SVR: Sustained virological response; SSM: Spleen stiffness measurement.

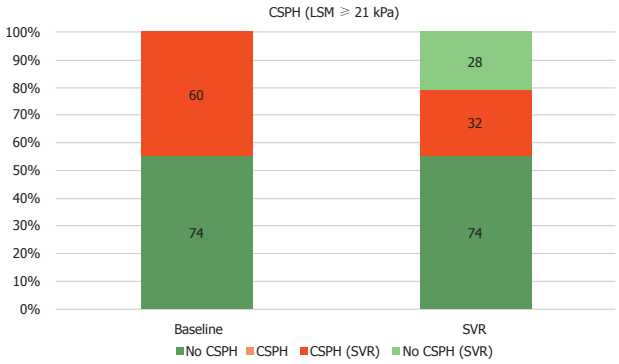


Figure 4 Clinically significant portal hypertension presence, according to Baveno Ⅵ (liver stiffness measurement ⩾ 21 kPa) at baseline and after SVR24. CSPH: Clinically significant portal hypertension; LSM: Liver stiffness measurement; SSM: Spleen stiffness measurement; SVR: Sustained virological response.

When looking at the bigger picture, SSM could represent a feasible tool to monitor therapy response and assess its benefit. This is also supported by a recent study by Buechter *et al*[47] that investigated LSM and SSM changes after TIPS placement.

The present study has some limitations: (1) its retrospective nature, even though SSM and LSM were prospectively collected according to the Italian Medicines Agency committee eligibility criteria for the treatment of HCV patients with DAAs, and (2) the absence of a gold-standard reference for PH assessment. However, according to the Baveno Ⅵ consensus[11], we could consider NITs, in addition to LSM, to be good surrogates of invasive methods, such as liver biopsy and HVPG. The time of follow-up was too short to fully correlate SSM changes with clinical outcomes after viral eradication, such as events of decompensation after SVR[48]. As in previous studies that include SSM, the upper limit of 75 kPa for SSM affects the possibility to detect changes in patients with severe PH[49,50]; in fact, both BL and SVR24 values were 75 kPa in seven (5.2%) patients.

In conclusion, SSM could be an accurate and useful NIT for the follow-up of patients after SVR, as it faithfully reflects changes in PH better than other NITs, including LSM. Further prospective studies are required in order to confirm the accuracy and usefulness of SSM and other NITs in the follow-up of patients with ACLD and its correlation with clinical outcomes.

ARTICLE HIGHLIGHTS

Research background

The long-term benefits of achieving sustained virological response (SVR) in cirrhotic patients are still to be established. Non-invasive tests (NITs), such

as liver (LSM) and especially spleen stiffness (SSM), are widely validated in hepatology as portal hypertension (PH) surrogates. However, their use in SVR patients and their changes after virus eradication is still under discussion.

Research motivation

Many studies have reported rapid LSM decrease after achieving SVR. However, only a few have investigated changes in SSM in such patients, with contrasting results. Given that there is a decrease in SSM after therapy, it means that SSM could be exploited to assess changes in PH and PH-driven complication after achieving SVR.

Research objectives

The main objective of the study was to investigate changes in PH after successful eradication of HCV infection, as reflected by its non-invasive assessment by SSM and other NITs.

Research methods

This is a retrospective study of prospectively collected data. Patients with available paired SSM assessment at baseline and 6 mo after end-of-therapy (SVR24) were included in the study.

Research results

Our main result is that a significant SSM decrease at SVR24 was demonstrated in a large cohort of 134 patients. This is the first study that also reveals a decrease in LSPS after SVR. SSM reduction differed according to the patient's clinical condition, especially when divided by the presence of clinically significant PH. An LSM decrease of > 20% was evident in the majority of patients, and also in patients in whom no SSM reduction was present. This finding likely reflects the reduction in liver necro-inflammation rather than PH improvement.

Research conclusions

PH, reflected by NITs, improves after achieving SVR in cirrhotic patients. SSM is a direct surrogate of PH and less influenced by liver necro-inflammation, as opposed to LSM. Its decrease (> 20%) could help the clinician to stratify the risk for PH-related complication after DAA therapy.

Research perspectives

Future prospective studies should investigate whether changes in SSM are predictive of clinical decompensation or other complications of cirrhosis after viral eradication. SSM could become a helpful and accurate method to assess therapy response and the risk of complications.

REFERENCES

1 **Hajarizadeh B**, Grebely J, Dore GJ. Epidemiology and natural history of HCV infection. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 553-562 [PMID: 23817321 DOI: 10.1038/nrgastro.2013.107]

2 **Millman AJ**, Nelson NP, Vellozzi C. Hepatitis C: Review of the Epidemiology, Clinical Care, and Continued Challenges in the Direct Acting Antiviral Era. *Curr Epidemiol Rep* 2017; **4**: 174-185 [PMID: 28785531 DOI: 10.1007/s40471-017-0108-x]

3 **Manns M**, Samuel D, Gane EJ, Mutimer D, McCaughan G, Buti M, Prieto M, Calleja JL, Peck-Radosavljevic M, Müllhaupt B, Agarwal K, Angus P, Yoshida EM, Colombo M, Rizzetto M, Dvory-Sobol H, Denning J, Arterburn S, Pang PS, Brainard D, McHutchison JG, Dufour JF, Van Vlierberghe H, van Hoek B, Forns X; SOLAR-2 investigators. Ledipasvir and sofosbuvir plus ribavirin in patients with genotype 1 or 4 hepatitis C virus infection and advanced liver disease: a multicentre, open-label, randomised, phase 2 trial. *Lancet Infect Dis* 2016; **16**: 685-697 [PMID: 26907736 DOI: 10.1016/S1473-3099(16)00052-9]

4 **Ferenci P**, Kozbial K, Mandorfer M, Hofer H. HCV targeting of patients with cirrhosis. *J Hepatol* 2015; **63**: 1015-1022 [PMID: 26100497 DOI: 10.1016/j.jhep.2015.06.003]

5 **Babatin MA**, Alghamdi AS, Albenmousa A, Alaseeri A, Aljarodi M, Albiladi H, Alsahafi A, Almugharbal M, Alothmani HS, Sanai FM, Bzeizi KI. Efficacy and Safety of Simeprevir or Daclatasvir in Combination With Sofosbuvir for the Treatment of Hepatitis C Genotype 4 Infection. *J Clin Gastroenterol* 2018; **52**: 452-457 [PMID: 28767462 DOI: 10.1097/MCG.0000000000000896]

6 **Charlton M**, Everson GT, Flamm SL, Kumar P, Landis C, Brown RS Jr, Fried MW, Terrault NA, O'Leary JG, Vargas HE, Kuo A, Schiff E, Sulkowski MS, Gilroy R, Watt KD, Brown K, Kwo P, Pungpapong S, Korenblat KM, Muir AJ, Teperman L, Fontana RJ, Denning J, Arterburn S, Dvory-Sobol H, Brandt-Sarif T, Pang PS, McHutchison JG, Reddy KR, Afdhal N; SOLAR-1 Investigators. Ledipasvir and Sofosbuvir Plus Ribavirin for Treatment of HCV Infection in Patients With Advanced Liver Disease. *Gastroenterology* 2015; **149**: 649-659 [PMID: 25985734 DOI: 10.1053/j.gastro.2015.05.010]

7 **Deterding K**, Höner Zu Siederdissen C, Port K, Solbach P, Sollik L, Kirschner J, Mix C, Cornberg J, Worzala D, Mix H, Manns MP, Cornberg M, Wedemeyer H. Improvement of liver function parameters in advanced HCV-associated liver cirrhosis by IFN-free antiviral therapies. *Aliment Pharmacol Ther* 2015; **42**: 889-901 [PMID: 26250762 DOI: 10.1111/apt.13343]

8 **Cammà C**, Di Bona D, Schepis F, Heathcote EJ, Zeuzem S, Pockros PJ, Marcellin P, Balart L, Alberti A, Craxì A. Effect of peginterferon alfa-2a on liver histology in chronic hepatitis C: a meta-analysis of individual patient data. *Hepatology* 2004; **39**: 333-342 [PMID: 14767986 DOI: 10.1002/hep.20073]

9 **van der Meer AJ**, Veldt BJ, Feld JJ, Wedemeyer H, Dufour JF, Lammert F, Duarte-Rojo A, Heathcote EJ, Manns MP, Kuske L, Zeuzem S, Hofmann WP, de Knegt RJ, Hansen BE, Janssen HL. Association between sustained virological response and all-cause mortality among patients with chronic hepatitis C and advanced hepatic fibrosis. *JAMA* 2012; **308**: 2584-2593 [PMID: 23268517 DOI: 10.1001/jama.2012.144878]

10 **D'Amico G**, Garcia-Tsao G, Pagliaro L. Natural history and prognostic indicators of survival in cirrhosis: a systematic review of 118 studies. *J Hepatol* 2006; **44**: 217-231 [PMID: 16298014 DOI: 10.1016/j.jhep.2005.10.013]

11 **de Franchis R**; Baveno VI Faculty. Expanding consensus in portal hypertension: Report of the Baveno VI Consensus Workshop: Stratifying risk and individualizing care for portal hypertension. *J Hepatol* 2015; **63**: 743-752 [PMID: 26047908 DOI: 10.1016/j.jhep.2015.05.022]

12 **Groszmann RJ**, Wongcharatrawee S. The hepatic venous pressure gradient: anything worth doing should be done right. *Hepatology* 2004; **39**: 280-282 [PMID: 14767976 DOI: 10.1002/hep.20062]

13 **Abraldes JG**, Tarantino I, Turnes J, Garcia-Pagan JC, Rodés J, Bosch J. Hemodynamic response to pharmacological treatment of portal hypertension and long-term prognosis of cirrhosis. *Hepatology* 2003; **37**: 902-908 [PMID: 12668985 DOI: 10.1053/jhep.2003.50133]

14 **Bosch J**, Abraldes JG, Berzigotti A, García-Pagan JC. The clinical use of HVPG measurements in chronic liver disease. *Nat Rev Gastroenterol Hepatol* 2009; **6**: 573-582 [PMID: 19724251 DOI: 10.1038/nrgastro.2009.149]

15 **Rincon D**, Ripoll C, Lo Iacono O, Salcedo M, Catalina MV, Alvarez E, Nuñez O, Matilla AM, Clemente G, Bañares R. Antiviral therapy decreases hepatic venous pressure gradient in patients with chronic hepatitis C and advanced fibrosis. *Am J Gastroenterol* 2006; **101**: 2269-2274 [PMID: 17032192 DOI: 10.1111/j.1572-0241.2006.00743.x]

16 **Roberts S**, Gordon A, McLean C, Pedersen J, Bowden S, Thomson K, Angus P. Effect of sustained viral response on hepatic venous pressure gradient in hepatitis C-related cirrhosis. *Clin Gastroenterol Hepatol* 2007; **5**: 932-937 [PMID: 17544878 DOI: 10.1016/j.cgh.2007.02.022]

17 **Reiberger T**, Payer BA, Ferlitsch A, Sieghart W, Breitenecker F, Aichelburg MC, Schmied B, Rieger A, Trauner M, Peck-Radosavljevic M; Vienna Hepatic Hemodynamic Lab and Vienna HIV & Liver Study Group. A prospective evaluation of pulmonary, systemic and hepatic haemodynamics in HIV-HCV-coinfected patients before and after antiviral therapy with pegylated interferon and ribavirin. *Antivir Ther* 2012; **17**: 1327-1334 [PMID: 22948263 DOI: 10.3851/IMP2349]

18 **Mandorfer M**, Kozbial K, Schwabl P, Freissmuth C, Schwarzer R, Stern R, Chromy D, Stättermayer AF, Reiberger T, Beinhardt S, Sieghart W, Trauner M, Hofer H, Ferlitsch A, Ferenci P, Peck-Radosavljevic M. Sustained virologic response to interferon-free therapies ameliorates HCV-induced portal hypertension. *J Hepatol* 2016; **65**: 692-699 [PMID: 27242316 DOI: 10.1016/j.jhep.2016.05.027]

19 **Mauro E**, Crespo G, Montironi C, Londoño MC, Hernández-Gea V, Ruiz P, Sastre L, Lombardo J, Mariño Z, Díaz A, Colmenero J, Rimola A, Garcia-Pagán JC, Brunet M, Forns X, Navasa M. Portal pressure and liver stiffness measurements in the prediction of fibrosis regression after sustained virological response in recurrent hepatitis C. *Hepatology* 2018; **67**: 1683-1694 [PMID: 28960366 DOI: 10.1002/hep.29557]

20 **Afdhal N**, Everson GT, Calleja JL, McCaughan GW, Bosch J, Brainard DM, McHutchison JG, De-Oertel S, An D, Charlton M, Reddy KR, Asselah T, Gane E, Curry MP, Forns X. Effect of viral suppression on hepatic venous pressure gradient in hepatitis C with cirrhosis and portal hypertension. *J Viral Hepat* 2017; **24**: 823-831 [PMID: 28295923 DOI: 10.1111/jvh.12706]

21 **Lens S**, Alvarado-Tapias E, Mariño Z, Londoño MC, LLop E, Martinez J, Fortea JI, Ibañez L, Ariza X, Baiges A, Gallego A, Bañares R, Puente A, Albillos A, Calleja JL, Torras X, Hernández-Gea V, Bosch J, Villanueva C, Forns X, García-Pagán JC. Effects of All-Oral Anti-Viral Therapy on HVPG and Systemic Hemodynamics in Patients With Hepatitis C Virus-Associated Cirrhosis. *Gastroenterology* 2017; **153**: 1273-1283.e1 [PMID: 28734831 DOI: 10.1053/j.gastro.2017.07.016]

22 **Berzigotti A**. Non-invasive evaluation of portal hypertension using ultrasound elastography. *J Hepatol* 2017; **67**: 399-411 [PMID: 28223101 DOI: 10.1016/j.jhep.2017.02.003]

23 **European Association for Study of Liver**; Asociacion Latino-americana para el Estudio del Higado. EASL-ALEH Clinical Practice Guidelines: Non-invasive tests for evaluation of liver disease severity and prognosis. *J Hepatol* 2015; **63**: 237-264 [PMID: 25911335 DOI: 10.1016/j.jhep.2015.04.006]

24 **Colecchia A**, Montrone L, Scaioli E, Bacchi-Reggiani ML, Colli A, Casazza G, Schiumerini R, Turco L, Di Biase AR, Mazzella G, Marzi L, Arena U, Pinzani M, Festi D. Measurement of spleen stiffness to evaluate portal hypertension and the presence of esophageal varices in patients with HCV-related cirrhosis. *Gastroenterology* 2012; **143**: 646-654 [PMID: 22643348 DOI: 10.1053/j.gastro.2012.05.035]

25 **Ma X**, Wang L, Wu H, Feng Y, Han X, Bu H, Zhu Q. Spleen Stiffness Is Superior to Liver Stiffness for Predicting Esophageal Varices in Chronic Liver Disease: A Meta-Analysis. *PLoS One* 2016; **11**: e0165786 [PMID: 27829057 DOI: 10.1371/journal.pone.0165786]

26 **Abraldes JG**, Bureau C, Stefanescu H, Augustin S, Ney M, Blasco H, Procopet B, Bosch J, Genesca J, Berzigotti A; Anticipate Investigators. Noninvasive tools and risk of clinically significant portal hypertension and varices in compensated cirrhosis: The "Anticipate" study. *Hepatology* 2016; **64**: 2173-2184 [PMID: 27639071 DOI: 10.1002/hep.28824]

27 **Voutilainen S**, Kivisaari R, Lohi J, Jalanko H, Pakarinen MP. A Prospective Comparison of Noninvasive Methods in the Assessment of Liver Fibrosis and Esophageal Varices in Pediatric Chronic Liver Diseases. *J Clin Gastroenterol* 2016; **50**: 658-663 [PMID: 27105175 DOI: 10.1097/MCG.0000000000000532]

28 **Chon YE**, Jung ES, Park JY, Kim DY, Ahn SH, Han KH, Chon CY, Jung KS, Kim SU. The accuracy of noninvasive methods in predicting the development of hepatocellular carcinoma and hepatic decompensation in patients with chronic hepatitis B. *J Clin Gastroenterol* 2012; **46**: 518-525 [PMID: 22688146 DOI: 10.1097/MCG.0b013e31825079f1]

29 **Colecchia A**, Ravaioli F, Marasco G, Colli A, Dajti E, Di Biase AR, Bacchi Reggiani ML, Berzigotti A, Pinzani M, Festi D. A combined model based on spleen stiffness measurement and Baveno Ⅵ criteria to rule out high-risk varices in advanced chronic liver disease. *J Hepatol* 2018; **69**: 308-317 [PMID: 29729368 DOI: 10.1016/j.jhep.2018.04.023]

30 **Knop V**, Hoppe D, Welzel T, Vermehren J, Herrmann E, Vermehren A, Friedrich-Rust M, Sarrazin C, Zeuzem S, Welker MW. Regression of fibrosis and portal hypertension in HCV-associated cirrhosis and sustained virologic response after interferon-free antiviral therapy. *J Viral Hepat* 2016; **23**: 994-1002 [PMID: 27500382 DOI: 10.1111/jvh.12578]

31 **Verlinden W**, Francque S, Michielsen P, Vanwolleghem T. Successful antiviral treatment of chronic hepatitis C leads to a rapid decline of liver stiffness without an early effect on spleen stiffness. *Hepatology* 2016; **64**: 1809-1810 [PMID: 27118145 DOI: 10.1002/hep.28610]

32 **Pons M**, Santos B, Simón-Talero M, Ventura-Cots M, Riveiro-Barciela M, Esteban R, Augustin S, Genescà J. Rapid liver and spleen stiffness improvement in compensated advanced chronic liver disease patients treated with oral antivirals. *Therap Adv Gastroenterol* 2017; **10**: 619-629 [PMID: 28835776 DOI: 10.1177/1756283X17715198]

33 **Llop E**, Berzigotti A, Reig M, Erice E, Reverter E, Seijo S, Abraldes JG, Bruix J, Bosch J, García-Pagan JC. Assessment of portal hypertension by transient elastography in patients with compensated cirrhosis and potentially resectable liver tumors. *J Hepatol* 2012; **56**: 103-108 [PMID: 21827733 DOI: 10.1016/j.jhep.2011.06.027]

34 **Vizzutti F**, Arena U, Romanelli RG, Rega L, Foschi M, Colagrande S, Petrarca A, Moscarella S, Belli G, Zignego AL, Marra F, Laffi G, Pinzani M. Liver stiffness measurement predicts severe portal hypertension in patients with HCV-related cirrhosis. *Hepatology* 2007; **45**: 1290-1297 [PMID: 17464971 DOI: 10.1002/hep.21665]

35 **European Association for Study of Liver**. EASL Clinical Practice Guidelines: management of hepatitis C virus infection. *J Hepatol* 2014; **60**: 392-420 [PMID: 24331294 DOI: 10.1016/j.jhep.2013.11.003]

36 **Dietrich CF**, Bamber J, Berzigotti A, Bota S, Cantisani V, Castera L, Cosgrove D, Ferraioli G, Friedrich-Rust M, Gilja OH, Goertz RS, Karlas T, de Knegt R, de Ledinghen V, Piscaglia F, Procopet B, Saftoiu A, Sidhu PS, Sporea I, Thiele M. EFSUMB Guidelines and Recommendations on the Clinical Use of Liver Ultrasound Elastography, Update 2017 (Long Version). *Ultraschall Med* 2017; **38**: e16-e47 [PMID: 28407655 DOI: 10.1055/s-0043-103952]

37 **Kim BK**, Han KH, Park JY, Ahn SH, Kim JK, Paik YH, Lee KS, Chon CY, Kim DY. A liver stiffness measurement-based, noninvasive prediction model for high-risk esophageal varices in B-viral liver cirrhosis. *Am J Gastroenterol* 2010; **105**: 1382-1390 [PMID: 20087336 DOI: 10.1038/ajg.2009.750]

38 **Chayama K**, Suzuki F, Karino Y, Kawakami Y, Sato K, Atarashi T, Naganuma A, Watanabe T, Eguchi Y, Yoshiji H, Seike M, Takei Y, Kato K, Alves K, Burroughs M, Redman R, Pugatch DL, Pilot-Matias TJ, Krishnan P, Oberoi RK, Xie W, Kumada H. Efficacy and safety of glecaprevir/pibrentasvir in Japanese patients with chronic genotype 1 hepatitis C virus infection with and without cirrhosis. *J Gastroenterol* 2018; **53**: 557-565 [PMID: 28948366 DOI: 10.1007/s00535-017-1391-5]

39 **Sack J**, Garcia-Tsao G. Variceal Hemorrhage in a Patient With Hepatitis C Virus Cirrhosis in Whom Liver Synthetic Function had Normalized After Viral Elimination. *Hepatology* 2016; **63**: 1733-1735 [PMID: 26806550 DOI: 10.1002/hep.28470]

40 **Dailey F**, Ayoub WS. Hepatitis C Virus Therapy for Decompensated and Posttransplant Patients. *J Clin Gastroenterol* 2017; **51**: 215-222 [PMID: 28178089 DOI: 10.1097/MCG.0000000000000701]

41 **Saadi T**, Khoury J. Is There a Relationship Between Treatment With Direct Antiviral Agents for HCV Infection and the Development of Malignancies? *J Clin Gastroenterol* 2018; **52**: 353-359 [PMID: 28590324 DOI: 10.1097/MCG.0000000000000853]

42 **Singh S**, Facciorusso A, Loomba R, Falck-Ytter YT. Magnitude and Kinetics of Decrease in Liver Stiffness After Antiviral Therapy in Patients With Chronic Hepatitis C: A Systematic Review and Meta-analysis. *Clin Gastroenterol Hepatol* 2018; **16**: 27-38.e4 [PMID: 28479504 DOI: 10.1016/j.cgh.2017.04.038]

43 **Facciorusso A**, Del Prete V, Turco A, Buccino RV, Nacchiero MC,

Muscatiello N. Long-term liver stiffness assessment in hepatitis C virus patients undergoing antiviral therapy: Results from a 5-year cohort study. *J Gastroenterol Hepatol* 2018; **33**: 942-949 [PMID: 28976021 DOI: 10.1111/jgh.14008]

44 **Pinzani M**. Liver Fibrosis in the Post-HCV Era. *Semin Liver Dis* 2015; **35**: 157-165 [PMID: 25974901 DOI: 10.1055/s-0035-1550056]

45 **Mejias M**, Garcia-Pras E, Gallego J, Mendez R, Bosch J, Fernandez M. Relevance of the mTOR signaling pathway in the pathophysiology of splenomegaly in rats with chronic portal hypertension. *J Hepatol* 2010; **52**: 529-539 [PMID: 20206401 DOI: 10.1016/j.jhep.2010.01.004]

46 **Castera L**. Non-invasive tests for liver fibrosis progression and regression. *J Hepatol* 2016; **64**: 232-233 [PMID: 26603523 DOI: 10.1016/j.jhep.2015.10.011]

47 **Buechter M**, Manka P, Theysohn JM, Reinboldt M, Canbay A, Kahraman A. Spleen stiffness is positively correlated with HVPG and decreases significantly after TIPS implantation. *Dig Liver Dis* 2018; **50**: 54-60 [PMID: 29102174 DOI: 10.1016/j.dld.2017.09.138]

48 **Colecchia A**, Colli A, Casazza G, Mandolesi D, Schiumerini R, Reggiani LB, Marasco G, Taddia M, Lisotti A, Mazzella G, Di Biase AR, Golfieri R, Pinzani M, Festi D. Spleen stiffness measurement can predict clinical complications in compensated HCV-related cirrhosis: a prospective study. *J Hepatol* 2014; **60**: 1158-1164 [PMID: 24607624 DOI: 10.1016/j.jhep.2014.02.024]

49 **Ravaioli F**, Montagnani M, Lisotti A, Festi D, Mazzella G, Azzaroli F. Noninvasive Assessment of Portal Hypertension in Advanced Chronic Liver Disease: An Update. *Gastroenterol Res Pract* 2018; **2018**: 4202091 [PMID: 29977287 DOI: 10.1155/2018/4202091]

50 **Colecchia A**, Ravaioli F, Marasco G, Festi D. Spleen Stiffness by Ultrasound Elastography. In: Diagnostic Methods for Cirrhosis and Portal Hypertension. Cham: Springer International Publishing; 2018: 113-137 [DOI: 10.1007/978-3-319-72628-1_8]

P- Reviewer: Ferraioli G, Furuichi Y, Kahraman A
S- Editor: Gong ZM **L- Editor**: Filipodia **E- Editor**: Song H

World Journal of Hepatology

Submit a Manuscript: http://www.f6publishing.com

World J Hepatol 2018 October 27; 10(10): 743-751

DOI: 10.4254/wjh.v10.i10.743

ISSN 1948-5182 (online)

EVIDENCE-BASED MEDICINE

Comparison of hepatitis C virus testing recommendations in high-income countries

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Author contributions: Irvin R, Nelson NP, Vellozzi C, Thomas DL and Millman AJ developed the concept and search strategy for the manuscript; Irvin R, Ward K and Agee T performed all relevant data searches; Irvin R, Ward K, Agee T, Nelson NP, Vellozzi C, Thomas DL and Millman AJ reviewed all data obtained from searches and participated in manuscript preparation.

Supported by Centers for Disease Control and Prevention, contract No. 200-2013-M-57552; additional support was provided to the primary author by the Johns Hopkins University Center for AIDS Research, No. 1P30AI094189 and NIDA, No. R37DA013806 (to Thomas DL).

Conflict-of-interest statement: Dr. Irvin has nothing to disclose. The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Manuscript source: Unsolicited manuscript

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Received: May 5, 2018
Peer-review started: May 5, 2018
First decision: June 5, 2018
Revised: June 27, 2018
Accepted: July 9, 2018
Article in press: July 10, 2018
Published online: October 27, 2018

Abstract

AIM

To investigate hepatitis C virus (HCV) testing recommendations from the United States and other high-income countries.

METHODS

A comprehensive search for current HCV testing recommendations from the top quartile of United Nations Human Development Index (HDI) countries (very high HDI) was performed using Google and reviewed from May 1 - October 30, 2014 and re-reviewed April 1 - October 2, 2017.

RESULTS

Of the 51 countries identified, 16 had HCV testing recommendations from a government body or recommendations issued collaboratively between a government and a medical organization. Of these 16 countries, 15 had HCV testing recommendations that were primarily risk-based and highlight behaviors, exposures, and conditions that are associated with HCV transmission in that region. In addition to risk-based testing, the HCV Guidance Panel (United States) incorporates recommendations for a

one-time test for individuals born during 1945-1965 (the birth cohort) without prior ascertainment of risk into their guidance. In addition to the United States, six other countries either have an age-based testing recommendation or recommend one-time testing for all adults independent of risk factors typical of the region.

CONCLUSION

This review affirmed the similarities of the HCV Guidance Panel's guidance with those of recommendations from very high HDI countries.

Key words: Hepatitis C; Testing; Recommendations; Mass screening; Guidelines

Core tip: This report investigates hepatitis C virus (HCV) testing recommendations from the United States and other high-income countries to assess any risk-based or universal screening categories that should be considered for updates to HCV testing guidance from the HCV Guidance Panel (United States). This review affirmed the similarities of the HCV Panel guidance with those of very high-income countries. No significant gaps in the guidance were identified. HCV testing recommendations from very high-income countries will be continually reviewed and as new risk categories or universal screening recommendations are identified, they will be considered for incorporation into the HCV Panel guidance when peer-reviewed evidence is available to support the incorporation of the HCV testing practices in the United States.

Irvin R, Ward K, Agee T, Nelson NP, Vellozzi C, Thomas DL, Millman AJ. Comparison of hepatitis C virus testing recommendations in high-income countries. *World J Hepatol* 2018; 10(10): 743-751 Available from: URL: http://www.wjgnet.com/1948-5182/full/v10/i10/743.htm DOI: http://dx.doi.org/10.4254/wjh.v10.i10.743

INTRODUCTION

Hepatitis C virus (HCV) infection, the leading cause of liver cancer and liver failure, is now curable with the recent emergence of short-duration, non-toxic, all-oral therapies[1-4]. This breakthrough in curative therapies for HCV infection has renewed interest in developing mechanisms to improve the HCV care continuum (testing, linkage to care, treatment initiation, cure). Notably, estimates suggest that 50% of HCV infected individuals remain undiagnosed[5]. Thus, it has become extremely important in infectious disease public health policy to identify the appropriate groups of individuals to test for HCV infection and to have this information readily available to healthcare professionals.

To provide healthcare professionals with a single web-based resource for evidence-based, expert-developed recommendations for hepatitis C testing and management, the American Association for the Study of Liver Diseases (AASLD) and the Infectious Diseases Society of America (IDSA) formed the HCV Guidance Panel[6]. The HCV Guidance Panel is also supported, in part, by the Centers for Disease Control and Prevention (CDC) and the International Antiviral Society United States (IAS-USA). The guidance published by the HCV Guidance Panel (the HCV Panel guidance) are updated periodically as new evidence is reviewed, including any updates to formal recommendations from the CDC and United States Preventive Services Task Force (USPSTF)[6-8]. In preparation for making updates to the HCV Panel guidance, HCV testing recommendations from the top quartile of United Nations Human Development Index (HDI) countries were evaluated for similarities and differences[9]. These data have been used periodically by the HCV Guidance Panel to explore HCV testing recommendations globally for comparison to the United States and for consideration in updating the HCV Panel guidance when additional peer-review data is available to support the inclusion of the category in the United States. For example, the periodic reviews of global HCV testing recommendations have served to inform discussion on categories that are not included in the HCV Panel guidance. Any new categories for consideration by panel members are reviewed and literature searches are conducted to ensure that the panel addresses all relevant data on the subject[6]. Each guidance statement is then rated in terms of the level and strength of evidence[6].

MATERIALS AND METHODS

The HDI is a summary measure of average achievement in health, education, and gross national income per capita[9]. Fifty-one countries including the United States were identified as having a very high HDI, defined as the top quartile of HDI countries[9]. A comprehensive search for current HCV testing recommendations from the top quartile of HDI countries was performed using a Google search with a combination of free text terms. Relevant terms included: country name, hepatitis C, HCV, screening, testing, recommendations, and guidelines. The Google results were then reviewed with experts in the field of hepatitis C (including email and in-person interviews) inquiring about any additional countries known to have HCV testing recommendations.

Testing recommendations were considered if they were from a government body or represented collaborative recommendations between a government and a medical organization. To be included in our analysis, recommendations needed to be available online May 1, 2014 - October 2, 2017. Non-English language documents were translated into English using Google Translate. When logical, testing categories were combined to make the tabulation of the results more manageable. Because in the United States both the CDC and USPSTF independently issue HCV testing recommendations, the HCV Guidance Panel's routine synthesis of these recom-

mendations into their guidance were used for the purpose of this report. From May 1 - October 30, 2014, two reviewers performed the initial searches and engaged consultants to identify HCV testing recommendations from very high HDI countries. Two reviewers re-reviewed HCV testing recommendations through Google searches and follow up with expert consults from April 1 - October 2, 2017 to identify and update any changes.

RESULTS

Of the 51 countries categorized as very high HDI, 16, including the United States were found to have HCV testing recommendations (Table 1)[6,10-26]. Of these 16 countries, 15 had HCV testing recommendations that were primarily risk-based and highlight behaviors, exposures, and conditions that are associated with HCV transmission in that region. In addition to risk-based testing, the HCV Panel guidance incorporates CDC and USPSTF recommendations for a one-time test for individuals born during 1945-1965 (the birth cohort) without prior ascertainment of risk[6-8]. Six additional countries have either an age-based testing recommendation or recommend one-time testing for all adults independent of risk factors. In the United States, individuals born from 1945-1965 are included in both CDC and USPSTF HCV testing recommendations as they account for 75% of all HCV infections and evidence confirmed that a risk-based strategy alone failed to identify more than 50% of HCV infections due to provider and patient barriers in correctly ascertaining risk[6,27].

The HCV Panel guidance recommends one-time testing for all HIV-positive individuals and for any persons about to start pre-exposure prophylaxis (PrEP) for HIV. The HCV Panel guidance also recommends annual HCV testing for persons who inject drugs and for HIV-infected men who have unprotected sex with men.

As of 2017, the HCV testing categories identified in other very high HDI countries not included in the HCV Panel guidance are: (1) Acute hepatitis or hepatitis symptoms; (2) Receiving an immunization or a medical procedure in a specified country or in a country where hepatitis C is common or where universal precautions are not in place; (3) Body piercing or tattoo history; (4) Hemophilia history; (5) Hepatitis A or B infection history; (6) Homeless persons; (7) Immigrants or visitors from countries where HCV is endemic; (8) Liver Cancer; (9) Living with, or sexual partner of, HCV-positive person; (10) Multiple sex partners, history of sexually transmitted infections (STIs), or high risk sexual behaviors; and (11) Attending STI clinic (± any risk factors)

DISCUSSION

The HCV Panel guidance is based on epidemiologic data and behaviors, exposures, and conditions associated with acquisition of HCV infection in the United States and are compiled by the expert panel and reviewed regularly[6]. As a result of this initial work in 2014, solid organ donors (deceased and living) were identified as a group not included in the guidance and were thus considered for review. After additional review of the available literature on the subject, donors were added to the HCV Panel guidance in 2014 and given an evidence rating of Class Ⅰ, Level B[6,28,29]. Prior to this, donors had not been explicitly named as a testing category but were discussed in the text below the testing guidance.

Currently, the HCV Panel guidance include the vast majority of HCV testing recommendation categories noted in other HDI countries and many of the people within risk categories not directly specified would likely receive testing nonetheless because of other associated risks or because of typical clinical care in the United States. For instance, acute hepatitis/hepatitis symptoms, hepatitis A or B history, and liver cancer would prompt a clinical evaluation for hepatitis C and might also be captured by the HCV Guidance Panel category of unexplained chronic liver disease and/or chronic hepatitis including elevated alanine aminotransferase levels[6]. Additionally, hemophilia alone is not recommended for testing by the HCV Guidance Panel; however, certain persons with hemophilia would be tested for HCV because the HCV Panel guidance incorporates recommendations to screen anyone who received blood components before 1992 or clotting factor concentrates produced before 1987 for HCV[6]. The remaining recommendation categories covered by other very high HDI countries but not included in the HCV Panel guidance include: body piercing or tattoo history; being homeless; living with, or the sexual partner of, an HCV-positive person; multiple sex partners, history of STIs, or high risk sexual behaviors; STI clinic populations; and immigrants/visitors from countries were HCV is endemic or those vaccinated or receiving medical procedures in those countries or where universal precautions are not in place. Although body piercings and tattoos are not specifically mentioned in the HCV Panel guidance, they do incorporate recommendations to test persons with percutaneous/parenteral exposures in an unregulated setting[6]. Hence, according to the HCV Guidance Panel, individuals with body piercings/tattoos obtained outside of licensed parlors should undergo HCV testing. Furthermore, this practice would also likely capture medical procedures where strict infection control may not have been followed, both domestically and internationally. While the higher prevalence of HCV in homeless populations is acknowledged by the HCV Guidance Panel, the homeless are not specifically named as a group for testing at this time. Although several studies have noted prevalence rates above 10% in several homeless populations in the United States, it is believed that the risk is often due to high rates of substance use disorders, which would be captured by the HCV Panel guidance to test injection and intranasal drug users[30]. Sexual transmission of HCV is generally considered inefficient except among HIV-infected MSM; therefore, guidance related to sexual transmission categories for the general United States population have not been included as an HCV testing

Table 1 Hepatitis C virus testing recommendations from the top quartile of human development index countries

Countries and approving bodies	Yr Recommendations approved	Abnormal Aminotransferases	Acute hepatitis or hepatitis symptoms	Being immunized/receiving a medical procedure in a specified country where hepatitis C is common or lack of universal precautions	Birth cohort/age recommendations or one-time testing for all	Blood/clotting factor transfusion Patients/ Transplant Patients	Body Piercing or Tattoo History	Children born to HCV-infected mothers (expectant mothers)
Argentina Dirección de Sida y ETS, Ministerio de Salud de la Nación	2016	X	X	X	X	X		X
Australia National HCV Testing Policy Expert Reference Committee	2016	X	X			X	X	X
Canada Canadian Task Force on Preventive Health Care	2017 and 2009	X	X	X		X	X	X
Chile Ministerio de Salud de Chile (Chilean Ministry of Health)	2015	X			X	X		X
Denmark Sundhedsst-yrelsen (Danish Health Authority)	2013	X				X		X
Finland Sosiaali- ja terveysministeriö (Social and Health Ministry)	2016			X	X	X	X	X
France ANRS/AFEF with Ministère des Affaires sociales et de la Santé (Ministry of Social Affairs and Health)	2014	X		X	X	X	X	X
Greece Υπουργειο ΥγειαΣ (Ministry of Health)	2017	X			X	X		X
Ireland Feidhmeannacht na Seirbhise Slainte	2012	X		X		X	X	X
Italy Istituto Superiore di Sanità	2005					X		
Japan Ministry of Health, Labour and Welfare	2011				X			
Russian Federation Министерство здравоохранения (Ministry of Health)	2017		X			X		X
Spain Ministeriode Sanidad, Servicios Socialese Igualdad (Ministry of Health, Social Services and Equality)	2015					X	X	X
Switzerland Bundesamt für Gesundheit (Swiss Federal Office of Public Health)	2013	X	X	X		X	X	X
United Kingdom NHS and National Inst for Health and Care Excellence	2015 and 2012	X		X		X	X	X
United States HCV Guidance Panel	2017	X			X	X		X

HCV: Hepatitis C virus; MSM: Men who have sex with men; X denotes that category is advised for testing.

Table 1 Hepatitis C virus testing recommendations from the top quartile of human development index countries

Countries and approving bodies	Chronic liver disease, cirrhosis, or fibrosis	Contact tracing /exposure to infected blood, shared hygiene materials with HCV + person	Donors - blood, blood product, tissue or organ	Drug users - injecting	BDrug users - intranasal	Healthcare workers (public safety) at risk and/or post-exposure	Hemodialysis history or repeated percutaneous injections	Hemophilia history	Hepatitis A or B history (or non-A/ non-B hepatitis)
Argentina Dirección de Sida y ETS, Ministerio de Salud de la Nación				X	X		X		X
Australia National HCV Testing Policy Expert Reference Committee	X	X	X	X		X	X		X
Canada Canadian Task Force on Preventive Health Care	X	X		X	X		X	X	X
Chile Ministerio de Salud de Chile (Chilean Ministry of Health)	X			X		X	X	X	
Denmark Sundhedsst-yrelsen (Danish Health Authority)	X			X	X	X	X	X	X
Finland Sosiaali- ja terveysministeriö (Social and Health Ministry)			X	X	X				
France ANRS/AFEF with Ministère des Affaires sociales et de la Santé (Ministry of Social Affairs and Health)		X	X	X	X		X		
Greece Υπουργειο ΥγειαΣ (Ministry of Health)				X			X		X
Ireland Feidhmeannacht na Seirbhise Slainte			X	X	X	X	X		X
Italy Istituto Superiore di Sanità				X			X		
Japan Ministry of Health, Labour and Welfare									
Russian Federation Министерство здравоохранения (Ministry of Health)	X	X	X	X		X	X		X
Spain Ministeriode Sanidad, Servicios Socialese Igualdad (Ministry of Health, Social Services and Equality)		X		X	X	X	X		X
Switzerland Bundesamt für Gesundheit (Swiss Federal Office of Public Health)	X			X	X	X	X		X
United Kingdom NHS and National Inst for Health and Care Excellence				X					
United States HCV Guidance Panel	X	X	X	X	X	X	X		

MSM: Men who have sex with men; X denotes that category is advised for testing.

Table 1 Hepatitis C virus testing recommendations from the top quartile of human development index countries

Countries and approving bodies	HIV-positive patients	Homeless	Immigrants or visitors from countries where HCV is endemic	Incarceration history alone or with additional risk factors	Liver cancer	Living with or sexual partner of HCV-positive person	MSM, limited to HIV + MSM (with or without unprotected sex), or sexually active starting PrEP	Multiple Sex Partners, history of STIs, or high risk sexual behaviors	Patient request, percutaneous-parenteral exposure, prior positive HCV test	STI clinic (+ /- any risk factors)
Argentina Dirección de Sida y ETS, Ministerio de Salud de la Nación						X		X	X	
Australia National HCV Testing Policy Expert Reference Committee	X		X	X	X	X	X		X	X
Canada Canadian Task Force on Preventive Health Care	X	X	X	X				X	X	
Chile Ministerio de Salud de Chile (Chilean Ministry of Health)	X				X	X				
Denmark Sundhedsst-yrelsen (Danish Health Authority)	X		X		X					
Finland Sosiaali- ja terveysministeriö (Social and Health Ministry)	X		X	X			X	X		
France ANRS/AFEF with Ministère des Affaires sociales et de la Santé (Ministry of Social Affairs and Health)	X		X	X		X	X			
Greece Υπουργειο ΥγειαΣ (Ministry of Health)	X		X	X		X		X	X	
Ireland Feidhmeannacht na Seirbhise Slainte	X		X	X		X	X		X	
Italy Istituto Superiore di Sanità						X		X	X	
Japan Ministry of Health, Labour and Welfare										
Russian Federation Министерство здравоохранения (Ministry of Health)				X		X	X	X		
Spain Ministeriode Sanidad, Servicios Socialese Igualdad (Ministry of Health, Social Services and Equality)	X			X		X	X		X	
Switzerland Bundesamt für Gesundheit (Swiss Federal Office of Public Health)	X		X	X	X	X	X		X	
United Kingdom NHS and National Inst for Health and Care Excellence		X	X	X		X	X		X	X
United States HCV Guidance Panel	X			X			X		X	

HIV: Human immunodeficiency virus; HCV: Hepatitis C virus; STI: Sexually transmitted infection; MSM: Men who have sex with men; X denotes that category is advised for testing.

category[31]. STI clinics are also not currently included in the HCV Panel guidance. Data suggest that STI clinic populations have higher prevalence rates of HCV infection than the general population due to overlapping risk factors of sexually transmitted infections and hepatitis C[32,33]. However, the higher prevalence of HCV infection in STI clinic populations is often attributed to the birth cohort (individuals born between 1945 and 1965) and injection drug use, risk groups captured elsewhere in the guidance[32-35]. Finally, while the HCV Guidance Panel continues to review evidence on immigrants/visitors from countries where HCV is endemic, any future guidance for HCV testing among foreign-born individuals would need to account for geographic disparities in HCV prevalence and practicalities in implementing this in clinical practice settings[36,37]. As an example of how this might be implemented, in 2008 the CDC recommended hepatitis B screening for persons born in regions of high and intermediate HBV endemicity (HBsAg prevalence > 2%)[38].

Our report has the following limitations. Our search methodology may have missed recommendations and/or classified recommendations incorrectly from some very high HDI countries due to search terms not capturing relevant information in different languages, recommendations not being accessible on the Internet at the time of the searches, or misinterpretation of the role of the government's involvement in development of the recommendations. Additionally, some governments may rely on international or national organizations for recommendations on hepatitis C testing without publishing their own recommendations.

This review affirmed the similarities of the HCV Panel guidance with those of very high HDI countries. No significant gaps in the guidance were identified. HCV testing recommendations from very high HDI countries will be continually reviewed and as new risk categories or universal screening recommendations are identified, they will be considered for incorporation into the HCV Panel guidance when peer-reviewed evidence is available to support the incorporation of the HCV testing practices in the United States.

ARTICLE HIGHLIGHTS

Research background

Hepatitis C virus (HCV) infection, the leading cause of liver cancer and liver failure, is now curable with the recent emergence of short-duration, non-toxic, all-oral therapies. This breakthrough in curative therapies for HCV infection has renewed interest in developing mechanisms to improve the HCV care continuum (testing, linkage to care, treatment initiation, cure). This renewed interest in HCV has led to many countries updating their HCV testing recommendations.

Research motivation

The United States HCV Guidance Panel provides healthcare professionals with a single web-based resource for evidence-based, expert-developed recommendations for hepatitis C testing and management. HCV recommendations in countries around the world have been recently updated due to advances in HCV treatment. However, this data is not compiled in a central location. This report investigates HCV testing recommendations from the United States and other high-income countries. In preparation for making updates to the HCV Panel guidance, HCV testing recommendations from the top quartile of United Nations Human Development Index (HDI) countries were evaluated for similarities and differences.

Research objectives

The main objective of this study was to identify HCV testing recommendations from the top quartile of United Nations HDI countries. The identified HCV recommendations were evaluated for similarities and differences. These data have been used periodically by the HCV Guidance Panel to explore HCV testing recommendations globally for comparison to the United States for consideration in updating the HCV Panel guidance when additional peer-review data is available to support inclusion of the category in the United States.

Research methods

A comprehensive search for current HCV testing recommendations from the top quartile of HDI countries was performed using a Google search with a combination of free text terms. Relevant terms included: country name, hepatitis C, HCV, screening, testing, recommendations, and guidelines. The Google results were then reviewed with experts in the field of hepatitis C (including email and in-person interviews) inquiring about any additional countries known to have HCV testing recommendations. Testing recommendations were considered if they were from a government body or represented collaborative recommendations between a government and a medical organization. To be included in our analysis, recommendations needed to be available online May 1, 2014-October 2, 2017. From May 1-October 30, 2014, two reviewers performed the initial searches and engaged consultants to identify HCV testing recommendations from very high HDI countries. Two reviewers re-reviewed HCV testing recommendations through Google searches and follow up with expert consults from April 1-October 2, 2017 to identify and update any changes.

Research results

Of the 51 countries identified, 16 had HCV testing recommendations from a government body or recommendations issued collaboratively between a government and a medical organization. Of these 16 countries, 15 had HCV testing recommendations that were primarily risk-based and highlight behaviors, exposures, and conditions that are associated with HCV transmission in that region. In addition to risk-based testing, the HCV Guidance Panel (United States) incorporates recommendations for a one-time test for individuals born during 1945-1965 (the birth cohort) without prior ascertainment of risk into their guidance. In addition to the United States, six other countries either have an age-based testing recommendation or recommend one-time testing for all adults independent of risk factors typical of the region.

Research conclusions

This review affirmed the similarities of the HCV Guidance Panel's guidance with those of recommendations from very high HDI countries. As a result of this initial work in 2014, solid organ donors (deceased and living) were identified as a group not included in the guidance and were thus considered for review. After additional review of the available literature on the subject, donors were added to the HCV Panel guidance in 2014 and given an evidence of rating of Class I, Level B. Prior to this, donors had not been explicitly named as a testing category but were discussed in the text below the testing guidance.

Research perspectives

HCV testing recommendations from very high HDI countries will be continually reviewed and as new risk categories or universal screening recommendations are identified, they will be considered for incorporation into the HCV Panel guidance when peer-reviewed evidence is available to support the incorporation of the HCV testing practices in the United States.

ACKNOWLEDGMENTS

The authors would like to thank the HCV Guidance Panel, the American Association for the Study of Liver Diseases, the Infectious Diseases Society of America, the

International Antiviral Society United States, the Centers for Disease Control and Prevention, the Johns Hopkins Division of Infectious Diseases, and all expert consults involved in this work.

REFERENCES

1 **Centers for Disease Control and Prevention**. HIV and Viral Hepatitis 2016. Available from: URL: http://www.cdc.gov/hiv/pdf/library_factsheets_hiv_and_viral_hepatitis.pdf

2 **Alqahtani S**, Sulkowski M. Current and Evolving Treatments of Genotype 1 Hepatitis C Virus. *Gastroenterol Clin North Am* 2015; **44**: 825-843 [PMID: 26600222 DOI: 10.1016/j.gtc.2015.06.001]

3 **Alqahtani SA**, Afdhal N, Zeuzem S, Gordon SC, Mangia A, Kwo P, Fried M, Yang JC, Ding X, Pang PS, McHutchison JG, Pound D, Reddy KR, Marcellin P, Kowdley KV, Sulkowski M. Safety and tolerability of ledipasvir/sofosbuvir with and without ribavirin in patients with chronic hepatitis C virus genotype 1 infection: Analysis of phase III ION trials. *Hepatology* 2015; **62**: 25-30 [PMID: 25963890 DOI: 10.1002/hep.27890]

4 **Foster GR**, Afdhal N, Roberts SK, Bräu N, Gane EJ, Pianko S, Lawitz E, Thompson A, Shiffman ML, Cooper C, Towner WJ, Conway B, Ruane P, Bourlière M, Asselah T, Berg T, Zeuzem S, Rosenberg W, Agarwal K, Stedman CA, Mo H, Dvory-Sobol H, Han L, Wang J, McNally J, Osinusi A, Brainard DM, McHutchison JG, Mazzotta F, Tran TT, Gordon SC, Patel K, Reau N, Mangia A, Sulkowski M; ASTRAL-2 Investigators; ASTRAL-3 Investigators. Sofosbuvir and Velpatasvir for HCV Genotype 2 and 3 Infection. *N Engl J Med* 2015; **373**: 2608-2617 [PMID: 26575258 DOI: 10.1056/NEJMoa1512612]

5 **Yehia BR**, Schranz AJ, Umscheid CA, Lo Re V 3rd. The treatment cascade for chronic hepatitis C virus infection in the United States: a systematic review and meta-analysis. *PLoS One* 2014; **9**: e101554 [PMID: 24988388 DOI: 10.1371/journal.pone.0101554]

6 **American Association for the Study of Liver Diseases**. HCV Guidance: Recommendations for Testing, Managing, and Treating Hepatitis C 2016. Available from: URL: http://hcvguidelines.org/

7 **Centers for Disease Control and Prevention**. Testing Recommendations for Hepatitis C Virus Infection 2015. Available from: URL: https://www.cdc.gov/hepatitis/hcv/guidelinesc.htm

8 **U.S. Preventive Services Task Force**. Hepatitis C: Screening 2013. Available from: URL: https://www.uspreventiveservicestaskforce.org/Page/Document/UpdateSummaryFinal/hepatitis-c-screening

9 **United Nations Development Programme**. Human Development Report 2016. Available from: URL: http://hdr.undp.org/en/2016-report

10 **Ministerio De Sanidad, Servicios Sociales E Igualdad**. Hepatitis Virales 2016. Available from: URL: http://www.msal.gob.ar/images/stories/bes/graficos/0000000780cnt-2016-09_hepatitis-virales-equipos-de-salud.pdf

11 **National HCV Testing Policy Expert Committee (Australian Government Department of Health)**. National Hepatitis C Testing Policy Commonwealth of Australia2017. Available from: URL: http://testingportal.ashm.org.au/images/HCV/HCV_TESTING_POLICY_Mar_2017.pdf

12 **Grad R**, Thombs BD, Tonelli M, Bacchus M, Birtwhistle R, Klarenbach S, Singh H, Dorais V, Holmes NM, Martin WK, Rodin R, Jaramillo Garcia A; Canadian Task Force on Preventive Health Care. Recommendations on hepatitis C screening for adults. *CMAJ* 2017; **189**: E594-E604 [PMID: 28438952 DOI: 10.1503/cmaj.161521]

13 **The College of Family Physicians of Canada**, Public Health Agency of Canada. Primary Care Management of Hepatitis C 2009. Available from: URL: http://www.cfpc.ca/uploadedFiles/Resources/Resource_Items/HEP_C_Guide_eng_2.pdf

14 **Ministerio de Salud de Chile**. Guía Clínica Auge Manejo Y Tratamiento De La Infección Crónica Por Virus De La Hepatitis C (VHC) 2015. Available from: URL: http://web.minsal.cl/wp-content/uploads/2016/04/GUIA-VHC.-2015-Editada.pdf

15 **Denmark National Board of Health**. Guidance on HIV (human immunodeficiency virus) and hepatitis B and C virus 2013. Available from: URL: https://sundhedsstyrelsen.dk/~/media/AD9E0753B12546B8AEA323BF02AC3D2C.ashx

16 **Social and Health Ministry of Finland**. Suomen C-hepatiittistrategia 2017-2019, 2016. Available from: URL: https://julkaisut.valtioneuvosto.fi/handle/10024/78923

17 **Ministère des Affaires sociales et de la Santé**. Management of Persons with Hepatitis B or C Virus Infection 2014. Available from: URL: http://www.afef.asso.fr/ckfinder/userfiles/files/actualites/veille/French-report-Dhumeaux.pdf

18 **Greece Ministry of Health**. National Action Plan for the confrontation of Hepatitis C 2017. Available from: URL: http://www.moh.gov.gr/articles/ministry/grafeio-typoy/press-releases/4865-ethniko-sxedio-drashs-gia-thn-antimetwpish-ths-hpatitidas-c

19 **Inclusion HNS**. Feidhmeannacht na Seirbhise Slainte Health Service Executive. National Hepatitis C Strategy 2011-2014. 2012. Available from: URL: http://www.lenus.ie/hse/handle/10147/243033

20 **Istituto Superiore Di Sanità**. Lo screening per infezione da virus dell' epatite C negli adulti in Italia 2005. Available from: URL: http://www.iss.it/binary/publ/cont/06-47.1171283608.pdf

21 **Ministry of Health Labour and Welfare**. Basic guidelines for promotion of control measures for hepatitis 2011. Available from: URL: http://www.mhlw.go.jp/bunya/kenkou/kekkaku-kansenshou09/pdf/hourei-17e.pdf

22 **Ministerio De Sanidad Servicios Sociales E Igualdad**. Plan Estratégico Para El Abordaje De La Hepatitis C En El Sistema Nacional De Salud 2015. Available from: URL: https://www.msssi.gob.es/ciudadanos/enfLesiones/enfTransmisibles/docs/plan_estrategico_hepatitis_C.pdf

23 **Fretz R**, Negro F, Bruggmann P, Lavanchy D, De Gottardi A, Pache I, Masserey Spicher V, Cerny A. Hepatitis B and C in Switzerland - healthcare provider initiated testing for chronic hepatitis B and C infection. *Swiss Med Wkly* 2013; **143**: w13793 [PMID: 23740193 DOI: 10.4414/smw.2013.13793]

24 **National Institute of Health and Care Excellence**. Hepatitis B and C Testing 2012. Available from: URL: https://www.nice.org.uk/guidance/ph43

25 **National Health Service**. Hepatitis C Quick reference guide for primary care 2015. Available from: URL: http://www.nhs.uk/hepatitisc/SiteCollectionDocuments/pdf/Hepatitis_C_quick_reference_for_PC_April2011.pdf

26 **MACOM**. Recommendations for diagnosis and treatment of adults with hepatitis C 2017. Available from: URL: http://www.iia-rf.ru/upload/iblock/6d9/6d96c71786128fcab695c11afea3338c.pdf

27 **Smith BD**, Morgan RL, Beckett GA, Falck-Ytter Y, Holtzman D, Teo CG, Jewett A, Baack B, Rein DB, Patel N, Alter M, Yartel A, Ward JW; Centers for Disease Control and Prevention. Recommendations for the identification of chronic hepatitis C virus infection among persons born during 1945-1965. *MMWR Recomm Rep* 2012; **61**: 1-32 [PMID: 22895429]

28 **Lai JC**, Kahn JG, Tavakol M, Peters MG, Roberts JP. Reducing infection transmission in solid organ transplantation through donor nucleic acid testing: a cost-effectiveness analysis. *Am J Transplant* 2013; **13**: 2611-2618 [PMID: 24034208 DOI: 10.1111/ajt.12429]

29 **Seem DL**, Lee I, Umscheid CA, Kuehnert MJ. Excerpt from PHS guideline for reducing HIV, HBV and HCV transmission through organ transplantation. *Am J Transplant* 2013; **13**: 1953-1962 [PMID: 23890284 DOI: 10.1111/ajt.12386]

30 **Noska AJ**, Belperio PS, Loomis TP, O'Toole TP, Backus LI. Prevalence of Human Immunodeficiency Virus, Hepatitis C Virus, and Hepatitis B Virus Among Homeless and Nonhomeless United States Veterans. *Clin Infect Dis* 2017; **65**: 252-258 [PMID: 28379316 DOI: 10.1093/cid/cix295]

31 **Schmidt AJ**, Falcato L, Zahno B, Burri A, Regenass S, Müllhaupt B, Bruggmann P. Prevalence of hepatitis C in a Swiss sample of men who have sex with men: whom to screen for HCV infection? *BMC Public Health* 2014; **14**: 3 [PMID: 24393532 DOI: 10.1186/1471-2458-14-3]

32 **Falade-Nwulia O**, Mehta SH, Lasola J, Latkin C, Niculescu A, O' Connor C, Chaulk P, Ghanem K, Page KR, Sulkowski MS, Thomas DL. Public health clinic-based hepatitis C testing and linkage to care in Baltimore. *J Viral Hepat* 2016; **23**: 366-374 [PMID: 26840570 DOI:

10.1111/jvh.12507]
33 **Thomas DL**, Cannon RO, Shapiro CN, Hook EW 3rd, Alter MJ, Quinn TC. Hepatitis C, hepatitis B, and human immunodeficiency virus infections among non-intravenous drug-using patients attending clinics for sexually transmitted diseases. *J Infect Dis* 1994; **169**: 990-995 [PMID: 8169429 DOI: 10.1093/infdis/169.5.990]
34 **Subiadur J**, Harris JL, Rietmeijer CA. Integrating viral hepatitis prevention services into an urban STD clinic: Denver, Colorado. *Public Health Rep* 2007; **122** Suppl 2: 12-17 [PMID: 17542447 DOI: 10.1177/00333549071220S203]
35 **Zimmerman R**, Finley C, Rabins C, McMahon K. Integrating viral hepatitis prevention into STD clinics in Illinois (excluding Chicago), 1999-2005. *Public Health Rep* 2007; **122** Suppl 2: 18-23 [PMID: 17542448 DOI: 10.1177/00333549071220S204]
36 **Ditah I**, Ditah F, Devaki P, Ewelukwa O, Ditah C, Njei B, Luma HN, Charlton M. The changing epidemiology of hepatitis C virus infection in the United States: National Health and Nutrition Examination Survey 2001 through 2010. *J Hepatol* 2014; **60**: 691-698 [PMID: 24291324 DOI: 10.1016/j.jhep.2013.11.014]
37 **Averhoff FM**, Glass N, Holtzman D. Global burden of hepatitis C: considerations for healthcare providers in the United States. *Clin Infect Dis* 2012; **55** Suppl 1: S10-S15 [PMID: 22715208 DOI: 10.1093/cid/cis361]
38 **Weinbaum CM**, Williams I, Mast EE, Wang SA, Finelli L, Wasley A, Neitzel SM, Ward JW; Centers for Disease Control and Prevention (CDC). Recommendations for identification and public health management of persons with chronic hepatitis B virus infection. *MMWR Recomm Rep* 2008; **57**: 1-20 [PMID: 18802412]

P- Reviewer: Abushady EAE, Bouare N, Chen EQ, Luo GH, Milovanovic T **S- Editor**: Ji FF **L- Editor**: A **E- Editor**: Yin SY

World Journal of Hepatology

Submit a Manuscript: http://www.f6publishing.com

World J Hepatol 2018 October 27; 10(10): 752-760

DOI: 10.4254/wjh.v10.i10.752

ISSN 1948-5182 (online)

SYSTEMATIC REVIEW

Thrombosis prophylaxis in pediatric liver transplantation: A systematic review

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Author contributions: Nacoti M was responsible for the concept, design, analysis of data and drafting; Ruggeri GM and Colombo G were responsible for selection of the papers and variables and drafting; Bonanomi E was responsible for concept and critical revision; Lussana F was responsible for the concept, design, searching strategy, drafting and critical revision.

Conflict-of-interest statement: The authors of this manuscript have no conflicts of interest to disclose.

PRISMA 2009 Checklist statement: The manuscript was prepared and revised according to the PRISMA 2009 Checklist.

Manuscript source: Invited manuscript

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Received: May 2, 2018
Peer-review started: May 4, 2018
First decision: May 23, 2018
Revised: July 13, 2018
Accepted: August 1, 2018
Article in press: August 1, 2018
Published online: October 27, 2018

Abstract

AIM

To review current literature of thrombosis prophylaxis in pediatric liver transplantation (PLT) as thrombosis remains a critical complication.

METHODS

Studies were identified by electronic search of MEDLINE, EMBASE and Cochrane Library (CENTRAL) databases until March 2018. The search was supplemented by manually reviewing the references of included studies and the references of the main published systematic reviews on thrombosis and PLT. We excluded from this review case report, small case series, commentaries, conference abstracts, papers which describing less than 10 pediatric liver transplants/year and articles published before 1990. Two reviewers performed study selection independently, with disagreements solved through discussion and by the opinion of a third reviewer when necessary.

RESULTS

Nine retrospective studies were included in this review. The overall quality of studies was poor. A pooled analysis of results from studies was not possible due to the retrospective design and heterogeneity of included studies. We found an incidence of portal vein thrombosis (PVT) ranging from 2% to 10% in pediatric living donor

liver transplantation (LDLT) and from 4% to 33% in pediatric deceased donor liver transplantation (DDLT). Hepatic artery thrombosis (HAT) was observed mostly in mixed LDLT and DDLT pediatric population with an incidence ranging from 0% to 29%. In most of the studies Doppler ultrasonography was used as a first line diagnostic screening for thrombosis. Four different surgical techniques for portal vein anastomosis were reported with similar efficacy in terms of PVT reduction. Reduced size liver transplant was associated with a low risk of both PVT (incidence 4%) and HAT (incidence 0%, $P < 0.05$). Similarly, aortic arterial anastomosis without graft interposition and microsurgical hepatic arterial reconstruction were associated with a significant reduced HAT incidence (6% and 0%, respectively). According to our inclusion and exclusion criteria, we did not find eligible studies that evaluated pharmacological prevention of thrombosis.

CONCLUSION

Poor quality retrospective studies show the use of tailored surgical strategies might be useful to reduce HAT and PVT after PLT; prospective studies are urgently needed.

Key words: Pediatric liver transplantation; Prophylaxis; Hepatic artery thrombosis; Surgical technique; Portal vein thrombosis

Core tip: Graft loss and patient death after pediatric liver transplantation (PLT) are most frequently caused by hepatic artery thrombosis and portal vein thrombosis. For this reason, the prevention of hepatic artery and vein thrombosis represents a primary interest for clinicians and researchers, considering the scarcity of hepatic allografts. In our systematic review, we found only nine poor quality retrospective studies showing that tailored surgical strategies might be useful to reduce thrombosis. We did not find eligible studies evaluating pharmacological prevention strategies. Prospective studies are urgently needed to standardize thrombosis prevention in PLT.

Nacoti M, Ruggeri GM, Colombo G, Bonanomi E, Lussana F. Thrombosis prophylaxis in pediatric liver transplantation: A systematic review. *World J Hepatol* 2018; 10(10): 752-760 Available from: URL: http://www.wjgnet.com/1948-5182/full/v10/i10/752.htm DOI: http://dx.doi.org/10.4254/wjh.v10.i10.752

INTRODUCTION

Vascular complications are relevant causes of poor outcome for patient and allograft after pediatric liver transplantation (PLT)[1-5]. Among vascular complications of PLT hepatic artery thrombosis (HAT) and portal vein thrombosis (PVT) are one of the most frequent[1-7] and serious causes of graft loss and also patient death[1,2,6-9]. In particular HAT is an extremely serious complication resulting in bile duct necrosis and often requiring retransplantation[10,11]. Thrombosis of other intra-abdominal vessels, such as the hepatic vein and inferior vena cava occurs less frequently[6,11,12]. In the first years of PLT the observed incidence of thrombosis was very high, up to 42%[13-15]. In the last years, an improvement of perioperative care has significantly decreased the thrombosis incidence[1-3]. More recently, an incidence rate of HAT ranging from 2% to 10% after liver transplantation in the pediatric population has been reported[1,6,7,11]; likewise, the incidence rate of PVT ranged from 2% to 10%[1,6,7,9].

In this context, the prevention of HAT and PVT remains very important for PLT outcome and it should be a matter of primary interest for clinicians and researchers, considering the ongoing scarcity of hepatic allografts[1,2,11,12,16-18]. In clinical practice, there is not a standardized approach for thrombosis prevention in PLT. Different surgical techniques and pharmacological prophylaxis have been purposed in several studies[1,5,6,12,14,19-27]. Therefore, we performed a systematic review of current literature about surgical and pharmacological prophylaxis for prevention of thrombosis after PLT to evaluate the current evidence available.

MATERIALS AND METHODS

Search strategy

The publications were selected through an electronic search of the MEDLINE and EMBASE and Cochrane Library (CENTRAL) databases up to March 2018. The search strategy used the following Medical Subject Headings (MeSH) and EMTREE terms and text words: ("liver transplantation"/exp OR "liver transplantation") AND ("thromboembolism"/exp OR "thromboembolism" OR "ischemia"/exp OR "ischemia" OR "vascular disease" /exp OR "vascular disease") AND ("prophylaxis"/exp OR "prophylaxis" OR "prevention"/exp OR "prevention") AND ([newborn]/lim OR [infant]/lim OR [child]/lim OR [preschool]/lim OR [school]/lim OR [adolescent]/lim). In addition the references of the selected studies and two systematic reviews on thrombosis and PLT[11,17] were screened to identify further relevant studies.

Two reviewers (Giulia Maria Ruggeri and Giovanna Colombo) performed an independent study selection, solving any disagreements through discussion and the opinion of a third reviewer (Mirco Nacoti). They obtained study characteristics like year of publication, design, study centre, patients' characteristics like number, mean age and gender, treatments and number of arterial and venous thrombotic complications.

The following criteria were needed in order to be considered potentially eligible for this systematic review: (1) phase Ⅲ randomized clinical trials or cohorts including case series with more than 10 patients undergoing

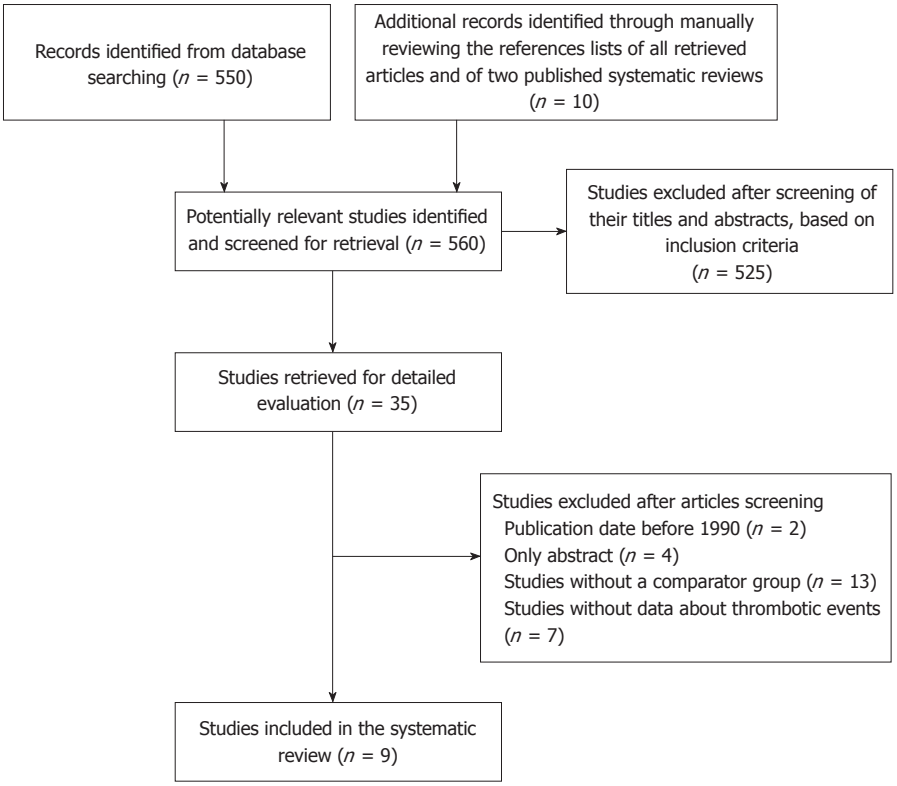

Figure 1 Flow-chart of study selection process.

elective PLT (age ranged from 0 to 18 years); and (2) reporting arterial and venous thrombosis as primary or secondary events in all different groups according to the used prophylaxis strategy. If data from a study were reported in several publications, data from the most recent paper were used. Studies–describing less than 10 PLT/year were excluded as significant high mortality is associated with low volume center[28,29]. Articles published before 1990 were also excluded because new developments in perioperative PLT have dramatically improved the survival[1-3].

Risk of bias assessment

Although the evaluation of quality for observational studies is controversial[30], Giulia Maria Ruggeri and Giovanna Colombo assessed the risk of bias using the following items for cohort studies: Type of study (prospective or retrospective); selection of the patients (consecutive or not); thrombosis as pre-specified outcome; quality of measurements (studies in which thrombotic events were measured in an objective way were considered higher quality than studies without these characteristics). A scoring system was put in place to identify the following two quality categories: Studies at low-risk of bias (4 points) and studies at high-risk of bias ($\leqslant$ 3 points).

RESULTS

Results of the search strategy

The study process is presented in Figure 1. A total of 560 publications (550 retrieved with the electronic search strategy and 10 by manually reviewing the reference lists of all retrieved articles) were identified. After reading their titles and abstracts 525 were excluded, according to inclusion and exclusion criteria. The remaining 35 publications were analyzed in full for detailed evaluation. Twenty-six papers were eliminated for the following reasons: Publication date before 1990 (n = 2), only abstract (n = 4), studies without a comparator group (n = 13) and studies (n = 7) which did not contain explicit data about pediatric thrombotic events.

Nine manuscripts[31-39], which included a total of 1034 PLT in 991 subjects, were included in this systematic review (Table 1). None of the all included studies were at high risk of bias according to pre-defined requirements (Table 2). In particular, no study were prospective, only 7 studies reported thrombosis as a pre-defined outcome, and 8 studies detailed how the diagnosis of thrombosis had been made. The poor quality and the heterogeneity of included studies did not allow us to perform a pooled analysis of results.

Incidence of the artery and PVT

Table 1 shows detailed incidence of artery and PVT as reported in the included studies. Most studies reported a total incidence (early and late) of thrombosis. The incidence of PVT varies from 2% to 10% in pediatric living donor liver transplantation (LDLT)[37,39] and from 33% in pediatric LDLT to 4% in pediatric deceased donor liver transplantation (DDLT) with reduced graft[33]. HAT is presented mostly in mixed LDLT and DDLT pedia-

Table 1 Summary of findings of the nine included studies

Reference	Country	Population (*n*)	Study design	Method used for diagnosis and follow-up duration	Intervention	Outcome	Main results
Sabra *et al*[37]	Japan	113 pediatric LDLT	Retrospective	Doppler US twice daily till 1st week. If any PV complications were found, specific tests such as angiography were performed 1 yr of follow-up	PV reconstruction with VG (31 pts) PV reconstruction with EEA (82 pts)	Preoperative recipient factors PVT incidence Pt survival Graft survival	Global incidence PVT (2.6%) in the first 3 mo after OLT 1 PVT in 31 VGs *vs* 2 PVT in 82 without VGs No significant difference for PVT, Pt survival, Graft survival In the two groups
Julka *et al*[38]	Taiwan	87 pediatric LDLT	Retrospective	Routine doppler US post LT; CT angiography for HAT confirmation 5 yr of follow-up	HA reconstruction with two arterial stumps. 2 HA stumps with 2 HA reconstruction = 20 pts 2 HA stumps with 1 HA reconstruction = 22 pts 1 HA stump with 1 HA reconstruction = 45 pts	HAT incidence BC incidence	Overall HAT incidence 6.9% The incidence of HA thrombosis and biliary complications was similar in the three groups
Saad *et al*[39]	Japan	110 LDLT in pediatric pts	Retrospective LDLT	Doppler US, performed routinely before, during and after surgery Follow-up not defined	Different types of portal vein reconstructions Type 1: End- to- end anastomosis = 36 pts Type 2: Branch patch anastomosis = 27 pts Type 3: Anastomosis to the confluence (superior mesenteric vein-splenic vein) = 16 pts Type 4: Vein graft = 32 pts Chosen according to the surgical evaluation	C TC SC Survival rate	Type 1: 1 SC / 36 pts Type 2: 2 TC / 27 pts Type 3: 0 / 16 pts Type 4: 1 TC / 32 pts Overall survival rate 86%
Shackleton *et al*[31]	California	194 pediatric OLT for biliary atresia (mixed LDLT and DDLT)	Retrospective	Clinical suspect confirmed by angiography and/or surgical exploration. 3 yr of follow-up	Gr 1: Conventional artery reconstruction (*n* = 166) Gr 2: MHR (*n* = 28)	Risk factors for HAT Impact of MHR on incidence of HAT, need of re-OLT, patient and graft survival	Impact of MHR HAT incidence: Gr 1 32/166 (19%) *vs* Gr 2 0/28 (0%), *P* = 0.006 Re-OLT: Gr 1 31/166 (19%) *vs* Gr 2 1/28 (4%), *P* = 0.05 1 yr actuarial survival: Gr 1 81% *vs* Gr 2 100%, *P* = 0.02 (univariate analysis) BUT *P* = 0.076 in step wise Cox regression for patient survival
López *et al*[32]	Spain	104 OLT in 82 pediatric pts (mixed LDLT and DDLT)	Retrospective	Doppler US routinely and selective arteriography for confirmation. 3 yr of follow-up	Arterial revascularization technique: Gr 1 (*n* = 48) AhG Gr 2 (*n* = 56) EEA Chosen according to the surgical evaluation	HAT incidence Survival rate	HAT incidence Gr 1. (AhG): 6.25% Gr 2. (EEA): 8.92% (*P* not significant) Graft Survival rate (1 yr) 61.5% (AhG) *vs* 60% (EEA) (*P* < 0.05) Graft survival rate (5 yr): 77.5% (AhG) *vs* 75.1% (EEA) (*P* < 0.05)
Millis *et al*[33]	Illinois	66 pediatric LDLT and 48 pediatric cadaveric RLT	Retrospective	Doppler US every day for the first 3 d and at 1, 3, 6, 12, 18, and 24 mo after transplantation + angiography for confirmation 5 yr of follow-up	Portal anastomosis with venous graft conduit in LDLT Gr 1 (*n* = 18): Native reconstructed vein Gr 2 (*n* = 37): Cryopreserved iliac vein; Gr 3 (*n* = 11):	Incidence of PVC Graft survival Patient survival	Incidence PVC LDLT 33/66 (50%) *vs* RLT 4/48 (8%) *P* < 0.0001 Early PVT LDLT Gr 1: 6 (33%)[a] LDLT Gr 2: 3 (8%) LDLT Gr 3: 1 (9%) RLT: 2 (4%)

					Cryopreserved femoral vein		[a]$P < 0.005$ *vs* RLT Late PVC LDLT Gr 1: 3 (16%) LDLT Gr 2: 19 (51%)[a] LDLT Gr 3: 1 (9%) RLT: 2 (4%) [a]$P < 0.005$ *vs* RLT; $P < 0.02$ *vs* Gr 1 and Gr 3 Graft survival PVC: 61% No PVC: 67%, P = NS Patient survival: PVC: 67% No PVC: 71%, P = NS
Jurim *et al*[34]	California	35 pediatric OLT Emergency transplants only (type of donor not specified)	Retrospective	Not reported. Follow-up not defined	Gr 1: RLT = 7 pts Gr 2: Whole graft = 18 pts	HAT incidence Incidence of other complications: Biliary; bleeding; chronic rejection	HAT: Gr 1:0 (0%)/Gr 2:5 (29%) ($P < 0.05$) The incidence of biliary complications, bleeding (requiring surgical exploration) and chronic rejection were similar between the groups
Yandza *et al*[35]	France	143 DDLT in 122 pediatric pts	Retrospective	Doppler US daily the first 15 d, twice/wk until discharge Follow-up not defined	Gr 1 (n = 41 pts, n = 50 grafts) children < 10 kg Gr 2 (n = 81 pts, n = 93 grafts) children > 10 kg Surgical technique: EEA *vs* AhG	Effect of the site of liver graft arterial inflow on HAT incidence according to the recipient weight	Overall HAT incidence: 14/143 (10%) HAT incidence between the 2 groups: Gr 1: 6/50 (12%) *vs* Gr 2: 8/93 (9%), P not significant; Gr 1 EEA 5/31 (16%) *vs* Gr 1 AhG 1/19 (5%); P not significant Gr 2 EEA 4/60 (6%) *vs* Gr 2 AhG 4/32 (12%) P not significant
Stevens *et al*[36]	Chicago	134 OLT in 100 pediatric pts < 2 yr : mixed LDLT and DDLT	Retrospective	Doppler US, frequency not defined Follow-up	60 standard whole liver *vs* 74 RLT (13 LDLT) Surgical technique: Arterial inflow with 83 hepatic artery *vs* 32 celiac artery *vs* 5 supraceliac aorta *vs* 27 infrarenal aorta *vs* 7 unusual reconstruction	Effect of the graft type and site of arterial inflow on the Incidence of HAT	HAT incidence in 25% whole liver transplant *vs* 23% in LDLT *vs* 15% RLT (P = 0.06) Aortic anastomosis (supraceliac and infrarenal) reduces incidence of HAT (6% *vs* 24%, P = 0.02)

BA: Biliary atresia; BW: Body weight; CTA: CT angiography; Gr: Group; GRWR: Graft-to-recipient weight ratio; HAG: Hepatic artery graft; LT: Liver transplantation; OLT: Orthotopic liver transplantation; Pt: Patient; RLT: Reduced size liver transplantation; re-OLT: Re-transplantation; SC: Stenotic complication; TC: Thrombosis complication; US: Ultrasonography; LDLT: Living donor liver transplantation; PV: Portal vein; VG: Vein graft; EEA: End-to-end anastomosis; PVT: Portal vein thrombosis; HAT: Hepatic artery thrombosis; HA: Hepatic artery; BC: Biliary complications; C: Complications; MHR: Microsurgical hepatic arterial reconstruction; AhG: Aortohepatic interposition graft; PVC: Portal vein complications; DDLT: Deceased donor liver transplantation.

tric population; incidence of HAT varies from 0% to 29%[31,32,34-36,38].

Screening protocol for thrombosis detection

Most of the studies used Doppler ultrasonography (US) as a first line diagnostic screening for thrombosis[32,33,35-39]; frequency and duration of the screening is quite variable. Confirmation of the thrombosis detected by a second level diagnostic test, such as computer CT angiography, surgery or other methods is rarely specified[31-33,37,38].

Intraoperative surgical prophylaxis

Table 1 summarizes the main results of the studies analyzed. In LDLT there are four different modalities to perform portal vein anastomosis: (1) standard reconstruction with end to end anastomosis[37,39]; (2) reconstruction with anastomosis to the bifurcation of the recipient left and right vein[39]; (3) reconstruction with anastomosis to the confluence of the recipient mesenteric vein[39]; and (4) reconstruction with an interposition of vein graft[33,37,39]. The overall results of different techniques were similar. The choice of the type of reconstruction depended on the size (length and diameter) and quality of the portal vein and size mismatch between donor and recipient portal vein[33,37,39]. Millis *et al*[33] showed a low PVT incidence with reduced size liver transplant (RLT) [4%

Table 2 Risk of bias and quality of the studies

Reference	Study design	Consecutive enrolment	Thrombosis as pre-defined outcome	Methods use to the diagnosis of thrombosis
Shackleton *et al*[31]	Retrospective	Yes	Yes, HAT	Clinical grounds and angiography and/or surgical exploration for confirmation
López *et al*[32]	Retrospective	Yes	No	Doppler US and angiography for confirmation, post mortem second confirmation
Millis *et al*[33]	Retrospective	Yes	Yes, PVT	Doppler US and angiography for confirmation
Jurim *et al*[34]	Retrospective	Yes	Yes, HAT	Not reported
Yandza *et al*[35]	Retrospective	Yes	Yes, HAT	Doppler US
Stevens *et al*[36]	Retrospective	Yes	Yes, HAT	Doppler US
Sabra *et al*[37]	Retrospective	Yes	Yes, PVT	Doppler US
Julka *et al*[38]	Retrospective	Yes	No	Doppler US and angiography for confirmation
Saad *et al*[39]	Retrospective	Yes	Yes, PVT	Doppler US

PVT: Portal vein thrombosis; HAT: Hepatic artery thrombosis; US: Ultrasonography.

vs 33% with whole liver transplant (WLT) and native reconstructed vein, $P < 0.005$]; RLT was developed in attempt to resolve the mismatch size liver between donor and recipient and was applied to split liver transplantation and LDLT.

Three different surgical procedures seemed to reduce HAT incidence: RLT with cadaveric left lobe [incidence 0% *vs* 29% with WLT, $P < 0.05$[34]]; aortic arterial anastomosis without graft interposition [incidence 6% *vs* 24% in celiac-hepatic artery anastomosis, $P = 0.02$[36]] and microsurgical hepatic arterial reconstruction (MHR) [incidence 0% *vs* conventional artery reconstruction, $P = 0.006$[31]].

López *et al*[32] and Yandza *et al*[35] did not find significant HAT difference between end-to-end anastomosis and aortohepatic interposition graft; Julka *et al*[38] showed that single hepatic artery reconstruction did not increase the HAT incidence in pediatric LDLT having dual hepatic arterial stump in the liver graft.

Post-operative pharmacological prophylaxis

No studies on pharmacological prophylaxis compared clinical outcomes according to different treatments used[1,9,11-13,17,22-24,27].

DISCUSSION

Vascular thrombotic complications were a serious life-threatening complication in the first year of PLT with an incidence up to 42% associated with mortality up to 50%[13–15]. Although factors causing thrombotic complications are not fully understood[15], a global improvement of perioperative care has significantly decreased the thrombosis incidence in the last 20 years[1-3,6,7,9,11]. Several retrospective studies without control group tried to identify factors for thrombosis; among them should be mentioned medical factors, such as administration of fresh frozen plasma, elevated hematocrit, protein C deficiency[1,14,40,41] and surgical factors, such as cold ischemia time, technique of anastomosis, small vessel diameter, the use of aortic grafts, donor arterial anatomy and reconstruction[1,7,14,31,36], but without any definitive conclusions.

Accordingly, the aim of this systematic review was to identify evidence based methods both surgical and pharmacological for the prevention of thrombosis after PLT. In this systematic review, we found no prospective studies and only 9 retrospective studies with a control group referred to surgical prevention.

RLT (with left lobe or segment of left lobe)[34] direct aortic anastomosis and MHR[31] seem the best surgical options for reducing thrombotic complications in PLT, but the impact of RLT and aortic anastomosis on HAT were not confirmed by Stevens[36] and López-Yandza[32,35] respectively. MHR is an arterial reconstruction performed with an operating microscope; it was introduced by the Kyoto group for the fine graft arteries (less than 2 mm in diameter) in LDLT[42]. The amazing results of MHR (0 HAT in 28 PLT)[31] need to be confirmed in a larger clinical trial. It is worth noting that one of the most extensive studies about incidence and risk factors for vascular complication in liver transplantation was excluded from this systematic review because it included a mixed adult and pediatric population, without an appropriate control group[1].

Pharmacological prophylaxis is a relevant topic in PLT. Several studies[1,9,11-13,17,22-24,26] reported their experience using different drugs, such as unfractionated heparin, low molecular weight heparin, vitamin K antagonist fresh frozen plasma, aspirin, dipyridamole, antithrombin concentrate, dextran 40, thrombin inhibitor, prostaglandin. Unfortunately, in these studies there was not a comparator group, necessary in order to achieve formal proof of efficacy and safety and according to the inclusion criteria of this systematic review. In this regard, for example, aspirin is one of the most extensive drugs used for HAT prevention[11,14,17], but without formal evidence derived from prospective clinical trials.

The careful search of the literature and the inclusion of different types of studies are the main strengths of this review. Nevertheless, our study presents some weaknesses. First, the risk of thrombosis might have been underestimated because we assumed not all

authors systematically reported thrombotic events. Second, the description of methods for preventing vascular thromboses may be incomplete because only studies reporting the outcome were considered.

Although, HAT and PVT incidence has decreased in the last decades[1-3,6,7,9,11], they remain one of the more frequent and serious complications causing a poor outcome after PLT[1,2]. Furthermore, the old question "thrombosis after PLT - a medical or surgical event?"[14] remains an unresolved issue. Concerning this, our systematic review of studies, in which different prophylaxis strategies were tested for the prevention of HAT and PVT failed to provide enough evidence for a definitive conclusion due to the poor quality of studies found[31-39]. However, our analysis emphasizes the need of developing well-designed clinical studies in order to correctly determine PLT-associated thrombosis risk and to define an evidence-based antithrombotic prophylactic strategy. The recent "single ventricle trial"[43] showed that randomized clinical trials are possible also in the pediatric surgery area.

ARTICLE HIGHLIGHTS

Research background

Hepatic artery thrombosis (HAT) and portal vein thrombosis (PVT) commonly occur after pediatric liver transplantation (PLT) that may cause graft loss and patient death. Different surgical techniques and pharmacological prophylaxis have been purposed in several studies; nevertheless, there is not a standardized approach for thrombosis prevention in PLT.

Research motivation

Prevention of HAT and PVT remains very important for PLT outcome and it should be a matter of primary interest for clinicians and researchers, considering the ongoing scarcity of hepatic allografts.

Research objective

We performed a systematic review of current literature about surgical and pharmacological prophylaxis for prevention of thrombosis after PLT to evaluate the current evidence available.

Research methods

Studies were identified by electronic search of MEDLINE, EMBASE and Cochrane Library (CENTRAL) databases until March 2018. We excluded from this review case report, small case series, commentaries, conference abstracts, papers which describe less than 10 pediatric liver transplants/year and articles published before 1990. Two reviewers performed an independent study selection, solving any disagreements through discussion and the opinion of a third reviewer.

Research results

Nine retrospective studies were included in this review. They showed the use of tailored surgical strategies might be useful to reduce thrombosis. We did not find eligible studies evaluating pharmacological prevention strategies. The overall quality of studies was poor. A pooled analysis of results from studies was not possible due to the retrospective design and heterogeneity of included studies.

Research conclusions

This systematic review in which different prophylaxis strategies were tested for the prevention of HAT and PVT failed to provide enough evidence for a definitive conclusion due to the poor quality of studies found.

Research perspective

This systematic review showed there is no evidence based strategy for thrombosis prevention in PLT. Prospective studies are urgently needed. The recent "single ventricle trial" showed that randomized clinical trials are possible also in the pediatric surgery area.

ACKNOWLEDGMENTS

The authors acknowledge Dr. Squizzato A for his help in developing the search strategy and Ms. Anne Brown for language revision.

REFERENCES

1 **Duffy JP**, Hong JC, Farmer DG, Ghobrial RM, Yersiz H, Hiatt JR, Busuttil RW. Vascular complications of orthotopic liver transplantation: experience in more than 4,200 patients. *J Am Coll Surg* 2009; **208**: 896-903; discussion 903-905 [PMID: 19476857 DOI: 10.1016/j.jamcollsurg.2008.12.032]

2 **Kamath BM**, Olthoff KM. Liver transplantation in children: update 2010. *Pediatr Clin North Am* 2010; **57**: 401-414, table of contents [PMID: 20371044 DOI: 10.1016/j.pcl.2010.01.012]

3 **Goss JA**, Shackleton CR, McDiarmid SV, Maggard M, Swenson K, Seu P, Vargas J, Martin M, Ament M, Brill J, Harrison R, Busuttil RW. Long-term results of pediatric liver transplantation: an analysis of 569 transplants. *Ann Surg* 1998; **228**: 411-420 [PMID: 9742924 DOI: 10.1097/00000658-199809000-00014]

4 **Yilmaz A**, Arikan C, Tumgor G, Kilic M, Aydogdu S. Vascular complications in living-related and deceased donation pediatric liver transplantation: single center's experience from Turkey. *Pediatr Transplant* 2007; **11**: 160-164 [PMID: 17300495 DOI: 10.1111/j.1399-3046.2006.00601.x]

5 **Orlandini M**, Feier FH, Jaeger B, Kieling C, Vieira SG, Zanotelli ML. Frequency of and factors associated with vascular complications after pediatric liver transplantation. *J Pediatr* (Rio J) 2014; **90**: 169-175 [PMID: 24370174 DOI: 10.1016/j.jped.2013.08.010]

6 **Ooi CY**, Brandão LR, Zolpys L, De Angelis M, Drew W, Jones N, Ling SC, Fecteau A, Ng VL. Thrombotic events after pediatric liver transplantation. *Pediatr Transplant* 2010; **14**: 476-482 [PMID: 19849808 DOI: 10.1111/j.1399-3046.2009.01252.x]

7 **Spada M**, Riva S, Maggiore G, Cintorino D, Gridelli B. Pediatric liver transplantation. *World J Gastroenterol* 2009; **15**: 648-674 [PMID: 19222089 DOI: 10.3748/wjg.15.648]

8 **Unal B**, Gonultas F, Aydin C, Otan E, Kayaalp C, Yilmaz S. Hepatic artery thrombosis-related risk factors after living donor liver transplantation: single-center experience from Turkey. *Transplant Proc* 2013; **45**: 974-977 [PMID: 23622602 DOI: 10.1016/j.transproceed.2013.02.070]

9 **Corno V**, Torri E, Bertani A, Guizzetti M, Lucianetti A, Maldini G, Pinelli D, Zambelli M, Aluffi A, Alberti D, Spada M, Gridelli B, Torre G, Colledan M. Early portal vein thrombosis after pediatric split liver transplantation with left lateral segment graft. *Transplant Proc* 2005; **37**: 1141-1142 [PMID: 15848649 DOI: 10.1016/j.transproceed.2004.11.034]

10 **Heffron TG**, Emond JC, Whitington PF, Thistlethwaite JR Jr, Stevens L, Piper J, Whitington S, Broelsch CE. Biliary complications in pediatric liver transplantation. A comparison of reduced-size and whole grafts. *Transplantation* 1992; **53**: 391-395 [PMID: 1738934 DOI: 10.1097/00007890-199202010-00024]

11 **Bekker J**, Ploem S, de Jong KP. Early hepatic artery thrombosis after liver transplantation: a systematic review of the incidence, outcome and risk factors. *Am J Transplant* 2009; **9**: 746-757 [PMID: 19298450 DOI: 10.1111/j.1600-6143.2008.02541.x]

12 **Sieders E**, Peeters PM, TenVergert EM, de Jong KP, Porte RJ, Zwaveling JH, Bijleveld CM, Slooff MJ. Early vascular complications after pediatric liver transplantation. *Liver Transpl* 2000; **6**:

326-332 [PMID: 10827234 DOI: 10.1053/lv.2000.6146]
13 **Wozney P**, Zajko AB, Bron KM, Point S, Starzl TE. Vascular complications after liver transplantation: a 5-year experience. *AJR Am J Roentgenol* 1986; **147**: 657-663 [PMID: 3529892 DOI: 10.2214/ajr.147.4.657]
14 **Mazzaferro V**, Esquivel CO, Makowka L, Belle S, Kahn D, Koneru B, Scantlebury VP, Stieber AC, Todo S, Tzakis AG. Hepatic artery thrombosis after pediatric liver transplantation--a medical or surgical event? *Transplantation* 1989; **47**: 971-977 [PMID: 2472028 DOI: 10.1097/00007890-198906000-00011]
15 **Mazzaferro V**, Esquivel CO, Makowka L, Kahn D, Belle S, Kahn D, Scantlebury VP, Ferla G, Koneru B, Scotti-Foglieni CL. Factors responsible for hepatic artery thrombosis after pediatric liver transplantation. *Transplant Proc* 1989; **21**: 2466-2467 [PMID: 2652807]
16 **Warnaar N**, Polak WG, de Jong KP, de Boer MT, Verkade HJ, Sieders E, Peeters PM, Porte RJ. Long-term results of urgent revascularization for hepatic artery thrombosis after pediatric liver transplantation. *Liver Transpl* 2010; **16**: 847-855 [PMID: 20583091 DOI: 10.1002/lt.22063]
17 **Robertson JD**. Pediatric transplantation: preventing thrombosis. *J Thromb Haemost* 2015; **13** Suppl 1: S351-S361 [PMID: 26149047 DOI: 10.1111/jth.12968]
18 **Nacoti M**, Cazzaniga S, Colombo G, Corbella D, Fazzi F, Fochi O, Gattoni C, Zambelli M, Colledan M, Bonanomi E. Postoperative complications in cirrhotic pediatric deceased donor liver transplantation: Focus on transfusion therapy. *Pediatr Transplant* 2017; **21** [PMID: 28681471 DOI: 10.1111/petr.13020]
19 **Neto JS**, Pugliese R, Fonseca EA, Vincenzi R, Pugliese V, Candido H, Stein AB, Benavides M, Ketzer B, Teng H, Porta G, Miura IK, Baggio V, Guimaraes T, Porta A, Rodrigues CA, Carnevale FC, Carone E, Kondo M, Chapchap P. Four hundred thirty consecutive pediatric living donor liver transplants: variables associated with posttransplant patient and graft survival. *Liver Transpl* 2012; **18**: 577-584 [PMID: 22271646 DOI: 10.1002/lt.23393]
20 **Englesbe MJ**, Kelly B, Goss J, Fecteau A, Mitchell J, Andrews W, Krapohl G, Magee JC, Mazariegos G, Horslen S, Bucuvalas J. Reducing pediatric liver transplant complications: a potential roadmap for transplant quality improvement initiatives within North America. *Am J Transplant* 2012; **12**: 2301-2306 [PMID: 22883313 DOI: 10.1111/j.1600-6143.2012.04204.x]
21 **Nacoti M**, Corbella D, Fazzi F, Rapido F, Bonanomi E. Coagulopathy and transfusion therapy in pediatric liver transplantation. *World J Gastroenterol* 2016; **22**: 2005-2023 [PMID: 26877606 DOI: 10.3748/wjg.v22.i6.2005]
22 **McLin VA**, Rimensberger P, Belli DC, Wildhaber BE. Anticoagulation following pediatric liver transplantation reduces early thrombotic events. *Pediatr Transplant* 2011; **15**: 117-118 [PMID: 21159111 DOI: 10.1111/j.1399-3046.2010.01426.x]
23 **Quintero J**, Ortega J, Miserachs M, Bueno J, Bilbao I, Charco R. Low plasma levels of antithrombin III in the early post-operative period following pediatric liver transplantation: should they be replaced? A single-center pilot study. *Pediatr Transplant* 2014; **18**: 185-189 [PMID: 24438318 DOI: 10.1111/petr.12217]
24 **Hardikar W**, Poddar U, Chamberlain J, Teo S, Bhat R, Jones B, Ignjatovic V, Campbell J, Newall F, Monagle P. Evaluation of a post-operative thrombin inhibitor replacement protocol to reduce haemorrhagic and thrombotic complications after paediatric liver transplantation. *Thromb Res* 2010; **126**: 191-194 [PMID: 20541794 DOI: 10.1016/j.thromres.2010.05.015]
25 **Uchida Y**, Sakamoto S, Egawa H, Ogawa K, Ogura Y, Taira K, Kasahara M, Uryuhara K, Takada Y, Kamiyama Y, Tanaka K, Uemoto S. The impact of meticulous management for hepatic artery thrombosis on long-term outcome after pediatric living donor liver transplantation. *Clin Transplant* 2009; **23**: 392-399 [PMID: 19191812 DOI: 10.1111/j.1399-0012.2008.00924.x]
26 **Borst AJ**, Sudan DL, Wang LA, Neuss MJ, Rothman JA, Ortel TL. Bleeding and thrombotic complications of pediatric liver transplant. *Pediatr Blood Cancer* 2018; **65**: e26955 [PMID: 29350493 DOI: 10.1002/pbc.26955]
27 **Haberal M**, Sevmis S, Karakayali H, Moray G, Ozcay F, Torgay A, Arslan G. Outcome of pediatric liver transplant in grafts with multiple arteries. *Pediatr Transplant* 2008; **12**: 407-411 [PMID: 18266797 DOI: 10.1111/j.1399-3046.2008.00888.x]
28 **Burroughs AK**, Sabin CA, Rolles K, Delvart V, Karam V, Buckels J, O'Grady JG, Castaing D, Klempnauer J, Jamieson N, Neuhaus P, Lerut J, de Ville de Goyet J, Pollard S, Salizzoni M, Rogiers X, Muhlbacher F, Garcia Valdecasas JC, Broelsch C, Jaeck D, Berenguer J, Gonzalez EM, Adam R; European Liver Transplant Association. 3-month and 12-month mortality after first liver transplant in adults in Europe: predictive models for outcome. *Lancet* 2006; **367**: 225-232 [PMID: 16427491 DOI: 10.1016/S0140-6736(06)68033-1]
29 **Rana A**, Pallister Z, Halazun K, Cotton R, Guiteau J, Nalty CC, O' Mahony CA, Goss JA. Pediatric Liver Transplant Center Volume and the Likelihood of Transplantation. *Pediatrics* 2015; **136**: e99-e107 [PMID: 26077479 DOI: 10.1542/peds.2014-3016]
30 **Stroup DF**, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, Moher D, Becker BJ, Sipe TA, Thacker SB. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. *JAMA* 2000; **283**: 2008-2012 [PMID: 10789670 DOI: 10.1001/jama.283.15.2008]
31 **Shackleton CR**, Goss JA, Swenson K, Colquhoun SD, Seu P, Kinkhabwala MM, Rudich SM, Markowitz JS, McDiarmid SV, Busuttil RW. The impact of microsurgical hepatic arterial reconstruction on the outcome of liver transplantation for congenital biliary atresia. *Am J Surg* 1997; **173**: 431-435 [PMID: 9168083 DOI: 10.1016/S0002-9610(97)00066-4]
32 **López SM**, Vazquez J, Gamez M, Murcia J, Bueno J, Martinez L, Paz Cruz JA, Reinoso F, Bourgeois P, Diaz MC, Hierro L, Camarena C, de la Vega A, Frauca E, Jara P, Tovar JA. Donor vascular grafts for arterial reconstruction in pediatric liver transplantation. *J Pediatr Surg* 1996; **31**: 600-603 [PMID: 8801323 DOI: 10.1016/S0022-3468(96)90506-0]
33 **Millis JM**, Seaman DS, Piper JB, Alonso EM, Kelly S, Hackworth CA, Newell KA, Bruce DS, Woodle ES, Thistlethwaite JR, Whitington PF. Portal vein thrombosis and stenosis in pediatric liver transplantation. *Transplantation* 1996; **62**: 748-754 [PMID: 8824471 DOI: 10.1097/00007890-199609270-00008]
34 **Jurim O**, Csete M, Gelabert HA, Millis JM, Olthoff K, Imagawa D, Shaked A, McDiarmid SV, Busuttil RW. Reduced-size grafts--the solution for hepatic artery thrombosis after pediatric liver transplantation? *J Pediatr Surg* 1995; **30**: 53-55 [PMID: 7722830 DOI: 10.1016/0022-3468(95)90609-6]
35 **Yandza T**, Hamada H, Gauthier F, Pariente D, Lababidi A, de Dreuzy O, Valayer J. Pediatric liver transplantation: effect of the site of arterial inflow on the incidence of hepatic artery thrombosis according to recipient weight. *Transplant Proc* 1994; **26**: 169-170 [PMID: 8108923]
36 **Stevens LH**, Emond JC, Piper JB, Heffron TG, Thistlethwaite JR Jr, Whitington PF, Broelsch CE. Hepatic artery thrombosis in infants. A comparison of whole livers, reduced-size grafts, and grafts from living-related donors. *Transplantation* 1992; **53**: 396-399 [PMID: 1738935 DOI: 10.1097/00007890-199202010-00025]
37 **Sabra TA**, Okajima H, Yoshizawa A, Okamoto T, Anazawa T, Ygi S, Hata K, Yasuchika K, Taura K, Hatano E, Kaido T, Uemoto S. Portal vein reconstruction using vein grafts in pediatric living donor liver transplantation: Current status. *Pediatr Transplant* 2017; **21** [PMID: 28111865 DOI: 10.1111/petr.12888]
38 **Julka KD**, Lin TS, Chen CL, Wang CC, Komorowski AL. Reconstructing single hepatic artery with two arterial stumps: biliary complications in pediatric living donor liver transplantation. *Pediatr Surg Int* 2014; **30**: 39-46 [PMID: 24292409 DOI: 10.1007/s00383-013-3436-z]
39 **Saad S**, Tanaka K, Inomata Y, Uemoto S, Ozaki N, Okajima H, Egawa H, Yamaoka Y. Portal vein reconstruction in pediatric liver transplantation from living donors. *Ann Surg* 1998; **227**: 275-281 [PMID: 9488527 DOI: 10.1097/00000658-199802000-00018]
40 **Harper PL**, Edgar PF, Luddington RJ, Seaman MJ, Carrell RW,

Salt AT, Barnes N, Rolles K, Calne RY. Protein C deficiency and portal thrombosis in liver transplantation in children. *Lancet* 1988; **2**: 924-927 [PMID: 2902380 DOI: 10.1016/S0140-6736(88)92597-4]

41 **Hesselink EJ**, Klompmaker IJ, Grond J, Gouw AS, van Schilfgaarde R, Sloof MJ. Hepatic artery thrombosis (HAT) after orthotopic transplantation (OLT)--the influence of technical factors and rejection episodes. *Transplant Proc* 1989; **21**: 2468 [PMID: 2652808]

42 **Inomoto T**, Nishizawa F, Sasaki H, Terajima H, Shirakata Y, Miyamoto S, Nagata I, Fujimoto M, Moriyasu F, Tanaka K, Yamaoka Y. Experiences of 120 microsurgical reconstructions of hepatic artery in living related liver transplantation. *Surgery* 1996; **119**: 20-26 [PMID: 8560381 DOI: 10.1016/S0039-6060(96)80208-X]

43 **Ohye RG**, Sleeper LA, Mahony L, Newburger JW, Pearson GD, Lu M, Goldberg CS, Tabbutt S, Frommelt PC, Ghanayem NS, Laussen PC, Rhodes JF, Lewis AB, Mital S, Ravishankar C, Williams IA, Dunbar-Masterson C, Atz AM, Colan S, Minich LL, Pizarro C, Kanter KR, Jaggers J, Jacobs JP, Krawczeski CD, Pike N, McCrindle BW, Virzi L, Gaynor JW; Pediatric Heart Network Investigators. Comparison of shunt types in the Norwood procedure for single-ventricle lesions. *N Engl J Med* 2010; **362**: 1980-1992 [PMID: 20505177 DOI: 10.1056/NEJMoa0912461]

P- Reviewer: Mikulic D, Morimatsu H, Tchilikidi KY
S- Editor: Ji FF **L- Editor**: A **E- Editor**: Song H

World Journal of Hepatology

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World J Hepatol 2018 October 27; 10(10): 761-771

DOI: 10.4254/wjh.v10.i10.761

ISSN 1948-5182 (online)

META-ANALYSIS

Liver transplantation and atrial fibrillation: A meta-analysis

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Conflict-of-interest statement: The authors deny any conflict of interest.

PRISMA 2009 Checklist statement: The authors have read the PRISMA 2009 Checklist, and the manuscript was prepared and revised according to the PRISMA 2009 Checklist.

Manuscript source: Invited manuscript

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Received: May 2, 2018
Peer-review started: May 3, 2018
First decision: May 17, 2018
Revised: June 24, 2018
Accepted: June 28, 2018
Article in press: June 29, 2018
Published online: October 27, 2018

Abstract

AIM

To assess prevalence of pre-existing atrial fibrillation (AF) and/or incidence of AF following liver transplantation, and the trends of patient's outcomes overtime; to evaluate impact of pre-existing AF and post-operative AF on patient outcomes following liver transplantation.

METHODS

A literature search was conducted utilizing MEDLINE, EMBASE and Cochrane Database from inception through

March 2018. We included studies that reported: (1) prevalence of pre-existing AF or incidence of AF following liver transplantation; or (2) outcomes of liver transplant recipients with AF. Effect estimates from the individual study were extracted and combined utilizing random-effect, generic inverse variance method of DerSimonian and Laird. The protocol for this meta-analysis is registered with PROSPERO (International Prospective Register of Systematic Reviews, No. CRD42018093644).

RESULTS

Twelve observational studies with a total of 38586 liver transplant patients were enrolled. Overall, the pooled estimated prevalence of pre-existing AF in patients undergoing liver transplantation was 5.4% (95%CI: 4.9%-5.9%) and pooled estimated incidence of AF following liver transplantation was 8.5% (95%CI: 5.2%-13.6%). Meta-regression analyses were performed and showed no significant correlations between year of study and either prevalence of pre-existing AF (P = 0.08) or post-operative AF after liver transplantation (P = 0.54). The pooled OR of mortality among liver transplant recipients with pre-existing AF was 2.34 (2 studies; 95%CI: 1.10-5.00). In addition, pre-existing AF is associated with postoperative cardiovascular complications among liver transplant recipients (3 studies; OR: 5.15, 95%CI: 2.67-9.92, I^2 = 64%). With limited studies, two studies suggested significant association between new-onset AF and poor clinical outcomes including mortality, cerebrovascular events, post-transplant acute kidney injury, and increased risk of graft failure among liver transplant recipients (P < 0.05).

CONCLUSION

The overall estimated prevalence of pre-existing AF and incidence of AF following liver transplantation are 5.4% and 8.5%, respectively. Incidence of AF following liver transplant does not seem to decrease overtime. Pre-existing AF and new-onset AF are potentially associated with poor clinical outcomes post liver transplantation.

Key words: Atrial fibrillation; Liver; Hepatic; Transplant; Transplantation; Systematic reviews; Meta-analysis

Core tip: Atrial fibrillation (AF) occurs in a substantial number of postoperative and post-transplantation patients. In addition, postoperative AF confers both short-term and long-term morbidity and mortality in liver transplant patients. However, the incidence of postoperative AF in patients undergoing liver transplantation and its impacts remain unclear. To further investigate, we conducted a meta-analysis to assess the rates of preexisting AF and AF following liver transplantation as well as the outcomes of liver transplant patients with AF. Incidence of AF following liver transplant does not seem to decrease overtime. Pre-existing AF and new-onset AF are potentially associated with poor clinical outcomes post liver transplantation.

Chokesuwattanaskul R, Thongprayoon C, Bathini T, Ungprasert P, Sharma K, Wijarnpreecha K, Pachariyanon P, Cheungpasitporn W. Liver transplantation and atrial fibrillation: A meta-analysis. *World J Hepatol* 2018; 10(10): 761-771 Available from: URL: http://www.wjgnet.com/1948-5182/full/v10/i10/761.htm DOI: http://dx.doi.org/10.4254/wjh.v10.i10.761

INTRODUCTION

Atrial fibrillation (AF) is one of the most common heart diseases, affecting 3 to 6 million populations in the United States, almost 30 million people worldwide, which is expected to reach 50 million peoples worldwide in 2050[1-4]. Patients with AF carry a higher risk of adverse cardiovascular events and reduced survival[5,6]. Incidence of AF increases with age. At the same time, aging population is likely to develop other chronic diseases and one of them is end-stage liver disease or cirrhosis[7-9]. This treatment of cirrhosis comprises of multidisciplinary approach ranging from very simple, symptomatic treatment with diuretic or treatment of primary cause, down the road to the most advanced treatment; liver transplantation[10-13].

Liver transplantation is the treatment of choice for end-stage liver diseases[10,13]. In 2017, around 8000 patients all over the United State suffered from end-stage liver disease receiving liver transplantation and the number trends to increase 3% to 5% annually in the past 20 years along with the excellent outcomes with almost 95% survival rate at 1-year post-procedure and some patients could live even more than 30 years after liver transplantation[14-17]. Recent advances in basic and clinical sciences, including surgical technique, immunosuppressive therapy and postoperative supportive care, have led to the substantial improvement in quality of life and survival after liver transplantation[18,19]. In addition, higher risk patients tend to receive transplantation in a higher proportion than they did before. In the view of higher risk patients, they tend to carry the risk factors that accompany with older age such as cardiovascular diseases.

In transplant centers, AF and liver transplantation are entities that we commonly encounter in the practice[20-23]. However, the occurrence rates of preexisting AF and AF following liver transplantation as well as clinical outcomes of liver transplant patients with AF remain unclear[20-31]. Thus, we conduct this meta-analysis: (1) to assess prevalence of pre-existing AF and/or incidence of AF following liver transplantation, and the trends of patient's outcomes overtime; and (2) to evaluate impact of pre-existing AF and post-operative AF on patient outcomes following liver transplantation.

MATERIALS AND METHODS

Search strategy and literature review

We registered this systematic review protocol with International Prospective Register of Systematic Reviews, No. CRD42018093644 (PROSPERO). We conducted a systematic literature search of EMBASE (between January 1988 and March 2018), Ovid MEDLINE (between January 1946 and March 2018), and the Cochrane Database of Systematic Reviews (from database inception to March 2018): (1) to estimate prevalence of pre-existing AF and/or incidence of AF following liver transplantation; and (2) to evaluate impact of pre-existing AF and post-operative AF on patient outcomes following liver transplantation. Ronpichai Chokesuwattanaskul and Charat Thongprayoon, two investigators, independently performed the systematic literature review using the search strategy that consolidated the terms of "liver" OR "hepatic" AND "transplant" OR "transplantation" AND "atrial fibrillation", described in online supplementary data 1. No language restriction was implemented. We followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE)[32] and the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement[33].

Study selection

Our inclusion criteria comprised: (1) clinical trials or observational studies such as cohort, cross-sectional, or case-control studies; (2) available data on prevalence of pre-existing AF or incidence of AF following liver transplantation or outcomes of liver transplant recipients with AF; and (3) available data on prevalence, incidence, odds ratios (OR), hazard ratios, or relative risks. Retrieved articles were individually reviewed for eligibility by the two investigators as mentioned prior. Inclusion was not restricted by the size of study. Contrarieties were discussed and solved through joint agreement. We used Newcastle-Ottawa quality assessment scale to assess the quality of study for cohort and case-control studies[34], as shown in Table 1.

Data items and data collection process

We used a structured information collecting form to collect the data from individual article including last name of the first investigator, title, year of publication, country that the research was carried out, baseline characteristics of liver transplant patients, processes utilized to diagnose AF, prevalence of pre-existing AF, incidence of post-operative AF, patient outcomes following liver transplantation.

Statistical analysis

We used Comprehensive Meta-Analysis Version 3.3.070 software (Biostat Inc., Englewood, NJ, United States) for all analyses. Estimated prevalence, incidence and estimated risks from each study were incorporated by the random-effect, generic inverse-variance approach of DerSimonian and Laird[35]. Given the possibility of between-study variance, we used a random-effect model rather than a fixed-effect model. Cochran's *Q* test and I^2 statistic were implemented to assess heterogeneity caused by between-study differences. I^2 values of 0%-25% indicate insignificant heterogeneity. I^2 values of 26%-50% indicate low heterogeneity. I^2 values of 51%-75% indicate moderate heterogeneity and I^2 value of 76%-100% indicate high heterogeneity[36]. Egger test was used to evaluate publication bias[37].

RESULTS

Study selection and characteristics

Applying our search strategy, 121 potential studies were selected. Following the elimination of 83 studies (title and abstract clearly not meeting inclusion criteria due to study design, type of study, patient population or reported outcomes), 38 studies were included for complete examination. After the complete review, twenty articles were omitted because the outcome of interest was not provided and six articles were excluded since they were descriptive studies without data of interest. Hence, we included 12 articles[20-31] into the final analysis including 9 cohort studies[20,22-24,26-30] and 3 case-control studies[21,25,31] with 38586 liver transplant recipients were enrolled, as demonstrated in Figure 1. Study characteristics and quality appraisal of studies are shown in Table 1[20-31].

Prevalence of pre-existing AF and incidence of AF following liver transplantation

Overall, the pooled estimated prevalence of pre-existing AF in patients undergoing liver transplantation was 5.4% [95% confidence intervals (CI): 4.9%-5.9%, I^2 = 66%, Figure 2]. The pooled estimated prevalence of pre-existing AF in patients undergoing liver transplantation was 5.4% (95%CI: 4.4%-6.5%, I^2 = 8%) in case-control studies and 5.4% (95%CI: 4.9%-6.0%, I^2 = 75) in cohort studies, respectively, when analysis was conducted based on type of study. The pooled estimated incidence of AF following liver transplantation was 8.5% (95%CI: 5.2%-13.6%, I^2 = 99%, Figure 3). When analysis was performed based on type of study, the pooled estimated incidence of AF following liver transplantation was 9.4% (95%CI: 5.5%-15.6%, I^2= 73%) in case-control studies and 5.3% (95%CI: 1.6%-16.3%, I^2= 99%) in cohort studies, respectively.

Meta-regression analyses were performed and showed no significant correlations between year of study and either prevalence of pre-existing AF (P = 0.08) or post-operative AF after liver transplantation (P = 0.54), as shown in Figures 4 and 5.

Outcomes of liver transplant recipients with AF

Data on the association between pre-existing AF and the risk of mortality were limited in two studies[20,21].

Table 1 Main characteristic of studies included in meta-analysis of atrial fibrillation and liver transplantation

	Fouad *et al*[24]	VanWagner *et al*[25]	Nicalau-Raducu *et al*[26]	Josefsson *et al*[27]
Country	Canada	United States	United States	Sweden
Study design	Retrospective Cohort	Case-Control	Retrospective Cohort	Retrospective Cohort
Yr	2009	2012	2014	2014
Total number	197	242	389	186
Mean age ± SD	56	55	55	52
Duration (yr)	6 mo	1 yr	3.4	4
Outcome definition	Cardiac complication after LTx	CV complication after LTx	Early (< 1 yr) and Late (> 1 yr) post LTx AF	Incident cardiac event post LTx
Outcome ascertainment	Review EKG in medical records	EKG, Echo, LHC, RHC, DSE as indicated	Review medical records	Review medical records
Incidence of pre-operative AF	NA	All 12/242 (5.0%) NASH 7/115 (6.1%) Alcohol 5/127 (3.9%)	NA	Atrial fibrillation/flutter 4/186 (2.2%)
Incidence of post-operative AF	Intraoperative 1/197 (0.5%) Early postoperative (0-30 d) 3/197 (1.5%) Late postoperative (1-6 mo) 2/197 (1.0%)	All 21/242 (8.7%) NASH 11/115 (9.6%) Alcohol 10/127 (7.9%)	All 12/389 (3.1%) Early (< 1 yr after transplant) 10/389 (2.6%) Late (> 1 yr after transplant) 2/389 (0.5%)	Arrhythmia (mainly AF or flutter) All 36/186 (19.4%) Peri-transplant 24/186 (12.9%) Late 12/186 (6.5%)
Outcomes	NA (study aim to identify predictor of cardiac complication 6 mo after LTX)	NA (study aim to compare CV event between liver disease before liver transplant)	NA (study demonstrated target DSE prior liver transplant associated with increased risk of AF)	NA (study aim to assess pretransplant EKG as a predictor of post liver transplant event)
Confounder adjustment	NA	NA	NA	NA
Newcastle-Ottawa scale	S3 C0 O3	S4 C2 O3	S3 C3 O3	S4 C2 O3
	Vannucci *et al*[20]	**Bargehr *et al*[21]**	**Xia *et al*[22]**	**Piazza *et al*[23]**
Country	United States	United States	United States	Italy
Study design	Retrospective Cohort	Case-Control study	Retrospective Cohort	Retrospective Cohort
Yr	2014	2015	2015	2016
Total number	757	717	1387	143
Mean age ± SD	57.9 ± 6.8	58	54	55
Duration (yr)	1 yr	NA	30 d	3
Outcome definition	30 d and 1-yr survival after Liver Tx.	Cardiac complication after LTX	POAF (postoperative AF in LTX)	Incident AF (also other CVE) in NASH and alcoholic s/p LTx
Outcome ascertainment	Medical records	Review Medical records	EKG, Holter and medical records	Review medical records
Incidence of pre-operative AF	19/757 (2.5%)	32/717 (4.5%)	77/1387 (5.6%)	Alcoholic cirrhosis 2/65 (3.1%) NASH cirrhosis 3/78 (3.8%)
Incidence of post-operative AF	NA	1/63 (1.6%)	New onset AF within 30 d after LT 102/1387 (7.4%)	2/143 (1.4%)
Outcomes	1-mo mortality 5.29 (1.73-16.18) 1-yr mortality 3.28 (1.63-6.59)	Intraoperative cardiac complications 7.83 (1.94-31.49) Mortality 1.50 (0.61-3.69)	Median Hospital stays 31 d (16-67) in POAF *vs* 20 d (12-37) AKI 2.5 (1.06-5.70) Mortality 2.36 (1.45-3.85) Graft failure 2.28 (1.44-3.59)	NA (study aim to compare outcome as CV event after liver transplant between patients with NASH and those with alcoholic cirrhosis who receive liver transplant)
Confounder adjustment	NA	Age, MELD, donor risk index, DM	Age, MELD, intraoperative blood transfusion	NA
Newcastle-Ottawa scale	S3 C0 O3	S4 C2 E3	S4 C2 O3	S4 C2 O3

	VanWagner *et al*[28]	VanWagner *et al*[31]	VanWagner *et al*[29]	Wange *et al*[30]
Country	United States	United States	United States	Sweden
Study design	Retrospective Cohort	Case Control	Retrospective Cohort	Retrospective Cohort
Yr	2016	2017	2018	2018
Total number	32810	1024	671	63
Mean age ± SD	55 ± 10	56	Various by renal disease classification group	45
Duration (yr)	90 d	1 yr	NA	10
Outcome definition	MACE after Liver transplantation	CVD complication *vs* No CVD complication group	1-yr CV complication	Incident AF post LTx who survive > 3 yr (LTx ATTRm amyloidosis)
Outcome ascertainment	Medical record in patient admitted by MACE	EKG, Holter and medical records	Medical record	Echo and Holter every visit
Incidence of pre-operative AF	1969/32810 (6.0%)	62/1024 (6.1%)	2145/37322 (5.7%)	1/63 (1.6%)
Incidence of post-operative AF	204/32810 (0.6%)	130/1024 (12.7%)	65/671 (9.7%)	Incident AF 20/63 (31.7%) All AF post-op 21/63 (33.3%) (Median diagnosis 2 yr)
Outcomes	Pre-transplant AF and 30-d MACE (MI, HF, AF, cardiac arrest, PE, stroke) 6.9 (5.0-9.6) Pre-transplant AF and 90-d MACE 6.1 (4.5-8.3)	Pre-transplant AF and CVD complication 8.96 (3.70-22.0)	NA (study aim to assess degree of renal disease to 1-yr CV outcome in liver transplant patient)	Cerebrovascular events (TIA, ischemic stroke, intracerebral hemorrhage, subarachnoid hemorrhage) 3.8 (1.1-9.5)
Confounder adjustment	Sex, age, history of stroke, type of cirrhosis, and pre-transplant creatinine	Age, sex, race, working status, education, respiratory failure on ventilator at transplant, pulmonary hypertension, HCC, hypertension, DM, heart failure	NA	Cardiomyopathy, ischemic heart disease
Newcastle-Ottawa scale	S3 C0 O3	S4 C2 E3	S4 C2 O3	S3 C0 O3

ATTRm: Amyloid-forming variant Transthyretin proteins; CVD: Cardiovascular disease; HCC: Hepatocellular carcinoma; HF: Heart failure; MACE: Major adverse cardiovascular event; MI: Myocardial infarction; AF: Atrial fibrillation; DSE: Dobutamine stress echocardiogram; EKG: Electrocardiogram; LTX: Liver transplantation; NASH: Nonalcoholic steatohepatitis; NA: Not available; RA: Right atrium; S: Selection; C: Comparability; O: Outcome; AKI: Acute kidney injury; POAF: Post-operative atrial fibrillation; MELD: Model for End Stage Liver Disease.

The pooled OR of mortality among liver transplant recipients was 2.34 (95%CI: 1.10-5.00, I^2 = 45%). In addition, pre-existing AF is associated with postoperative cardiovascular complications among liver transplant recipients (3 studies[21,28,31]; OR: 5.15, 95%CI: 2.67-9.92, I^2 = 64%). New onset AF is associated with poor outcomes after liver transplantation[22,30]. Wange *et al*[30] demonstrated a significant association between incident AF and cerebrovascular events in liver transplant patients with OR of 3.80 (95%CI: 1.10-9.50). In addition to increased mortality risk, Xia *et al*[22] demonstrated significant associations of new-onset AF with post-transplant acute kidney injury (OR: 2.50, 95%CI: 1.06-5.70), and increased risk of graft failure (OR: 2.28, 95%CI: 1.44-3.59) among liver transplant recipients.

Risk of bias across studies

Funnel plots, as demonstrated in Supplementary Figures 1 and 2, and Egger tests were conducted to assess for possibility of publication bias in analyses evaluating prevalence of pre-existing AF and incidence of postoperative AF in liver transplant patients, respectively. The graph is somewhat asymmetric and implies the possibility of publication bias towards negative studies in analysis of prevalence of pre-existing AF (P = 0.01). However, we found no significant publication bias in analysis evaluating incidence of postoperative AF in liver transplant patients, P = 0.32.

DISCUSSION

In this meta-analysis, we demonstrated that end stage liver disease patients who received liver transplantation had a prevalence of AF of 5.6%, which was higher than prevalence of AF in general patient population of 2.5%[38]. This number of higher prevalence may imply that patients who received liver transplantation appeared to carry the higher risk profiles. In addition, our study showed the pooled incidence of post-liver transplant AF of 8.5%, which is lower incidence, when compared to those patients who underwent heart transplantation (incidence of AF up to 40%)[39-44] or other open-heart surgeries (incidence of AF up to 50%)[5,45,46].

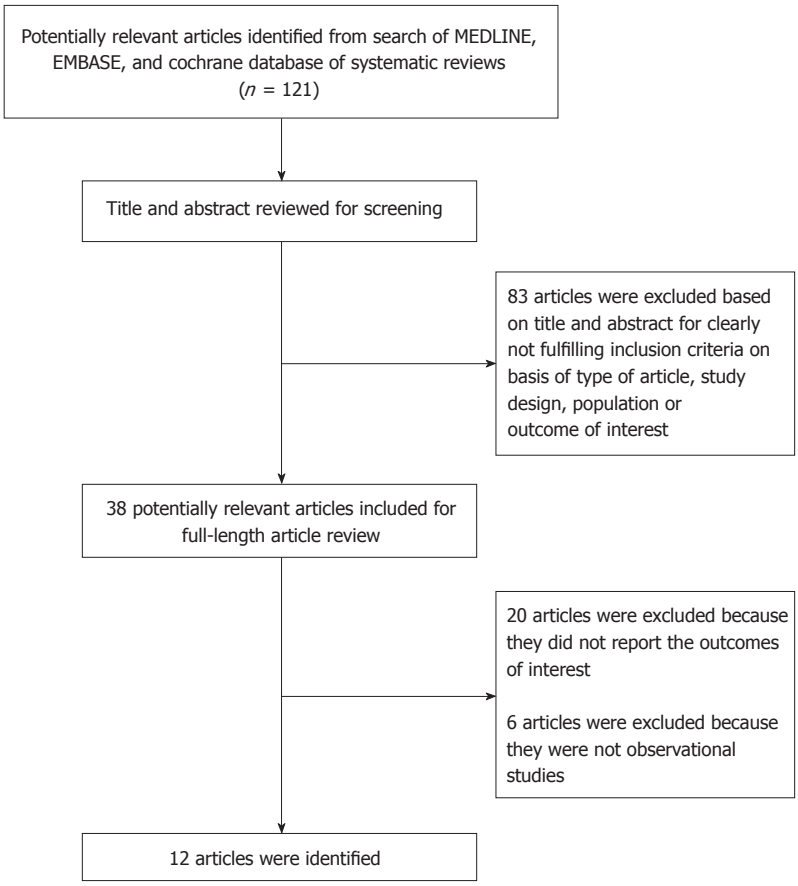

Figure 1 Outline of our search methodology.

Group by Type of study	Study name	Event rate	Lower limit	Upper limit	Z-Value	*P*-Value	Relative weight
Case-Control	Bargehr *et al*	0.045	0.032	0.062	-16.940	0.000	31.66
Case-Control	VanWagner *et al* (1)	0.050	0.028	0.085	-9.973	0.000	12.51
Case-Control	VanWagner *et al* (3)	0.061	0.047	0.077	-20.926	0.000	55.83
Case-Control		0.054	0.044	0.065	-26.917	0.000	
Cohort	Josefsson *et al*	0.022	0.008	0.056	-7.553	0.000	1.32
Cohort	Piazza	0.035	0.015	0.081	-7.288	0.000	1.61
Cohort	Vannucci	0.025	0.016	0.039	-15.750	0.000	5.55
Cohort	VanWagner *et al* (2)	0.060	0.057	0.063	-118.365	0.000	37.75
Cohort	VanWagner *et al* (4)	0.057	0.055	0.060	-125.774	0.000	37.94
Cohort	Wange *et al*	0.016	0.002	0.104	-4.094	0.000	0.34
Cohort	Xia *et al*	0.056	0.045	0.069	-24.168	0.000	15.50
Cohort		0.054	0.049	0.060	-48.512	0.000	
Overall		0.054	0.049	0.059	-55.479	0.000	

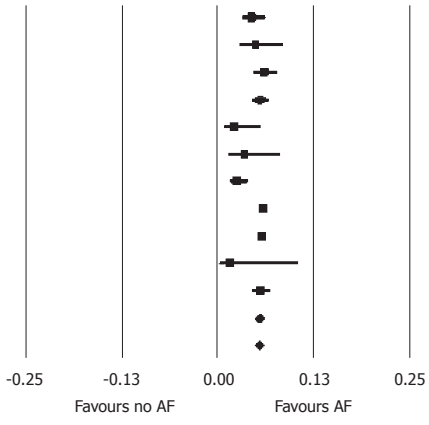

Figure 2 Forest plots of the included studies assessing prevalence of pre-existing atrial fibrillation in patients undergoing liver transplantation. AF: Atrial fibrillation.

This mitigated number of incidence of postoperative AF in liver transplantation could be explained by the use of intensive postoperative hemodynamic care and, immunosuppressive therapy, the surgical technique, and not physically direct impact to the heart[28-31].

In general population, AF can put the patients at higher mortality risk, compared to those without AF[47]. In addition to mortality risk, our study also revealed the association of pre-existing AF and incident AF with poor clinical outcomes following liver transplantation. New-onset AF following liver transplantation is also associated with post-transplant acute kidney injury, cerebrovascular events, and increased risk of graft failure among liver transplant recipients. There are several mechanisms that put the liver transplant patients with AF at higher risk of postoperative morbidity and mortality compared

Group by Type of study	Study name	Statistics for each study					Event rate and 95%CI	Relative weight
		Event rate	Lower limit	Upper limit	Z-Value	*P*-Value		
Case-Control	Bargehr *et al*	0.016	0.002	0.104	-4.094	0.000		7.32
Case-Control	VanWagner *et al* (1)	0.087	0.057	0.129	-10.307	0.000		41.00
Case-Control	VanWagner *et al* (3)	0.127	0.108	0.149	-20.542	0.000		51.69
Case-Control		0.094	0.055	0.156	-7.724	0.000		
Cohort	Fouad *et al*	0.025	0.011	0.060	-8.053	0.000		12.15
Cohort	Josefsson *et al*	0.194	0.143	0.257	-7.690	0.000		12.79
Cohort	Nicolau-raducu *et al*	0.031	0.018	0.054	-11.756	0.000		12.59
Cohort	Piazza *et al*	0.014	0.004	0.054	-5.976	0.000		11.15
Cohort	VanWagner *et al* (2)	0.006	0.005	0.007	-72.247	0.000		12.91
Cohort	VanWagner *et al* (4)	0.097	0.077	0.122	-17.105	0.000		12.86
Cohort	Wange *et al*	0.333	0.228	0.458	-2.594	0.009		12.65
Cohort	Xia *et al*	0.074	0.061	0.089	-24.629	0.000		12.89
Cohort		0.053	0.016	0.163	-4.518	0.000		
Overall		0.085	0.052	0.136	-8.903	0.000		

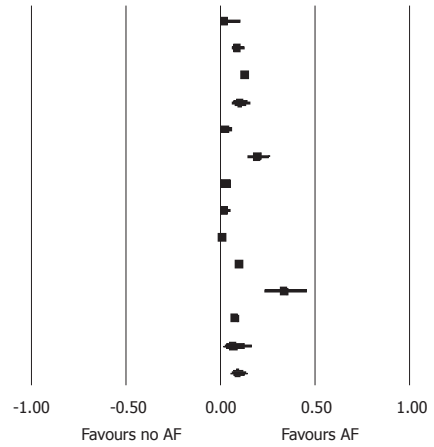

Figure 3 Forest plots of the included studies assessing incidence of atrial fibrillation following liver transplantation. AF: Atrial fibrillation.

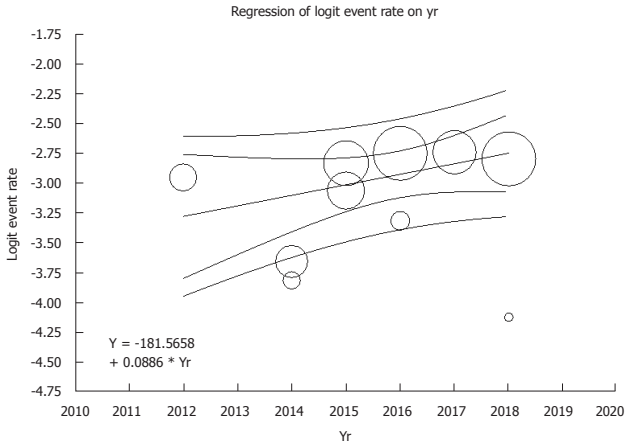

Figure 4 Meta-regression analysis showed no significant correlations between year of study and prevalence of pre-existing atrial fibrillation (*P* = 0.08).

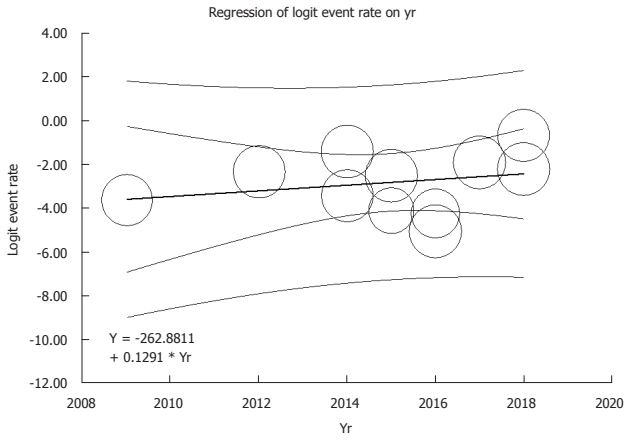

Figure 5 Meta-regression analysis showed no significant correlations between year of study and incidence of post-operative atrial fibrillation after liver transplantation (*P* = 0.54).

to those without AF[24,48]. Patients with AF reflect that they are frail and have already been at higher risk profiles accompanying with other cardiovascular risks (left ventricular hypertrophy, heart failure, stroke, *etc*.) at the time even before liver transplantation, so that they will inevitably develop higher complication rates at postoperative period[21,49,50]. Furthermore, AF itself plays a critical role as marker of underlying heart diseases that make patients vulnerable to perioperative hemodynamic challenges[51,52].

There are also several mechanisms explained why liver transplantation promotes the occurrence of AF during postoperative period (Figure 6). Firstly, conventional postoperative hemodynamic challenge could provoke AF through hemodynamic instability or inotropic administration[50]. Also, some preexisting liver diseases, such as nonalcoholic fatty liver disease (NAFLD), share a common risk factor, that is diabetes and obesity, with the AF patients[53]. In addition, NAFLD could also occur as de novo after liver transplantation and subsequently enhances the postoperative complications, contributed by systematic inflammatory mechanism[54-56]. Furthermore, immunosuppressive therapy increases the risk to develop insulin resistance which eventually leads to metabolic syndrome[57]. Various kind of cirrhosis-specific heart diseases, such as a well-known entity called congestive hepatopathy, prior to transplantation play a substantial arrhythmogenesis role as a substrate for pathogenesis of AF[50,58]. Various underlying medical problems including AF would, in the future, be used to identify high-risk patient population that needs to be optimized the treatment to achieve higher outcome after liver transplantation.

Leading cause of long term mortality in patients with liver transplantation is cardiovascular complications which, other than AF, include heart failure and myocardial infarction. These complications are predominantly driven by the development of metabolic syndrome after liver transplantation. However, this topic of interest is beyond the scope of our study and

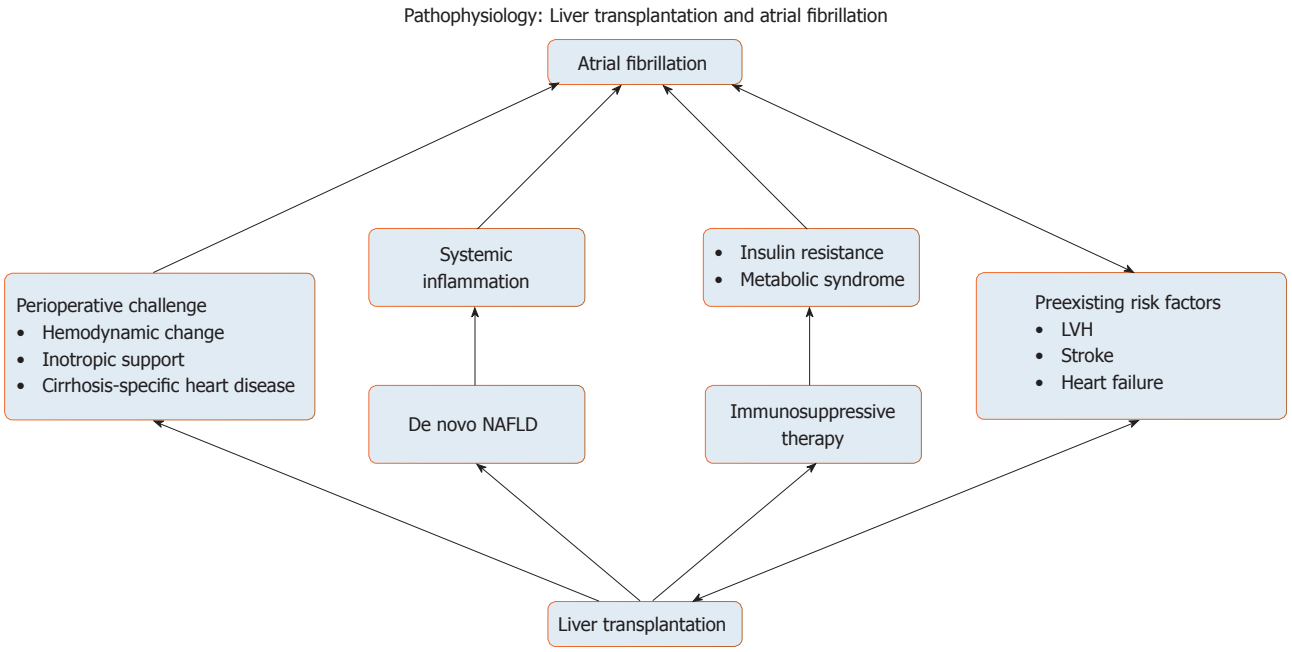

Figure 6 Potential mechanisms of atrial fibrillation in liver transplantation. NAFLD: Nonalcoholic fatty liver disease; LVH: Left ventricular hypertrophy.

could be explained elsewhere[50]. More or less, these cardiovascular complications were also considered as potential risk modification strategy that should not be overlooked. Our study has noteworthy limitations. Firstly, an inconsistent in definition, for an example how to define the timing of AF as an early or late onset, among the different studies preclude to draw the generalized conclusion. Such this limitation, data use needs tailoring to the individual patient. Secondly, duration of follow up during the postoperative period by some study prospectively monitored a cardiovascular event for just 30 d post-transplantation, which this time frame does not long enough to reveal the long-term morbidity and mortality outcome. However, with the potential of higher morbidity and mortality in liver transplant patients with AF by our meta-analysis, future studies, preferable with population-based or national database studies, are required to discover whether focused AF cares for liver transplanted patients can improve patient outcomes after liver transplantation. Finally, since our study is a meta-analysis of observational studies, it could entirely prove association, but could not demonstrate a cause-effect (causal) relationship, between liver transplantation and AF.

In conclusion, our study demonstrated the actual prevalence of preexisting AF in patient underwent liver transplantation, incidence of AF post-liver transplantation. Our study also highlighted the association of AF with higher morbidity and mortality among liver transplant recipients. Further well-designed studies are needed to explore the impact of AF in liver transplant patients, which we strongly believe that AF management, specified to liver transplant patients, would be an important strategy to augment standard of care in this particular population.

ARTICLE HIGHLIGHTS

Research background

Among liver transplant patients with atrial fibrillation (AF), there are lacks of data about incidence, prevalence and prognosis of AF in this specific group of patients. In spite of improvement of liver transplant care to the point of achieving almost 90% of 1-year survival rate, outcomes of liver transplantation related to AF remain unclear.

Research motivation

With excellent results of liver transplantation in term of survival, current indications of the transplantation have been extending into higher risk candidates due to higher amount of donors and more advanced treatment, which include preoperative preparation, surgical technique, immunosuppressive therapy and post-transplantation care. The high-risk liver transplant candidates tend to experience the adverse effects throughout perioperative period and worse outcomes, compared to those with less comorbidity. AF is one of the most common cardiac rhythm abnormalities and its prevalence increases with older age and higher comorbidities. Therefore, a number of patients with AF who received liver transplantation would definitely increase.

Research objectives

To examine outcomes of liver transplant recipients with AF, we performed this meta-analysis: (1) to assess prevalence of pre-existing AF and/or incidence of AF following liver transplantation, and the trends of patient's outcomes overtime; and (2) to evaluate impact of pre-existing AF and post-operative AF on patient outcomes following liver transplantation. Innovations and breakthroughs.

Research methods

We conducted a systematic literature search of EMBASE, Ovid MEDLINE, and the Cochrane Database (from database inception to March 2018): (1) to estimate prevalence of pre-existing AF and/or incidence of AF following liver transplantation; and (2) to evaluate impact of pre-existing AF and post-operative AF on patient outcomes following liver transplantation. Estimated prevalence, incidence and estimated risks from each study were incorporated by the random-effect, generic inverse-variance approach of DerSimonian and Laird.

Research results

There were significant associations of AF with worse clinical outcomes following liver transplantation including 2.3-fold higher risk of death and 5.1-fold higher risk of postoperative cardiovascular complications, and poor clinical outcomes such as stroke, acute kidney injury and graft failure. We also showed the incidence of postoperative AF, namely 8.5%, consistently across different type of studies without the change overtime by meta-regression.

Research conclusions

The overall estimated prevalence of pre-existing AF and incidence of AF following liver transplantation are 5.4% and 8.5%, respectively. Incidence of AF following liver transplant does not seem to decrease overtime. Pre-existing AF and new-onset AF are potentially associated with poor clinical outcomes post liver transplantation.

Research perspectives

This systematic review confirmed higher risks of death and postoperative complications in liver transplant patients with AF. Our findings indicate that AF may be an independent predictor for worse clinical outcomes following liver transplantation.

REFERENCES

1 **Lip GYH**, Brechin CM, Lane DA. The global burden of atrial fibrillation and stroke: a systematic review of the epidemiology of atrial fibrillation in regions outside North America and Europe. *Chest* 2012; **142**: 1489-1498 [PMID: 22459778 DOI: 10.1378/chest.11-2888]

2 **Schnabel RB**, Yin X, Gona P, Larson MG, Beiser AS, McManus DD, Newton-Cheh C, Lubitz SA, Magnani JW, Ellinor PT, Seshadri S, Wolf PA, Vasan RS, Benjamin EJ, Levy D. 50 year trends in atrial fibrillation prevalence, incidence, risk factors, and mortality in the Framingham Heart Study: a cohort study. *Lancet* 2015; **386**: 154-162 [PMID: 25960110 DOI: 10.1016/S0140-6736(14)61774-8]

3 **Zoni-Berisso M**, Lercari F, Carazza T, Domenicucci S. Epidemiology of atrial fibrillation: European perspective. *Clin Epidemiol* 2014; **6**: 213-220 [PMID: 24966695 DOI: 10.2147/CLEP.S47385]

4 **Andrade J**, Khairy P, Dobrev D, Nattel S. The clinical profile and pathophysiology of atrial fibrillation: relationships among clinical features, epidemiology, and mechanisms. *Circ Res* 2014; **114**: 1453-1468 [PMID: 24763464 DOI: 10.1161/CIRCRESAHA.114.303211]

5 **January CT**, Wann LS, Alpert JS, Calkins H, Cigarroa JE, Cleveland JC Jr, Conti JB, Ellinor PT, Ezekowitz MD, Field ME, Murray KT, Sacco RL, Stevenson WG, Tchou PJ, Tracy CM, Yancy CW; American College of Cardiology/American Heart Association Task Force on Practice Guidelines. 2014 AHA/ACC/HRS guideline for the management of patients with atrial fibrillation: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines and the Heart Rhythm Society. *J Am Coll Cardiol* 2014; **64**: e1-76 [PMID: 24685669 DOI: 10.1016/j.jacc.2014.03.022]

6 **Odutayo A**, Wong CX, Hsiao AJ, Hopewell S, Altman DG, Emdin CA. Atrial fibrillation and risks of cardiovascular disease, renal disease, and death: systematic review and meta-analysis. *BMJ* 2016; **354**: i4482 [PMID: 27599725 DOI: 10.1136/bmj.i4482]

7 **Lee H**, Choi EK, Rhee TM, Lee SR, Lim WH, Kang SH, Han KD, Cha MJ, Oh S. Cirrhosis is a risk factor for atrial fibrillation: A nationwide, population-based study. *Liver Int* 2017; **37**: 1660-1667 [PMID: 28432810 DOI: 10.1111/liv.13459]

8 **Mwalitsa JP**, Maimone S, Filomia R, Alibrandi A, Saitta C, Caccamo G, Cacciola I, Spinella R, Oliva G, Lembo T, Vadalà D, Gambino G, Raimondo G, Squadrito G. Atrial fibrillation in patients with cirrhosis. *Liver Int* 2016; **36**: 395-400 [PMID: 26235424 DOI: 10.1111/liv.12928]

9 **Biviano AB**, Nazif T, Dizon J, Garan H, Abrams M, Fleitman J, Hassan D, Kapadia S, Babaliaros V, Xu K, Rodes-Cabau J, Szeto WY, Fearon WF, Dvir D, Dewey T, Williams M, Kindsvater S, Mack MJ, Webb JG, Craig Miller D, Smith CR, Leon MB, Kodali S. Atrial Fibrillation is Associated with Increased Pacemaker Implantation Rates in the Placement of AoRTic Transcatheter Valve (PARTNER) Trial. *J Atr Fibrillation* 2017; **10**: 1494 [PMID: 29250217 DOI: 10.4022/jafib.1494]

10 **Foundation AL**. Liver Transplantation. 2018. Available from: URL: https://www.liverfoundation.org/for-patients/about-the-liver/the-progression-of-liver-disease/liver-transplant/#information-for-the-newly-diagnosed

11 **Martin P**, DiMartini A, Feng S, Brown R Jr, Fallon M. Evaluation for liver transplantation in adults: 2013 practice guideline by the American Association for the Study of Liver Diseases and the American Society of Transplantation. *Hepatology* 2014; **59**: 1144-1165 [PMID: 24716201 DOI: 10.1002/hep.26972]

12 **Kling CE**, Perkins JD, Carithers RL, Donovan DM, Sibulesky L. Recent trends in liver transplantation for alcoholic liver disease in the United States. *World J Hepatol* 2017; **9**: 1315-1321 [PMID: 29359014 DOI: 10.4254/wjh.v9.i36.1315]

13 **Dutkowski P**, Linecker M, DeOliveira ML, Müllhaupt B, Clavien PA. Challenges to liver transplantation and strategies to improve outcomes. *Gastroenterology* 2015; **148**: 307-323 [PMID: 25224524 DOI: 10.1053/j.gastro.2014.08.045]

14 **Organ Procurement and Transplantation Network**. National data on liver transplantation. 2018. Available from: URL: https://optn.transplant.hrsa.gov/data/view-data-reports/national-data

15 **Reddy KR**, Ellerbe C, Schilsky M, Stravitz RT, Fontana RJ, Durkalski V, Lee WM; Acute Liver Failure Study Group. Determinants of outcome among patients with acute liver failure listed for liver transplantation in the United States. *Liver Transpl* 2016; **22**: 505-515 [PMID: 26421889 DOI: 10.1002/lt.24347]

16 **Young K**, Liu B, Bhuket T, Younossi Z, Saab S, Ahmed A, Wong RJ. Long-term trends in chronic hepatitis B virus infection associated liver transplantation outcomes in the United States. *J Viral Hepat* 2017; **24**: 789-796 [PMID: 28273387 DOI: 10.1111/jvh.12703]

17 **Urrunaga NH**, Rachakonda VP, Magder LS, Mindikoglu AL. Outcomes of living versus deceased donor liver transplantation for acute liver failure in the United States. *Transplant Proc* 2014; **46**: 219-224 [PMID: 24507055 DOI: 10.1016/j.transproceed.2013.08.111]

18 **Wan P**, Yu X, Xia Q. Operative outcomes of adult living donor liver transplantation and deceased donor liver transplantation: a systematic review and meta-analysis. *Liver Transpl* 2014; **20**: 425-436 [PMID: 24478109 DOI: 10.1002/lt.23836]

19 **Mathur AK**, Talwalkar J. Quality measurement and improvement in liver transplantation. *J Hepatol* 2018; **68**: 1300-1310 [PMID: 29559346 DOI: 10.1016/j.jhep.2018.02.034]

20 **Vannucci A**, Rathor R, Vachharajani N, Chapman W, Kangrga I. Atrial fibrillation in patients undergoing liver transplantation-a single-center experience. *Transplant Proc* 2014; **46**: 1432-1437 [PMID: 24935310 DOI: 10.1016/j.transproceed.2014.02.020]

21 **Bargehr J**, Trejo-Gutierrez JF, Patel T, Rosser B, Aranda-Michel J, Yataco ML, Taner CB. Preexisting atrial fibrillation and cardiac complications after liver transplantation. *Liver Transpl* 2015; **21**: 314-320 [PMID: 25488693 DOI: 10.1002/lt.24060]

22 **Xia VW**, Worapot A, Huang S, Dhillon A, Gudzenko V, Backon A, Agopian VG, Aksoy O, Vorobiof G, Busuttil RW, Steadman RH. Postoperative atrial fibrillation in liver transplantation. *Am J Transplant* 2015; **15**: 687-694 [PMID: 25657037 DOI: 10.1111/ajt.13034]

23 **Piazza NA**, Singal AK. Frequency of Cardiovascular Events and Effect on Survival in Liver Transplant Recipients for Cirrhosis Due to Alcoholic or Nonalcoholic Steatohepatitis. *Exp Clin Transplant* 2016; **14**: 79-85 [PMID: 26581602 DOI: 10.6002/ect.2015.0089]

24 **Fouad TR**, Abdel-Razek WM, Burak KW, Bain VG, Lee SS. Prediction of cardiac complications after liver transplantation. *Transplantation* 2009; **87**: 763-770 [PMID: 19295324 DOI: 10.1097/TP.0b013e318198d734]

25 **Vanwagner LB**, Bhave M, Te HS, Feinglass J, Alvarez L, Rinella ME. Patients transplanted for nonalcoholic steatohepatitis are at

increased risk for postoperative cardiovascular events. *Hepatology* 2012; **56**: 1741-1750 [PMID: 22611040 DOI: 10.1002/hep.25855]
26 **Nicolau-Raducu R**, Gitman M, Ganier D, Loss GE, Cohen AJ, Patel H, Girgrah N, Sekar K, Nossaman B. Adverse cardiac events after orthotopic liver transplantation: a cross-sectional study in 389 consecutive patients. *Liver Transpl* 2015; **21**: 13-21 [PMID: 25213120 DOI: 10.1002/lt.23997]
27 **Josefsson A**, Fu M, Björnsson E, Kalaitzakis E. Prevalence of pre-transplant electrocardiographic abnormalities and post-transplant cardiac events in patients with liver cirrhosis. *BMC Gastroenterol* 2014; **14**: 65 [PMID: 24708568 DOI: 10.1186/1471-230X-14-65]
28 **VanWagner LB**, Serper M, Kang R, Levitsky J, Hohmann S, Abecassis M, Skaro A, Lloyd-Jones DM. Factors Associated With Major Adverse Cardiovascular Events After Liver Transplantation Among a National Sample. *Am J Transplant* 2016; **16**: 2684-2694 [PMID: 26946333 DOI: 10.1111/ajt.13779]
29 **VanWagner LB**, Montag S, Zhao L, Allen NB, Lloyd-Jones DM, Das A, Skaro AI, Hohmann S, Friedewald JJ, Levitsky J. Cardiovascular Disease Outcomes Related to Early Stage Renal Impairment Following Liver Transplantation. *Transplantation* 2018 [PMID: 29557907 DOI: 10.1097/TP.0000000000002175]
30 **Wange N**, Anan I, Ericzon BG, Pennlert J, Pilebro B, Suhr OB, Wixner J. Atrial Fibrillation and Central Nervous Complications in Liver Transplanted Hereditary Transthyretin Amyloidosis Patients. *Transplantation* 2018; **102**: e59-e66 [PMID: 29019809 DOI: 10.1097/TP.0000000000001975]
31 **VanWagner LB**, Ning H, Whitsett M, Levitsky J, Uttal S, Wilkins JT, Abecassis MM, Ladner DP, Skaro AI, Lloyd-Jones DM. A point-based prediction model for cardiovascular risk in orthotopic liver transplantation: The CAR-OLT score. *Hepatology* 2017; **66**: 1968-1979 [PMID: 28703300 DOI: 10.1002/hep.29329]
32 **von Elm E**, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP; STROBE Initiative. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *PLoS Med* 2007; **4**: e296 [PMID: 17941714 DOI: 10.1371/journal.pmed.0040296]
33 **Moher D**, Liberati A, Tetzlaff J, Altman DG; PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med* 2009; **6**: e1000097 [PMID: 19621072 DOI: 10.1371/journal.pmed.1000097]
34 **Stang A**. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. *Eur J Epidemiol* 2010; **25**: 603-605 [PMID: 20652370 DOI: 10.1007/s10654-010-9491-z]
35 **DerSimonian R**, Laird N. Meta-analysis in clinical trials. *Control Clin Trials* 1986; **7**: 177-188 [PMID: 3802833 DOI: 10.1016/0197-2456(86)90046-2]
36 **Higgins JP**, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ* 2003; **327**: 557-560 [PMID: 12958120 DOI: 10.1136/bmj.327.7414.557]
37 **Easterbrook PJ**, Berlin JA, Gopalan R, Matthews DR. Publication bias in clinical research. *Lancet* 1991; **337**: 867-872 [PMID: 1672966 DOI: 10.1016/0140-6736(91)90201-Y]
38 **Chugh SS**, Havmoeller R, Narayanan K, Singh D, Rienstra M, Benjamin EJ, Gillum RF, Kim YH, McAnulty JH Jr, Zheng ZJ, Forouzanfar MH, Naghavi M, Mensah GA, Ezzati M, Murray CJ. Worldwide epidemiology of atrial fibrillation: a Global Burden of Disease 2010 Study. *Circulation* 2014; **129**: 837-847 [PMID: 24345399 DOI: 10.1161/CIRCULATIONAHA.113.005119]
39 **Brandt M**, Harringer W, Hirt SW, Walluscheck KP, Cremer J, Sievers HH, Haverich A. Influence of bicaval anastomoses on late occurrence of atrial arrhythmia after heart transplantation. *Ann Thorac Surg* 1997; **64**: 70-72 [PMID: 9236337 DOI: 10.1016/S0003-4975(97)82822-6]
40 **Cohn WE**, Gregoric ID, Radovancevic B, Wolf RK, Frazier OH. Atrial fibrillation after cardiac transplantation: experience in 498 consecutive cases. *Ann Thorac Surg* 2008; **85**: 56-58 [PMID: 18154778 DOI: 10.1016/j.athoracsur.2007.07.037]
41 **el Gamel A**, Yonan NA, Grant S, Deiraniya AK, Rahman AN, Sarsam MA, Campbell CS. Orthotopic cardiac transplantation: a comparison of standard and bicaval Wythenshawe techniques. *J Thorac Cardiovasc Surg* 1995; **109**: 721-729; discussion 729-730 [PMID: 7715220 DOI: 10.1016/S0022-5223(95)70354-3]
42 **Dasari TW**, Pavlovic-Surjancev B, Patel N, Williams AA, Ezidinma P, Rupani A, Sinacore JL, Heroux AL. Incidence, risk factors, and clinical outcomes of atrial fibrillation and atrial flutter after heart transplantation. *Am J Cardiol* 2010; **106**: 737-741 [PMID: 20723655 DOI: 10.1016/j.amjcard.2010.04.035]
43 **Rivinius R**, Helmschrott M, Ruhparwar A, Erbel C, Gleissner CA, Darche FF, Thomas D, Bruckner T, Katus HA, Doesch AO. The influence of surgical technique on early posttransplant atrial fibrillation - comparison of biatrial, bicaval, and total orthotopic heart transplantation. *Ther Clin Risk Manag* 2017; **13**: 287-297 [PMID: 28331331 DOI: 10.2147/TCRM.S126869]
44 **Sattiraju S**, Vats S, Krishnan B, K Kim S, Austin E, Can I, Tholakanahalli V, G Benditt D, Y Chen L. Operative Technique and Atrial Tachyarrhythmias After Orthotopic Heart Transplantation. *J Atr Fibrillation* 2012; **5**: 690 [PMID: 28496794 DOI: 10.4022/jafib.690.eCollection]
45 **Auer J**, Weber T, Berent R, Ng CK, Lamm G, Eber B. Risk factors of postoperative atrial fibrillation after cardiac surgery. *J Card Surg* 2005; **20**: 425-431 [PMID: 16153272 DOI: 10.1111/j.1540-8191.2005.2004123.x]
46 **Nisanoglu V**, Erdil N, Aldemir M, Ozgur B, Berat Cihan H, Yologlu S, Battaloglu B. Atrial fibrillation after coronary artery bypass grafting in elderly patients: incidence and risk factor analysis. *Thorac Cardiovasc Surg* 2007; **55**: 32-38 [PMID: 17285471 DOI: 10.1055/s-2006-924711]
47 **January CT**, Wann LS, Alpert JS, Calkins H, Cigarroa JE, Cleveland JC Jr, Conti JB, Ellinor PT, Ezekowitz MD, Field ME, Murray KT, Sacco RL, Stevenson WG, Tchou PJ, Tracy CM, Yancy CW; ACC/AHA Task Force Members. 2014 AHA/ACC/HRS guideline for the management of patients with atrial fibrillation: executive summary: a report of the American College of Cardiology/American Heart Association Task Force on practice guidelines and the Heart Rhythm Society. *Circulation* 2014; **130**: 2071-2104 [PMID: 24682348 DOI: 10.1161/CIR.0000000000000040]
48 **Keeffe EB**. Liver transplantation at the millennium. Past, present, and future. *Clin Liver Dis* 2000; **4**: 241-255 [PMID: 11232187 DOI: 10.1016/S1089-3261(05)70106-9]
49 **Bargehr J**, Trejo-Gutierrez JF, Rosser BG, Patel T, Yataco ML, Pungpapong S, Taner CB, Aranda-Michel J. Liver transplantation in patients with atrial fibrillation. *Transplant Proc* 2013; **45**: 2302-2306 [PMID: 23953542 DOI: 10.1016/j.transproceed.2013.02.130]
50 **Gallegos-Orozco JF**, Charlton MR. Predictors of Cardiovascular Events After Liver Transplantation. *Clin Liver Dis* 2017; **21**: 367-379 [PMID: 28364819 DOI: 10.1016/j.cld.2016.12.009]
51 **Benjamin EJ**, Levy D, Vaziri SM, D'Agostino RB, Belanger AJ, Wolf PA. Independent risk factors for atrial fibrillation in a population-based cohort. The Framingham Heart Study. *JAMA* 1994; **271**: 840-844 [PMID: 8114238 DOI: 10.1001/jama.271.11.840]
52 **Stewart S**, Hart CL, Hole DJ, McMurray JJ. A population-based study of the long-term risks associated with atrial fibrillation: 20-year follow-up of the Renfrew/Paisley study. *Am J Med* 2002; **113**: 359-364 [PMID: 12401529 DOI: 10.1016/S0002-9343(02)01236-6]
53 **Tanner RM**, Baber U, Carson AP, Voeks J, Brown TM, Soliman EZ, Howard VJ, Muntner P. Association of the metabolic syndrome with atrial fibrillation among United States adults (from the REasons for Geographic and Racial Differences in Stroke [REGARDS] Study). *Am J Cardiol* 2011; **108**: 227-232 [PMID: 21530935 DOI: 10.1016/j.amjcard.2011.03.026]
54 **Pagadala M**, Dasarathy S, Eghtesad B, McCullough AJ. Posttransplant metabolic syndrome: an epidemic waiting to happen. *Liver Transpl* 2009; **15**: 1662-1670 [PMID: 19938136 DOI: 10.1002/lt.21952]
55 **Laryea M**, Watt KD, Molinari M, Walsh MJ, McAlister VC, Marotta PJ, Nashan B, Peltekian KM. Metabolic syndrome in

liver transplant recipients: prevalence and association with major vascular events. *Liver Transpl* 2007; **13**: 1109-1114 [PMID: 17663411 DOI: 10.1002/lt.21126]

56 **Moon JI**, Barbeito R, Faradji RN, Gaynor JJ, Tzakis AG. Negative impact of new-onset diabetes mellitus on patient and graft survival after liver transplantation: Long-term follow up. *Transplantation* 2006; **82**: 1625-1628 [PMID: 17198248 DOI: 10.1097/01.tp.0000250361.60415.96]

57 **Pisano G**, Fracanzani AL, Caccamo L, Donato MF, Fargion S. Cardiovascular risk after orthotopic liver transplantation, a review of the literature and preliminary results of a prospective study. *World J Gastroenterol* 2016; **22**: 8869-8882 [PMID: 27833378 DOI: 10.3748/wjg.v22.i40.8869]

58 **Møller S**, Bernardi M. Interactions of the heart and the liver. *Eur Heart J* 2013; **34**: 2804-2811 [PMID: 23853073 DOI: 10.1093/eurheartj/eht246]

P- Reviewer: Cerwenka H, Chiu KW, Gencdal G, Kanda T
S- Editor: Ji FF **L- Editor**: A **E- Editor**: Song H

World Journal of Hepatology

Submit a Manuscript: http://www.f6publishing.com

World J Hepatol 2018 October 27; 10(10): 772-779

DOI: 10.4254/wjh.v10.i10.772

ISSN 1948-5182 (online)

CASE REPORT

Proton beam therapy in apneic oxygenation treatment of an unresectable hepatocellular carcinoma: A case report and review of literature

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Author contributions: Lin YL performed all research and interpretive functions for this case report.

Supported by Rinecker Proton Therapy Center (RPTC), Munich, Germany.

Informed consent statement: After contacting the patient about the planned publication of his case, he provided written authorization.

Conflict-of-interest statement: The author states there are no potential conflicts of interest relevant to this publication.

CARE Checklist (2013) statement: The author has read the CARE Checklist (2013), and the manuscript was prepared and revised according to the CARE Checklist (2013).

Manuscript source: Unsolicited manuscript

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Received: April 5, 2018
Peer-review started: April 6, 2018
First decision: May 16, 2018
Revised: June 14, 2018
Accepted: June 27, 2018
Article in press: June 28, 2018
Published online: October 27, 2018

Abstract

Presented here is the clinical course of a 63-year-old patient with a central, large and unresectable hepatocellular carcinoma (HCC) with liver metastases and tumor invasion of the portal and hepatic veins. After the tumor had been diagnosed, the patient was immediately treated with proton beam therapy (PBT), at a total dose of 60 Gy (relative biological effectiveness) in 20 fractions administered within 4 wk. To manage the respiratory movements, at the Rinecker Proton Therapy Center, apneic oxygenation was given daily, under general anesthesia. The patient tolerated both the PBT and general anesthesia very well, and did now show any signs of acute or late toxicity. The treatment was followed by constant reductions in the tumor marker alpha-fetoprotein and the cholestatic parameters gamma-glutamyltransferase and alkaline phosphatase. The patient commenced an adjuvant treatment with sorafenib, given at 6-wk intervals, after the PBT. Follow-up with regular magnetic resonance imaging has continued for 40 mo so far, demonstrating remarkable shrinkage of the HCC (maximal diameter dropping from approximately 13 cm to 2 cm). To date, the patient remains free of tumor recurrence. PBT served as a safe and effective treatment method for an unresectable HCC with vascular invasion.

Key words: Particle therapy; Proton beam therapy; Apneic oxygenation; Unresectable; Vascular invasion; Hepatocellular carcinoma; Intrahepatic metastasis

Core tip: Hepatocellular carcinoma (HCC) is one of the most common cancers in Asia. Patients with unresectable tumor disease require more options for in-principle curative therapies. We report here a patient with a large unresectable HCC due to vascular invasion and satellite metastases, who showed remarkable tumor shrinkage after completing proton beam therapy 4 years ago and who is still free of tumor recurrence to date.

Lin YL. Proton beam therapy in apneic oxygenation treatment of an unresectable hepatocellular carcinoma: A case report and review of literature. *World J Hepatol* 2018; 10(10): 772-779 Available from: URL: http://www.wjgnet.com/1948-5182/full/v10/i10/772.htm DOI: http://dx.doi.org/10.4254/wjh.v10.i10.772

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common tumor diseases worldwide, with particularly high incidence in the Asia-Pacific region, and is often associated with liver cirrhosis owing to alcohol abuse and chronic viral hepatitis[1]. Surgical resection and liver transplantation are the first-line curative treatments for large HCC, while small HCC (preferably < 2 cm in diameter) in an accessible location, away from critical structures, can be cured by local ablative techniques. For patients with large unresectable tumors, such as those with advanced tumor extension and vascular invasion, other locoregional treatment modalities are usually considered as palliative options; these include arterial catheter-based treatments, ablative techniques and radiation therapy.

In past years, many convincing results were published on the effectiveness and tolerability of charged-particle therapy with dose-escalating proton and heavy ion beam as a curative-intent treatment of unresectable HCC[2-4]. Based on the physical property of Bragg peak, proton beams deposit the dose maximum at a predefined depth in the tumor. Behind the maximal deposit, the dose drops rapidly, having no exit dose. This is a significant advantage in comparison to photon radiotherapy and enables the dose escalation to improve the local control. Because the surrounding uninvolved liver parenchyma can be spared from the unnecessary radiation dose, even larger tumor volume can be treated with proton beam therapy (PBT) without risk of fatal radiation-induced liver disease (RILD)[5,6].

CASE REPORT

In November 2013, a routine abdominal sonography of a 63-year-old German male revealed a huge tumor in the liver. Subsequent magnetic resonance imaging (MRI) and computed tomography (CT) scan of the abdomen revealed the main tumor mass to be of about 13.4 cm × 7.1 cm in size, encompassing segments Ⅰ, Ⅳ, Ⅴ and Ⅷ, as well as satellite metastases in segments Ⅱ and Ⅲ. The tumor involved all three hepatic veins and the portal vein, compressing the inferior vena cava (Figure 1). Liver biopsy, taken in January 2014, confirmed a diagnosis of HCC, Edmondson-Steiner-grade Ⅱ, with partially cirrhotic parenchymal modification. The additional esophagogastroduodenoscopy and colonoscopy demonstrated chronic gastritis with helicobacter pylori infection and sigmoid diverticulosis, but no evidence of other gastrointestinal malignancy. The patient reported not feeling any discomfort but having lost 12 kg of weight, which he had attributed to a dietary prescription to address his decompensated diabetes mellitus. Blood test (hepatic function panel) showed remarkably increased levels of gamma-glutamyltransferase (GGT) and alkaline phosphatase (AP), and moderately elevated levels of total bilirubin, glutamic-oxalocetic transaminase (GOT), glutamic-pyruvic transaminase (GPT) and cholesterol.

Because the initial consulting hospital classified this case of HCC as Child-Pugh score A, BCLC stage B, palliative treatments with transarterial chemoembolization (TACE) and sorafenib were recommended in the tumor board review. However, extended vascular invasion and tumor size > 10 cm are contraindications for TACE. The patient was informed about best supportive care and approximate survival time of 6 mo. He contacted our institute, the Rinecker Proton Therapy Center (RPTC), and was treated with PBT from February to March 2014. A total dose of 60.00 Gy (relative biological effectiveness, RBE) administered in 20 fractions within 4 wk was applied to both the main liver tumor and the satellite metastases, with a safety margin of 3 mm.

For precise targeting by PBT, special techniques to control the respiratory movements are required. At RPTC, we use apneic oxygenation (AO) with total intravenous anesthesia and oral intubation, which prolongs the safe apnea time during the irradiation (Figure 2). After preoxygenation, the artificial ventilation is stopped during the apneic phase. The patient stays connected, with delivery of 1 L/min oxygen and constant airway pressure. Due to the disproportion between the rate of oxygen removal from the alveoli compared to the rate of carbon dioxide delivery to them, the barometric pressure in the alveoli sinks, pushing the oxygen to move from the upper airway to the alveoli. That means, the consumed oxygen is replaced but the carbon dioxide is not removed, and the arterial partial pressure of carbon dioxide ($PaCO_2$) rises in the time following, in a range of 2-4 mmHg/min[7,8]. According to our standard operating procedure, the oxygen saturation during AO should not fall below 97%, and $PaCO_2$ is not allowed to exceed 61 mmHg.

The patient tolerated the PBT under general anesthesia daily, without any considerable toxicity

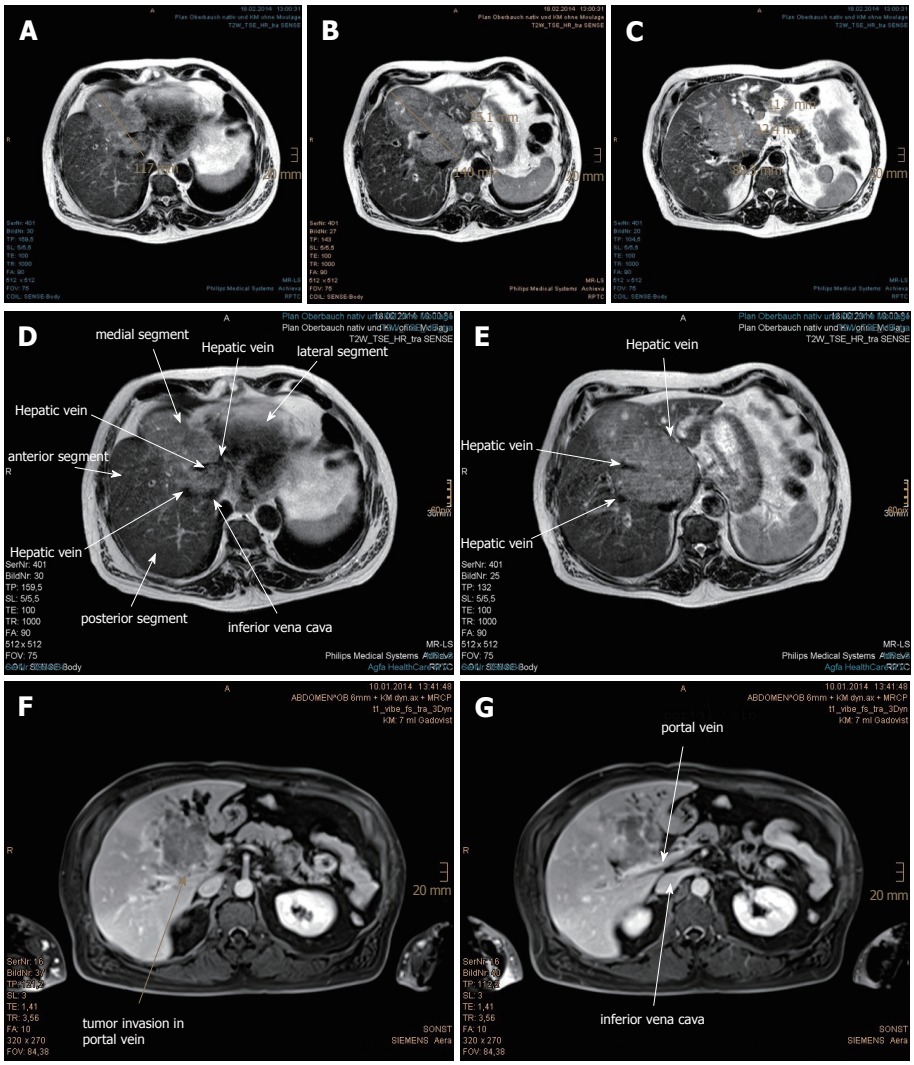

Figure 1 Large hepatocellular carcinoma with vascular invasion and satellite metastases in the magnetic resonance imaging scan prior to proton beam therapy. A: The upper portion of the main tumor abutting the heart in axial plane is shown; B: Extended hepatocellular carcinoma (HCC) with invasion of portal and hepatic veins in the axial plane is shown; C: The lower portion of the main tumor abutting the inferior vena cava is shown, with dilated segmental bile ducts and 2 liver metastases in the left hepatic lobe; D-G: The HCC involved all three hepatic veins as well as the portal vein. The inferior vena cava was also compressed.

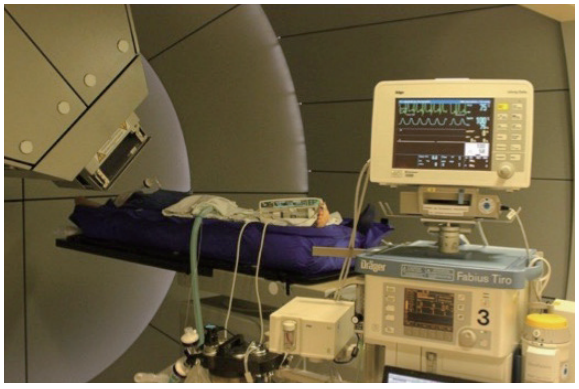

Figure 2 Proton beam therapy in use with apenic oxygenation under general anesthesia.

(Common Terminology Criteria for Adverse Events, grade 0). At the first follow-up (after 6 wk of treatment), the patient showed remarkable reduction in the tumor marker alpha-fetoprotein (AFP) (from 109 μg/L to 34 μg/L; normal range: < 15 ng/mL) and decrease in GGT (from 64 μkat/L to 25 μkat/L; normal range: < 1.00 μkat/L). From this time forward, the patient also commenced with targeted therapy, *i.e.*, tyrosine kinase inhibitor (sorafenib) and continued in his normal occupational activities (working as a driver). At 5 mo after the PBT, routine blood test showed leukocytosis (12.7 Gpt/L; normal range: 4.0-10.9 Gpt/L) with elevated C-reactive protein (101 mg/L; normal range: < 5.0 mg/L). Respiratory or urinary infection was excluded. The patient denied experience of fever but complained of night sweats, and was treated with antibiotics by his oncologist. Because of diarrhea, the sorafenib was stopped for a few months and restarted with the dosage halved (to 400 mg daily). At the end of 2014, both the leukocyte count and C-reactive protein level finally decreased and the GGT continued to fall (to 3.3 μkat/L). At 20 mo after the PBT, the AFP

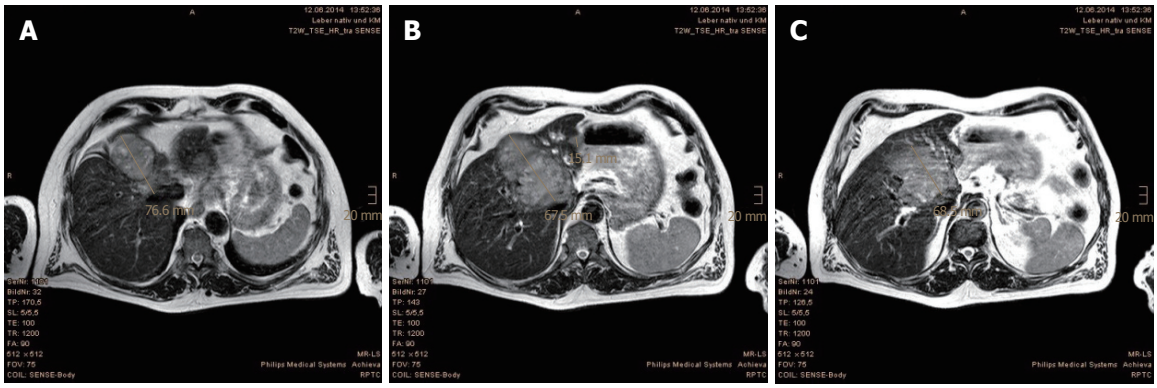

Figure 3 Tumor regression evidenced in the first follow-up magnetic resonance imaging scan at 3 mo after proton beam therapy. A-C: Size reductions of the main tumor and the liver metastases are shown, measured in three levels of axial plane.

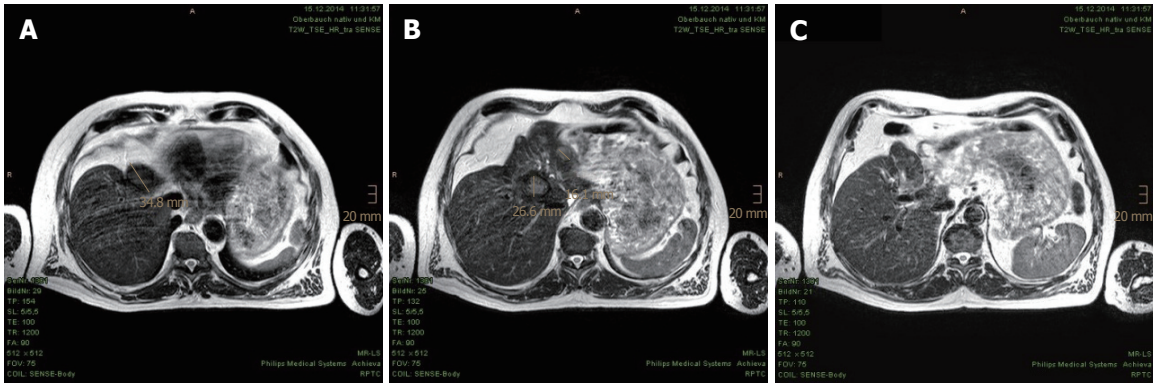

Figure 4 Continuous tumor shrinkage evidenced in the magnetic resonance imaging scan at 9 mo after proton beam therapy. A-C: The hepatocellular carcinoma and liver metastases presented as residual nodules without contrast enhancement.

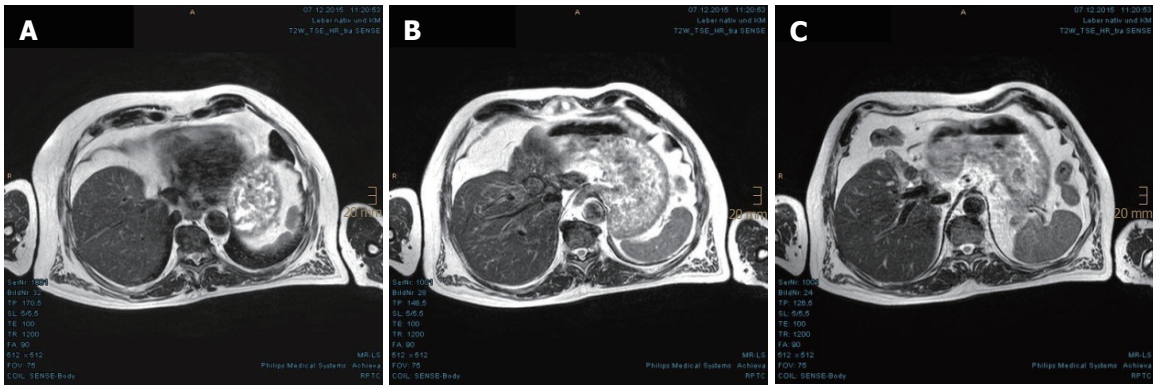

Figure 5 Continuous tumor shrinkage evidenced in the magnetic resonance imaging scan at 21 mo after proton beam therapy. A-C: Further size reductions of the residual tumor nodules in the axial plane are shown.

was within normal range (2.8 μg/L), remaining stable through the last measurement in June 2017 (2.5 μg/L). The thoracic CT scan at 19 mo after the PBT also did not reveal any suspicious metastasis.

In the first MRI control, taken 3 mo after the PBT, significant size reductions of the main tumor in the hepatic hilum and the satellite metastases in the left hepatic lobe were observed. The segmental cholestasis had also regressed, consistent with the falling GGT and AP (Figure 3). In the subsequent check-ups, continuous tumor shrinkage with indentation of the liver contour was observed, as if the patient had undergone a liver resection (Figure 4 and Figure 5). The latest MRI scan, performed in August 2017, revealed a residual nodule of approximately 2 cm × 2 cm without pathological enhancement in the hilar region (Figure 6). No signs of ascites, or new distant or lymph node metastases have been found in the 40-mo period following the PBT. To date, the patient is still receiving semi-dosed sorafenib and participating in

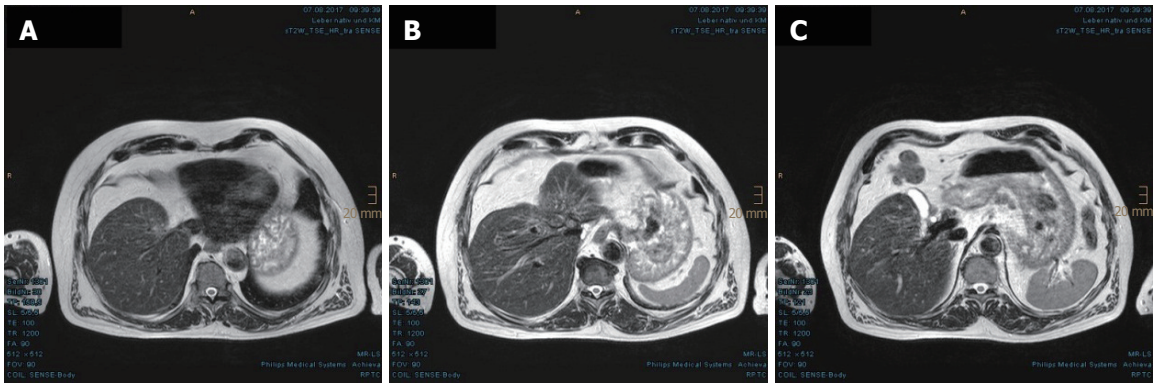

Figure 6 Residual nodules in the hepatic hilum without enhancement evidenced in the latest magnetic resonance imaging scan, at 40 mo after proton beam therapy. A: Complete regression of the upper portion of the hepatocellular carcinoma is shown; B: Pronounced indentation of the right-sided liver contour is shown, as if the patient had undergone a liver resection; C: The portal vein was able to be delimited again.

regular follow-up visits with his oncologist as well as in an annual MRI scan performed at the RPTC.

DISCUSSION

Patients with unresectable HCC are usually not deemed to be curable because surgical resection and liver transplantation are considered as the only curative options. According to guidelines, TACE and sorafenib are the standard of palliative care for unresectable HCC. Other local treatment methods, such as radiofrequency ablation, cryoablation and radioembolization, are often combined with the TACE and sorafenib regimen to improve the total response[9]. Yet, the therapeutic efficacy of combined treatments, such as that of radiofrequency ablation after TACE, is limited by the tumor size and morphology[10].

Katsanos *et al*[11] analyzed 55 randomized controlled trials on mono- or combined treatments with TACE for unresectable HCC and concluded that TACE in combination with external radiotherapy or local liver ablation may distinctly improve tumor response and the survival rates of patients. Choi *et al*[12] reviewed the different radiotherapy techniques and summarized the results of radiotherapy, partially combined with liver transplantation, TACE and concomitant chemotherapy, for HCC in each tumor stage. Radiotherapy can amend the total therapeutic assortments and outcomes of HCC by improving local control, enabling downstaging and treating unresectable HCC with vascular invasion or multiple intrahepatic metastases. The latest National Comprehensive Cancer Network (NCCN) guidelines[13] consider external-beam radiation therapy as a category 2B option for patients with unresectable HCC or those with contraindication for operation due to comorbidity. Besides, stereotactic body radiation therapy can be recommended as alternative to ablation and embolization techniques, particularly after their failure or in case of their contraindication. Because prospective randomized controlled trials evaluating the outcome of various techniques of external-beam radiation therapy *vs* ablation and arterially directed therapies are still pending or ongoing, clear guidelines of treatment recommendations for large unresectable HCC, specifically with vascular invasion and intrahepatic metastasis, are still missing. Mostly, only when further interventional treatments are no longer possible, will radiation therapy be considered.

The aim of modern radiotherapy techniques is to achieve delivery of an effective high-dose in the tumor, while sparing the surrounding structures as much as possible. If the liver function is restricted by coexistent liver disease, such as cirrhosis and/or viral hepatitis, the more essential the role of sparing the surrounding noncancerous liver tissues becomes. Crane *et al*[14] pointed out the challenges of radiotherapy for large HCC due to proximity of stomach and intestines, underlying liver disease, radiosensitivity of liver parenchyma and respiratory and interfractional motions. Because partial resection of the liver (with only 25% remnant) has been demonstrated as well tolerated by noncirrhotic patients[15], it allows radiation therapy of partial liver with higher doses if there is enough functional liver parenchyma left.

In order to prevent RILD, our institute uses the following dose constraints of liver: at least 700 cc of uninvolved liver parenchyma should receive less than 15 Gy (RBE), and the mean dose of liver should be less than 13 Gy (RBE). This is the Quantitative Analyses of Normal Tissue Effects in the Clinic (commonly known as QUANTEC) recommendation for stereotactic body radiation therapy of liver tumor with three fractions[16]. In the case of our patient, presented herein, the total liver volume prior to PBT was 2789 cc (including 638 cc gross tumor volume), and the mean dose of uninvolved liver parenchyma (*i.e.*, total liver volume with subtraction of clinical target volume) was 12.77 Gy (RBE). The volume of uninvolved liver that received less than 15 Gy (RBE) was approximately 1370 cc (Figure 7), while the remnant liver volume after tumor regression was 1470 cc, as measured by MRI scan at 40 mo after the PBT.

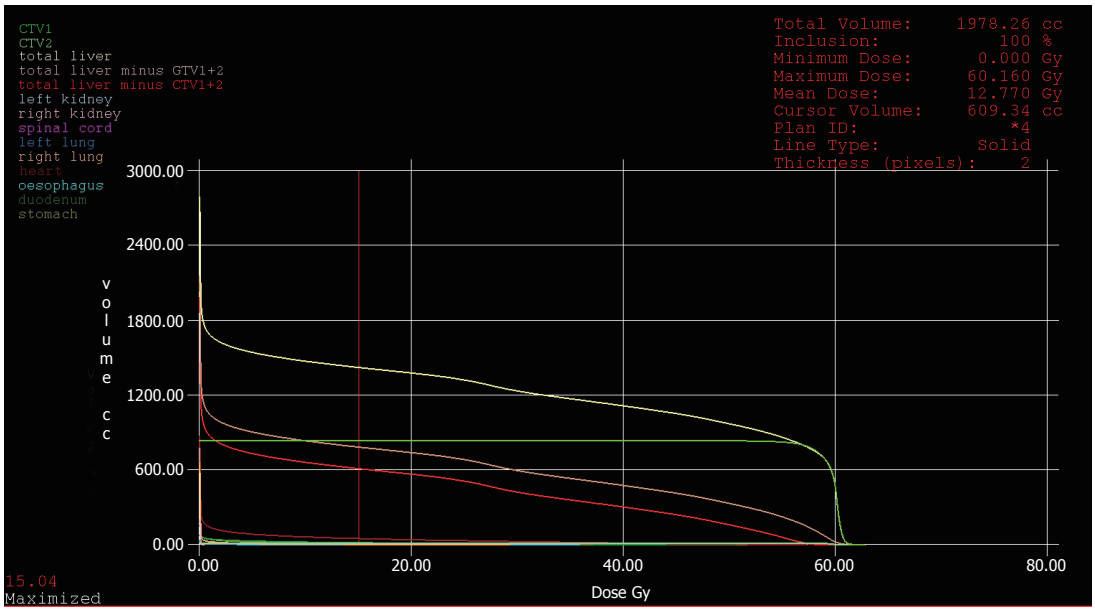

Figure 7 Dose-volume histogram of the clinical target volume and organs at risk.

Unlike photon radiotherapy, PBT requires less irradiation fields to encompass the target. Therefore, the dose burden of uninvolved liver parenchyma and other surrounding organs at risk, such as heart, stomach, intestines, spinal cord and kidney, as well as the risk of radiation-induced secondary malignancies is significantly reduced by the dosimetric advantage of proton beams[17,18]. Leung *et al*[19] compared the cost-utility of stereotactic radiation therapy and PBT, assuming PBT is a cost-effective therapy for inoperable advanced HCC due to the improved quality-adjusted life years. The spot scanning technique used at RPTC also facilitates more conformity to the extent of the target, in comparison to common scattering PBT[20]. In our case, we only used two irradiation fields, from 0 and 300 degrees, to embrace the main tumor in the central hepatic region and the satellite metastases in the left lobe (Figure 8). Irradiation of each field lasted 2.5-3.5 min.

The main challenge of radiotherapy for movable organs, in particular lungs and liver, is to manage the intra- and interfractional motion of the tumor. Measures like implantation of fiducial markers, respiratory gating or tracking, breath-hold method, abdominal compression and 4-dimensional CT simulation are applied in different institutions[21,22]. A unique feature of our center is the use of AO to control respiratory motion and to reduce the safety margin. To assess whether the patient can withstand apneic oxygenation, besides general preanesthesia evaluation, additional tests, such as body plethysmography, echocardiography and arterial blood gas test, can be required in case of relevant cardiopulmonary comorbidity. Since the inauguration of RPTC in 2009, we have treated over 500 patients with the apneic method. Up to the end of 2017, the total number of general anesthesia sessions was more than 6000, while the total time, including introduction and discharge, was 55 min on average. The longest period for AO was over 9 min, and the average apneic duration was approximately 3 min. Statistically speaking, the most treated tumor entities for the use of AO were thoracic malignancies and liver metastases.

Although the patient presented in our case report had several adverse prognostic factors, his case demonstrates that large unresectable HCC with vascular invasion and intrahepatic metastases can be treated excellently with PBT. RPTC has long-standing experience in PBT of movable tumors with AO, which securely solves the problem of organ motion, permits the reduction of safety margin and consequently the side effects, like RILD, and requires the least compliance of patients concerning breath control and abandonment of fiducial markers.

ARTICLE HIGHLIGHTS

Case characteristics

In a routine abdominal sonography of a 63-year-old asymptomatic male, a large central tumor in the liver was detected.

Clinical diagnosis

According to the abdominal sonography, the magnetic resonance imaging and the pathologically increased tumor marker alpha-fetoprotein and liver function panel, a hepatic malignancy was urgently suspected.

Differential diagnosis

Liver cirrhosis, cholangiocellular carcinoma, liver angiosarcoma, liver metastases of non-hepatic origin.

Laboratory diagnosis

The laboratory tests showed significant increase of the tumor marker alpha-fetoprotein and the cholestatic parameters gamma-glutamyltransferase and

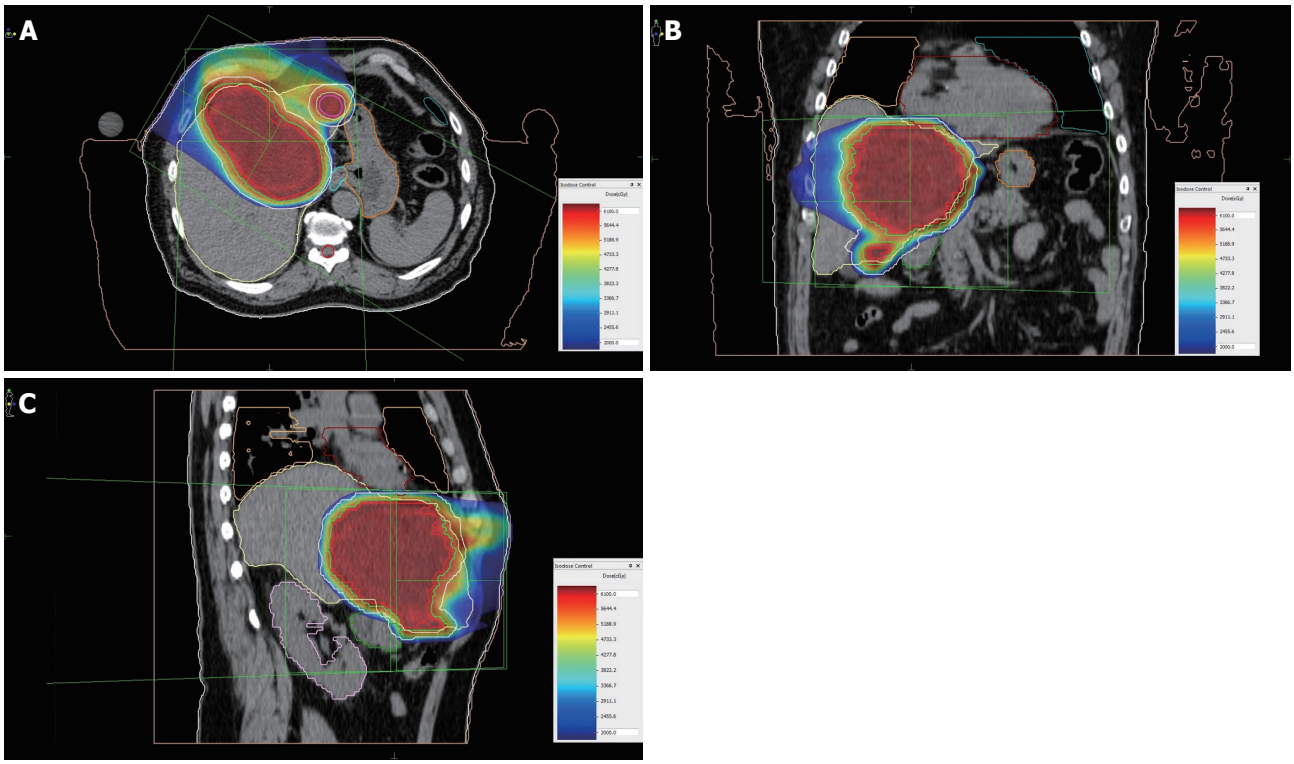

Figure 8 Treatment plan of proton beam therapy with isodose distributions. A: Axial plane; B: Coronal plane; C: Sagittal plane. Red line: Gross tumor volume; Green line: Clinical target volume.

alkaline phosphatase.

Imaging diagnosis

Magnetic resonance imaging and computed tomography of the abdomen revealed the main tumor mass to be of about 13.4 cm × 7.1 cm in size, encompassing segments Ⅰ, Ⅳ, Ⅴ and Ⅷ, as well as satellite metastases in segments Ⅱ and Ⅲ. The tumor involved all three hepatic veins and the portal vein, compressing the inferior vena cava.

Pathological diagnosis

A liver biopsy confirmed a diagnosis of hepatocellular carcinoma (HCC), Edmondson-Steiner-grade Ⅱ, with partially cirrhotic parenchymal modification.

Treatment

The patient was treated with proton beam therapy (PBT) in apneic oxygenation at the Rinecker Proton Therapy Center, at a total dose of 60 Gy (relative biological effectiveness) in 20 fractions.

Term explanation

We use the apneic oxygenation to manage respiratory motion of the tumor and to reduce the safety margin, which enables the sparing of uninvolved liver parenchyma and other surrounding organs at risk as well as a dose escalation.

Experiences and lessons

The patient is free of tumor recurrence in a period of 4 years after the treatment. PBT is a safe and effective therapy for large unresectable HCC with vascular invasion and intrahepatic metastases.

REFERENCES

1 **Omata M**, Cheng AL, Kokudo N, Kudo M, Lee JM, Jia J, Tateishi R, Han KH, Chawla YK, Shiina S, Jafri W, Payawal DA, Ohki T, Ogasawara S, Chen PJ, Lesmana CRA, Lesmana LA, Gani RA, Obi S, Dokmeci AK, Sarin SK. Asia-Pacific clinical practice guidelines on the management of hepatocellular carcinoma: a 2017 update. *Hepatol Int* 2017; **11**: 317-370 [PMID: 28620797 DOI: 10.1007/s12072-017-9799-9]

2 **Skinner HD**, Hong TS, Krishnan S. Charged-particle therapy for hepatocellular carcinoma. *Semin Radiat Oncol* 2011; **21**: 278-286 [PMID: 21939857 DOI: 10.1016/j.semradonc.2011.05.007]

3 **Hong TS**, Wo JY, Yeap BY, Ben-Josef E, McDonnell EI, Blaszkowsky LS, Kwak EL, Allen JN, Clark JW, Goyal L, Murphy JE, Javle MM, Wolfgang JA, Drapek LC, Arellano RS, Mamon HJ, Mullen JT, Yoon SS, Tanabe KK, Ferrone CR, Ryan DP, DeLaney TF, Crane CH, Zhu AX. Multi-Institutional Phase Ⅱ Study of High-Dose Hypofractionated Proton Beam Therapy in Patients With Localized, Unresectable Hepatocellular Carcinoma and Intrahepatic Cholangiocarcinoma. *J Clin Oncol* 2016; **34**: 460-468 [PMID: 26668346 DOI: 10.1200/JCO.2015.64.2710]

4 **Fukuda K**, Okumura T, Abei M, Fukumitsu N, Ishige K, Mizumoto M, Hasegawa N, Numajiri H, Ohnishi K, Ishikawa H, Tsuboi K, Sakurai H, Hyodo I. Long-term outcomes of proton beam therapy in patients with previously untreated hepatocellular carcinoma. *Cancer Sci* 2017; **108**: 497-503 [PMID: 28012214 DOI: 10.1111/cas.13145]

5 **Pan CC**, Kavanagh BD, Dawson LA, Li XA, Das SK, Miften M, Ten Haken RK. Radiation-associated liver injury. *Int J Radiat Oncol Biol Phys* 2010; **76**: S94-100 [PMID: 20171524 DOI: 10.1016/j.ijrobp.2009.06.092]

6 **Thomas TO**, Hasan S, Small W Jr, Herman JM, Lock M, Kim EY, Mayr NA, Teh BS, Lo SS. The tolerance of gastrointestinal organs to stereotactic body radiation therapy: what do we know so far? *J Gastrointest Oncol* 2014; **5**: 236-246 [PMID: 24982772 DOI: 10.3978/j.issn.2078-6891.2014.024]

7 **Frumin MJ**, Epstein RM, Cohen G. Apneic oxygenation in man. *Anesthesiology* 1959; **20**: 789-798 [PMID: 13825447 DOI: 10.1097/00000542-195911000-00007]

8 **Kolettas A**, Grosomanidis V, Kolettas V, Zarogoulidis P, Tsakiridis K, Katsikogiannis N, Tsiouda T, Kiougioumtzi I, Machairiotis N,

Drylis G, Kesisis G, Beleveslis T, Zarogoulidis K. Influence of apnoeic oxygenation in respiratory and circulatory system under general anaesthesia. *J Thorac Dis* 2014; **6** Suppl 1: S116-S145 [PMID: 24672687 DOI: 10.3978/j.issn.2072-1439.2014.01.17]

9 **Murata S**, Mine T, Sugihara F, Yasui D, Yamaguchi H, Ueda T, Onozawa S, Kumita S. Interventional treatment for unresectable hepatocellular carcinoma. *World J Gastroenterol* 2014; **20**: 13453-13465 [PMID: 25309076 DOI: 10.3748/wjg.v20.i37.13453]

10 **Yamakado K**, Nakatsuka A, Ohmori S, Shiraki K, Nakano T, Ikoma J, Adachi Y, Takeda K. Radiofrequency ablation combined with chemoembolization in hepatocellular carcinoma: treatment response based on tumor size and morphology. *J Vasc Interv Radiol* 2002; **13**: 1225-1232 [PMID: 12471186 DOI: 10.1016/S1051-0443(07)61969-1]

11 **Katsanos K**, Kitrou P, Spiliopoulos S, Maroulis I, Petsas T, Karnabatidis D. Comparative effectiveness of different transarterial embolization therapies alone or in combination with local ablative or adjuvant systemic treatments for unresectable hepatocellular carcinoma: A network meta-analysis of randomized controlled trials. *PLoS One* 2017; **12**: e0184597 [PMID: 28934265 DOI: 10.1371/journal.pone.0184597]

12 **Choi SH**, Seong J. Strategic Application of Radiotherapy for Hepatocellular Carcinoma. *Clin Mol Hepatol* 2018; Epub ahead of print [PMID: 29439305 DOI: 10.3350/cmh.2017.0073]

13 **Benson AB 3rd**, D'Angelica MI, Abbott DE, Abrams TA, Alberts SR, Saenz DA, Are C, Brown DB, Chang DT, Covey AM, Hawkins W, Iyer R, Jacob R, Karachristos A, Kelley RK, Kim R, Palta M, Park JO, Sahai V, Schefter T, Schmidt C, Sicklick JK, Singh G, Sohal D, Stein S, Tian GG, Vauthey JN, Venook AP, Zhu AX, Hoffmann KG, Darlow S. NCCN Guidelines Insights: Hepatobiliary Cancers, Version 1.2017. *J Natl Compr Canc Netw* 2017; **15**: 563-573 [PMID: 28476736 DOI: 10.6004/jnccn.2017.0059]

14 **Crane CH**, Koay EJ. Solutions that enable ablative radiotherapy for large liver tumors: Fractionated dose painting, simultaneous integrated protection, motion management, and computed tomography image guidance. *Cancer* 2016; **122**: 1974-1986 [PMID: 26950735 DOI: 10.1002/cncr.29878]

15 **Guglielmi A**, Ruzzenente A, Conci S, Valdegamberi A, Iacono C. How much remnant is enough in liver resection? *Dig Surg* 2012; **29**: 6-17 [PMID: 22441614 DOI: 10.1159/000335713]

16 **Marks LB**, Yorke ED, Jackson A, Ten Haken RK, Constine LS, Eisbruch A, Bentzen SM, Nam J, Deasy JO. Use of normal tissue complication probability models in the clinic. *Int J Radiat Oncol Biol Phys* 2010; **76**: S10-S19 [PMID: 20171502 DOI: 10.1016/j.ijrobp.2009.07.1754]

17 **Lischalk JW**, Repka MC, Unger K. Radiation therapy for hepatobiliary malignancies. *J Gastrointest Oncol* 2017; **8**: 279-292 [PMID: 28480067 DOI: 10.21037/jgo.2016.08.02]

18 **Mondlane G**, Gubanski M, Lind PA, Ureba A, Siegbahn A. Comparative study of the calculated risk of radiation-induced cancer after photon- and proton-beam based radiosurgery of liver metastases. *Phys Med* 2017; **42**: 263-270 [PMID: 28366554 DOI: 10.1016/j.ejmp.2017.03.019]

19 **Leung HWC**, Chan ALF. Cost-utility of stereotactic radiation therapy versus proton beam therapy for inoperable advanced hepatocellular carcinoma. *Oncotarget* 2017; **8**: 75568-75576 [PMID: 29088891 DOI: 10.18632/oncotarget.17369]

20 **McGowan SE**, Burnet NG, Lomax AJ. Treatment planning optimisation in proton therapy. *Br J Radiol* 2013; **86**: 20120288 [PMID: 23255545 DOI: 10.1259/bjr.20120288]

21 **Hong TS**, DeLaney TF, Mamon HJ, Willett CG, Yeap BY, Niemierko A, Wolfgang JA, Lu HM, Adams J, Weyman EA, Arellano RS, Blaszkowsky LS, Allen JN, Tanabe KK, Ryan DP, Zhu AX. A prospective feasibility study of respiratory-gated proton beam therapy for liver tumors. *Pract Radiat Oncol* 2014; **4**: 316-322 [PMID: 25194100 DOI: 10.1016/j.prro.2013.10.002]

22 **Lock MI**, Klein J, Chung HT, Herman JM, Kim EY, Small W, Mayr NA, Lo SS. Strategies to tackle the challenges of external beam radiotherapy for liver tumors. *World J Hepatol* 2017; **9**: 645-656 [PMID: 28588749 DOI: 10.4254/wjh.v9.i14.645]

P- Reviewer: Iliescu L, Lin ZY **S- Editor**: Ji FF
L- Editor: A **E- Editor**: Song H

World Journal of Hepatology

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World J Hepatol 2018 October 27; 10(10): 780-784

DOI: 10.4254/wjh.v10.i10.780

ISSN 1948-5182 (online)

CASE REPORT

Neuroendocrine tumor incidentally detected during living donor hepatectomy: A case report and review of literature

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Author contributions: Akbulut S, Isik B, and Cicek E designed the report; Akbulut S collected the patient's clinical data; Samdanci E provided histopathological information; Akbulut S and Yilmaz S analyzed the data and wrote the paper.

Informed consent statement: The patient involved in this study gave his informed written consent prior to study enrollment, authorizing the use and disclosure of his protected health information.

Conflict-of-interest statement: The author declares no potential conflict of interest.

CARE Checklist (2013) statement: Guidelines of the CARE Checklist (2013) have been adopted while writing this manuscript.

Manuscript source: Invited manuscript

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Received: May 18, 2018
Peer-review started: May 18, 2018
First decision: June 6, 2018
Revised: June 19, 2018
Accepted: June 27, 2018
Article in press: June 28, 2018
Published online: October 27, 2018

Abstract

To our best knowledge, no case of a tumor that was incidentally detected during living donor hepatectomy (LDH) has been reported in the English language medical literature. We present two cases in which grade I neuroendocrine tumors (NET) were incidentally detected during our twelve-year LDH experience. First Case: A 26-year-old male underwent LDH for his brother suffering from HBV-related chronic liver disease (CLD). After right lobe LDH, intestinal length was measured as part of a study concerning the relationship between small intestinal lengths and surgical procedure. At this stage, a mass lesion with a size of 10 mm × 10 mm was detected on the antimesenteric surface, approximately 90 cm proximal to the ileocecal valve. A wedge resection with primary intestinal anastomosis was performed. Second Case: A 29-year-old male underwent right lobe LDH for his father with hepatitis B virus (HBV)-related CLD. An abdominal exploration immediately prior to the closure of the incision revealed that the appendix vermiformis was edematous and had firmness with a size of 8-10 mm at its tip. An appendectomy was performed. The pathological examinations of the specimens of both patients revealed

grade 1 NET. In conclusion, even if patients undergoing LDH are healthy individuals, whole abdominal cavity should be gently palpated and all findings recorded after completing laparotomy. Suspected masses or lesions should be confirmed by frozen section examination. Such an approach would avert potential medicolegal issues.

Key words: Living donor hepatectomy; Incidental tumor; Neuroendocrine tumor; Chronic liver disease; Hepatitis B virus

Core tip: To our best knowledge, no case of a tumor that was incidentally detected during living donor hepatectomy (LDH) has been reported in the English language medical literature. Herein, we present two cases in which grade I neuroendocrine tumors were incidentally detected during our twelve-year LDH experience. Even if patients undergoing LDH are healthy individuals, the whole abdominal cavity should be gently palpated and all findings be recorded after completing laparotomy. Suspected masses or lesions should be confirmed by frozen section examination. Such an approach would avert potential medicolegal issues.

Akbulut S, Isik B, Cicek E, Samdanci E, Yilmaz S. Neuroendocrine tumor incidentally detected during living donor hepatectomy: A case report and review of literature. *World J Hepatol* 2018; 10(10): 780-784 Available from: URL: http://www.wjgnet.com/1948-5182/full/v10/i10/780.htm DOI: http://dx.doi.org/10.4254/wjh.v10.i10.780

INTRODUCTION

While deceased donors are an important part of the liver donor pool in western countries, living donors constitute an important portion of the donor pool in many Asian countries including Turkey[1]. The most important problem with living donor liver transplantation is the mortality and morbidity risk faced by completely healthy donor candidates due to a major surgery like liver resection. In order to minimize those risks, all living liver donor (LLD) candidates undergo an examination according to an algorithm consisting of biochemical blood tests and advanced radiological instruments[2]. Incidental hemangioma, focal nodular hyperplasia, cystic lesions, median arcuate ligament, and ventricular septal defect have been rarely reported to be detected during examinations of LLD candidates[3-5]. On the other hand, no publication other than our study has ever reported unusual findings such as cancer detected incidentally in the liver or other intraabdominal organs during either preoperative investigations or in a living donor hepatectomy (LDH) procedure[6]. In this study we report two neuroendocrine tumor (NET) cases detected incidentally during our 12-year LDH experience.

CASE REPORT

Case 1

A 26-year-old healthy man applied to our transplant center for being a LLD to his 37-year-old brother with chronic liver disease (HBV). The donor candidate was taken into the operating room for LDH in May 2017. A laparotomy was performed through an incision starting from xiphoid to umblicus and extending laterally on the right side. As no macroscopic finding was detected in the liver, a right lobe LDH was performed. As part of a study conducted in our department investigating the relationship of mesenteric and antimesenteric lengths of small intestine with the surgical procedure, intestinal lengths of this patient were also measured. At this time, a mass lesion measuring approximately 10 mm was detected on the antimesenteric face of the intestine, approximately 90 cm proximal to the ileocecal valve (Figure 1). The mass was resected together with the mesentery of the adjacent intestinal segment. A wedge resection with primary intestinal anastomosis was performed. Intestinal mucosa and submucosa were closed with polyglactin 910 suture material while the seromuscular layer was closed with polypropylene. The patient experienced no complications during his postoperative follow-up and was discharged. A histopathological examination revealed a grade I neuroendocrine tumor (carcinoid tumor) with a size of 7 mm × 5 mm, which had intact surgical margins (Figure 2). An immunohistochemical analysis showed that it was NSE (+), Chromogranin (+), Synaptophysin (+), and Ki67 proliferation index (1%-2%) positive (Figures 3 and 4). The patient was put under follow-up by the medical oncology department, and a thoracoabdominal computerized tomography taken in the first controls revealed no additional lesions.

Case 2

A 29-year-old healthy man applied to our transplant center for being a LLD candidate for his 51-year-old father who had chronic liver disease (HBV + HCC). His preoperative biochemical tests and radiological studies were normal. The patient was taken into the operating room for right donor hepatectomy in May 2008. A laparotomy was performed through an incision starting from xiphoid to umblicus and extending laterally on the right side. As no macroscopic finding was detected in the liver, right lobe LDH was performed. At the time when the surgeon suspended the anterior abdominal wall in order to place a drain into the surgical field, it was noticed that the appendix vermiformis was edematous and had prominent superficial arterioles. In addition, a firmness measuring 8-10 cm in size was palpated at the tip of the appendix. Therefore, an appendectomy was performed. As no complications developed at the postoperative follow-up, the patient was discharged to be seen at

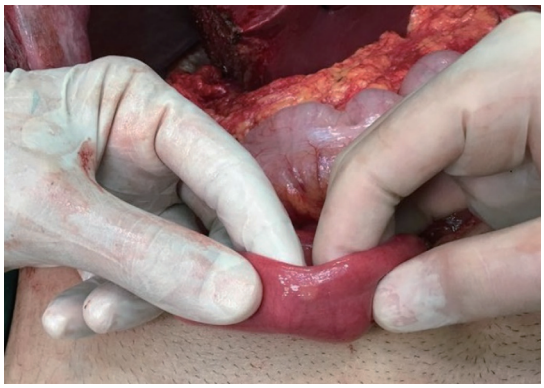

Figure 1 Intraoperative view of the tumor located in the antimesenteric border of the small intestine.

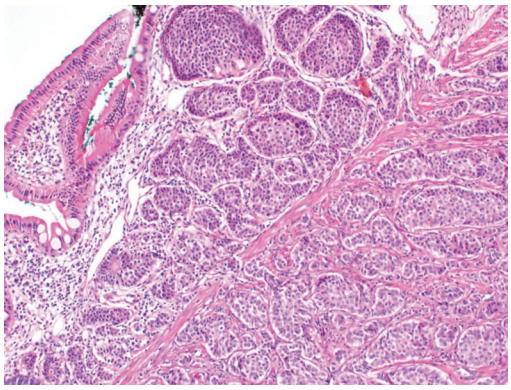

Figure 2 Tumor cells are seen in the submucosa with insulary pattern (HE × 100).

control visits. The pathology report of the appendix vermiformis showed a grade Ⅰ neuroendocrine tumor (NET) with a diameter of 10 cm at the tip of the appendix vermiformis, which showed full-thickness invasion but did not spare the mesoappendix (Figure 2). In addition, the lesion was NSE (+), Chromogranin (+), Synaptophysin (+), and Ki67 (1%) positive upon immunohistochemical analysis (Figures 3 and 4). As the patient was living in another city, he is currently under follow-up of another center there. He has recently informed us that no problems have occurred in his final control visit.

DISCUSSION

The World Health Organization has classified NETs into two main categories: well differentiated (low grade, intermediate grade, high grade neuroendocrine tumor) and poorly differentiated (high grade neuroendocrine carcinoma). The basic criteria for this classification are the Ki67 proliferation index (< 3%, 3%-20%, > 20%) and number of mitosis (< 2 mitosis, 2-20 mitosis, > 20 mitosis).

NETs most commonly involve the small intestine. NETs are most commonly located in the first 60 cm segment before the ileocecal valve. Unlike appendix NETs, small intestinal NETs have the potential to make both nodal and distant metastasis independent of their size. Therefore, the most appropriate surgical approach is to perform a partial small intestinal resection to involve lymph nodes and mesentery, independent of the tumor size. As small intestinal NETs have the potential of being multiple, all intestinal loops should be carefully inspected during the operation[7-9].

Appendix vermiformis is the second most commonly involved organ by NETs in the gastrointestinal system[10]. The majority of appendix NETs are incidentally detected in patients taken into operating room for a presumed diagnosis of acute appendicitis. NETs are diagnosed at a rate of 0.3%-2.27% in patients diagnosed with acute appendicitis while they are detected in 1.8%-2.3% of patients undergoing incidental appendectomy[7-9]. The prognosis and metastasis potential of appendix NETs depend on their size. Ninety-five percent of appendix NETs are smaller than 2 cm at the time of diagnosis and have a low metastasis potential (4%). In tumors with a diameter ⩾ 2 cm or patients with mesoappendix invasion, right hemicolectomy is the most appropriate approach. In tumors with a diameter ranging between 1.0 cm and 1.9 cm, adjunct hemicolectomy is the most appropriate approach when one or more of the following parameters exists: mesoappendix invasion, vascular invasion, high proliferation index (grade 2), suspicious/positive surgical margins, and mixed histology (goblet cell carcinoid, adenocarcinoid). Simple appendectomy is sufficient for patients without mesoappendix invasion despite a diameter of 1.0-1.9 cm and appendix NETs with a diameter < 1 cm. No adjunctive surgical procedure was undertaken since the patient presented here had a grade Ⅰ NET.

The follow-up of patients with NET depends on tumor size and grade. One or a combination of several diagnostic modalities of biochemical (5-hydroxyindoleacetic acid, serum chromogranin), endoscopic (pan endoscopy, colonoscopy), radiological (computed tomography, magnetic resonance imaging, Indium-111 pentetreotide (OctreoScan), and functional PET imaging with 68-Ga DOTA-TATE can be used for diagnosis. Patients with an appendix NET having a diameter of ⩾ 2 cm should be evaluated clinically and with biochemical/radiological studies whenever indicated at three and twelve months postoperation. Controls should be carried out after one year and advanced radiological tests should be performed as needed. In cases with a tumor size of less than 2 cm routine controls are not needed. The follow-up protocol of small intestinal NETs and appendix NETs should be in the form of close follow-up.

This case study brings into question whether it is necessary to palpate the whole abdominal cavity and especially gastrointestinal organs in healthy individuals undergoing laparotomy for LDH. Many studies so far have recommended not to touch the gastrointestinal tract unless indicated because even powder on surgical gloves may trigger the development of postoperative adhesions. In addition, handling, palpating, or removing the bowel

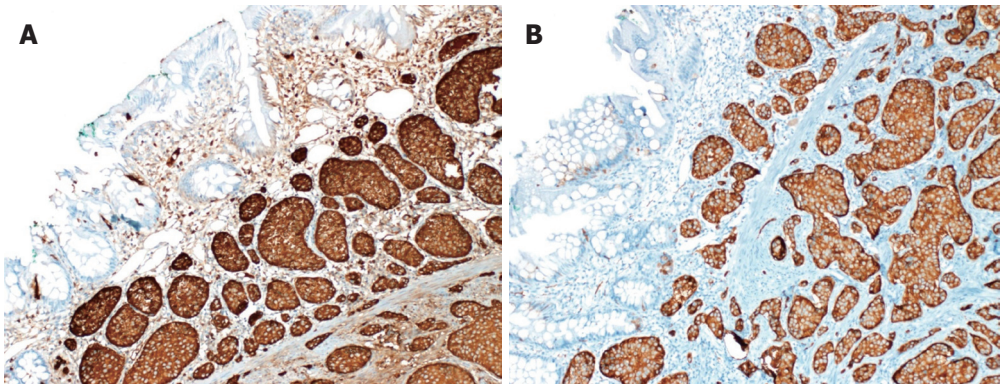

Figure 3 Positive immunostaining of tumor cells with chromogranin or synaptophysin antibody (HE × 100). A: Chromogranin; B: Synaptophysin.

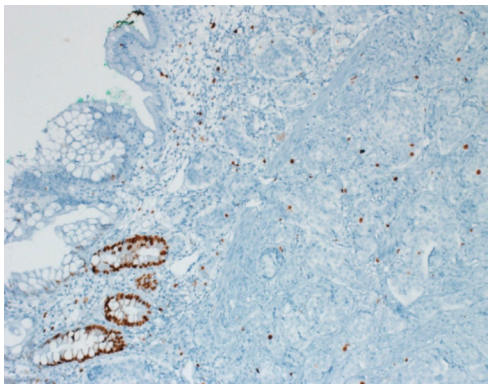

Figure 4 The low proliferation index in the tumor with Ki67 antibody (HE × 100).

loops may delay the return of intestinal motility during the postoperative period. Another question is what to do when a mass lesion is encountered in the gastrointestinal system. We suggest two methods: (1) when a tumoral lesion is detected in the gastrointestinal system before the LDH procedure, the lesion's gross macroscopic features should be evaluated and the entire mass or part of it should be resected and sent for frozen examination. When the result of the latter is a benign pathology, the hepatectomy procedure should be resumed. Whenever the result of the frozen examination is a malignant condition, hepatectomy procedure should be aborted and a definitive surgery should be performed against the mass; and (2) when a tumoral lesion is encountered incidentally at the exploration after the completion of the LDH procedure, a frozen examination of the tumoral lesion should be performed. In these patients, too, the result of the frozen examination should be obtained and surgical treatment should be carried out on the basis of oncological principles. However, an important caveat is what would be the fate of the liver graft harvested from these patients. In our opinion, ultrasonography should be performed for the living liver graft on the back table, and it should be transplanted when no hepatic lesion is identified. We assume that preoperative hepatic MDCT and dynamic MRI examinations are already negative for hepatic lesions in such patients. Recipients of such a liver should still be closely followed.

Unfortunately, we did not perform frozen examination in both patients presented here. The lesion of the patient with the appendix NET was both very small and located at the tip of the appendix. Hence, the frozen report would not affect our surgical strategy. As for the intestinal NET case, the lesion was of completely benign appearance, as can be seen in Figure 1. Despite this, we resected the segment with the lesion on the basis of the oncological principles. The recipients of both donors were put under close follow-up, and neither developed any lesion suspected to be a tumor to the date of the writing of this article.

In patients undergoing LDH operation, the abdominal cavity may be explored very gently using powderless gloves. This approach both creates a chance for early detection of some unexpected lesions and avoids potential medicolegal problems.

Whenever a suspicious lesion is detected before starting the LDH procedure, a frozen examination should be done from that lesion. When the frozen report indicates a malignant lesion, the LDH procedure should be aborted and the lesion should be resected on the basis of oncological principles. However, the question whether donors with NETs having a diameter smaller than 2 cm that are located to the tip of the appendix should be used is open to discussion.

ARTICLE HIGHLIGHTS

Case characteristics

We aimed to present two cases in which grade Ⅰ neuroendocrine tumors were incidentally detected during our twelve-year living donor hepatectomy experience.

Clinical diagnosis

Intraoperative appearance of the lesions of both patients was compatible with benign disease.

Differential diagnosis

Differential diagnosis of the first case included calcified nodule, benign intestinal tumor, and early stage malignant tumor. Differential diagnosis of the second

case includes acute appendicitis, mucocele, and carcinoid tumor

Laboratory diagnosis
No abnormal findings were detected in preoperative biochemical blood tests in both living liver donor candidates.

Imaging diagnosis
No abnormal findings were detected in preoperative radiological examinations in both living liver donor candidates.

Pathological diagnosis
The immunohistochemical examinations of the specimens of both patients were reported as grade Ⅰ neuroendocrine tumor.

Treatment
While a wedge resection with primary intestinal anastomosis was performed in the first case, simple appendectomy was performed in the second case.

Related reports
To our knowledge, no publication other than our study has ever reported unusual findings, such as cancer, detected incidentally during a living donor hepatectomy procedure.

Experiences and lessons
Even if patients undergoing living donor hepatectomy are healthy individuals, the whole abdominal cavity should be gently palpated and all findings recorded after completing laparotomy. Suspected masses or lesions should be confirmed by frozen section examination.

REFERENCES

1 **Akbulut S**, Yilmaz S. Liver transplantation in Turkey: historical review and future perspectives. *Transplant Rev* (Orlando) 2015; **29**: 161-167 [PMID: 25535023 DOI: 10.1016/j.trre.2014.12.002]

2 **Sapisochin G**, Goldaracena N, Laurence JM, Levy GA, Grant DR, Cattral MS. Right lobe living-donor hepatectomy-the Toronto approach, tips and tricks. *Hepatobiliary Surg Nutr* 2016; **5**: 118-126 [PMID: 27115005 DOI: 10.3978/j.issn.2304-3881.2015.07.03]

3 **Salama IA**, Dessouky BA, Korayem EM, Aal SA. Impact of multislice spiral computed tomography on donor selection and surgical planning in living-related liver transplant. *Exp Clin Transplant* 2010; **8**: 111-124 [PMID: 20565367]

4 **Zirpe D**, Muthukumaran CS, Vaidya A, Ramamurthy A. Incidental Ventricular Septal Defect (VSD) in the Donor of a Live Donor Liver Transplant: Tackle and Proceed. *J Clin Diagn Res* 2016; **10**: PD06-PD07 [PMID: 27790513 DOI: 10.7860/JCDR/2016/20839.8448]

5 **Akamatsu N**, Sugawara Y, Tamura S, Kaneko J, Togashi J, Makuuchi M. Impact of celiac axis stenosis on living donor hepatectomy. *Transplant Proc* 2006; **38**: 2948-2950 [PMID: 17112871 DOI: 10.1016/j.transproceed.2006.08.107]

6 **Yilmaz M**, Olmez A, Piskin T, Unal B, Ersan V, Sarici KB, Dirican A, Yilmaz S. Incidental appendectomy in donors undergoing hepatectomy for living-donor liver transplantation. *Transplant Proc* 2012; **44**: 1630-1634 [PMID: 22841232 DOI: 10.1016/j.transproceed.2012.04.012]

7 **Akbulut S**, Tas M, Sogutcu N, Arikanoglu Z, Basbug M, Ulku A, Semur H, Yagmur Y. Unusual histopathological findings in appendectomy specimens: a retrospective analysis and literature review. *World J Gastroenterol* 2011; **17**: 1961-1970 [PMID: 21528073 DOI: 10.3748/wjg.v17.i15.1961]

8 **Tartaglia D**, Bertolucci A, Galatioto C, Palmeri M, Di Franco G, Fantacci R, Furbetta N, Chiarugi M. Incidental appendectomy? Microscopy tells another story: A retrospective cohort study in patients presenting acute right lower quadrant abdominal pain. *Int J Surg* 2016; **28**: 149-152 [PMID: 26931338 DOI: 10.1016/j.ijsu.2016.02.085]

9 **Lee JH**, Choi JS, Jeon SW, Son CE, Bae JW, Hong JH, Lee KW, Lee YS. Laparoscopic incidental appendectomy during laparoscopic surgery for ovarian endometrioma. *Am J Obstet Gynecol* 2011; **204**: 28.e1-28.e5 [PMID: 20887970 DOI: 10.1016/j.ajog.2010.08.042]

10 **Caldarella A**, Crocetti E, Paci E. Distribution, incidence, and prognosis in neuroendocrine tumors: a population based study from a cancer registry. *Pathol Oncol Res* 2011; **17**: 759-763 [PMID: 21476126 DOI: 10.1007/s12253-011-9382-y]

P- Reviewer: Maida M, Ozdemir F, Sazcı A, Sugimura H
S- Editor: Ma YJ **L- Editor**: Filipodia **E- Editor**: Yin SY

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