World Journal of Hepatology

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

World J Hepatol 2018 November 27; 10(11): 837-848

DOI: 10.4254/wjh.v10.i11.837

ISSN 1948-5182 (online)

MINIREVIEWS

Decision modelling for economic evaluation of liver transplantation

Zhi Qu, Christian Krauth, Volker Eric Amelung, Alexander Kaltenborn, Jill Gwiasda, Lena Harries, Jan Beneke, Harald Schrem, Sebastian Liersch

Zhi Qu, Christian Krauth, Volker Eric Amelung, Alexander Kaltenborn, Jill Gwiasda, Lena Harries, Jan Beneke, Harald Schrem, Sebastian Liersch, Core Facility Quality Management and Health Technology Assessment in Transplantation, Integrated Research and Treatment Facility Transplantation (IFB-Tx), Hannover Medical School, Hannover 30625, Germany

Zhi Qu, Christian Krauth, Volker Eric Amelung, Lena Harries, Sebastian Liersch, Institute for Epidemiology, Social Medicine and Health Systems Research, Hannover Medical School, Hannover 30625, Germany

Harald Schrem, General, Visceral and Transplant Surgery, Hannover Medical School, Hannover 30625, Germany

ORCID number: Zhi Qu (0000-0003-0578-939X); Christian Krauth (0000-0003-0836-9737); Volker Eric Amelung (0000-0001-5721-2459); Alexander Kaltenborn (0000-0001-5885-0786); Jill Gwiasda (0000-0002-1749-5690); Lena Harries (0000-0003-3044-7906); Jan Beneke (0000-0003-2834-7164); Harald Schrem (0000-0002-5527-7555); Sebastian Liersch (0000-0002-0065-6101).

Author contributions: Qu Z, Krauth C and Schrem H conceptualized the overview. Qu Z, Gwiasda J and Liersch S drafted the manuscript. Amelung VE, Kaltenborn A, Harries L, Beneke J and Schrem H critically reviewed the manuscript and contributed important intellectual contents.

Supported by a grant from the German Federal Ministry of Education and Research, No. 01EO1302.

Conflict-of-interest statement: The authors of this manuscript declare no conflicts of interest.

Manuscript source: Unsolicited manuscript

Correspondence to: Zhi Qu, PhD, Doctor, Core Facility Quality Management and Health Technology Assessment in Transplantation, Integrated Research and Treatment Facility Transplantation (IFB-Tx), Hannover Medical School, Carl-Neuberg-Str. 1, Hannover 30625, Germany. qu.zhi@mh-hannover.de
Telephone: +49-511-5324453
Fax: +49-511-5325347

Received: May 25, 2018
Peer-review started: May 25, 2018
First decision: June 13, 2018
Revised: July 2, 2018
Accepted: October 9, 2018
Article in press: October 9, 2018
Published online: November 27, 2018

Abstract

As the gap between a shortage of organs and the immense demand for liver grafts persists, every available donor liver needs to be optimized for utility, urgency and equity. To overcome this challenge, decision modelling might allow us to gather evidence from previous studies as well as compare the costs and consequences of alternative options. For public health policy and clinical intervention assessment, it is a potentially powerful tool. The most commonly used types of decision analytical models include decision trees, the Markov model, microsimulation, discrete event simulation and the system dynamic model. Analytic models could support decision makers in the field of liver transplantation when facing specific problems by synthesizing evidence, comprising all relevant options, generalizing results

to other contexts, extending the time horizon and exploring the uncertainty. For modeling studies of economic evaluation for transplantation, understanding the current nature of the disease is crucial, as well as the selection of appropriate modelling techniques. The quality and availability of data is another key element for the selection and development of decision analytical models. In addition, good practice guidelines should be complied, which is important for standardization and comparability between economic outputs.

Key words: Cost benefit analysis; Decision tree; Liver transplantation; Decision analysis; Decision support models; Resource allocation; Cost effectiveness

Core tip: This overview focuses on providing an understanding of decision modelling approaches and their application to liver transplantation, outlining the major characteristics of decision analytic models as well as the individual strengths and weaknesses of several main techniques for modelling. We believe decision modelling may be able to provide tools by bringing all evidence from other studies together and comparing the costs and consequences of alternative options to reach a decision. It is a particularly powerful tool for public health policy and clinical intervention assessment.

Qu Z, Krauth C, Amelung VE, Kaltenborn A, Gwiasda J, Harries L, Beneke J, Schrem H, Liersch S. Decision modelling for economic evaluation of liver transplantation. *World J Hepatol* 2018; 10(11): 837-848 Available from: URL: http://www.wjgnet.com/1948-5182/full/v10/i11/837.htm DOI: http://dx.doi.org/10.4254/wjh.v10.i11.837

INTRODUCTION

The improvement of immunosuppression, innovation of splitting technique and growing clinical experience in liver transplantation have increased the utilization of available donor organs and survival rates[1,2]. Nevertheless, the crisis of organ shortage is subsisting. In 2013, 5921 livers were donated for transplantation in the US, while 12407 patients were waiting for an appropriate donor[3]. In 2016, 1567 patients received liver grafts, while 1704 remained on the waiting list in eight European countries[4]. The situation in Germany has been under particularly increasing pressure due to publicly discussed transplant scandals. Furthermore, the gap between donated organs and the necessity of transplants has been widening due to regulatory issues highlighting the relevance of public trust[5]. In 2011, 1191 liver transplantations were performed in Germany, but 1792 patients were listed for liver transplantation[6]. Although living donation and split-liver transplantation have been established to relieve the shortage of organs, the immense demand for liver grafts constantly increases[7-10]. Managing this widening gap remains a major challenge both ethically as well as economically. Decision modelling, based on real clinical and economic data, might provide the tools to overcome these challenges.

Due to this scarcity, every available donor liver should be allocated in a manner that maximizes its utility, urgency and equity. Required resources, funding and coverage by health care insurance for transplant systems need reliable information based on validated economic models to support political and practical decisions. The recent liver allocation system has been urgently confined in the past two decades, and prioritizes candidates by the Child-Turcotte-Pugh score or Model for End-Stage Liver Disease (MELD) score and its adaptions[11]. However, MELD scores and similar systems lack the predictive power for short- and long-term outcome of liver transplantation[12], in addition to the consideration of utility and transplant benefit. Furthermore, care management interventions and extensive treatment of liver transplant recipients are commonly required and consume considerable financial healthcare resources[13]. Therefore, selection and evaluation in this lifesaving procedure is an important topic in health economics[14].

Economic evaluation involves different aspects of transplantation. Evaluations of donor organ quality, recipient characteristics, as well as strategies for organ allocation demand an economically-based decision evaluation. Considerations of alternative therapies other than transplantation, as well as adequate immunosuppression therapy regimes after transplantation, require intensive evaluation. In addition, comorbidities and complications play an important role in the estimation of the cost-effectiveness of transplantation[13].

Decision analytical models combine information from various sources to assess the implications of different decisions, and could therefore generalize evidence from other contexts when local data and studies are unavailable. This sets them apart from statistical models[15]. Furthermore, when randomized clinical trials (RCT) cannot be performed due to practical or ethical issues, the power of decision analytical models lies in their ability to generate results without primary data[16]. This review focuses on providing an understanding of decision modelling approaches and their application to liver transplantation.

WHAT IS DECISION MODELLING?

Decision analytic modelling uses mathematical relationships to define a series of consequences that derive from a set of options[17]. Although it shares a common theoretical foundation with statistic models and has a

close association with Bayesian statistics[18], the key feature of decision analytic modelling accounts for the variability and uncertainty in all possible decisions. Moreover, it combines evidence from other studies like clinical-, cost- and health-related quality of life (QOL) data as utility values and compares the cost and consequences of alternative options. This generates a framework to reflect on the key differences of possible end points from all the alternative options in terms of cost and effect. It is thus a powerful tool for public health policy and clinical intervention assessment.

Even though the methods of decision analysis have been applied to medicine for over 40 years, their rather modest impact on real-world decision-making[19] has only recently been on the rise. To illuminate decision analytic models, we introduce the most commonly used types of models: Decision trees, the Markov model, microsimulation, discrete event simulation and the system dynamic model, illustrating how decision analytical models perform in the context of liver transplantation.

Decision trees

A decision tree model is recognized as the simplest structural decision analytical model and represents both the clinical decision procedure as well as consequential results in aggregate levels[15,20]. All clinical outcomes of patients in a decision tree model are visualized as a series of decision nodes and follow pathways with probabilities for each respective branch.

An example is given by Kantola *et al*[21] in Figure 1. This study was designed to determine cost-utility of molecular adsorbent recirculating system treatment in acute liver failure. The square node at the start of the tree represents the decision between alternative treatment strategies. The circular chance node shows the possible alternative events for a patient. Pathways (the "branches") following each node represent a series of alternative events, which are mutually exclusive. Probabilities show the likelihood of certain events, multiplying along the nodes and branches to estimate the overall probability of reaching the distinct outcome. Probabilities for all events assessed sum up to a total of one.

Following these branches and nodes, a total cost can be derived for the distinct combination of therapy options and compared to the potential benefit, such as in this case Quality Adjusted Life Years. Nevertheless, interpretation needs to account for clinical reason and include a careful discussion when assessing the most beneficial choice for combining therapies.

The simplicity and transparency of decision trees are their main advantages and may illustrate which possible set of options may be most promising. However, decision tree models can be very complex when used to model complicated long-term prognoses[18]. In other words, when they are used to model a chronic disease, decision trees can have numerous lengthy pathways representing recurring events, which is very time-consuming to analyze and interpret.

Markov models

Markov models are commonly used to provide a framework that represents sequences of events as a large number of complexity modelling options over time. Certain events lead to different health states (patients with different probabilities of transitioning from one state to another) given a defined period of time (cycle length). They commonly include large numbers of complexity modelling options. The number of states and the association among them are pre-defined in accordance with the decision problem, as well as the transition probabilities and cycle length[17,22].

Sarasin and his colleagues[23] showed an example of using Markov models to compare the gain of life expectancy and the cost-effectiveness of living and deceased donor liver transplantation in Figure 2. Each patient starts at the state of "cirrhosis, hepatocellular carcinoma (HCC), with no contraindications to cadaveric liver transplantation". With this initial state of health, they can then make a transition into several other states with different probabilities for each transition state for each defined, discrete time interval or cycle. They also might stay in their current state. The chances of transferring between different states are a set of defined transition probabilities derived from the appropriate transaction of longitudinal research data. Lengths of these cycles (one month in this example) depend on the disease or interventions of interest. To end the transition process in this Markov model, an absorbing state "death" was set that the patients obviously cannot leave once reached. Then, the Markov process modeled an integrity profile of both donor and recipient life expectancy over a lifetime-long horizon. The application of this Markov model handled the complexity of patients with early HCC and revealed that living donor liver transplantation is cost-effective compared to deceased donor liver transplantation under certain conditions[23].

A major advantage of Markov models is that they account for time dependency and can model changing probabilities over time. Therefore, Markov models are eligible to analyze chronic and complex conditions and clinical matters[24], such as the transplant field, relatively quickly and easily[13]. The important limitation of Markov models is the "Markov property", also called "Markov assumption", which assumes that transition probabilities only depend on the current health status but not on past history. Moreover, the Markov assumption might over-simplify the nature of disease, as it handles patient cohorts homogenously[18]. For higher resolution in this regard, an alternative approach known as patient-leveled simulation can be applied.

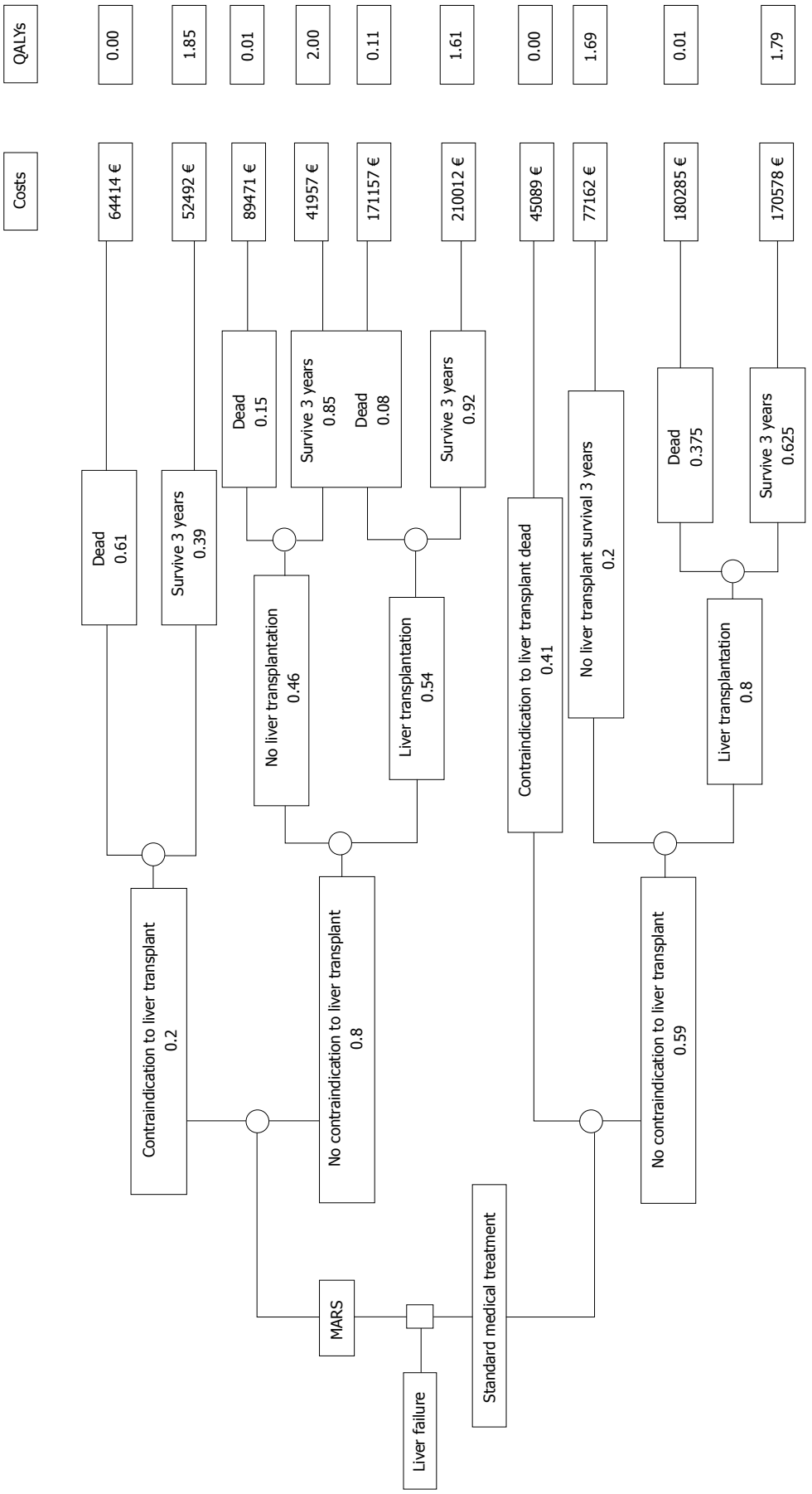

Figure 1 Decision tree for determining the short-term cost-utility of treatment in acute liver failure. Choice between strategies (decision node) and occurrence of chance events (chance node). Kantola *et al*[21]. MARS: Molecular adsorbent recirculating system.

Patient level simulation (microsimulation)

The microsimulation model is featured by "individual sampling", which means to simulate one individual at a time, rather than the whole cohort.

Perkins *et al*[25] developed a Markov-based microsimulation model to compare results under the present liver allocation policy in the United States (Figure 3). This model simulates how each patient proceeds through the model with the chance of multiple parallel events. One individual case is randomly selected from all patients.

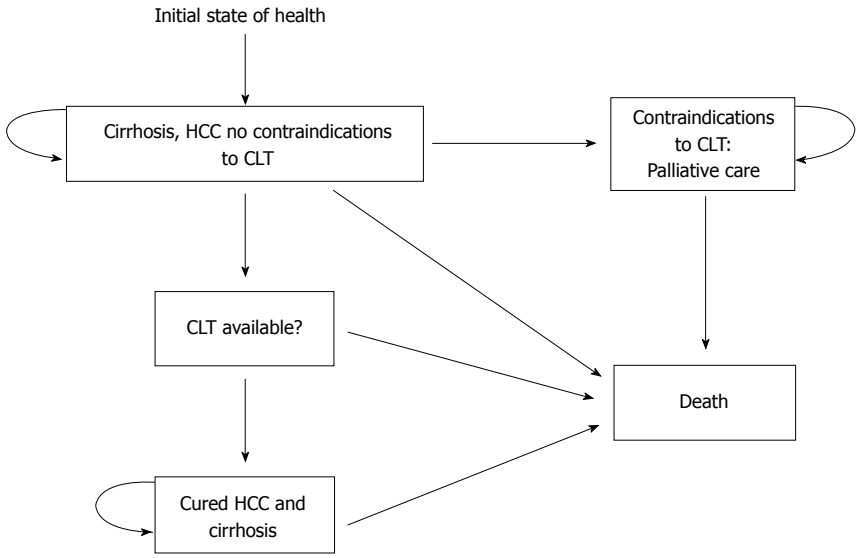

Figure 2 States of health in the decision model. Each square represents a state of health. Straight arrows represent the changes that may occur during each month. Curved arrows mean that the patient may remain in the same state of health. Sarasin *et al*[23]. HCC: Hepatocellular carcinoma.

The initial state is "alive". Patients can enter other states based on fixed transition probabilities, which simulate events in one cycle (three months in this study) until reaching the state "death". This study modeled the changes of the allocation policy, which demonstrated the survival benefit for the patients who have a MELD score $\leqslant$ 14 from transplantation, among the highly diverse patient population in the waiting list. The results appear more reliable than models based on aggregated data and could also be validated.

The advantage of microsimulation models is flexibility, in regards to different patterns of disease processes and intervention, because these models keep track of each individual's history[19]. Moreover, it can be useful when accumulating the history of each patient to determine the different transitions, costs and health benefits. However, there are also disadvantages in using microsimulation: First, outcome effective determinants in patients' history demand more detailed data, which challenge simply structured database research. Secondly, the simulation and computation of patient level simulation are time-consuming. Consequentially, the uncertainty assessment is not flexible when compared with other types of decision analytic models.

Discrete event simulations

Discrete event simulations (DES) can represent the competition for resources and investigate the changes in stochastic systems[26,27], and are mainly used to evaluate health care systems. The capacity and utility of allocation systems have previously been assessed before and after policy changes[28-31]. Another example reported by Shechter *et al*[26] is a biologically-based discrete-event simulation model, which represents the biological progression of end stage liver disease (ESLD) and examines the impact of changing allocation policies on this issue. The model was comprised of five modules: The patient generator, organ generator, pretransplant natural history, matching algorithm, and post-transplant survival (Figure 4). DES allows different modules to run independently, as this study shows, where pre-transplant history and allocation policy stand in parallel and individual patient attributes may influence the pathway, costs and outcomes. Unlike patient-level simulation models, DES is appropriate to model situations where constraints on resources could affect treatment options[15,32].

DES has several methodological advantages compared to other commonly used models, because it simulates the time until the next event for a given patient, which reduces the amount of time required for model construction and interim computations[18]. The output is not limited to survival only, but also allows estimations of event counts and sub-group analyses[33]. Moreover, statistical processing tools for relevant input parameters can be deployed. In contrast, structural complexity is the most prominent disadvantage of DES, which makes it difficult to apply to clinical research[34]. The complicated structure also makes computations more extensive, in regards to time and resources compared to Markov models when dealing with the same decision problem[35].

System dynamic models

System dynamic models allow modeling interactions within a population and with their environment over time; hence, they are especially suited for studies related to infectious diseases. The theoretic background of a system dynamic model is that complex behaviors of systems are a result of ongoing accumulations of

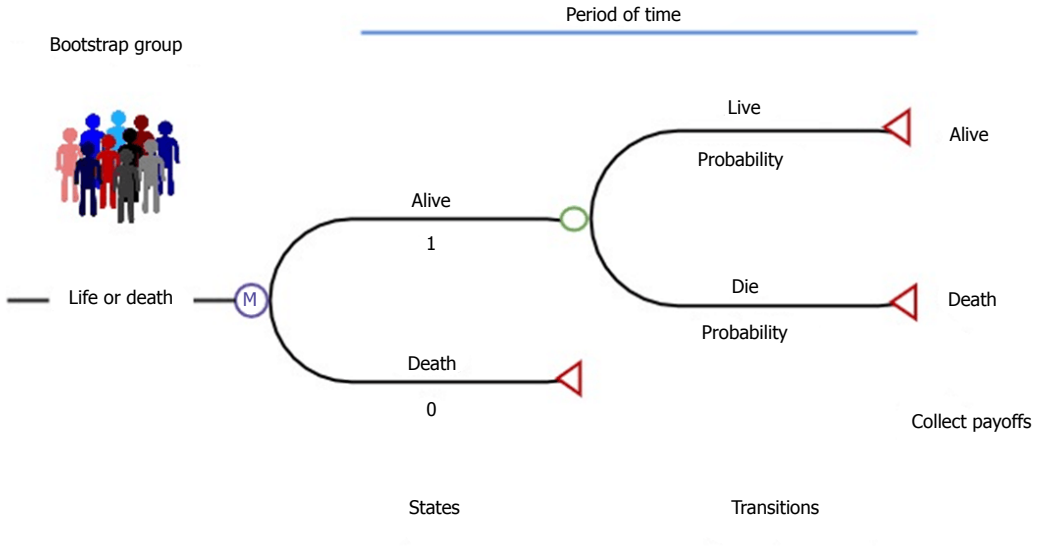

Figure 3 Simple example of a Markov microsimulation model. Perkins *et al*[25].

people, resources as well as biological and physiological states[36]. The probabilities of events can change through feedback of such accumulations. To the best of our knowledge, there is no application example of systematic dynamic models in the context of liver transplantation. However, examples of kidney[37] and corneal[38] transplantation showed that the predicted number of transplantations are consistent with observed results, which indicates the potential usefulness of system dynamic models for this field.

The advantage of system dynamic models is the information on interactions between individuals, which may quantify the impact of intervention on outcomes more accurately. Disadvantages are similar to DES, where information and interaction on individual level exponentially increase the complexity of model structure, computational burden, as well as lower transparency more than Markov models[19].

HOW DECISION ANALYTIC MODELLING CAN BE USED IN THE FIELD OF LIVER TRANSPLANTATION

Decision analytic models, in contrast to statistical models, incorporate decision-making into analysis[19]. Established in economic evaluations within other fields, decision analytic models in liver transplantation aim to inform decision-makers in two main areas: Decision analysis and measurement[18]. There are several aspects in which decision analytical modelling could help decision-makers in this field.

Combining different sources of evidence

In concordance with the principle of evidence-based medicine, decision-making on the basis of economic evaluation also requires the use of all accessible evidence related to the intervention effectiveness[39]. A decision analytical model offers a logic framework for the integration of data from very different sources, such as clinical trials, observational studies, insurance claim databases, case registries, public health statistics, and preference surveys[40]. In addition, more parameters related to resource utilization and utilities like unit cost, health-related QOL and preferences of patients are important evidence in economic evaluation[18]. This series of non-clinical indicators complement clinical data within the framework of decision analytics and support a much more complete picture of expectations from various parties involved. In particular, the specific data for patients with ESLD should also be organized into informative resources. Cillo *et al*[41] recommend a prospective assessment, which will substantially help decision analysis and support the decision-making process[42].

Comprising relevant options

In most instances, a single study cannot compare all the relevant alternative options for treatment paths for diseases such as ESLD. Decision-makers might therefore be challenged by a lacking comparison of all potentially effective interventions. New techniques like network meta-analysis extend the concept of indirect comparison by including multiple pairwise comparison information from clinical trials to constitute a network of evidence[43,44]. However, system dynamic models are more eligible to combine different types and sources of evidence, like clinical trials and patient questionnaires, and therefore adds fundamental information to the shared decision-making process.

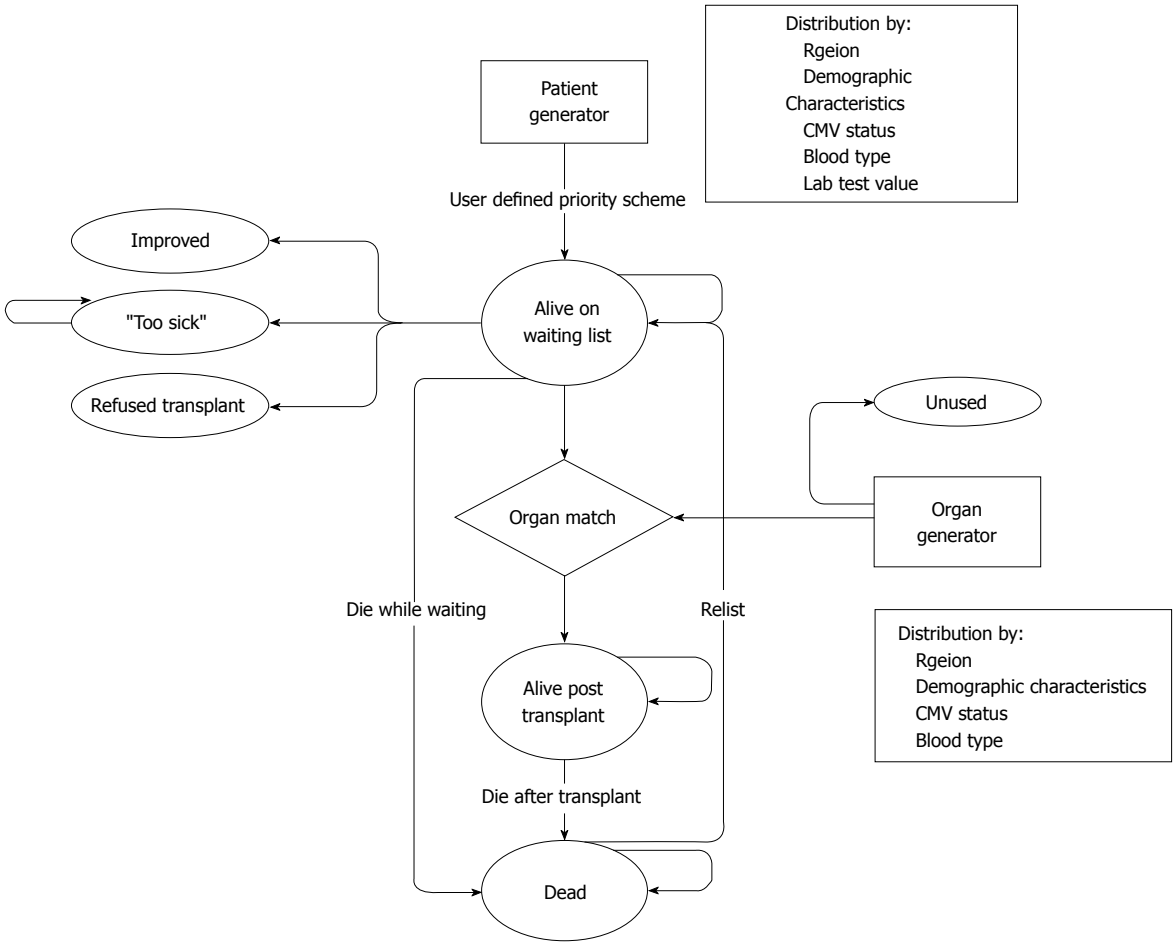

Figure 4 Model structure for patients entering the liver transplantation program. Shechter *et al*[26].

Applying results to other context/subgroups

The differences between patient subgroups can, for example, derive from either baseline characteristics like age, gender, comorbidity severity or variations in the healthcare context. The application of findings from one context can be difficult to transfer to other situations. Cucchetti *et al*[45] performed a study to measure the risk of age for salvaging transplantation in patients resected for HCC. A Markov model was developed to quantify the effect of patient's age above Milan criteria. Next, the risk of resection at two or three years below the age limit could be evaluated. The clinical evidence may not be able to show the difference between subgroups in heterogeneous patients over a long horizon[46]. However, in this research, the reduction of life expectancy of hepatic resection in different patient groups was clearly shown using a decision model.

Extending the time horizon

Many of the interventions for liver transplant patients require long time periods, and the weight of personal value added by these therapy options takes a long time for patients to assess. Therefore, models that evaluate the benefits of interventions for patients should cover sufficient time horizons. Long-term consequences, as well as costs of alternative options and interventions, are substantially affected by time. Even lifetime horizons are often needed for many models and are almost always required for models in which options have different time-varying survival rates[40]. Decision models offer the framework to include the effect and cost over time by adding respective results, and can evaluate the effects of main interventions beyond primary data sources and their continuous treatment effects[40].

Exploring the uncertainty

A key interest in liver transplantation is weighing the probabilities of risk and success between different options, especially in organ resource allocation and the decision of appropriate time-point selection for certain interventions. Not only are patients affected, but so are other potential organ recipients. Transplantation itself is not a definitively curing option, but leads to a life-long immunosuppressive treatment. Population variation, parametric imprecision as well as modelling selections and other aspects challenge predictive modelling, with uncertainty in different layers[47]. Clinical and economic data accessibility and validity also contribute to this

uncertainty[16].

In the face of these challenges, decision modelling methods are not only for reflecting this uncertainty but also to assess their influence so that the decision-makers can make choices with the relevant possibilities known. Analyses estimating the uncertainty due to parameters of interest is the most common approach to perform in modeling, which could be represented *via* deterministic sensitivity analysis (DSA) or probabilistic sensitivity analysis (PSA)[48]. In DSA, parameters in modelling are specified as multiple point estimates, and are varied manually to test the sensitivity of modeling results. In PSA, model inputs are specified as a distribution and varied to predefined probability distributions accordingly.

Along with the probabilistic analysis mentioned above, expected value of perfect information analysis is argued to be the most appropriate presentational technique for representing decision uncertainty. Jay and colleagues[49] showed the cost-effectiveness of organ donation after cardiac death versus after brain death. This novel sensitivity analysis represents both the probability of whether a decision is appropriate and its consequence, which is important for comparing the incremental net benefits under different accessibilities with the information of probabilities.

KEY POINT IN DECISION ANALYTICAL MODEL DEVELOPMENT

Understanding the nature of disease history

Model construction should combine efforts from multiple parties, including clinical and economic experts as well as decision-makers from the context of interest, and make best utilization of all available evidence. Neither the modeler nor the clinician alone can complete the task that conceptualizes an accuracy-simplicity balanced model. The accuracy of the model depends on whether the structure accounts for all important events or transitions and probabilities[25]. A thorough understanding of the disease is crucial for defining the possible health states in the model as well as capturing the occurrence of clinical events beyond follow-up[50].

Model characteristics and techniques

The key consideration of decision analytical model selection is the acceptance of the modelling technique, model "error", model appropriateness, dimensionality, and ease and speed of model development[32]. Decision trees are typically used when the process is not complicated, the recurrence of disease is not important and the time frame is short. Markov models are more feasible when simple chronic interventions are conducted. When the interaction is important, discrete event time and system dynamics could construct a more comprehensive and interactive system, but the development time and cost may significantly increase[51].

Figure 5 shows a flow chart for selecting the appropriate decision models based on the mentioned summaries and guidelines recommended by Barton *et al*[15] and Cooper *et al*[32].

Data quality and availability

The quality and availability of data is another key element for the selection and development of decision analytical models. Without sufficient and high quality data, the development of models will be difficult and result in low validity. As discussed above, synthesizing evidence is one of the most important fields that decision analytical modelling could help with during economic evaluation. In general, the information needed as input parameters for economic evaluation is derived from different kinds of data sources[52], including RCT, observational studies, secondary data analysis (*e.g.,* Meta-analysis) and expert opinions[50]. For topics of interest in liver transplantation, ethical considerations may additionally constrain the option of performing RCT. Therefore, data from published literature needs to be consolidated and considered in this context. In particular, reviews and reports from both the European Liver Transplant Registry, Organ Procurement and Transplantation Network as well as the Scientific Registry of Transplant Recipients database are valuable sources.

However, the consolidated data may cause incorrect estimations of parameters within the models, especially when multiple inputs are derived from single publications. In this situation, individual data from electronic medical databases or re-analysis of available published individual level data will be more appropriate. When several studies provide results on the same parameters of interest, researchers usually need to combine different results using meta-analytical methods[53] or adopt the results reported in meta-analyses. When potential biases in the original research or meta-analyses are handled appropriately, the inclusion of these results might increase uncertainty, which will be noted when constructing the model. Although expert opinion is the least preferable data source due to its subjectivity, it may still play an important role in the evaluation of cost and resource use when other sources of evidence are absent[54].

Good practice guidelines for modelling

The development of decision analytical models is a sophisticated task requiring the modelers to have sufficient experience, as well as the ability to evaluate, present and interpret the model's output. The complexity of the clinical pathway for complex interventions such as liver transplantation and the differences of health care environments between transplant centers and countries (*e.g.,* organ availability, allocation strategy, financial assistance for transplantation, post-

Table 1 Summary of types of decision models in liver transplantation

Model type	Model description	Type of scenario most suited for
Decision tree	Clinical outcomes are modelled as a series of decision nodes and follow pathways with probabilities for each respective branch.	Disease without relapse or recurrence.
Markov model	Represents sequences of events that lead to different health states with different probabilities of transitioning from one state to another over a defined period of time.	Chronic conditions involving recurrent events over time.
Microsimulation	Simulates one individual patient proceeding through the model with the chance of multiple parallel events.	Individual level information is important.
Discrete event simulation	Represents the competition for resources and investigates the changes in stochastic systems.	Interactions of resource allocation between individuals are of importance.
System dynamic model	Modeling interactions within a population and with their environment over time.	Spread of infectious diseases.

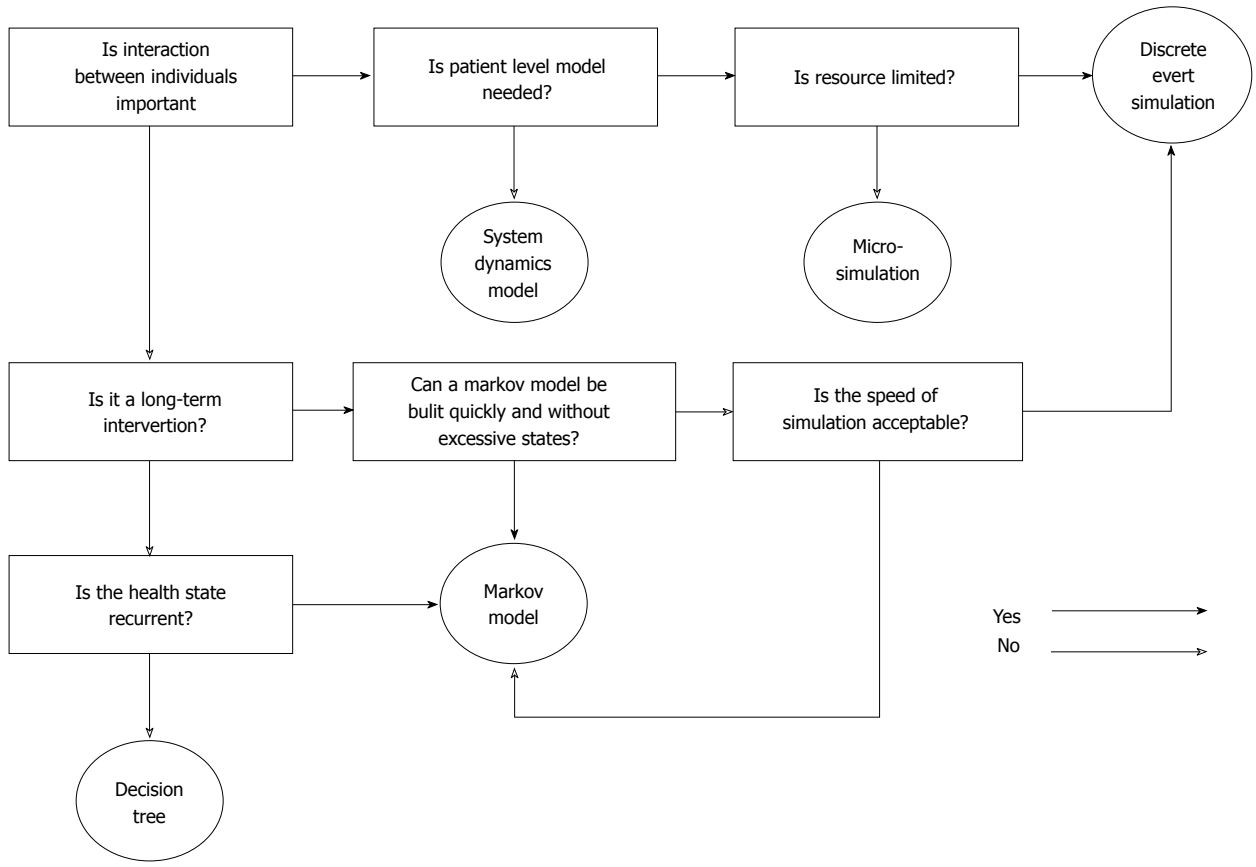

Figure 5 Scheme of selecting the appropriate model type.

transplant management and the consequential influence on QOL) challenge even the most experienced modeler to develop a model of economic outcomes of interest[51]. The best practice guidelines have therefore been significantly improving the process of model development, which is important for standardizing and comparing economic outputs.

The International Society for Pharmaco-economics and Outcomes Research Task Force group published a series of guidelines on good practice standards for modeling research, which set the standards for modeling practice[48,55-60]. However, these very detailed guidelines may not be well-understood in practice when performing a modeling study for the first time. To bridge this gap, Rautenberg *et al*[61] developed a beginner's guide to support modelers alongside the development of decision analytical cost-effectiveness models. This guide is especially helpful for researchers who are interested in utilizing this economic evaluation instrument, which is an easy-to-use practical guideline recommended for elementary modelers to initiate studies in this field.

CONCLUSION

This review demonstrates the major characteristics of decision analytic models (Table 1) as well as the

individual strengths and weaknesses of several main techniques for modelling. Decision trees are fit for disease interventions without relapse or recurrence. Markov models are suitable for interventions for chronic conditions involving recurring events over time. When individual level information is important, microsimulation models should be considered. If interactions between individuals are of importance, discrete event time models are suitable for simulation of the interaction of resource allocation. Dynamic models are fit to simulate the spread of infectious diseases.

Besides this, choosing the best depends on advanced understanding of the disease and its related interventions. Inter-professional cooperation is likely needed to combine methodological and clinical knowledge into a purposeful model. Furthermore, data availability and quality must be taken into account, which is as important as the definition and measurement of critical model components. The availability, weight and information of details for interventions, alternatives, target populations, health outcomes and time horizons have to be considered when conceptualizing the model. This is in regards to the modelling technique, model appropriateness and both the ease and speed of model development.

This framework of methods guides the analysis and interpretation of various data sources that further conclusions and a more advanced understanding of various elements and aspects of liver transplantation.

REFERENCES

1 **Zarrinpar A**, Busuttil RW. Liver transplantation: past, present and future. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 434-440 [PMID: 23752825 DOI: 10.1038/nrgastro.2013.88]

2 **Kim JS**, Broering DC, Tustas RY, Fischer L, Ganschow R, Burdelski M, Rogiers X. Split liver transplantation: past, present and future. *Pediatr Transplant* 2004; **8**: 644-648 [PMID: 15598341 DOI: 10.1111/j.1399-3046.2004.00264.x]

3 **Kim WR**, Lake JR, Smith JM, Skeans MA, Schladt DP, Edwards EB, Harper AM, Wainright JL, Snyder JJ, Israni AK, Kasiske BL. OPTN/SRTR 2013 Annual Data Report: liver. *Am J Transplant* 2015; **15** Suppl 2: 1-28 [PMID: 25626341 DOI: 10.1111/ajt.13197]

4 **Branger P,** Samuel U. Eurotransplant International Foundation. Annual Report 2016. Eurotransplant International Foundation 2017

5 **Schrem H**, Kaltenborn A. Germany: Avoid more organ transplant scandals. *Nature* 2013; **498**: 37 [PMID: 23739417 DOI: 10.1038/498037b]

6 **Manns MP**. Liver cirrhosis, transplantation and organ shortage. *Dtsch Arztebl Int* 2013; **110**: 83-84 [PMID: 23450999 DOI: 10.3238/arztebl.2013.0083]

7 **Schrem H**, Kleine M, Lankisch TO, Kaltenborn A, Kousoulas L, Zachau L, Lehner F, Klempnauer J. Long-term results after adult ex situ split liver transplantation since its introduction in 1987. *World J Surg* 2014; **38**: 1795-1806 [PMID: 24414197 DOI: 10.1007/s00268-013-2444-4]

8 **Chen CL**, Kabiling CS, Concejero AM. Why does living donor liver transplantation flourish in Asia? *Nat Rev Gastroenterol Hepatol* 2013; **10**: 746-751 [PMID: 24100300 DOI: 10.1038/nrgastro.2013.194]

9 **Ng KK**, Lo CM. Liver transplantation in Asia: past, present and future. *Ann Acad Med Singapore* 2009; **38**: 322-310 [PMID: 19434335]

10 **Yersiz H,** Renz JF, Busuttil RW. Split-liver transplantation: Past, present, and future. *Transplantation rev* 2004; **18**:164-170 [DOI: 10.1016/j.trre.2004.02.001]

11 **Cholongitas E**, Germani G, Burroughs AK. Prioritization for liver transplantation. *Nat Rev Gastroenterol Hepatol* 2010; **7**: 659-668 [PMID: 21045793 DOI: 10.1038/nrgastro.2010.169]

12 **Kaltenborn A**, Salinas R, Jäger MD, Lehner F, Sakirow L, Klempnauer J, Schrem H. Model of End-Stage Liver Disease Score and Derived Variants Lack Prognostic Ability after Liver Transplantation. *Ann Transplant* 2015; **20**: 441-448 [PMID: 26242315 DOI: 10.12659/AOT.893967]

13 **Machnicki G,** Seriai L, Schnitzler MA. Economics of transplantation: a review of the literature. *Transplantation rev* 2006; **20**: 61-75 [DOI: 10.1016/j.trre.2006.05.001]

14 **Jarl J,** Gerdtham U-G. Economic evaluations of organ transplantations-a systematic literature review. *Nordic Journal of Health Economics* 2011; **1** [DOI: 10.5617/njhe.168]

15 **Barton P**, Bryan S, Robinson S. Modelling in the economic evaluation of health care: selecting the appropriate approach. *J Health Serv Res Policy* 2004; **9**: 110-118 [PMID: 15099459 DOI: 10.1258/135581904322987535]

16 **Siebert U**. When should decision-analytic modeling be used in the economic evaluation of health care? *The European Journal of Health Economics, Springer*; 2003; **4**: 143-150 [DOI: 10.1007/s10198-003-0205-2]

17 **Briggs A,** Sculpher M, Claxton K. Decision modelling for health economic evaluation: OUP Oxford; 2006

18 **Drummond MF,** Sculpher MJ, Claxton K, Stoddart GL, Torrance GW. Methods for the economic evaluation of health care programmes: Oxford university press; 2015

19 **Kuntz K**, Sainfort F, Butler M, Taylor B, Kulasingam S, Gregory S, Mann E, Anderson JM, Kane RL. 2013 [PMID: 23534078]

20 **Petrou S**, Gray A. Economic evaluation using decision analytical modelling: design, conduct, analysis, and reporting. *BMJ* 2011; **342**: d1766 [PMID: 21482590 DOI: 10.1136/bmj.d1766]

21 **Kantola T**, Mäklin S, Koivusalo AM, Räsänen P, Rissanen A, Roine R, Sintonen H, Höckerstedt K, Isoniemi H. Cost-utility of molecular adsorbent recirculating system treatment in acute liver failure. *World J Gastroenterol* 2010; **16**: 2227-2234 [PMID: 20458759 DOI: 10.3748/wjg.v16.i18.2227]

22 **Sonnenberg FA**, Beck JR. Markov models in medical decision making: a practical guide. *Med Decis Making* 1993; **13**: 322-338 [PMID: 8246705 DOI: 10.1177/0272989X9301300409]

23 **Sarasin FP**, Majno PE, Llovet JM, Bruix J, Mentha G, Hadengue A. Living donor liver transplantation for early hepatocellular carcinoma: A life-expectancy and cost-effectiveness perspective. *Hepatology* 2001; **33**: 1073-1079 [PMID: 11343234 DOI: 10.1053/jhep.2001.23311]

24 **Briggs A**, Sculpher M. An introduction to Markov modelling for economic evaluation. *Pharmacoeconomics* 1998; **13**: 397-409 [PMID: 10178664 DOI: 10.2165/00019053-199813040-00003]

25 **Perkins JD**, Halldorson JB, Bakthavatsalam R, Fix OK, Carithers RL Jr, Reyes JD. Should liver transplantation in patients with model for end-stage liver disease scores <or= 14 be avoided? A decision analysis approach. *Liver Transpl* 2009; **15**: 242-254 [PMID: 19177441 DOI: 10.1002/lt.21703]

26 **Shechter SM**, Bryce CL, Alagoz O, Kreke JE, Stahl JE, Schaefer AJ, Angus DC, Roberts MS. A clinically based discrete-event simulation of end-stage liver disease and the organ allocation process. *Med Decis Making* 2005; **25**: 199-209 [PMID: 15800304 DOI: 10.1177/0272989X04268956]

27 **Brennan A**, Chick SE, Davies R. A taxonomy of model structures for economic evaluation of health technologies. *Health Econ* 2006; **15**: 1295-1310 [PMID: 16941543 DOI: 10.1002/hec.1148]

28 **Koizumi N**, Ganesan R, Gentili M, Chen CH, Waters N, DasGupta D, Nicholas D, Patel A, Srinivasan D, Melancon K. Redesigning Organ Allocation Boundaries for Liver Transplantation in the United

States. *Proc Int Conf Health Care Syst Eng* (2013) 2014; **61**: 15-27 [PMID: 26029745 DOI: 10.1007/978-3-319-01848-5_2]
29 **Iyer AK,** Schaefer AJ, Bryce CL, Zenarosa GL, Chang C-CH, Roberts MS, editors. A biologically based discrete-event simulation model of liver transplantation in the United States for pediatric and adult patients: Winter Simulation Conference; 2011 [DOI: 10.1109/wsc.2011.6147848]
30 **Toro-Díaz H**, Mayorga ME, Barritt AS, Orman ES, Wheeler SB. Predicting Liver Transplant Capacity Using Discrete Event Simulation. *Med Decis Making* 2015; **35**: 784-796 [PMID: 25391681 DOI: 10.1177/0272989X14559055]
31 **Orman ES**, Mayorga ME, Wheeler SB, Townsley RM, Toro-Diaz HH, Hayashi PH, Barritt AS 4th. Declining liver graft quality threatens the future of liver transplantation in the United States. *Liver Transpl* 2015; **21**: 1040-1050 [PMID: 25939487 DOI: 10.1002/lt.24160]
32 **Cooper K,** Brailsford SC, Davies R. Choice of modelling technique for evaluating health care interventions. *J Oper Res Soc* 2007; **58**: 168-176 [DOI: 10.1057/palgrave.jors.2602230]
33 **Comas M**, Castells X, Hoffmeister L, Román R, Cots F, Mar J, Gutiérrez-Moreno S, Espallargues M. Discrete-event simulation applied to analysis of waiting lists. Evaluation of a prioritization system for cataract surgery. *Value Health* 2008; **11**: 1203-1213 [PMID: 18494754 DOI: 10.1111/j.1524-4733.2008.00322.x]
34 **Caro JJ**, Möller J. Advantages and disadvantages of discrete-event simulation for health economic analyses. *Expert Rev Pharmacoecon Outcomes Res* 2016; **16**: 327-329 [PMID: 26967022 DOI: 10.1586/14737167.2016.1165608]
35 **Karnon J**. Alternative decision modelling techniques for the evaluation of health care technologies: Markov processes versus discrete event simulation. *Health Econ* 2003; **12**: 837-848 [PMID: 14508868 DOI: 10.1002/hec.770]
36 **Homer JB**, Hirsch GB. System dynamics modeling for public health: background and opportunities. *Am J Public Health* 2006; **96**: 452-458 [PMID: 16449591 DOI: 10.2105/AJPH.2005.062059]
37 **Patricio I,** Figal J. A System Dynamics Model of the Kidney Transplants in the U.S.; 2015
38 **Devi SP,** Rao KS, Krishnaswamy S, Wang S. System dynamics model for simulation of the dynamics of corneal transplants. *Opsearch* 2010; **47**: 284-92 [DOI: 10.1007/s12597-010-0023-0]
39 **Sackett DL**, Rosenberg WM, Gray JA, Haynes RB, Richardson WS. Evidence based medicine: what it is and what it isn't. *BMJ* 1996; **312**: 71-72 [PMID: 8555924 DOI: 10.1136/bmj.312.7023.71]
40 **Weinstein MC**, O'Brien B, Hornberger J, Jackson J, Johannesson M, McCabe C, Luce BR; ISPOR Task Force on Good Research Practices--Modeling Studies. Principles of good practice for decision analytic modeling in health-care evaluation: report of the ISPOR Task Force on Good Research Practices--Modeling Studies. *Value Health* 2003; **6**: 9-17 [PMID: 12535234 DOI: 10.1046/j.1524-4733.2003.00234.x]
41 **Cillo U**, Burra P, Mazzaferro V, Belli L, Pinna AD, Spada M, Nanni Costa A, Toniutto P; I-BELT (Italian Board of Experts in the Field of Liver Transplantation). A Multistep, Consensus-Based Approach to Organ Allocation in Liver Transplantation: Toward a "Blended Principle Model". *Am J Transplant* 2015; **15**: 2552-2561 [PMID: 26274338 DOI: 10.1111/ajt.13408]
42 **Sapisochin G**, Bruix J. Liver transplantation for hepatocellular carcinoma: outcomes and novel surgical approaches. *Nat Rev Gastroenterol Hepatol* 2017; **14**: 203-217 [PMID: 28053342 DOI: 10.1038/nrgastro.2016.193]
43 **Jansen JP**, Trikalinos T, Cappelleri JC, Daw J, Andes S, Eldessouki R, Salanti G. Indirect treatment comparison/network meta-analysis study questionnaire to assess relevance and credibility to inform health care decision making: an ISPOR-AMCP-NPC Good Practice Task Force report. *Value Health* 2014; **17**: 157-173 [PMID: 24636374 DOI: 10.1016/j.jval.2014.01.004]
44 **Greco T**, Biondi-Zoccai G, Saleh O, Pasin L, Cabrini L, Zangrillo A, Landoni G. The attractiveness of network meta-analysis: a comprehensive systematic and narrative review. *Heart Lung Vessel* 2015; **7**: 133-142 [PMID: 26157739]
45 **Cucchetti A**, Cescon M, Trevisani F, Morelli MC, Ercolani G, Pellegrini S, Erroi V, Bigonzi E, Pinna AD. What is the probability of being too old for salvage transplantation after hepatocellular carcinoma resection? *Dig Liver Dis* 2012; **44**: 523-529 [PMID: 22387286 DOI: 10.1016/j.dld.2012.01.018]
46 **Fuks D**, Dokmak S, Paradis V, Diouf M, Durand F, Belghiti J. Benefit of initial resection of hepatocellular carcinoma followed by transplantation in case of recurrence: an intention-to-treat analysis. *Hepatology* 2012; **55**: 132-140 [PMID: 21932387 DOI: 10.1002/hep.24680]
47 **Briggs AH**. Handling uncertainty in cost-effectiveness models. *Pharmacoeconomics* 2000; **17**: 479-500 [PMID: 10977389 DOI: 10.2165/00019053-200017050-00006]
48 **Briggs AH**, Weinstein MC, Fenwick EA, Karnon J, Sculpher MJ, Paltiel AD; ISPOR-SMDM Modeling Good Research Practices Task Force. Model parameter estimation and uncertainty: a report of the ISPOR-SMDM Modeling Good Research Practices Task Force--6. *Value Health* 2012; **15**: 835-842 [PMID: 22999133 DOI: 10.1016/j.jval.2012.04.014]
49 **Jay CL**, Skaro AI, Ladner DP, Wang E, Lyuksemburg V, Chang Y, Xu H, Talakokkla S, Parikh N, Holl JL, Hazen GB, Abecassis MM. Comparative effectiveness of donation after cardiac death versus donation after brain death liver transplantation: Recognizing who can benefit. *Liver Transpl* 2012; **18**: 630-640 [PMID: 22645057 DOI: 10.1002/lt.23418]
50 **Saramago P**, Manca A, Sutton AJ. Deriving input parameters for cost-effectiveness modeling: taxonomy of data types and approaches to their statistical synthesis. *Value Health* 2012; **15**: 639-649 [PMID: 22867772 DOI: 10.1016/j.jval.2012.02.009]
51 **Sun X**, Faunce T. Decision-analytical modelling in health-care economic evaluations. *Eur J Health Econ* 2008; **9**: 313-323 [PMID: 17943332 DOI: 10.1007/s10198-007-0078-x]
52 **Sculpher MJ**, Claxton K, Drummond M, McCabe C. Whither trial-based economic evaluation for health care decision making? *Health Econ* 2006; **15**: 677-687 [PMID: 16491461 DOI: 10.1002/hec.1093]
53 **Whitehead A**. Meta-analysis of controlled clinical trials. John Wiley & Sons, Ltd 2002: 175-196 [PMID: 11813282 DOI: 10.1002/0470854200.ch7]
54 **Tanajewski L**, Harris R, Harman DJ, Aithal GP, Card TR, Gkountouras G, Berdunov V, Guha IN, Elliott RA. Economic evaluation of a community-based diagnostic pathway to stratify adults for non-alcoholic fatty liver disease: a Markov model informed by a feasibility study. *BMJ Open* 2017; **7**: e015659 [PMID: 28679676 DOI: 10.1136/bmjopen-2016-015659]
55 **Caro JJ**, Briggs AH, Siebert U, Kuntz KM; ISPOR-SMDM Modeling Good Research Practices Task Force. Modeling good research practices--overview: a report of the ISPOR-SMDM Modeling Good Research Practices Task Force--1. *Value Health* 2012; **15**: 796-803 [PMID: 22999128 DOI: 10.1016/j.jval.2012.06.012]
56 **Roberts M**, Russell LB, Paltiel AD, Chambers M, McEwan P, Krahn M; ISPOR-SMDM Modeling Good Research Practices Task Force. Conceptualizing a model: a report of the ISPOR-SMDM Modeling Good Research Practices Task Force-2. *Med Decis Making* 2012; **32**: 678-689 [PMID: 22990083 DOI: 10.1177/0272989X12454941]
57 **Karnon J**, Stahl J, Brennan A, Caro JJ, Mar J, Möller J; ISPOR-SMDM Modeling Good Research Practices Task Force. Modeling using discrete event simulation: a report of the ISPOR-SMDM Modeling Good Research Practices Task Force--4. *Value Health* 2012; **15**: 821-827 [PMID: 22999131 DOI: 10.1016/j.jval.2012.04.013]
58 **Siebert U**, Alagoz O, Bayoumi AM, Jahn B, Owens DK, Cohen DJ, Kuntz KM; ISPOR-SMDM Modeling Good Research Practices Task Force. State-transition modeling: a report of the ISPOR-SMDM Modeling Good Research Practices Task Force--3. *Value Health* 2012; **15**: 812-820 [PMID: 22999130 DOI: 10.1016/j.jval.2012.06.014]

59 **Pitman R**, Fisman D, Zaric GS, Postma M, Kretzschmar M, Edmunds J, Brisson M; ISPOR-SMDM Modeling Good Research Practices Task Force. Dynamic transmission modeling: a report of the ISPOR-SMDM Modeling Good Research Practices Task Force--5. *Value Health* 2012; **15**: 828-834 [PMID: 22999132 DOI: 10.1016/j.jval.2012.06.011]
60 **Eddy DM**, Hollingworth W, Caro JJ, Tsevat J, McDonald KM, Wong JB; ISPOR−SMDM Modeling Good Research Practices Task Force. Model transparency and validation: a report of the ISPOR-SMDM Modeling Good Research Practices Task Force--7. *Value Health* 2012; **15**: 843-850 [PMID: 22999134 DOI: 10.1016/j.jval.2012.04.012]
61 **Rautenberg T**, Hulme C, Edlin R. Methods to construct a step-by-step beginner's guide to decision analytic cost-effectiveness modeling. *Clinicoecon Outcomes Res* 2016; **8**: 573-581 [PMID: 27785080 DOI: 10.2147/CEOR.S113569]

P- Reviewer: Hilmi I, Ho MC, Gad EH **S- Editor**: Dou Y
L- Editor: Filipodia **E- Editor**: Tan WW

ISSN 1948-5182 (online)

World Journal of *Hepatology*

World J Hepatol 2018 November 27; 10(11): 785-891

Published by Baishideng Publishing Group Inc

World Journal of Hepatology

Contents

Monthly Volume 10 Number 11 November 27, 2018

World Journal of Hepatology

Contents

Monthly Volume 10 Number 11 November 27, 2018

ABOUT COVER

Editorial Board Member of *World Journal of Hepatology*, Hie-Won Hann, MD, Professor, Department of Medicine, Division of Gastroenterology and Hepatology, Jefferson Medical College of Thomas Jefferson University, Philadelphia, PA 19107, United States

AIM AND SCOPE

World Journal of Hepatology (*World J Hepatol*, *WJH*, online ISSN 1948-5182, DOI: 10.4254), is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

WJH covers topics concerning liver biology/pathology, cirrhosis and its complications, liver fibrosis, liver failure, portal hypertension, hepatitis B and C and inflammatory disorders, steatohepatitis and metabolic liver disease, hepatocellular carcinoma, biliary tract disease, autoimmune disease, cholestatic and biliary disease, transplantation, genetics, epidemiology, microbiology, molecular and cell biology, nutrition, geriatric and pediatric hepatology, diagnosis and screening, endoscopy, imaging, and advanced technology. Priority publication will be given to articles concerning diagnosis and treatment of hepatology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

We encourage authors to submit their manuscripts to *WJH*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great basic and clinical significance.

INDEXING/ABSTRACTING

World Journal of Hepatology (*WJH*) is now abstracted and indexed in PubMed, PubMed Central, Emerging Sources Citation Index (Web of Science), Scopus, China National Knowledge Infrastructure (CNKI), and Superstar Journals Database.

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

EDITORIAL OFFICE
Jin-Lei Wang, Director
World Journal of Hepatology
Baishideng Publishing Group Inc
7901 Stoneridge Drive, Suite 501,
Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: editorialoffice@wjgnet.com
Help Desk: http://www.f6publishing.com/helpdesk
http://www.wjgnet.com

PUBLISHER
Baishideng Publishing Group Inc
7901 Stoneridge Drive, Suite 501,
Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.f6publishing.com/helpdesk
http://www.wjgnet.com

PUBLICATION DATE
November 27, 2018

INSTRUCTIONS TO AUTHORS
http://www.wjgnet.com/bpg/gerinfo/204

ONLINE SUBMISSION
http://www.f6publishing.com

World Journal of Hepatology

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

World J Hepatol 2018 November 27; 10(11): 785-789

DOI: 10.4254/wjh.v10.i11.785

ISSN 1948-5182 (online)

EDITORIAL

Exosomal microRNAs as a potential therapeutic strategy in hepatocellular carcinoma

Angélique Gougelet

Angélique Gougelet, Inserm, U1016, Institut Cochin, Paris 75014, France

Angélique Gougelet, Cnrs, UMR8104, Paris 75014, France

Angélique Gougelet, Université Paris Descartes, Sorbonne Paris Cité, Paris 75006, France

ORCID number: Angélique Gougelet (0000-0001-7464-9804).

Author contributions: Gougelet A constructed and wrote the manuscript.

Conflict-of-interest statement: The author has no conflict of interest to declare. No financial support.

Manuscript source: Invited manuscript

Correspondence to: Angélique Gougelet, PhD, Research Scientist, Inserm, U1016, Institut Cochin, 24, rue du Faubourg Saint Jacques, Paris 75014, France. angelique.gougelet@inserm.fr
Telephone: +33-14-4412446
Fax: +33-14-4412421

Received: August 2, 2018
Peer-review started: August 3, 2018
First decision: August 24, 2018
Revised: September 5, 2018
Accepted: October 10, 2018
Article in press: October 10, 2018
Published online: November 27, 2018

Abstract

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the second cause of cancer-related death worldwide. The incidence of HCC is constantly increasing in correlation with the rise in diabetes and obesity, arguing for an urgent need for new developments in the treatment of this lethal cancer. Exosomes are small double-membrane vesicles loaded with distinct cargos, particularly small non-coding RNAs called microRNAs, representative of each donor cell and secreted to affect the features of neighboring cells or recipient cells located further away, like in the case of metastasis. A better understanding of the role of exosomes with a microRNA signature in cancer pathogenesis gave rise to the concept of their use as a non-invasive diagnostic biomarker and in the treatment of cancer, including HCC. In this communication, we review recent works that demonstrate that hepatic stellate cells establish an epigenetic communication with liver cancer cells, which affects their pro-malignant features. If naturally secreted patient-derived exosomes show major limitations concerning their clinical use, bio-engineered exosome mimetics that incorporate controlled components and exhibit no protumoral properties could be promising carriers for the treatment of liver cancers, which is the organ preferentially targeted by systemic injection of exosomes.

Key words: MicroRNAs; Hepatocellular carcinoma; Targeted therapy; Exosomes

Core tip: Despite the intensive research efforts to identify the molecular events responsible for the emergence of liver cancer, hepatocellular carcinoma (HCC) remains a major health problem worldwide. Thus, the identification of new therapeutic opportunities to

counteract the challenging issues linked to HCC heterogeneity and resistance to conventional treatments is a short-term necessity. Over the last few decades, microRNAs have appeared as interesting therapeutic strategies with their pleiotropic inhibitory action, but the use of a delivery system is a requirement for miRNA mimic administration. Exosomes, which are small vesicles naturally produced by immune cells and aberrantly by cancer cells, have recently emerged as a promising vehicle.

Gougelet A. Exosomal microRNAs as a potential therapeutic strategy in hepatocellular carcinoma. *World J Hepatol* 2018; 10(11): 785-789 Available from: URL: http://www.wjgnet.com/1948-5182/full/v10/i11/785.htm DOI: http://dx.doi.org/10.4254/wjh.v10.i11.785

INTRODUCTION

Hepatocellular carcinoma (HCC) is the second cause of cancer-related death worldwide for which therapeutic options are very limited. Indeed, because of its heterogeneity, the development of effective therapies against this cancer remains a challenging issue. HCC is considered to be a paradigm of inflammation-associated cancer, since 80% of HCC emerges following vast liver remodeling. Briefly, HCC primarily affects men with cirrhosis due to hepatitis B and C viruses, alcohol abuse, genotoxic exposure and metabolic disorders, and the incidence is increasing due to diabetes and obesity[1]. Efforts in the molecular and genetic profiling of HCC revealed that among the mutational landscape of HCC, the Wnt/β-catenin, p53 and Ras pathways are the most frequently mutated. Other prevalent mutations occur in epigenetic modifiers such as chromatin remodelers and imprinted clusters[2].

Despite these molecular findings, the molecular pathogenesis of HCC is still not fully understood, and novel strategies are urgently needed to cure this lethal disease with high incidence. Over the last decades, other crucial epigenetic regulators, the small non-coding RNAs named microRNAs (miRNAs), have been largely found to be disturbed during hepatocarcinogenesis[3]. Promisingly, due to their large spectrum of action on proliferation, inflammation and metabolism, miRNAs have emerged as robust therapeutic opportunities in various cancers. Regarding liver diseases, miRNA-based therapies have been successfully tested[4,5] - this type of molecule is preferentially delivered to the liver[6]. Despite promising results obtained with miRNA-based therapies, a number of challenges remain to improve the efficiency of this type of treatments. Their limitations are similar to those which have delayed the use of therapies based on small interfering RNAs: Improvement of stability, free or encapsulated administration, problems of specificity, tissue distribution, response persistence and secondary effects.

Recently, new therapeutic candidates for anticancer drug delivery have been proposed that are based on a biological system of transporting active cargos called exosomes. Exosomes are double membrane cell-derived microvesicles defined by a diameter of 30 to 100 nm that contain a great diversity of nucleic acids, protein and lipids - 3408 mRNAs, 2838 miRNAs and 9769 proteins according to the Exocarta database based on 286 studies[7]. Most cells, and particularly immune cells, are physiologically secreting exosomes that originate from multivesicular bodies. Multivesicular body fusion with the plasma membrane is orchestrated by Rab, soluble N-ethylmaleimide-sensitive-factor attachment protein (commonly known as SNAP) and SNAP receptor (commonly known as SNARE) proteins[8]. In response to different activating signals like antigenic, cytokinic or mitogenic stimuli, immune cells are able to increasingly release these small vesicles[9]. Recent studies revealed that disequilibrium in exosome formation and/or delivery contributes to pathological processes leading to immunological disorders and cancers. Indeed, during tumorigenesis, tumor cells aberrantly secrete exosomes to communicate with stromal cells and to modify secondary sites favoring metastasis[10]. In consequence, the detection of exosomal miRNAs in body fluids has appeared as a potent non-invasive diagnosis tool for cancer, including HCC[11], but also as a new therapeutic opportunity.

Exosome-based therapies have emerged over the past decade as an attractive strategy for tissue repair, immune vaccine and against cancer, firstly because of their biocompatibility. Second, their small size facilitates their crossing through biological barriers, notably the blood-brain barrier, and limits their renal clearance. These microvesicles might prove to be suitable for liver disease, especially for liver cancer treatment, since exosomes accumulate in the liver after systemic injection. In particular, exosomes preferentially target the resident macrophages, the Kupffer cells. Interestingly, the uptake of extracellular vesicles by Kupffer cells increases in the case of liver damage[12]. Over the past two years, the Selaru laboratory published two compelling manuscripts studying exosomes carrying miRNAs as a way to dialog between stromal cells, in particular stellate cells, and cancer cells in cholangiocarcinoma (CCA)[13] and in HCC[14]. Both studies revealed the clinical potential of miRNAs loaded in stellate cell-derived exosomes for *in vivo* delivery in mice and, on a longer-term perspective, CCA or HCC treatment.

STUDY ANALYSIS

The work from Wang *et al*[14] focused on miR-335-5p, a microRNA already described as a tumor suppressor in HCC[15] and that is known to gradually decrease in activated hepatic stellate cells (HSCs) during hepatic fibrosis[16]. This miRNA is also reduced in the serum of

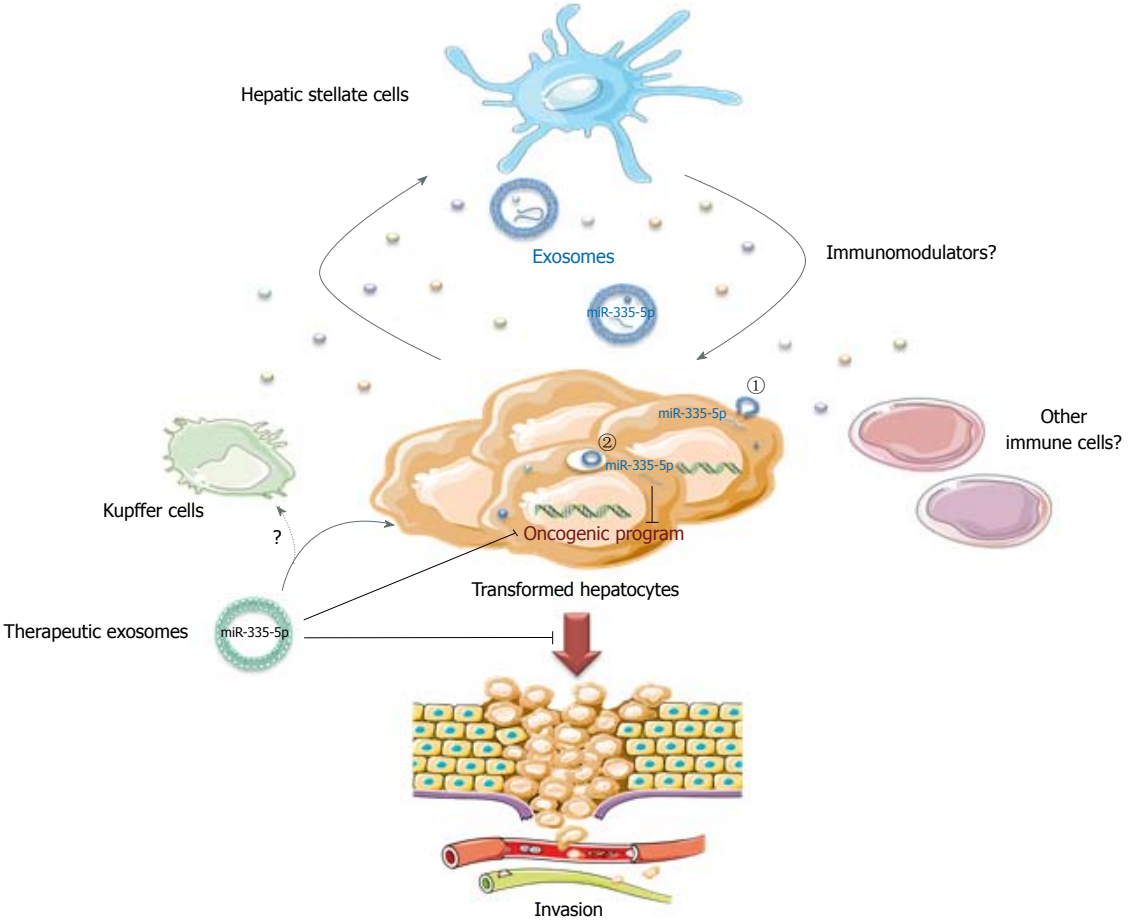

Figure 1 An exosomal miR-335-5p-based therapy for hepatocellular carcinoma. In the case of hepatocellular carcinoma, miR-335-5p is lost in cancer cells, favoring cell proliferation and invasion. The hepatic stellate cells could counteract these pro-malignant features by secreting exosomes containing nucleic acids and miRNAs, including miR-335-5p, which are captured by HCC cells by a direct fusion with recipient cell membrane (1) or by endocytosis (2). Mimicking this biological process, therapeutic exosomes, either isolated from patients or bioengineered exosome mimetics, loaded with miR-335-5p might slow cell proliferation, promote apoptosis and limit cell invasion. It remains to be determined whether other immune cells could participate in this material transfer and which immunomodulators could regulate this exchange.

HCC patients in association with progressive features and is predictive of chemo-embolization response[17]. For their study, the authors used different HCC cell lines either mutated for p53 (HuH-7) or β-catenin (HepG2), or infected with HBV (MHCC97) with low or high metastatic features. They confirmed a global tumor suppressive role of miR-335-5p associated with miR-335-5p loss in all cell lines compared to the LX-2 HSC line[14]. A miR-335-5p mimic inhibited HCC cell proliferation and invasion upon transfection, but also, crucially, when transfected in LX-2 cells seeded in co-culture with HCC cells. This suggests that a miRNA dialog between HCC and stromal cells might be established to favor tumor progression and invasion (Figure 1).

To confirm the material transfer *via* the exosomal route between HSC and HCC cell lines, the authors used an elegant system with two fluorophores coupled to a stop signal based on the Cre-lox strategy (loxP-dsRED-loxP-stop-eGFP) transfected in HCC cells. Using this tool, they demonstrated that LX-2 cells transferred Cre recombinase to neighboring HCC cells, as evidenced by green labeling. An efficient transfer was also observed when exosomes were purified from LX-2 cells and later added to HCC cell culture. *In vivo*, intra-tumoral injection of LX-2 Cre-positive exosomes to a subcutaneous HCC xenograft also led to GFP signal in the tumors. This means that the construct was efficiently recombined in HCC *via* exosome capture. Finally, with a view to treatment, Wang *et al*[14] showed that LX-2 isolated exosomes, extemporaneously enriched with miR-335-5p, reduce HCC growth after intra-tumoral injection every 2 d for 4 wk in MHCC97H cell xenografts.

The intra-tumoral administration was preferred to concentrate exosomes into the tumor mass and to mimic the historically intra-arterial administration of chemotherapeutic agents. Exosome treatment led to overexpression of miR-335-5p by 30-fold in the tumors and modification of its target landscape, resulting in an attenuation of proliferation and an increase in apoptosis. This study confirmed the previous observations

performed by the Selaru laboratory showing that LX2 cells secrete miR-195-enriched exosomes, which can communicate with CCA cells and decrease *in vivo* tumor growth in a CCA rat model after intravenous injections every 2 d[13].

PERSPECTIVES

In conclusion, these proof of concept studies performed in two different models of liver cancer support the existence of an epigenetic dialog between cancer cells and stromal cells driven by exosomes to favor tumor progression. In particular, HSCs play an important part in this dialog, but other non-parenchymal cells like Kupffer cells could also probably participate. They also highlight that HSC-derived exosome manipulation succeeds in restoring a more physiological expression of miRNAs found deregulated in cancer cells *in vivo*. The restoration of miRNA expression modifies gene expression, and subsequently limits cell proliferation and favors apoptosis. These results, and others generated in various cancer models, support extracellular vesicles as an attractive modality for personalized treatment for liver cancer, especially since this type of particle primarily targeted the liver after systemic injection.

Several studies have used exosomes as a targeted delivery system for chemotherapeutic agents, leading to cancer cell death and promoting a domino effect through the release of secondary cytotoxic vesicles[18]. Additionally, exosomes produced by mesenchymal stem cells have been largely studied in liver disease and found to be modulators of the immune response, favored by their engulfment by resident macrophages. They are also key modulators of oxidative stress and fibrotic processes[19]. Despite all these encouraging features, a number of limitations for their clinical feasibility are currently a brake for using these delivery systems. Indeed, the production of patient-derived exosomes for clinical application appears expensive, time-consuming and complex (preparation method, loading, characterization, *etc*). Since these biological carriers present pro-tumoral characteristics, the pro-malignant factors have to be preliminarily identified and removed before re-injection. The reproducibility also remains a major barrier, since a previous study suggested that three independent preparations of exosomes from mesenchymal stem cells only shared 20% of their proteome[20].

A promising alternative for extracellular vesicle-based therapeutics is the synthesis of bioengineered exosome mimetics, which could allow the production of exosome preparations suitable for clinical use (sterile, characterized, reproducible)[21]. In conclusion, even if about a hundred clinical trials are currently testing the benefit of exosomes as therapeutic agents, a gold standard method for their isolation and loading has to be approved. A better characterization of their specificity, functionality and safety is required, for which liver cancer, characterized by its pro-inflammatory microenvironment and its refractoriness to conventional treatments, undoubtedly constitutes a model of choice.

REFERENCES

1 **El-Serag HB**, Rudolph KL. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. *Gastroenterology* 2007; **132**: 2557-2576 [PMID: 17570226 DOI: 10.1053/j.gastro.2007.04.061]

2 **Schulze K**, Imbeaud S, Letouzé E, Alexandrov LB, Calderaro J, Rebouissou S, Couchy G, Meiller C, Shinde J, Soysouvanh F, Calatayud AL, Pinyol R, Pelletier L, Balabaud C, Laurent A, Blanc JF, Mazzaferro V, Calvo F, Villanueva A, Nault JC, Bioulac-Sage P, Stratton MR, Llovet JM, Zucman-Rossi J. Exome sequencing of hepatocellular carcinomas identifies new mutational signatures and potential therapeutic targets. *Nat Genet* 2015; **47**: 505-511 [PMID: 25822088 DOI: 10.1038/ng.3252]

3 **Gougelet A**, Colnot S. [microRNA: new diagnostic and therapeutic tools in liver disease?]. *Med Sci* (Paris) 2013; **29**: 861-867 [PMID: 24148124 DOI: 10.1051/medsci/20132910013]

4 **Gougelet A**, Sartor C, Bachelot L, Godard C, Marchiol C, Renault G, Tores F, Nitschke P, Cavard C, Terris B, Perret C, Colnot S. Antitumour activity of an inhibitor of miR-34a in liver cancer with β-catenin-mutations. *Gut* 2016; **65**: 1024-1034 [PMID: 25792709 DOI: 10.1136/gutjnl-2014-308969]

5 **Shibata C**, Otsuka M, Kishikawa T, Ohno M, Yoshikawa T, Takata A, Koike K. Diagnostic and therapeutic application of noncoding RNAs for hepatocellular carcinoma. *World J Hepatol* 2015; **7**: 1-6 [PMID: 25624991 DOI: 10.4254/wjh.v7.i1.1]

6 **Roberts J**, Palma E, Sazani P, Ørum H, Cho M, Kole R. Efficient and persistent splice switching by systemically delivered LNA oligonucleotides in mice. *Mol Ther* 2006; **14**: 471-475 [PMID: 16854630 DOI: 10.1016/j.ymthe.2006.05.017]

7 **Keerthikumar S**, Chisanga D, Ariyaratne D, Al Saffar H, Anand S, Zhao K, Samuel M, Pathan M, Jois M, Chilamkurti N, Gangoda L, Mathivanan S. ExoCarta: A Web-Based Compendium of Exosomal Cargo. *J Mol Biol* 2016; **428**: 688-692 [PMID: 26434508 DOI: 10.1016/j.jmb.2015.09.019]

8 **Théry C**, Zitvogel L, Amigorena S. Exosomes: composition, biogenesis and function. *Nat Rev Immunol* 2002; **2**: 569-579 [PMID: 12154376 DOI: 10.1038/nri855]

9 **Blanchard N**, Lankar D, Faure F, Regnault A, Dumont C, Raposo G, Hivroz C. TCR activation of human T cells induces the production of exosomes bearing the TCR/CD3/zeta complex. *J Immunol* 2002; **168**: 3235-3241 [PMID: 11907077 DOI: 10.4049/jimmunol.168.7.3235]

10 **Kalluri R**. The biology and function of exosomes in cancer. *J Clin Invest* 2016; **126**: 1208-1215 [PMID: 27035812 DOI: 10.1172/JCI81135]

11 **Gougelet A**, Colnot S. Hepatocellular carcinoma diagnosis: Circulating microRNAs emerge as robust biomarkers. *Clin Res Hepatol Gastroenterol* 2016; **40**: 367-369 [PMID: 26823043 DOI: 10.1016/j.clinre.2015.12.010]

12 **Haga H**, Yan IK, Takahashi K, Matsuda A, Patel T. Extracellular Vesicles from Bone Marrow-Derived Mesenchymal Stem Cells Improve Survival from Lethal Hepatic Failure in Mice. *Stem Cells Transl Med* 2017; **6**: 1262-1272 [PMID: 28213967 DOI: 10.1002/sctm.16-0226]

13 **Li L**, Piontek K, Ishida M, Fausther M, Dranoff JA, Fu R, Mezey E, Gould SJ, Fordjour FK, Meltzer SJ, Sirica AE, Selaru FM. Extracellular vesicles carry microRNA-195 to intrahepatic cholangiocarcinoma and improve survival in a rat model. *Hepatology* 2017; **65**: 501-514 [PMID: 27474881 DOI: 10.1002/hep.28735]

14 **Wang F**, Li L, Piontek K, Sakaguchi M, Selaru FM. Exosome miR-335 as a novel therapeutic strategy in hepatocellular carcinoma. *Hepatology* 2018; **67**: 940-954 [PMID: 29023935 DOI: 10.1002/hep.29586]

15 **Liu H**, Li W, Chen C, Pei Y, Long X. MiR-335 acts as a potential

tumor suppressor miRNA via downregulating ROCK1 expression in hepatocellular carcinoma. *Tumour Biol* 2015; **36**: 6313-6319 [PMID: 25804796 DOI: 10.1007/s13277-015-3317-2]

16 **Chen C**, Wu CQ, Zhang ZQ, Yao DK, Zhu L. Loss of expression of miR-335 is implicated in hepatic stellate cell migration and activation. *Exp Cell Res* 2011; **317**: 1714-1725 [PMID: 21586285 DOI: 10.1016/j.yexcr.2011.05.001]

17 **Cui L**, Hu Y, Bai B, Zhang S. Serum miR-335 Level is Associated with the Treatment Response to Trans-Arterial Chemoembolization and Prognosis in Patients with Hepatocellular Carcinoma. *Cell Physiol Biochem* 2015; **37**: 276-283 [PMID: 26305026 DOI: 10.1159/000430352]

18 **Tang K**, Zhang Y, Zhang H, Xu P, Liu J, Ma J, Lv M, Li D, Katirai F, Shen GX, Zhang G, Feng ZH, Ye D, Huang B. Delivery of chemotherapeutic drugs in tumour cell-derived microparticles. *Nat Commun* 2012; **3**: 1282 [PMID: 23250412 DOI: 10.1038/ncomms2282]

19 **Borrelli DA**, Yankson K, Shukla N, Vilanilam G, Ticer T, Wolfram J. Extracellular vesicle therapeutics for liver disease. *J Control Release* 2018; **273**: 86-98 [PMID: 29373816 DOI: 10.1016/j.jconrel.2018.01.022]

20 **Lai RC**, Tan SS, Teh BJ, Sze SK, Arslan F, de Kleijn DP, Choo A, Lim SK. Proteolytic Potential of the MSC Exosome Proteome: Implications for an Exosome-Mediated Delivery of Therapeutic Proteasome. *Int J Proteomics* 2012; **2012**: 971907 [PMID: 22852084 DOI: 10.1155/2012/971907]

21 **Kim OY**, Lee J, Gho YS. Extracellular vesicle mimetics: Novel alternatives to extracellular vesicle-based theranostics, drug delivery, and vaccines. *Semin Cell Dev Biol* 2017; **67**: 74-82 [PMID: 27916566 DOI: 10.1016/j.semcdb.2016.12.001]

P- Reviewer: Bogdanos DP, El Din NGB **S- Editor**: Ji FF
L- Editor: Filipodia **E- Editor**: Tan WW

World Journal of Hepatology

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

World J Hepatol 2018 November 27; 10(11): 790-794

DOI: 10.4254/wjh.v10.i11.790

ISSN 1948-5182 (online)

EDITORIAL

Treating nonalcoholic steatohepatitis with antidiabetic drugs: Will GLP-1 agonists end the struggle?

Maria Kalogirou, Emmanouil Sinakos

Maria Kalogirou, Emmanouil Sinakos, 4th Department of Internal Medicine, Hippocrates Hospital, Aristotle University of Thessaloniki, Thessaloniki 54642, Greece

ORCID number: Maria Kalogirou (0000-0001-9985-4038); Emmanouil Sinakos (0000-0003-0923-050X).

Author contributions: Kalogirou M and Sinakos E conceived the study and drafted the manuscript; both authors approved the final version of the article.

Conflict-of-interest statement: Emmanouil Sinakos reports personal fees from Novo Nordisk, outside the submitted work.

Manuscript source: Invited manuscript

Correspondence to: Emmanouil Sinakos, MD, PhD, Assistant Professor, 4th Department of Internal Medicine, Hippocrates Hospital, Aristotle University of Thessaloniki, 49, Konstantinopoleos Street, Thessaloniki 54642, Greece. esinakos@auth.gr
Telephone: +30-69-44912668
Fax: +30-23-10992940

Received: July 31, 2018
Peer-review started: July 31, 2018
First decision: August 20, 2018
Revised: September 10, 2018
Accepted: October 9, 2018
Article in press: October 10, 2018
Published online: November 27, 2018

Abstract

Nonalcoholic fatty liver disease (NAFLD) is highly associated with insulin resistance (IR), type 2 diabetes mellitus and metabolic syndrome, being characterized as the hepatic component of metabolic syndrome. Despite its high prevalence, no pharmacological treatment has been established, as of yet. A growing body of evidence, however, shows that reducing IR can result in improvement of the biochemical and histological features of nonalcoholic steatohepatitis (NASH)-the aggressive form of NAFLD that can lead to cirrhosis and hepatocellular carcinoma. Unfortunately, the several trials that have assessed the effect of various antidiabetic agents to date have failed to establish an effective and safe treatment regimen for patients with NAFLD. Glucagon-like peptide-1 (commonly known as GLP-1) agonists are a novel class of antidiabetic drugs that improve insulin sensitivity and promote weight loss. They also appear to have a direct effect on the lipid metabolism of hepatocytes, reducing hepatic steatosis. Several trials have demonstrated that GLP-1 agonists can reduce aminotransferase levels and improve liver histology in patients with NAFLD, suggesting that these agents could serve as an alternative treatment option for these patients. This manuscript discusses the role and potential mechanisms of GLP-1 agonists in the treatment of NASH.

Key words: Nonalcoholic steatohepatitis; Cirrhosis; Glucagon-like peptide-1 receptor agonists; Nonalcoholic fatty liver disease

Core tip: There is an urgent need for an effective treatment of nonalcoholic fatty liver disease (NAFLD). Growing evidence indicates that reducing insulin resistance can result in improvement of the biochemical

and histological features of patients with nonalcoholic steatohepatitis (NASH). However, no antidiabetic agent to date has been proven as both safe and effective for the treatment of patients with NASH. Recent studies have demonstrated that glucagon-like peptide-1 agonists, a novel class of antidiabetic drugs, may be effective in slowing the progression of NAFLD, highlighting their potential role in the treatment of this complex disease.

Kalogirou M, Sinakos E. Treating nonalcoholic steatohepatitis with antidiabetic drugs: Will GLP-1 agonists end the struggle? *World J Hepatol* 2018; 10(11): 790-794 Available from: URL: http://www.wjgnet.com/1948-5182/full/v10/i11/790.htm DOI: http://dx.doi.org/10.4254/wjh.v10.i11.790

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) encompasses a wide spectrum of clinical and histopathological conditions, ranging from simple steatosis [*i.e.,* nonalcoholic fatty liver (NAFL)] to liver injury [*i.e.,* nonalcoholic steatohepatitis (NASH), the aggressive form of NAFLD that can lead to cirrhosis and hepatocellular carcinoma][1,2]. NAFLD is highly associated with metabolic syndrome and type 2 diabetes mellitus (T2DM)[3]. In fact, the prevalence of NAFLD in T2DM has been estimated to be around 60%[4].

The pathophysiology of NAFLD is not yet fully elucidated; however, it is widely believed that insulin resistance (IR) may play a critical role in the pathogenesis of the disease. Several studies have shown that patients with NAFL and NASH are characterized by IR and hyperinsulinemia, irrespective of glucose tolerance or body mass index[5,6]. The multi-hit hypothesis, initially described by Day and James[7], claims that IR is the key factor in the pathogenesis of steatosis. IR causes dysregulation of peripheral lipolysis and increases *de novo* lipogenesis, leading to elevated levels of circulating fatty acids and lipid accumulation within hepatocytes-the "first hit" that predisposes to liver injury, inflammation and fibrosis[8]. Disrupted insulin signaling is also involved in inflammatory cascade activation, lipid peroxidation and liver injury-"the second hit" leading to NASH[9].

Currently, NAFLD is reported to be the most common chronic liver disease worldwide[10]. However, despite huge efforts, there is still no established pharmacotherapy. Lifestyle modifications remain the sole therapeutic approach[11]. Given that IR is considered as the main pathogenetic factor for the development of NAFLD, drugs targeting IR have been investigated the most as potential treatment options for NAFLD, but the studies have yielded conflicting results.

Metformin, the most widely used insulin-sensitizing agent, improves insulin sensitivity by mechanisms that are not yet fully understood[12]. A meta-analysis assessing the effect of metformin in NAFLD revealed that, while it can improve the biochemical and metabolic features of NAFLD, it does not improve the patients' histological response[13]. Metformin is not currently recommended for the treatment of NAFLD by either the American Association for the Study of Liver Diseases or the European Association for the Study of Liver (commonly referred to by their acronyms, AASLD and EASL, respectively)[14,15].

Thiazolidinediones, another class of insulin-sensitizers, act by redistributing fat from ectopic tissues to the adipose tissue, and by increasing levels of adiponectin-an adipokine that has insulin - sensitizing properties[16,17]. Several studies have evaluated the efficacy of thiazolidinediones in patients with NAFLD. The "Pioglitazone *vs* vitamin E *vs* placebo for the treatment of nondiabetic patients with nonalcoholic steatohepatitis" trial (published as the PIVENS trial) was the largest one performed, involving 247 nondiabetic patients with biopsy-proven NASH[18]. The patients were randomized to receive either pioglitazone (30 mg/d) or vitamin E (800 IU/d) or placebo. The pioglitazone treatment was associated with a significant reduction in steatosis and lobular inflammation compared to placebo; however, it did not improve fibrosis. A randomized, placebo-controlled trial performed in patients with NASH and prediabetes or T2DM showed that pioglitazone achieved the primary endpoint of an $\geqslant$ 2-point decrease in NAFLD activity score without worsening fibrosis, and was associated with improvement in steatosis, inflammation and ballooning necrosis[19]. The AASLD and EASL have suggested the use of pioglitazone in patients with biopsy - proven NASH[14,15], although concerns about the side effects and long-term safety of this drug have limited its widespread use. Pioglitazone has been associated with weight gain that is persistent (even after discontinuation of the treatment), fluid retention, deterioration of heart failure, bone fractures, and increased risk of bladder cancer[20-23].

ROLE OF GLUCAGON-LIKE PEPTIDE-1 AGONISTS IN NAFLD

Glucagon-like peptide-1 (GLP-1) agonists represent a novel class of antidiabetic drugs. They mimic the action of endogenous GLP-1, a gastrointestinal hormone of the incretin class of proteins that is secreted from Langerhans cells in response to nutrient ingestion[24]. This hormone has several metabolic effects, including the stimulation of glucose-dependent insulin secretion, inhibition of glucagon release, induction of pancreatic β-cell proliferation, and delay of gastric emptying[25]. While native GLP-1 is rapidly degraded by the enzyme dipeptidy1 peptidase-4 (otherwise known as DPP-4), GLP-1 agonists have increased resistance to DPP-4, thus prolonging the half-life time[26]. These agents have been shown to have beneficial effects on IR and weight control[25]. Several studies have demonstrated the

presence of GLP-1 receptor in hepatocytes, implying that GLP-1 agonists may also exert a direct effect on the liver. Gupta *et al*[27] found that the GLP-1 receptor plays a key role in the decrease of hepatic steatosis *in vitro*, by modulating elements of the insulin signaling pathway. GLP-1 agonists have demonstrated protection of hepatocytes from fatty acid-related death by prohibition of a dysfunctional endoplasmic reticulum stress response. They also appear to reduce fatty acid accumulation by activation of both macroautophagy and chaperone-mediated autophagy[28]. Evidence suggests that GLP-1 secretion is impaired in patients with NAFLD and NASH, highlighting the role of GLP-1 agonists as potential candidates for NAFLD treatment[29].

Liraglutide

Multiple trials have evaluated the efficacy of GLP-1-based therapies in NAFLD. Among the GLP-1 agonists, liraglutide is the most widely studied drug. In the "Liraglutide Efficacy and Action in NASH" study (published as the LEAN study), a double-blind randomized control trial, Armstrong *et al*[30] assessed the effect of 48 wk of treatment with liraglutide in patients with biopsy-proven NASH. Fifty-two patients with (n = 17) or without (n = 35) T2DM were randomly allocated to receive either liraglutide (1.8 mg/d) or placebo. The primary endpoint of the study was the resolution of definite steatohepatitis without worsening fibrosis. Secondary histological endpoints included change in the overall NAFLD activity score (steatosis, ballooning, lobular inflammation) and its individual components. Overall, 9/23 patients in the liraglutide group showed resolution of NASH with no worsening fibrosis compared to 2/22 patients in the placebo group (P = 0.019), successfully meeting the primary endpoint. Regarding the secondary outcomes, fewer patients in the liraglutide group showed progression in fibrosis compared to the placebo group (2/23 *vs* 8/22, P = 0.04). However, results concerning lobular inflammation and overall NAFLD activity score were not statistically significant when compared between the two groups. The authors used histological primary endpoints, being able to evaluate the direct effect of liraglutide on the liver. The study was performed on patients with biopsy-proven NASH, avoiding the inclusion of those without definite NASH. Their findings suggested that liraglutide led to the histological resolution of NASH, with the small sample size being, however, a major limitation.

Ohki *et al*[31] performed a retrospective cohort study evaluating the efficacy of liraglutide compared to sitagliptin and pioglitazone in patients with NAFLD. They reported a significant reduction in serum aminotransferase levels for all groups, while the aspartate aminotransferase (AST)-to-platelet counts ratio index was significantly reduced only for the liraglutide and pioglitazone groups. Body weight significantly decreased in the liraglutide group, while it increased in the pioglitazone group and did not retain a statistically significant difference for the sitagliptin group. Administration of liraglutide was identified as an independent factor for body weight reduction in multivariate analysis.

In a recent open-label trial by Feng *et al*[32], 87 patients with NAFLD were randomized to receive liraglutide, metformin or gliclazide for 24 wk. All three groups showed reduced intrahepatic fat, but the liraglutide group had the greatest reduction. In addition, the researchers found a statistically significant decrease in serum AST and alanine aminotransferase levels only in the liraglutide and metformin group, reporting slightly better results for the liraglutide group. However, a study by Khoo *et al*[33] demonstrated that liraglutide was as effective as structured lifestyle modification for reduction of liver fat fraction and serum aminotransferase levels.

Exenatide

Two trials examined the use of exenatide in patients with NAFLD and T2DM[34,35]. In the first, Shao and colleagues[34] studied 60 patients with NAFLD and T2DM[34]. The patients were randomized to receive exenatide plus insulin glargine U-100 (exenatide group) or insulin glargine U-100 plus insulin aspart (intensive insulin group) for 12 wk. The levels of alanine aminotransferase, AST, and gamma-glutamyl transferase were significantly lower in the exenatide group than in the intensive insulin group. The exenatide plus insulin glargine treatment was also found to be superior to the intensive insulin therapy concerning the reversal rate of fatty liver (93.3% *vs* 66.7%, respectively). The second study, conducted by Fan *et al*[35], compared the efficacy of exenatide *versus* metformin in patients with NAFLD and T2DM. The results revealed that exenatide was more effective than metformin in reducing body weight and improving liver enzymes. Nevertheless, the efficacy of exenatide has not been evaluated in randomized trials with histological outcomes in patients with NASH, as of yet. Lastly, a recent meta-analysis of six studies assessing the efficacy of GLP-1 agonists (liraglutide and exenatide) in NAFLD, revealed that these agents improve liver histology and reduce serum aminotransferase levels, indicating that they might be effective in patients with biopsy-proven NASH[36].

Semaglutide

Semaglutide is a novel long-acting GLP-1 analogue, and has been recently approved for T2DM[37]. It has 94% sequence homology to human GLP-1 and a half-life of 165 h, supporting a once weekly scheme of administration[38]. Semaglutide has shown beneficial effects on glucose control and weight loss compared to placebo and other antidiabetic drugs in patients with T2DM in the "SUSTAIN" trial program[37,38]. It is currently under investigation for its potential as a treatment option for patients with NASH. A 72-wk, randomized, double-blind trial of 372 patients comparing the effi-

cacy and safety of three dose levels of subcutaneous semaglutide once daily *vs* placebo in NASH patients is ongoing (NCT02970942). This trial is expected to be completed during 2019 and will provide additional information on the effectiveness of GLP-1 agonists in patients with NAFLD.

CONCLUSION

GLP-1 agonists are not currently recommended by the AASLD and EASL for the treatment of NAFLD. In their latest guidelines, it was pointed out that it is still premature to consider these agents as a specific treatment for patients with NASH without diabetes, due to inadequate evidence[14,15]. Future research is, therefore, needed to confirm their efficacy in these patients.

In conclusion, current evidence suggests that GLP-1 agonists may be an attractive therapeutic option for patients with NAFLD. However, larger studies of longer duration with histological endpoints are still required to establish their exact role in the management of NAFLD.

Perspective for future study

GLP-1 agonists have been shown to be effective in improving liver histology and reducing aminotransferase levels in patients with NASH. So, the question arises as to whether these agents could serve as a treatment option for such patients. While data are promising, they are still limited. Large-scale randomized, placebo-controlled trials with complete histological outcomes are warranted to elucidate the efficacy of GLP-1 agonists in treating NASH. Another major limitation of the currently available studies is the lack of long-term outcomes. Studies of longer duration are required to properly evaluate the histological improvement in NASH. What is more, it would be interesting if future trials would include both diabetic and nondiabetic patients, in order to clarify the effect of GLP-1 agonists in NASH, regardless of changes in glycemic control. It will also be significant to assess whether GLP-1 agonists affect NAFLD in a dose-dependent manner, in order to search for preferred doses.

REFERENCES

1 **Benedict M**, Zhang X. Non-alcoholic fatty liver disease: An expanded review. *World J Hepatol* 2017; **9**: 715-732 [PMID: 28652891 DOI: 10.4254/wjh.v9.i16.715]

2 **Michelotti GA**, Machado MV, Diehl AM. NAFLD, NASH and liver cancer. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 656-665 [PMID: 24080776 DOI: 10.1038/nrgastro.2013.183]

3 **Blaslov K**, Bulum T, Zibar K, Duvnjak L. Incretin based therapies: a novel treatment approach for non-alcoholic fatty liver disease. *World J Gastroenterol* 2014; **20**: 7356-7365 [PMID: 24966606 DOI: 10.3748/wjg.v20.i23.7356]

4 **Dai W**, Ye L, Liu A, Wen SW, Deng J, Wu X, Lai Z. Prevalence of nonalcoholic fatty liver disease in patients with type 2 diabetes mellitus: A meta-analysis. *Medicine* (Baltimore) 2017; **96**: e8179 [PMID: 28953675 DOI: 10.1097/MD.0000000000008179]

5 **Marchesini G**, Brizi M, Morselli-Labate AM, Bianchi G, Bugianesi E, McCullough AJ, Forlani G, Melchionda N. Association of nonalcoholic fatty liver disease with insulin resistance. *Am J Med* 1999; **107**: 450-455 [PMID: 10569299 DOI: 10.1016/S0002-9343(99)00271-5]

6 **Chitturi S**, Abeygunasekera S, Farrell GC, Holmes-Walker J, Hui JM, Fung C, Karim R, Lin R, Samarasinghe D, Liddle C, Weltman M, George J. NASH and insulin resistance: Insulin hypersecretion and specific association with the insulin resistance syndrome. *Hepatology* 2002; **35**: 373-379 [PMID: 11826411 DOI: 10.1053/jhep.2002.30692]

7 **Day CP**, James OF. Steatohepatitis: a tale of two "hits"? *Gastroenterology* 1998; **114**: 842-845 [PMID: 9547102 DOI: 10.1016/S0016-5085(98)70599-2]

8 **Buzzetti E**, Pinzani M, Tsochatzis EA. The multiple-hit pathogenesis of non-alcoholic fatty liver disease (NAFLD). *Metabolism* 2016; **65**: 1038-1048 [PMID: 26823198 DOI: 10.1016/j.metabol.2015.12.012]

9 **Malaguarnera M**, Di Rosa M, Nicoletti F, Malaguarnera L. Molecular mechanisms involved in NAFLD progression. *J Mol Med* (Berl) 2009; **87**: 679-695 [PMID: 19352614 DOI: 10.1007/s00109-009-0464-1]

10 **Athyros VG**, Katsiki N, Karagiannis A. Editorial: Can Glucagon Like Peptide 1 (GLP1) Agonists or Sodium-Glucose Co-Transporter 2 (SGLT2) Inhibitors Ameliorate Non-Alcoholic Steatohepatitis in People with or without Diabetes? *Curr Vasc Pharmacol* 2016; **14**: 494-497 [PMID: 27633289 DOI: 10.2174/1570161114666160909161811]

11 **Cernea S**, Cahn A, Raz I. Pharmacological management of nonalcoholic fatty liver disease in type 2 diabetes. *Expert Rev Clin Pharmacol* 2017; **10**: 535-547 [PMID: 28276774 DOI: 10.1080/17512433.2017.1300059]

12 **Pernicova I**, Korbonits M. Metformin--mode of action and clinical implications for diabetes and cancer. *Nat Rev Endocrinol* 2014; **10**: 143-156 [PMID: 24393785 DOI: 10.1038/nrendo.2013.256]

13 **Li Y**, Liu L, Wang B, Wang J, Chen D. Metformin in non-alcoholic fatty liver disease: A systematic review and meta-analysis. *Biomed Rep* 2013; **1**: 57-64 [PMID: 24648894 DOI: 10.3892/br.2012.18]

14 **Chalasani N**, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, Harrison SA, Brunt EM, Sanyal AJ. The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American Association for the Study of Liver Diseases. *Hepatology* 2018; **67**: 328-357 [PMID: 28714183 DOI: 10.1002/hep.29367]

15 **European Association for the Study of the Liver (EASL)**; European Association for the Study of Diabetes (EASD); European Association for the Study of Obesity (EASO). EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. *J Hepatol* 2016; **64**: 1388-1402 [PMID: 27062661 DOI: 10.1016/j.jhep.2015.11.004]

16 **Yki-Järvinen H**. Thiazolidinediones. *N Engl J Med* 2004; **351**: 1106-1118 [PMID: 15356308 DOI: 10.1056/NEJMra041001]

17 **Yu JG**, Javorschi S, Hevener AL, Kruszynska YT, Norman RA, Sinha M, Olefsky JM. The effect of thiazolidinediones on plasma adiponectin levels in normal, obese, and type 2 diabetic subjects. *Diabetes* 2002; **51**: 2968-2974 [PMID: 12351435 DOI: 10.2337/diabetes.51.10.2968]

18 **Sanyal AJ**, Chalasani N, Kowdley KV, McCullough A, Diehl AM, Bass NM, Neuschwander-Tetri BA, Lavine JE, Tonascia J, Unalp A, Van Natta M, Clark J, Brunt EM, Kleiner DE, Hoofnagle JH, Robuck PR; NASH CRN. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. *N Engl J Med* 2010; **362**: 1675-1685 [PMID: 20427778 DOI: 10.1056/NEJMoa0907929]

19 **Cusi K**, Orsak B, Bril F, Lomonaco R, Hecht J, Ortiz-Lopez C, Tio F, Hardies J, Darland C, Musi N, Webb A, Portillo-Sanchez P. Long-Term Pioglitazone Treatment for Patients With Nonalcoholic Steatohepatitis and Prediabetes or Type 2 Diabetes Mellitus: A Randomized Trial. *Ann Intern Med* 2016; **165**: 305-315 [PMID: 27322798 DOI: 10.7326/M15-1774]

20 **Balas B**, Belfort R, Harrison SA, Darland C, Finch J, Schenker S, Gastaldelli A, Cusi K. Pioglitazone treatment increases whole body fat but not total body water in patients with non-alcoholic

steatohepatitis. *J Hepatol* 2007; **47**: 565-570 [PMID: 17560678 DOI: 10.1016/j.jhep.2007.04.013]
21 **Hernandez AV**, Usmani A, Rajamanickam A, Moheet A. Thiazolidinediones and risk of heart failure in patients with or at high risk of type 2 diabetes mellitus: a meta-analysis and meta-regression analysis of placebo-controlled randomized clinical trials. *Am J Cardiovasc Drugs* 2011; **11**: 115-128 [PMID: 21294599 DOI: 10.2165/11587580-000000000-00000]
22 **Schwartz AV**, Sellmeyer DE, Vittinghoff E, Palermo L, Lecka-Czernik B, Feingold KR, Strotmeyer ES, Resnick HE, Carbone L, Beamer BA, Park SW, Lane NE, Harris TB, Cummings SR. Thiazolidinedione use and bone loss in older diabetic adults. *J Clin Endocrinol Metab* 2006; **91**: 3349-3354 [PMID: 16608888 DOI: 10.1210/jc.2005-2226]
23 **Ferwana M**, Firwana B, Hasan R, Al-Mallah MH, Kim S, Montori VM, Murad MH. Pioglitazone and risk of bladder cancer: a meta-analysis of controlled studies. *Diabet Med* 2013; **30**: 1026-1032 [PMID: 23350856 DOI: 10.1111/dme.12144]
24 **Liu Y**, Wei R, Hong TP. Potential roles of glucagon-like peptide-1-based therapies in treating non-alcoholic fatty liver disease. *World J Gastroenterol* 2014; **20**: 9090-9097 [PMID: 25083081]
25 **Dhir G**, Cusi K. Glucagon like peptide-1 receptor agonists for the management of obesity and non-alcoholic fatty liver disease: a novel therapeutic option. *J Investig Med* 2018; **66**: 7-10 [PMID: 28918389 DOI: 10.1136/jim-2017-000554]
26 **Drucker DJ**, Nauck MA. The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. *Lancet* 2006; **368**: 1696-1705 [PMID: 17098089 DOI: 10.1016/S0140-6736(06)69705-5]
27 **Gupta NA**, Mells J, Dunham RM, Grakoui A, Handy J, Saxena NK, Anania FA. Glucagon-like peptide-1 receptor is present on human hepatocytes and has a direct role in decreasing hepatic steatosis in vitro by modulating elements of the insulin signaling pathway. *Hepatology* 2010; **51**: 1584-1592 [PMID: 20225248 DOI: 10.1002/hep.23569]
28 **Wang XC**, Gusdon AM, Liu H, Qu S. Effects of glucagon-like peptide-1 receptor agonists on non-alcoholic fatty liver disease and inflammation. *World J Gastroenterol* 2014; **20**: 14821-14830 [PMID: 25356042 DOI: 10.3748/wjg.v20.i40.14821]
29 **Bernsmeier C**, Meyer-Gerspach AC, Blaser LS, Jeker L, Steinert RE, Heim MH, Beglinger C. Glucose-induced glucagon-like Peptide 1 secretion is deficient in patients with non-alcoholic fatty liver disease. *PLoS One* 2014; **9**: e87488 [PMID: 24489924 DOI: 10.1371/journal.pone.0087488]
30 **Armstrong MJ**, Gaunt P, Aithal GP, Barton D, Hull D, Parker R, Hazlehurst JM, Guo K; LEAN trial team, Abouda G, Aldersley MA, Stocken D, Gough SC, Tomlinson JW, Brown RM, Hübscher SG, Newsome PN. Liraglutide safety and efficacy in patients with non-alcoholic steatohepatitis (LEAN): a multicentre, double-blind, randomised, placebo-controlled phase 2 study. *Lancet* 2016; **387**: 679-690 [PMID: 26608256 DOI: 10.1016/S0140-6736(15)00803-X]
31 **Ohki T**, Isogawa A, Iwamoto M, Ohsugi M, Yoshida H, Toda N, Tagawa K, Omata M, Koike K. The effectiveness of liraglutide in nonalcoholic fatty liver disease patients with type 2 diabetes mellitus compared to sitagliptin and pioglitazone. *ScientificWorldJournal* 2012; **2012**: 496453 [PMID: 22927782 DOI: 10.1100/2012/496453]
32 **Feng W**, Gao C, Bi Y, Wu M, Li P, Shen S, Chen W, Yin T, Zhu D. Randomized trial comparing the effects of gliclazide, liraglutide, and metformin on diabetes with non-alcoholic fatty liver disease. *J Diabetes* 2017; **9**: 800-809 [PMID: 28332301 DOI: 10.1111/1753-0407.12555]
33 **Khoo J**, Hsiang J, Taneja R, Law NM, Ang TL. Comparative effects of liraglutide 3 mg vs structured lifestyle modification on body weight, liver fat and liver function in obese patients with non-alcoholic fatty liver disease: A pilot randomized trial. *Diabetes Obes Metab* 2017; **19**: 1814-1817 [PMID: 28503750 DOI: 10.1111/dom.13007]
34 **Shao N**, Kuang HY, Hao M, Gao XY, Lin WJ, Zou W. Benefits of exenatide on obesity and non-alcoholic fatty liver disease with elevated liver enzymes in patients with type 2 diabetes. *Diabetes Metab Res Rev* 2014; **30**: 521-529 [PMID: 24823873 DOI: 10.1002/dmrr.2561]
35 **Fan H**, Pan Q, Xu Y, Yang X. Exenatide improves type 2 diabetes concomitant with non-alcoholic fatty liver disease. *Arq Bras Endocrinol Metabol* 2013; **57**: 702-708 [PMID: 24402015 DOI: 10.1590/S0004-27302013000900005]
36 **Dong Y**, Lv Q, Li S, Wu Y, Li L, Li J, Zhang F, Sun X, Tong N. Efficacy and safety of glucagon-like peptide-1 receptor agonists in non-alcoholic fatty liver disease: A systematic review and meta-analysis. *Clin Res Hepatol Gastroenterol* 2017; **41**: 284-295 [PMID: 28065744 DOI: 10.1016/j.clinre.2016.11.009]
37 **Dhillon S**. Semaglutide: First Global Approval. *Drugs* 2018; **78**: 275-284 [PMID: 29363040 DOI: 10.1007/s40265-018-0871-0]
38 **Holst JJ**, Madsbad S. Semaglutide seems to be more effective the other GLP-1Ras. *Ann Transl Med* 2017; **5**: 505 [PMID: 29299466 DOI: 10.21037/atm.2017.11.10]

P- Reviewer: Kohla MAS, Pallav K, Reichert MCC **S- Editor**: Ji FF
L- Editor: A **E- Editor**: Tan WW

World Journal of Hepatology

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

World J Hepatol 2018 November 27; 10(11): 795-798

DOI: 10.4254/wjh.v10.i11.795

ISSN 1948-5182 (online)

EDITORIAL

Novel insights in the prevention of perinatal transmission of hepatitis B

Konstantinos Tziomalos, Georgios Neokosmidis, Georgios Mavromatidis, Konstantinos Dinas

Konstantinos Tziomalos, Georgios Neokosmidis, First Propedeutic Department of Internal Medicine, Medical School, Aristotle University of Thessaloniki, AHEPA Hospital, Thessaloniki 54636, Greece

Georgios Mavromatidis, Third Department of Obstetrics and Gynecology, Medical School, Aristotle University of Thessaloniki, Hippokration Hospital, Thessaloniki 54642, Greece

Konstantinos Dinas, Second Department of Obstetrics and Gynecology, Medical School, Aristotle University of Thessaloniki, Hippokration Hospital, Thessaloniki 54642, Greece

ORCID number: Konstantinos Tziomalos (0000-0002-3172-1594); Georgios Neokosmidis (0000-0003-1858-9098); Georgios Mavromatidis (0000-0003-3410-6826); Konstantinos Dinas (0000-0001-7144-2840).

Author contributions: Tziomalos K and Neokosmidis G drafted the editorial. Mavromatidis G and Dinas K critically revised the draft.

Conflict-of-interest statement: All authors declare no conflict of interest related to this publication.

Manuscript source: Invited manuscript

Correspondence to: Konstantinos Tziomalos, MD, MSc, PhD, Assistant Professor, First Propedeutic Department of Internal Medicine, Medical School, Aristotle University of Thessaloniki, AHEPA Hospital, 1 Stilponos Kyriakidi Street, Thessaloniki 54636, Greece. ktziomal@auth.gr
Telephone: +30-23-10994621
Fax: +30-23-10994773

Received: July 30, 2018
Peer-review started: July 30, 2018
First decision: August 8, 2018
Revised: August 14, 2018
Accepted: August 26, 2018
Article in press: August 27, 2018
Published online: November 27, 2018

Abstract

Perinatal transmission of hepatitis B virus (HBV) infection is major contributor to the growing burden of chronic hepatitis B worldwide. Administration of HBV immunoglobulin and HBV vaccination as soon after pregnancy as possible are the mainstay of prevention of perinatal transmission of HBV infection. In women with high viral loads, antiviral prophylaxis also appears to be useful. Lamivudine, telbivudine and tenofovir have been shown to be both safe and effective in this setting but tenofovir is the first-line option due to its low potential for resistance and more favorable safety profile.

Key words: Tenofovir; Perinatal transmission; Hepatitis B; Lamivudine; Telbivudine

Core tip: Administration of hepatitis B virus (HBV) immunoglobulin and HBV vaccination as soon after pregnancy as possible are the mainstay of prevention of perinatal transmission of HBV infection. In women with high viral loads, antiviral prophylaxis with tenofovir also appears to be useful.

Tziomalos K, Neokosmidis G, Mavromatidis G, Dinas K. Novel insights in the prevention of perinatal transmission of hepatitis B. *World J Hepatol* 2018; 10(11): 795-798 Available from: URL:

http://www.wjgnet.com/1948-5182/full/v10/i11/795.htm DOI: http://dx.doi.org/10.4254/wjh.v10.i11.795

INTRODUCTION

Perinatal transmission of hepatitis B virus (HBV) is a major healthcare problem, particularly in low-income countries with high prevalence of chronic hepatitis B (CHB)[1]. In regions where CHB is endemic, HBeAg(+) mothers transmit HBV in 70%-90% of their children if prophylaxis is not administered[1]. In addition, high CHB prevalence, poor compliance with medical care and barriers to health care among low-income population groups, especially in immigrants and Roma population, are associated with increased perinatal HBV transmission even in developed European countries[2,3]. Women with high viral loads are at particularly increased risk to transmit hepatitis B to their offspring[4-7]. In many CHB endemic areas, perinatal transmission of hepatitis B is the major cause of transmission of hepatitis B[8,9]. Moreover, progression from HBV infection to CHB is substantially more frequent in the offspring of HBeAg(+) women than in patients who are exposed to HBV during adulthood[10,11]. Indeed, approximately 90% of the former will progress to CHB[10,11].

ROLE OF HBV IMMUNOGLOBULIN AND HBV VACCINATION

Administration of HBV immunoglobulin and HBV vaccination prevents most cases of perinatal HBV transmission[1]. Nevertheless, children born from women with high viral load are still at considerable risk for acquiring HBV despite the administration of HBV immunoglobulin and HBV vaccination [8%-18% when HBV deoxyribonucleic acid (DNA) levels are > 10^7-10^8 copies/mL][4,12-14]. On the other hand, a recent study reported that prompt administration of HBV immunoglobulin (*i.e.* within 4 h after birth) and/or an increase in the number of HBV vaccination doses (at birth and at 1, 2, 4 and 6 mo) resulted in very low rates of perinatal HBV transmission (2%) in HBeAg-positive women with HBV DNA levels > 200000 IU/mL[15].

ROLE OF NUCLEOSIDE ANALOGUES

Several studies also showed that nucleoside analogues combined with administration of HBV immunoglobulin and HBV vaccination are more effective in the prevention of perinatal HBV transmission than administration of HBV immunoglobulin and HBV vaccination alone[16]. In a meta-analysis of 5 small randomized controlled trials (RCTs, *n* = 444 pregnant women), treatment with lamivudine combined with administration of HBV immunoglobulin and HBV vaccination reduced infant HBsAg seropositivity by 11.7% and infant HBV DNA positivity by 21.2% compared with administration of HBV immunoglobulin and HBV vaccination[17]. In a meta-analysis of 4 small RCTs (*n* = 293 pregnant women), telbivudine also reduced infant HBsAg seropositivity by 15.8% and infant HBV DNA positivity by 16.2% compared to the control group[17]. Three early small nonrandomized studies (*n* = 307 pregnant women) showed that tenofovir also reduces the risk for perinatal HBV transmission[18-20]. In a more recent RCT in HBeAg-positive mothers with viral load > 200000 IU/mL (*n* = 200), HBV transmission was observed in 5% of cases who received tenofovir in addition to HBV immunoglobulin/HBV vaccination compared with 18% in mothers treated with HBV immunoglobulin/HBV vaccination alone[14]. In contrast, in a larger RCT (*n* = 331), tenofovir combined with HBV immunoglobulin/HBV vaccination did not reduce the risk of HBV transmission compared with HBV immunoglobulin/HBV vaccination alone[15]. However, rates of HBV transmission in the latter group were very low (2%) and it is possible that the study was not powered to show superiority of tenofovir[15].

Very few studies compared the efficacy of different nucleoside analogues in the prevention of perinatal HBV transmission. In two non-randomized studies (*n* = 690 pregnant women), lamivudine was equally effective with telbivudine[21,22] and in another non-randomized study (*n* = 120 pregnant women), lamivudine was similarly effective with tenofovir[18]. Lamivudine, telbivudine and tenofovir also appear to be safe during pregnancy and do not increase the risk of congenital malformation, prematurity or maternal complications[17,23]. However, it should be emphasized that tenofovir and telbivudine are both Food and Drug Administration (FDA) pregnancy category B drugs (*i.e.,* no risk in animal studies, unknown in humans) whereas lamivudine is FDA pregnancy category C drug (*i.e.,* teratogenic in animal studies, unknown in humans)[24]. It has also been shown that in the United States, a country with very low prevalence of CHB, combining a nucleoside analogue with HBV immunoglobulin/HBV vaccination is more cost-effective than HBV immunoglobulin/HBV vaccination alone[25]. Nevertheless, it should be emphasized that none of these agents are licensed for use during pregnancy.

Current guidelines recommend screening of all pregnant women for CHB during the first trimester of pregnancy[24,26,27]. In all pregnant women with HBV DNA levels > 200000 IU/mL and/or > 6-7 $\log_{10}$ IU/mL or HBsAg levels > 4 $\log_{10}$ IU/mL, antiviral prophylaxis with tenofovir should start at week 24-32 of gestation and continue for up to 4-12 wk after delivery[24,26,27]. Tenofovir is preferred over lamivudine and telbivudine because of lower resistance rates and because it is a FDA pregnancy category B drug[24,26,27].

ROLE OF CAESAREAN SECTION

The role of caesarean section in the prevention of perinatal transmission of HBV infection is unclear. In a recent meta-analysis of 10 studies (*n* = 5091 new-

borns), caesarean section reduced the incidence HBV transmission by 38% compared with vaginal delivery (95%CI: 0.40-0.98; P = 0.04)[28]. However, the benefit of caesarean section was smaller in studies where hepatitis B immunoglobulin was administered to all women[28]. Moreover, caesarean section did not reduce the risk of vertical HBV transmission in HBeAg(+) women[28]. Accordingly, current guidelines do not recommend caesarean section for the prevention of perinatal transmission of HBV infection due to insufficient data[26].

BREASTFEEDING IN HBsAg(+) WOMEN

Regarding breastfeeding, current guidelines state that it is not contraindicated in HBsAg(+) women who are not receiving nucleoside analogues, since breast milk contains the lowest concentrations of HBV among body fluids and breast feeding does not increase the risk of HBV transmission in women who receive HBV immunoglobulin and HBV vaccination[24,26,27,29]. Moreover, breastfeeding is also not prohibited in women who are receiving prophylaxis with tenofovir, since this agent is excreted in very small amounts in breast milk[24,26,27,30,31].

CONCLUSION

Perinatal transmission of HBV infection is major contributor to the growing burden of CHB worldwide. Administration of HBV immunoglobulin and HBV vaccination as soon after pregnancy as possible are the mainstay of prevention of perinatal transmission of HBV infection. In women with high viral loads, antiviral prophylaxis with tenofovir also appears to be useful. Strategies to improve the awareness of this major healthcare problem are also needed to curb the rising incidence of CHB infection.

REFERENCES

1 **Lee C**, Gong Y, Brok J, Boxall EH, Gluud C. Effect of hepatitis B immunisation in newborn infants of mothers positive for hepatitis B surface antigen: systematic review and meta-analysis. *BMJ* 2006; **332**: 328-336 [PMID: 16443611 DOI: 10.1136/bmj.38719.435833.7C]

2 **Papaevangelou V**, Hadjichristodoulou C, Cassimos D, Theodoridou M. Adherence to the screening program for HBV infection in pregnant women delivering in Greece. *BMC Infect Dis* 2006; **6**: 84 [PMID: 16681862 DOI: 10.1186/1471-2334-6-84]

3 **Drazilova S**, Janicko M, Kristian P, Schreter I, Halanova M, Urbancikova I, Madarasova-Geckova A, Marekova M, Pella D, Jarcuska P; HepaMeta Team. Prevalence and Risk Factors for Hepatitis B Virus Infection in Roma and Non-Roma People in Slovakia. *Int J Environ Res Public Health* 2018; **15**: pii: E1047 [PMID: 29789486 DOI: 10.3390/ijerph15051047]

4 **Wiseman E**, Fraser MA, Holden S, Glass A, Kidson BL, Heron LG, Maley MW, Ayres A, Locarnini SA, Levy MT. Perinatal transmission of hepatitis B virus: an Australian experience. *Med J Aust* 2009; **190**: 489-492 [PMID: 19413519]

5 **Burk RD**, Hwang LY, Ho GY, Shafritz DA, Beasley RP. Outcome of perinatal hepatitis B virus exposure is dependent on maternal virus load. *J Infect Dis* 1994; **170**: 1418-1423 [PMID: 7995980 DOI: 10.1093/infdis/170.6.1418]

6 **Li XM**, Shi MF, Yang YB, Shi ZJ, Hou HY, Shen HM, Teng BQ. Effect of hepatitis B immunoglobulin on interruption of HBV intrauterine infection. *World J Gastroenterol* 2004; **10**: 3215-3217 [PMID: 15457579 DOI: 10.3748/wjg.v10.i21.3215]

7 **Zou H**, Chen Y, Duan Z, Zhang H, Pan C. Virologic factors associated with failure to passive-active immunoprophylaxis in infants born to HBsAg-positive mothers. *J Viral Hepat* 2012; **19**: e18-e25 [PMID: 22239517 DOI: 10.1111/j.1365-2893.2011.01492.x]

8 **Beasley RP**, Hwang LY, Lin CC, Leu ML, Stevens CE, Szmuness W, Chen KP. Incidence of hepatitis B virus infections in preschool children in Taiwan. *J Infect Dis* 1982; **146**: 198-204 [PMID: 7108271 DOI: 10.1093/infdis/146.2.198]

9 **Alter MJ**, Hadler SC, Margolis HS, Alexander WJ, Hu PY, Judson FN, Mares A, Miller JK, Moyer LA. The changing epidemiology of hepatitis B in the United States. Need for alternative vaccination strategies. *JAMA* 1990; **263**: 1218-1222 [PMID: 2304237 DOI: 10.1001/jama.1990.03440090052025]

10 **McMahon BJ**, Alward WL, Hall DB, Heyward WL, Bender TR, Francis DP, Maynard JE. Acute hepatitis B virus infection: relation of age to the clinical expression of disease and subsequent development of the carrier state. *J Infect Dis* 1985; **151**: 599-603 [PMID: 3973412 DOI: 10.1093/infdis/151.4.599]

11 **Tassopoulos NC**, Papaevangelou GJ, Sjogren MH, Roumeliotou-Karayannis A, Gerin JL, Purcell RH. Natural history of acute hepatitis B surface antigen-positive hepatitis in Greek adults. *Gastroenterology* 1987; **92**: 1844-1850 [PMID: 3569758 DOI: 10.1016/0016-5085(87)90614-7]

12 **Han GR**, Cao MK, Zhao W, Jiang HX, Wang CM, Bai SF, Yue X, Wang GJ, Tang X, Fang ZX. A prospective and open-label study for the efficacy and safety of telbivudine in pregnancy for the prevention of perinatal transmission of hepatitis B virus infection. *J Hepatol* 2011; **55**: 1215-1221 [PMID: 21703206 DOI: 10.1016/j.jhep.2011.02.032]

13 **del Canho R**, Grosheide PM, Mazel JA, Heijtink RA, Hop WC, Gerards LJ, de Gast GC, Fetter WP, Zwijneberg J, Schalm SW. Ten-year neonatal hepatitis B vaccination program, The Netherlands, 1982-1992: protective efficacy and long-term immunogenicity. *Vaccine* 1997; **15**: 1624-1630 [PMID: 9364693 DOI: 10.1016/S0264-410X(97)00080-7]

14 **Pan CQ**, Duan Z, Dai E, Zhang S, Han G, Wang Y, Zhang H, Zou H, Zhu B, Zhao W, Jiang H; China Study Group for the Mother-to-Child Transmission of Hepatitis B. Tenofovir to Prevent Hepatitis B Transmission in Mothers with High Viral Load. *N Engl J Med* 2016; **374**: 2324-2334 [PMID: 27305192 DOI: 10.1056/NEJMoa1508660]

15 **Jourdain G**, Ngo-Giang-Huong N, Harrison L, Decker L, Khamduang W, Tierney C, Salvadori N, Cressey TR, Sirirungsi W, Achalapong J, Yuthavisuthi P, Kanjanavikai P, Na Ayudhaya OP, Siriwachirachai T, Prommas S, Sabsanong P, Limtrakul A, Varadisai S, Putiyanun C, Suriyachai P, Liampongsabuddhi P, Sangsawang S, Matanasarawut W, Buranabanjasatean S, Puernngooluerm P, Bowonwatanuwong C, Puthanakit T, Klinbuayaem V, Thongsawat S, Thanprasertsuk S, Siberry GK, Watts DH, Chakhtoura N, Murphy TV, Nelson NP, Chung RT, Pol S, Chotivanich N. Tenofovir versus Placebo to Prevent Perinatal Transmission of Hepatitis B. *N Engl J Med* 2018; **378**: 911-923 [PMID: 29514030 DOI: 10.1056/NEJMoa1708131]

16 **Cholongitas E**, Tziomalos K, Pipili C. Management of patients with hepatitis B in special populations. *World J Gastroenterol* 2015; **21**: 1738-1748 [PMID: 25684938 DOI: 10.3748/wjg.v21.i6.1738]

17 **Brown RS Jr**, McMahon BJ, Lok AS, Wong JB, Ahmed AT, Mouchli MA, Wang Z, Prokop LJ, Murad MH, Mohammed K. Antiviral therapy in chronic hepatitis B viral infection during pregnancy: A systematic review and meta-analysis. *Hepatology* 2016; **63**: 319-333 [PMID: 26565396 DOI: 10.1002/hep.28302]

18 **Greenup AJ**, Tan PK, Nguyen V, Glass A, Davison S, Chatterjee U, Holdaway S, Samarasinghe D, Jackson K, Locarnini SA, Levy MT. Efficacy and safety of tenofovir disoproxil fumarate in pregnancy to prevent perinatal transmission of hepatitis B virus. *J Hepatol* 2014; **61**: 502-507 [PMID: 24801414 DOI: 10.1016/j.jhep.2014.04.038]

19 **Celen MK**, Mert D, Ay M, Dal T, Kaya S, Yildirim N, Gulsun S, Barcin T, Kalkanli S, Dal MS, Ayaz C. Efficacy and safety of tenofovir disoproxil fumarate in pregnancy for the prevention of vertical transmission of HBV infection. *World J Gastroenterol* 2013; **19**: 9377-9382 [PMID: 24409065 DOI: 10.3748/wjg.v19.i48.9377]

20 **Chen HL**, Lee CN, Chang CH, Ni YH, Shyu MK, Chen SM, Hu JJ, Lin HH, Zhao LL, Mu SC, Lai MW, Lee CL, Lin HM, Tsai MS, Hsu JJ, Chen DS, Chan KA, Chang MH; Taiwan Study Group for the Prevention of Mother-to-Infant Transmission of HBV (PreMIT Study); Taiwan Study Group for the Prevention of Mother-to-Infant Transmission of HBV PreMIT Study. Efficacy of maternal tenofovir disoproxil fumarate in interrupting mother-to-infant transmission of hepatitis B virus. *Hepatology* 2015; **62**: 375-386 [PMID: 25851052 DOI: 10.1002/hep.27837]

21 **Yu MM**, Jiang Q, Ji Y, Wu KH, Ju LL, Tang X, Yang YF. Comparison of telbivudine versus lamivudine in interrupting perinatal transmission of hepatitis B virus. *J Clin Virol* 2014; **61**: 55-60 [PMID: 24994007 DOI: 10.1016/j.jcv.2014.06.005]

22 **Zhang H**, Pan CQ, Pang Q, Tian R, Yan M, Liu X. Telbivudine or lamivudine use in late pregnancy safely reduces perinatal transmission of hepatitis B virus in real-life practice. *Hepatology* 2014; **60**: 468-476 [PMID: 25187919 DOI: 10.1002/hep.27034]

23 **Brown RS Jr**, Verna EC, Pereira MR, Tilson HH, Aguilar C, Leu CS, Buti M, Fagan EA. Hepatitis B virus and human immunodeficiency virus drugs in pregnancy: findings from the Antiretroviral Pregnancy Registry. *J Hepatol* 2012; **57**: 953-959 [PMID: 22766470 DOI: 10.1016/j.jhep.2012.06.031]

24 **Sarin SK**, Kumar M, Lau GK, Abbas Z, Chan HL, Chen CJ, Chen DS, Chen HL, Chen PJ, Chien RN, Dokmeci AK, Gane E, Hou JL, Jafri W, Jia J, Kim JH, Lai CL, Lee HC, Lim SG, Liu CJ, Locarnini S, Al Mahtab M, Mohamed R, Omata M, Park J, Piratvisuth T, Sharma BC, Sollano J, Wang FS, Wei L, Yuen MF, Zheng SS, Kao JH. Asian-Pacific clinical practice guidelines on the management of hepatitis B: a 2015 update. *Hepatol Int* 2016; **10**: 1-98 [PMID: 26563120 DOI: 10.1007/s12072-015-9675-4]

25 **Fan L**, Owusu-Edusei K Jr, Schillie SF, Murphy TV. Cost-effectiveness of active-passive prophylaxis and antiviral prophylaxis during pregnancy to prevent perinatal hepatitis B virus infection. *Hepatology* 2016; **63**: 1471-1480 [PMID: 26509655 DOI: 10.1002/hep.28310]

26 **Terrault NA**, Lok ASF, McMahon BJ, Chang KM, Hwang JP, Jonas MM, Brown RS Jr, Bzowej NH, Wong JB. Update on prevention, diagnosis, and treatment of chronic hepatitis B: AASLD 2018 hepatitis B guidance. *Hepatology* 2018; **67**: 1560-1599 [PMID: 29405329 DOI: 10.1002/hep.29800]

27 **European Association for the Study of the Liver**. EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection. *J Hepatol* 2017; **67**: 370-398 [PMID: 28427875 DOI: 10.1016/j.jhep.2017.03.021]

28 **Chang MS**, Gavini S, Andrade PC, McNabb-Baltar J. Caesarean section to prevent transmission of hepatitis B: a meta-analysis. *Can J Gastroenterol Hepatol* 2014; **28**: 439-444 [PMID: 25229465 DOI: 10.1155/2014/350179]

29 **Hill JB**, Sheffield JS, Kim MJ, Alexander JM, Sercely B, Wendel GD. Risk of hepatitis B transmission in breast-fed infants of chronic hepatitis B carriers. *Obstet Gynecol* 2002; **99**: 1049-1052 [PMID: 12052598]

30 **Benaboud S**, Pruvost A, Coffie PA, Ekouévi DK, Urien S, Arrivé E, Blanche S, Théodoro F, Avit D, Dabis F, Tréluyer JM, Hirt D. Concentrations of tenofovir and emtricitabine in breast milk of HIV-1-infected women in Abidjan, Cote d'Ivoire, in the ANRS 12109 TEmAA Study, Step 2. *Antimicrob Agents Chemother* 2011; **55**: 1315-1317 [PMID: 21173182 DOI: 10.1128/AAC.00514-10]

31 **Mirochnick M**, Taha T, Kreitchmann R, Nielsen-Saines K, Kumwenda N, Joao E, Pinto J, Santos B, Parsons T, Kearney B, Emel L, Herron C, Richardson P, Hudelson SE, Eshleman SH, George K, Fowler MG, Sato P, Mofenson L; HPTN 057 Protocol Team. Pharmacokinetics and safety of tenofovir in HIV-infected women during labor and their infants during the first week of life. *J Acquir Immune Defic Syndr* 2014; **65**: 33-41 [PMID: 23979002 DOI: 10.1097/QAI.0b013e3182a921eb]

P- Reviewer: Jarcuska P, Rodríguez-Perálvarez M **S- Editor**: Dou Y
L- Editor: A **E- Editor**: Tan WW

World Journal of Hepatology

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

World J Hepatol 2018 November 27; 10(11): 799-806

DOI: 10.4254/wjh.v10.i11.799

ISSN 1948-5182 (online)

REVIEW

Role of traditional Chinese medicine in the management of patients with hepatocellular carcinoma

Sheng-Yan Xi, Gerald Yosel Minuk

Sheng-Yan Xi, Department of Traditional Chinese Medicine, Medical College of Xiamen University, Cancer Research Center of Xiamen University, Xiamen 361102, Fujian Province, China

Gerald Yosel Minuk, Section of Hepatology, Department of Internal Medicine, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, MB R3E 3P4, Canada

ORCID number: Sheng-Yan Xi (0000-0002-8315-6742); Gerald Yosel Minuk (0000-0002-2687-940X).

Author contributions: Xi SY wrote the paper; Minuk GY gave guidance and critically revised the manuscript.

Conflict-of-interest statement: The authors declare that they have no conflict of interests.

Manuscript source: Unsolicited manuscript

Correspondence to: Gerald Yosel Minuk, MD, Professor, Head, Section of Hepatology, Department of Internal Medicine, Rady Faculty of Health Sciences, University of Manitoba, 715 McDermot Ave., Winnipeg, MB R3E 3P4, Canada. gerald.minuk@umanitoba.ca
Telephone: +1-204-7893204

Received: May 8, 2018
Peer-review started: May 8, 2018
First decision: June 5, 2018
Revised: September 13, 2018
Accepted: October 11, 2018
Article in press: October 12, 2018
Published online: November 27, 2018

Abstract

Traditional Chinese Medicines (TCMs) have been employed for centuries in the treatment of patients with hepatocellular carcinoma (HCC). Previous reviews of this topic have focused on certain aspects of TCM treatment rather than an overall assessment of their value and mechanisms of action. Both the Chinese and English medical literatures were reviewed to identify where TCM might be of value in the treatment of HCC and the justification for such treatment. TCM treatment corrects the "internal disequilibriums" thought to be responsible for the development, growth, and spread of the tumor. It has also been used to manage symptoms associated with HCC and the adverse effects of chemo- and radiation-therapies. Recent research has documented the precise effects of TCM on tumor biology. There are also increasing efforts to identify which of the many components of TCM herbal remedies are primarily responsible for these beneficial effects. This review outlines the benefits of TCM treatment of HCC and the laboratory data describing their anti-tumor properties.

Key words: Hepatoma; Herbal medicine; Liver disease; Liver; Cancer

Core tip: Traditional Chinese Medicines (TCMs) are commonly employed by patients with hepatocellular carcinoma (HCC). This review identifies which herbal concoctions are most frequently recommended by TCM authorities. TCMs serve to correct internal imbalances that contribute to HCC. TCMs favorably alter HCC cell biology.

Xi SY, Minuk GY. Role of traditional Chinese medicine in the management of patients with hepatocellular carcinoma. *World*

J Hepatol 2018; 10(11): 799-806 Available from: URL: http://www.wjgnet.com/1948-5182/full/v10/i11/799.htm DOI: http://dx.doi.org/10.4254/wjh.v10.i11.799

INTRODUCTION

Traditional Chinese Medicine (TCM) is a comprehensive medical system that utilizes herbal remedies, acupuncture, dietary therapy, exercise, and massage to prevent, treat, and rehabilitate disease states by restoring the internal environments of an individual to a state of equilibrium. It is based on traditional medical theories and the practice experiences of Chinese TCM physicians. The traditional medical theories describe two components of illness: "holism" (the concept of viewing the situation as a whole) and "syndrome differentiation" (the consequences of disrupted holism). Thus, rather than focusing on the tumor *per se*, TCM focuses on correcting the internal disequilibriums responsible for tumor development and progression.

Given the phylogeny of the oncogenic hepatitis B virus (HBV), it can be assumed that hepatocellular carcinoma (HCC) has been prevalent in the Chinese population for centuries[1]. Hence, Chinese TCM physicians have had extensive experience in identifying, developing, and refining treatments for this potentially lethal tumor. This longstanding experience and commitment to treating HCC is an important feature of TCM. Specifically, unlike "Western Medicine" where effective treatments are identified by the results of prospective, randomized, placebo-controlled trials, in TCM, the value of a particular herbal concoction is gauged by the number of recommendations it has received by TCM authorities over the course of centuries.

MOST COMMONLY EMPLOYED TCMS FOR HCC

TCM physicians have identified various Chinese herbal medicines that represent every category of the Chinese materia medica recognized by the International Organization for Standardization (ISO)[2]. The majority of these agents are deficiency-supplementing herbs, heat-clearing herbs, and blood-quickening stasis-transforming herbs (Table 1).

The ten most commonly employed individual herbs are provided in Table 2. They are: Poria (Fuling), Rhizoma Atractylodis Macrocephalae (Baizhu), Radix Astragali Mongolici (Huangqi), Herba Hedyotidis (Baihuasheshecao), Radix Glycyrrhizae (Gancao), Radix Bupleuri Chinensis (Chaihu), Radix Codonopsis (Dangshen), Radix Paeoniae Alba (Baishao), Radix Angelicae Sinensis (Danggui) and Carapax Trionycis (Biejia).

Often, combinations of herbs are advocated such as qi-boosting spleen-supplementing herbs being combined with heat-clearing toxin-resolving herbs, blood-quickening stasis-transforming herbs and/or liver-soothing qi-rectifying herbs (qi is the vital life force that is thought to animate the body internally)[3]. The ten most commonly advocated combinations of herbs are provided in Table 3.

TCMS FOR THE TREATMENT OF HCC SYMPTOMS

Anorexia, fatigue, weakness, and right upper quadrant discomfort are the most common symptoms of HCC while ascites and jaundice are the most common signs[4]. TCMs are often used in the treatment of these and the other features listed in Table 4. In a recent cluster analysis performed by Liu *et al*[4], Endothelium Coreneum Gigeriae Galli (Jineijin) and Fructus Hordei Germinatus (Maiya) were the most commonly-used herbal medicines for treating anorexia; Radix Astragali Mongolici (Huangqi) for fatigue and weakness; Rhizoma Corydalis Yanhusuo (Yanhusuo) and Fructus Toosendan (Chuanlianzi) for right upper quadrant discomfort; Pericarpium Arecae (Dafupi), Polyporus (Zhuling) and Poria (Fuling) for ascites; and Herba Artemisiae Capillaris (Yinchen) for jaundice[5]. Other herbal medicines used to treat less common symptoms and signs of HCC are also provided in Table 4.

TCM FOR IMPROVED QUALITY OF LIFE AND SURVIVAL IN HCC PATIENTS

The use of TCM to correct disequilibriums in a patient's internal environment has been associated with improved quality of life for HCC patients. For example, the Jianpi Jiedu Decoction has been reported to improve quality of life by attenuating symptoms in 30 patients with advanced HCC[6]. Similar results have been obtained with other combinations[7-10].

Other studies have described improved survival. Specifically, compared to untreated controls, treatment with a Ruanganlidan Decoction and Rhizoma Curcumae Longae increased median disease-free survival by approximately 12 mo in 78 HCC patients[11]. In another study, Qudu Huayu Xiaoji Formula not only improved the quality of life in 77 HCC patients after hepatic arterial chemoembolization, but also prolonged survival by 5-9 mo when compared to 76 patients treated with chemoembolization alone[12].

TCM AND ADVERSE REACTIONS TO CHEMOTHERAPEUTIC AGENTS

Side effects of chemotherapy are major concerns for cancer patients and often interfere with treatment.

Table 1 Types of herbal medicines and frequency of use in the treatment of patients with hepatocellular carcinoma

Category	Relative frequency	Category	Relative frequency
Herbs that supplement deficiency: Baizhu, Huangqi, Dangshen, Danggui, Shanyao, Gancao, Baishao, Biejia	27.70%	Herbs that drain downwards: Dahuang, Yuanhua	1.37%
Herbs that clear the heat: Baihuasheshecao, Banzhilian, Shengdihuang, Zhizi, Huangqin, Qinghao	19.26%	Herbs that astringe: Wuweizi, Shanzhuyu	1.01%
Herbs that invigorate blood and dissolve stasis: Ezhu, Danshen, Yujin, Tubiechong	13.67%	Herbs that counteract toxins, kill parasites and relieve itching: Fengfang	0.68%
Herbs that promote urination and percolate dampness: Fuling, Yiyiren, Yinchen, Cheqianzi, Yumixu	12.04%	Herbs that warm the interior: Wuyao	0.54%
Herbs that rectify qi: Zhiqiao, Chenpi	8.39%	Herbs that expel wind and damp: Sangjisheng, Qinjiao	0.46%
Herbs that release the exterior: Chaihu, Guizhi	4.14%	Herbs that calm the mind: Suanzaoren, Longgu	0.42%
Herbs that promote digestion: Jineijin	3.18%	Herbs that calm the liver and extinguish wind: Muli, Wugong	0.25%
Herbs that relieve cough, dissolve phlegm and calm panting: Banxia, Tinglizi, Walengzi	2.94%	Herbs that open the orifices: Shexiang	0.11%
Herbs that stanch bleeding: Sanqi, Xianhecao, Baimaogen	1.91%	Herbs that expel parasites: Binglang	0.08%
Herbs that transform dampness: Houpo	1.86%	Herbs that induce vomit: Changshan	0.02%

Table 2 The most frequently prescribed herbal medicines used in the treatment of patients with hepatocellular carcinoma

Herb name	Relative frequency	Herb name	Relative frequency
Poria (Fuling)	5.20%	Radix Angelicae Sinensis (Danggui)	2.35%
Rhizoma Atractylodis Macrocephalae (Baizhu)	5.20%	Carapax Trionycis (Biejia)	2.22%
Radix Astragali Mongolici (Huangqi)	4.07%	Radix Bupleuri Chinensis (Chaihu)	3.66%
Herba Hedyotidis (Baihuasheshecao)	3.75%	Radix Codonopsis (Dangshen)	3.26%
Radix Glycyrrhizae (Gancao)	3.71%	Radix Paeoniae Alba (Baishao)	3.03%

Numerous TCM herbs have been identified that reduce the side effects and non-tumor toxicity of chemotherapeutics. For example, Ciji Hua'ai Baosheng Granule Formula (CHBGF) attenuates the decreases in white blood cell and platelet counts of H_{22} hepatoma transplanted tumor caused by chemotherapy[13]. Combining Rhizoma Zingberis Recens (Shengjiang) and Rhizoma Phragmitis (Lugen) reduces the vomiting caused by chemotherapy in H_{22} hepatoma carcinoma-bearing mice[14], and Danggui Beimu Kushen attenuates cisplatin toxicity (in the same animal model). Other TCMs such as Panaxan, Fufang Ejiao Jiang, Lianqi Capsule, and the aqueous extract of Fructus Akebiae (Bayuezha) have also been reported to reduce side effects and improve the efficacy of chemotherapy for HCC in H_{22} hepatoma bearing mice[15-18]. Compared to chemotherapy alone, Tremella Polysaccharide, extracted from Polyporus (Zhuling), improved quality of life and physical activity and attenuated fatigue, nausea, vomiting, constipation, diarrhea, and white blood cell counts during chemotherapy in 50 patients[19]. Jianpi Jiedu Formula minimized hepatic dysfunction following transarterial chemoembolization (TACE) treatment in 16 patients[20]. Similarly, the Zipi Decoction was associated with improved hepatic function following TACE when compared to TACE alone[21]. Jian Pi Li Qi Decoction in 52 patients and Jiedu Granules combined with Cinobufacini in 60 patients alleviated signs and symptoms of the postembolization syndrome following TACE[22]. Finally, it should be noted that on occasion, TCM can adversely affect patient outcomes when TCM and chemotherapy drugs interact[23].

TCM AND HCC TUMOR BIOLOGY

Recent developments in molecular and cell biology have provided important insights into the pathogenesis and course of HCC. They have also provided investigators with an opportunity to identify the mechanisms whereby TCM impacts HCC. To date, such research has focused on HCC proliferative activity, apoptosis, metastasis, angiogenesis, immune reactivity, and multidrug resistance.

The effects of TCM on the proliferative activity and growth of malignant hepatocytes and tumors

A large number of herbs have been reported to inhibit malignant hepatocyte proliferation and tumor growth. In many instances, the precise mechanisms and signaling pathways have also been identified. For example, Akebia trifoliate (Thunb.) and Koidz (Sanyemutong) seed extract inhibited the proliferation of various human HCC cell lines *via* induction of endoplasmic reticulum stress *in vitro*[24] whereas the ethyl acetate extraction from a Chinese herbal formula, Jiedu Xiaozheng Yin inhibited proliferative activity by suppression of the

Table 3 Descending frequency of herbal medicine combinations used in the treatment of patients with hepatocellular carcinoma

Precedence	Herbal medicine combinations
1	Rhizoma Atractylodis Macrocephalae (Baizhu) and Poria (Fuling)
2	Radix Astragali Mongolici (Huangqi) and Rhizoma Atractylodis Macrocephalae Baizhu)
3	Radix Astragali Mongolici (Huangqi) and Radix Codonopsis (Dangshen)
4	Radix Astragali Mongolici (Huangqi) and Radix Angelicae Sinensis (Danggui)
5	Radix Astragali Mongolici (Huangqi) and Poria (Fuling)
6	Rhizoma Atractylodis Macrocephalae (Baizhu) and Radix Curcumae Wenyujin (Yujin)
7	Rhizoma Atractylodis Macrocephalae (Baizhu) and Radix Bupleuri Chinensis (Chaihu)
8	Rhizoma Atractylodis Macrocephalae (Baizhu) and Radix Glycyrrhizae (Gancao)
9	Rhizoma Atractylodis Macrocephalae (Baizhu) and Pericarpium Citri Reticulatae (Chenpi)
10	Rhizoma Atractylodis Macrocephalae (Baizhu) and Radix Codonopsis (Dangshen)

Table 4 Herbal medicines and the frequency of their use in treating symptoms and signs associated with hepatocellular carcinoma

Symptoms and signs	Herb and frequency of use (*n*)
Anorexia	Endothelium Coreneum Gigeriae Galli (Jineijin) (18), Fructus Hordei Germinatus (Maiya) (12), Fructus Amomi (Sharen) (9), stir-baking Fructus Hordei Germinatus et Massa Fer-mentata Medicinalis (Jiaosanxian) (7), Fructus Setariae Germinatus (Guya) (6), Massa Medicata Fermentata (Shenqu) (5) and Fructus Crataegi Pinnatifidae (Shanzha) (5)
Fatigue	Radix Astragali Mongolici (Huangqi) (23) and Radix Codonopsis (Dangshen) (14)
Discomfort	Rhizoma Corydalis Yanhusuo (Yanhusuo) (15), Fructus Toosendan (Chuanlianzi) (13), Radix Curcumae Wenyujin (Yujin) (10), Olibanum (Ruxiang) (9), Myrrha (Moyao) (7), Fructus Citri Sarcodactylis (Foshou) (7), Radix Aucklandiae (Muxiang) (5) and Rhizoma Cyperi (Xiangfu) (5)
Ascites	Pericarpium Arecae (Dafupi) (30), Polyporus (Zhuling) (22), Poria (Fuling) (18), Rhizoma Alismatis (Zexie) (13), Semen Plantaginis (Cheqianzi) (8) and Cortex Magnoliae Officinalis (Houpo) (5)
Jaundice	Herba Artemisiae Capillaris (Yinchen) (37), Rhizoma Polygoni Cuspidati (Huzhang) (13), Radix et Rhizoma Rhei Palmati (Dahuang) (11), Herba Hyperici Japonici (Tianjihuang) (8), Fructus Gradeniae (Zhizi) (8), Herba Lysimachiae (Jinqiancao) (7), Radix Paeoniae Rubra (Chishao) (6) and Radix Scutellariae Baicalensis (Huangqin) (6)
Abdominal distention	Fructus Aurantii Submaturus (Zhiqiao) (11), Cortex Magnoliae Officinalis (Houpo) (8), Semen Raphani Sativi (Laifuzi) (7), Pericarpium Citri Reticulatae Viride (Qingpi) (6), Radix Aucklandiae (Muxiang) (6), Fructus Amomi (Sharen) (5) and Fructus Aurantii Immaturus (Zhishi) (5)
Nausea and vomiting	Caulis Bambusae in Taeniam (Zhuru) (27), Rhizoma Pinelliae (Banxia) (19), Flos Inulae (Xuanfuhua) (17), Fructus Amomi (Sharen) (10), Ochra Haematitum (Daizheshi) (7) and Pericarpium Citri Reticulatae (Jupi) (6)
Fever	Gypsum Fibrosum (Shigao) (9), Cortex Moutan Radicis (Mudanpi) (8), Radix Bupleuri Chinensis (Chaihu) (8), Herba Artemisiae Annuae (Qinghao) (6), Rhizoma Anemarrhenae (Zhimu) (6) and Fructus Gradeniae (Zhizi) (6)
Diarrhea	Poria (Fuling) (7), Rhizoma Alismatis (Zexie) (7), Semen Euryales (Qianshi) (6) and Fructus Schisandrae Chinensis (Wuweizi) (5)
Constipation	Radix et Rhizoma Rhei Palmati (Dahuang) (12), Fructus et Semen Trichosanthis Kirilowii (Gualou) (6), Semen Pruni Japonicae (Yuliren) (5) and Fructus Cannabis (Huomaren) (5)

polycomb gene product Bmi1 and Wnt/β-catenin signaling and inducing G0/G1 phase arrest *in vitro* and *in vivo*[25,26]. Coptischinensis (Huanglian) restrained HepG2 cell proliferation through activation of the the NAG-1 gene enzyme *in vitro*[27].

Other TCM herbs have been reported to inhibit malignant hepatocyte proliferative activity and tumor growth through mechanisms that have yet to be identified. Of these, Bufalin, a component of Venenum Bufonis (Chansu), inhibited both proliferation and invasion of HCC cells *in vitro*[28], and Chaiqiyigan granula enhanced Taxol-induced growth inhibition of HCC xenografts in nude mice[29]. Other herbal medicine extracts that have been reported to possess tumor growth inhibiting properties *via* yet to be defined mechanisms include Jianpi Huayu Formula, which inhibited BEL7402 cell proliferation *in vitro*[30], Compound Recipe Kushen SMMC, which inhibited 7721 cell proliferation *in vitro*[31], and Fuzheng Yiliu Granule, which inhibited PLC tumor growth in H_{22} hepatoma-bearing ICR mice and the HepG2 cell line[32].

The effects of TCM on apoptosis and autophagy of malignant hepatocytes

Dysregulation of apoptosis and autophagy are important components of tumor development, often resulting from activation of oncogenes and/or mutations in tumor suppressor genes. Thus, much effort has been expended on identifying TCM herbs that induce malignant hepatocyte apoptosis. Kangai Fuzheng Prescription was found to promote apoptosis and inhibit the growth of human hepatoma SMMZ-7721 cells by downregulating *p53* gene expression *in vitro*[33]. TCM matrine, a component of Radix Sophorae Flavescentis (Kushen), induced apoptosis and cell arrest by altering Bcl-2, Bax, and miR122a expression in human HepG2 cells and murine HCC cells[34,35]. Quercetin, an extract

from multiple herbal medicines, promoted apoptosis in the same HepG2 cells by increasing the transcription of the apoptosis-related *fas* gene[36]. Ligustrumlucidum Aitfruit (Nüzhenzi) extract could induce apoptosis and cell senescence through upregulation of p21 in human HCC cell lines[37]. Finally, modified Yi Guan Jian, a Chinese herbal formula, induced apoptosis in Bel-7402 cells[38] and Rhizoma Panacis Majoria (Zhuzishen) in H22 hepatoma cells[39].

In addition to inducing apoptosis, Baicalein, from Radix Scutellariae Baicalensis (Huangqin), enhanced autophagy *via* increasing endoplasmic reticulum stress in HCC cells[40]. Similarly, Arenobufagin (Chansu), a natural bufadienolide from toad venom, induced apoptosis and autophagy in human HCC cells but through inhibition of the PI3K/Akt/mTOR pathway in human HCC cells[41].

The effects of TCM on malignant hepatocyte metastases

Controlling HCC metastases is an important strategy for preventing tumor recurrence. Various TCM herbs have been reported to possess this property. Specifically, Sini-San inhibited HBx-induced migration and invasiveness of HCC cells by inhibiting multiple signal transduction pathways including ERK/phosphatidylinositol 3-kinase/Akt upstream of NF-κB and AP-1 in human HCC cells[42] while Biejiajian Pill suppressed the invasiveness of HepG2 cells by inhibiting the Wnt/β-catenin pathway in HCC cells[43]. Jinlong Capsule decreased the adhesive ability of highly metastatic MHCC97H cells *in vitro* and thereby significantly inhibited their movement and invasion[44].

In animal studies, Ginsenoside Rg3 from Ginseng (Renshen) inhibited the growth and metastasis of the highly metastatic human LCI-D20 cells in nude mice. This affect was ascribed to regulating the expression of nm23 and CD44 proteins[45]. By inhibiting SMMC-7721 cell invasion, Radix Salviae Miltiorrhizae (Danshen) decreased intrahepatic and distant metastasis of these cells in nude mice[46]. Another TCM that inhibits malignant hepatocyte metastases is Berberine, which inhibited the growth and development of spontaneously developed lung metastases in an orthotopic model of HCC (MHCC-97L) in mice by suppressing Id-1 expression[47].

The effects of TCM on HCC angiogenesis

HCC survival, growth, and metastases are dependent on new blood vessel growth or angiogenesis (Figure 1). TCM herbs that inhibit HCC angiogenesis include the alkaloids of Rubusalceifolius Poir (Cuyexuangouzi) and Livistonachinensis seeds (Pukuizi), which interfere with Notch signaling in a mouse model of HCC[48,49]. Resveratrol [typically extracted from Rhizoma Polygoni Cuspidati (Huzhang) or Fructus Mori (Sangshen)] decreases microvessel density of transplanted hepatic tumors in nude mice and inhibits tumor growth[50]. By significantly reducing vascular endothelial growth factor expression, Celastrusorbiculatus Thunb (Nansheteng) inhibited Hep-G2 induced tumor growth in orthotopic nude mice[51]. Finally, Qinggan Huayu Formula has been reported to inhibit tumor development and growth by reducing vascular endothelial growth factor and transforming growth factor-β1 protein expression and neovascularization in HCC rats[52].

The effects of TCM on the immunologic response to HCC

In the absence or setting of a suboptimal immune response, tumor cell growth, metastasis, and rates of recurrence are enhanced. Thus, the status of natural killer cells, T lymphocyte subpopulations such as CD_3^+, CD_4^+ and CD_8^+, and pro- as well as anti-inflammatory cytokines are important, and the ability of TCM to enhance the immune response to HCC would be of therapeutic value. Ganodermalucidum polysaccharides (GLPS) is an extract from Ganoderma Lucidum (Lingzhi) that significantly increases the ratio of T effector to regulatory T cells and suppresses tumor growth in HCC-bearing mice[53]. Moreover, GLPS eliminates regulatory T cells suppression of T effector proliferation resulting in increased pro-inflammatory IL-2 secretion. GLPS has also been reported to inhibit T cell Notch1 and FoxP3 expression by increasing miR-125b expression in hepatoma-bearing mice[53]. Another TCM with immuno-modulant properties is Radix Astragali Mongolici (Huangqi), a polysaccharide, which inhibits the growth of mouse HCC HepA by promoting pro-inflammatory TNF-α and IFN-γ production[54]. Combining Jiedu Xiaozheng Yin and Fuzheng Yiliu Formula improved the immune function of mice with H_{22} HCC by increasing CD_3^+ and CD_3^+/CD_4^+[55]. Shaoyao Ruangan Recipe, Biejiajian Pill, Ginsenoside Rg3, Fructus Lycii (Gouqizi) polysaccharide, and Fructus Schisandrae Chinensis (Wuweizi) polysaccharides are other herbal medications that have been reported to inhibit HCC by enhancing the host's immune responsiveness in HCC-bearing mice[56-60].

The effects of TCM on the multidrug resistance of malignant hepatocytes

Increased expression of multidrug resistance (MDR) protein activity, the family of transporters responsible for exporting xenobiotics from within cells, is considered the principal explanation for the failure of chemotherapy in HCC treatment. Many TCM herbs have been reported to reverse MDR expression and/or activity. For example, Tetramethylpyrazine, a bioactive constituent isolated from the root of Ligusticum chuanxiong Hort (Chuanxiong) downregulated P-gp, MRP2, MRP3, and MRP5 expression in HCC BEL-7402/ADM cells[61]. Bufalin, extracted from Venenum Bufonis (Chansu) and Hedyoticdiffusa (Baihuasheshecao) injection, achieved the same effect in BEL-7402/5-FU cells[62-63], and Hirudo (Shuizhi) extract, Qizhu Decoction, Shehuang Xiaoliu

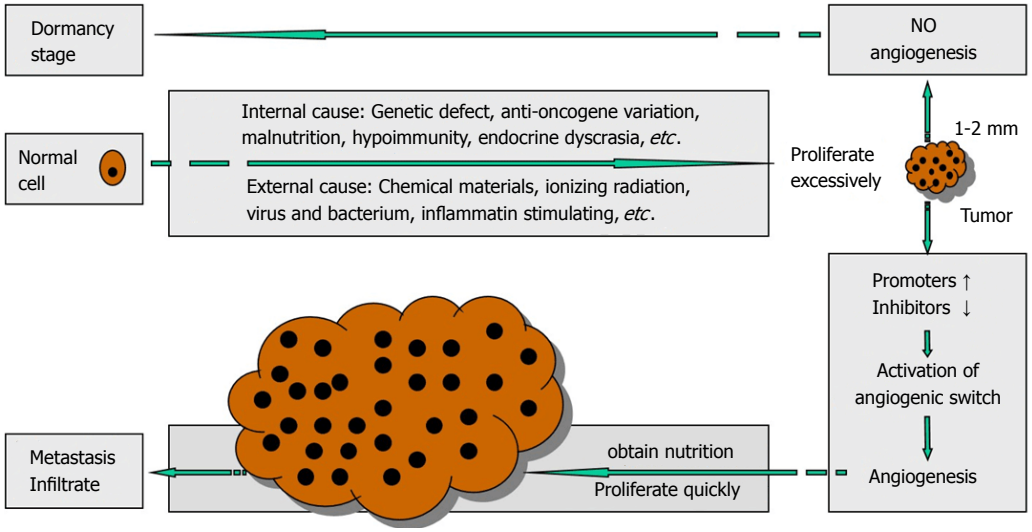

Figure 1 Solid tumor's occurrence and angiogenesis.

Decoction, Jianpi Huayu Formula and Quercetin all reversed MDR activity in HCC tissues[64-68].

CONCLUSION

Although much progress has been made in our utilization and understanding of TCMs for the treatment of HCC, additional experimentation and research is still required. Clearly, no single herbal medicine, active component, or compound recipe has been identified to be curative. Moreover, the mechanism(s) involved in achieving the benefits described are multiple and complex. Nonetheless, empiric and experimental data suggest that TCM is effective in limiting symptoms, reducing treatment associated side effects, inhibiting tumor growth, and altering key intracellular signaling pathways. While a combination of TCM and Western medicine may evolve as the optimal approach to treating HCC, certain challenges remain. Principal amongst these is the need for Western Medicine physicians to consider and where appropriate accept the concept of "holism" for cancer treatment. These physicians must also be willing to consider empiric findings, albeit of century's duration, as an additional measure of efficacy, particularly for compounds such as TCM herbs that due to their unique fragrance, do not always lend themselves to testing in placebo-controlled clinical trials.

ACKNOWLEDGEMENTS

The authors wish to thank Ms. R. Vizniak for her prompt and accurate typing of the manuscript and the Canadian Liver Foundation for their support.

REFERENCES

1 **Wallace MC**, Preen D, Jeffrey GP, Adams LA. The evolving epidemiology of hepatocellular carcinoma: a global perspective. *Expert Rev Gastroenterol Hepatol* 2015; **9**: 765-779 [PMID: 25827821 DOI: 10.1586/17474124.2015.1028363]

2 **Liao LP**, Xu MQ, Wu PK, Zeng QM, Yi BX, Xu GL. A study on classification of Chinese medicine by ISO and GB coding technology and the rules. *World Chin Med* 2015; **10**: 772-775 [DOI: 10.3969/j.issn.1673-7202.2015.05.035]

3 **Yang JM**, Han LT, Ren JG, Li JM, Li HH. Literature analysis of traditional Chinese medicine commonly used for treatment of liver cancer. *World Chin Med* 2013; **8**: 1150-1151

4 **Liu X**, Li N. [Regularity analysis on clinical treatment in primary liver cancer by traditional Chinese medicine]. *Zhongguo Zhongyao Zazhi* 2012; **37**: 1327-1331 [PMID: 22803386 DOI: 10.4268/cjcmm20120933]

5 **Sun M**, Chen Q. Investigate the relations between the TCM patterns of primary hepatic cancer and ultrasonography results. *Hubei J Tradit Chin Med* 2011; **33**: 20-21

6 **Lao GQ**, Chen F, Shi ZY, He XH, Luo JH, Huang RH, Liang DR, Chen JJ. Effect on life quality by Jianpi Jiedu Decoction in the treatment of advanced stage of hepatocellular carcinoma. *Chin J Chin Med* 2012; **27**: 1083-1084 [DOI: 10.16368/j.issn.1674-8999.2012.09.006]

7 **Li XL**, Lan MY, Wu LJT, Zhao XQ, Kan JG. Clinical therapeutic effect observation of Compound Recipe Kushen Injection treating the middle or advanced liver cancer patients. *J Chifeng Univ* (Nat Sci Edition) 2014; **30**: 135-136 [DOI: 10.13398/j.cnki.issn1673-260x.2014.22.057]

8 **Huang JD**, Wei AX. Impact of Jianpi Tiaogan Tang on life quality of patients with terminal primary liver cancer. *World Chin Med* 2014; **9**: 1319-1321 [DOI: 10.3969/j.issn.1673-7202.2014.10.019]

9 **Chen QS**, Chen Y, Pei RQ, Huang WZ, Li CY, Zhou B, Chen YY. Clinical observation of 30 cases of late-stage primary hepatic cancer treated with Jianpi Yiliu Decoction. *World J Integr Tradit West Med* 2013; **8**: 368-370 [DOI: 10.13935/j.cnki.sjzx.2013.04.012]

10 **Feng GF**, Chen R, Chen WZ. Observation on treating ascites in primary liver carcinoma by Ascending Lucidity-Descending Turbidity Decoction. *Liaoning J Tradit Chin Med* 2015; **42**: 1285-1286 [DOI: 10.13192/j.issn.1000-1719.2015.07.054]

11 **Si T**, Ning XJ, Yang JQ, Feng XB, Shi Y, Li R. Ruanganlidan decoction on disease-free survival after a radical liver resection. *J Changchun Univ Chin Med* 2015; **31**: 145-148 [DOI: 10.13463/j.cnki.cczyy.2015.01.050]

12 **Lu YX**, Lu XQ, Wu FS, Tan ZW, Mo YJ, Lai L. Effect of Qudu

Huayu Xiaoji formula on life span and life quality of patients with primary hepatocellular carcinoma in middle/advanced stage after interventional therapy. *J New Chin Med* 2016; **48**: 153-155 [DOI: 10.13457/j.cnki.jncm.2016.09.069]
13 **Xi S**, Hong R, Huang J, Lu D, Qian L, Li P, Wen L, Wang Y. Effects of Ciji Hua'ai Baosheng granule formula (CHBGF) on life time, pathology, peripheral blood cells of tumor chemotherapy model mouse with H22 hepatoma carcinoma cells. *Afr J Tradit Complement Altern Med* 2014; **11**: 94-100 [PMID: 25392588 DOI: 10.4314/ajtcam.v11i4.16]
14 **Hu J**, Wang HL, Zhang H, Li ZZ, Yue W, Zhang JG. The detoxification and synergistic effect of zingiber and rhizoma composite on radiotherapy and chemotherapy. *J Taishan Med College* 2014; **35**: 848-850 [DOI: 10.3969/j.issn.1004-7115.2014.09.003]
15 **Jia YP**, Zhou DS, Sun C, Qu BE. Efficiency-enhancing and toxicity-reducing effects of ginseng polysaccharide on cyclophosphamide treatment of mouse hepatoma. *Acta Lab Anim Sci Sin* 2013; **21**: 61-64
16 **Li M**, Ma HY, Shen JD, Li YC. Effects of Fufang Ejiao Jiang enhances efficacy and reduces toxicity of 5-Fu in hepatoma H22-bearing mice. *Chin J Exp Tradit Med Form* 2012; **18**: 216-219 [DOI: 10.13422/j.cnki.syfjx.2012.20.065]
17 **Wang HZ**, Mao HL. Lian Qi Capsules enhances efficacy and reduces toxicity of chemotherapy in hepatoma H22-bearing mice. *Chin J Med Guide* 2013; **15**: 1033-1034, 1037
18 **Bai X**, Guan BS, Sun YN, Zhang LY, Ji HT, Zhang T. Effect of aqueous extract of Fructus Akebiae (Bayuezha) on immune function of hepatoma H22-bearing mice. *Chin J Geront* 2015; **35**: 1946-1948 [DOI: 10.3969/j.issn.1005-9202.2015.07.096]
19 **Pang LF**. Clinical study about parenteral solution of Tremella Polysaccharide improving quality of life of patients with liver cancer during chemotherapy. *J Hubei Univ Chin Med* 2014; **16**: 85-86 [DOI: 10.3969/j.issn.1008-987x.2014.04.31]
20 **Xue WW**, Zhu CL. Clinical research of JianpiJiedu Formula preventing and treating hepatic functional lesion after chemoembolization for patients with primary hepatic carcinoma. *J Shandong Univ TCM* 2013; **37**: 392-394 [DOI: 10.16294/j.cnki.1007-659x.2013.05.011]
21 **Ding JP**. The retrospective study of advanced hepatocellular carcinoma by treated with Zipi decoction combined with TACE. *J Pract Tradit Chin Inter Med* 2015; **29**: 9-11 [DOI: 10.13729/j.issn.1671-7813.2015.02.04]
22 **Xu L**, Wang S, Zhuang L, Lin J, Chen H, Zhu X, Bei W, Zhao Q, Wu H, Meng Z. Jian Pi Li Qi Decoction Alleviated Postembolization Syndrome Following Transcatheter Arterial Chemoembolization for Hepatocellular Carcinoma: A Randomized, Double-Blind, Placebo-Controlled Trial. *Integr Cancer Ther* 2016; **15**: 349-357 [PMID: 26590124 DOI: 10.1177/1534735415617020]
23 **Yap KY**, See CS, Chan A. Clinically-relevant chemotherapy interactions with complementary and alternative medicines in patients with cancer. *Recent Pat Food Nutr Agric* 2010; **2**: 12-55 [PMID: 20653549 DOI: 10.2174/2212798411002010012]
24 **Lu WL**, Ren HY, Liang C, Zhang YY, Xu J, Pan ZQ, Liu XM, Wu ZH, Fang ZQ. Akebia trifoliate (Thunb.) Koidz Seed Extract Inhibits the Proliferation of Human Hepatocellular Carcinoma Cell Lines via Inducing Endoplasmic Reticulum Stress. *Evid Based Complement Alternat Med* 2014; **2014**: 192749 [PMID: 25389441 DOI: 10.1155/2014/192749]
25 **Chen XZ**, Cao ZY, Li JN, Hu HX, Zhang YQ, Huang YM, Liu ZZ, Hu D, Liao LM, Du J. Ethyl acetate extract from Jiedu Xiaozheng Yin inhibits the proliferation of human hepatocellular carcinoma cells by suppressing polycomb gene product Bmi1 and Wnt/β-catenin signaling. *Oncol Rep* 2014; **32**: 2710-2718 [PMID: 25333742 DOI: 10.3892/or.2014.3541]
26 **Cao Z**, Lin W, Huang Z, Chen X, Zhao J, Zheng L, Ye H, Liu Z, Liao L, Du J. Ethyl acetate extraction from a Chinese herbal formula, Jiedu Xiaozheng Yin, inhibits the proliferation of hepatocellular carcinoma cells via induction of G0/G1 phase arrest in vivo and in vitro. *Int J Oncol* 2013; **42**: 202-210 [PMID: 23165653 DOI: 10.3892/ijo.2012.1703]
27 **Auyeung KK**, Ko JK. Coptis chinensis inhibits hepatocellular carcinoma cell growth through nonsteroidal anti-inflammatory drug-activated gene activation. *Int J Mol Med* 2009; **24**: 571-577 [PMID: 19724899]
28 **Qiu DZ**, Zhang ZJ, Wu WZ, Yang YK. Bufalin, a component in Chansu, inhibits proliferation and invasion of hepatocellular carcinoma cells. *BMC Complement Altern Med* 2013; **13**: 185 [PMID: 23870199 DOI: 10.1186/1472-6882-13-185]
29 **You M**, Luo M, Liao W, Hu S, Xu W, Jing L. [Chaiqiyigan granula enhances Taxol-induced growth inhibition of hepatocellular carcinoma xenografts in nude mice: an in vivo fluorescence imaging study]. *Nanfang Yike Daxue Xuebao* 2012; **32**: 1042-1045 [PMID: 22820595]
30 **Wang CJ**, Liu YZ, Xu XM. Influence of JianpiHuayu Recipe serum on cell proliferation of human hepatocellular carcinoma Bel-7402. *China Clin Rehabil* 2006; **10**: 82-84
31 **Jiang ZY**, Hua HQ, Qin SK, Yang AZ. Effect of Compound Recipe Kushen Injection on cell proliferation and cycle of human hepatocellular carcinoma SMMC-7721. *Jilin J Tradit Chin Med* 2011; **31**: 690-692 [DOI: 10.13463/j.cnki.jlzyy.2011.07.049]
32 **Cao ZY**, Chen XZ, Liao LM, Peng J, Hu HX, Liu ZZ, Du J. Fuzheng Yiliu Granule inhibits the growth of hepatocellular cancer by regulating immune function and inducing apoptosis in vivo and in vitro. *Chin J Integr Med* 2011; **17**: 691-697 [PMID: 21910071 DOI: 10.1007/s11655-011-0847-3]
33 **Li LH**, Pi WX, Cheng HB, Yu JH, Zhang X, Zhang YH. Inhibiting effect and mechanism of Kangai Fuzheng Prescription (ALC) for human hepatoma SMMC-7721 cell and expression of P53. *Niaoning J Tradit Chin Med* 2010; **37**: 2215-2217 [DOI: 10.13192/j.ljtcm.2010.11.140.yelh.064]
34 **Zhou W**, Xu X, Gao J, Sun P, Li L, Shi X, Li J. TCM matrine inducescell arrest and apoptosis with recovery expression of the hepato-specific miR122a in human hepatocellular carcinomaHep G2cell line. *Int J Clin Exp Med* 2015; **8**: 9004-9012 [PMID: 26309553]
35 **Ma L**, Wen S, Zhan Y, He Y, Liu X, Jiang J. Anticancer effects of the Chinese medicine matrine on murine hepatocellular carcinoma cells. *Planta Med* 2008; **74**: 245-251 [PMID: 18283616 DOI: 10.1055/s-2008-1034304]
36 **Zhao XL**, Xu GC, He LM, Ma L. Apoptosis of human HepG2 cells induced by Guercetin. *Pract J Cardiac Cereb Pneum Vasc Dis* 2010; **18**: 310-311
37 **Hu B**, Du Q, Deng S, An HM, Pan CF, Shen KP, Xu L, Wei MM, Wang SS. Ligustrum lucidum Ait. fruit extract induces apoptosis and cell senescence in human hepatocellular carcinoma cells through upregulation of p21. *Oncol Rep* 2014; **32**: 1037-1042 [PMID: 25017491 DOI: 10.3892/or.2014.3312]
38 **Hu B**, An HM, Shen KP, Xu L, Du Q, Deng S, Wu Y. Modified Yi Guan Jian, a Chinese herbal formula, induces anoikis in Bel-7402 human hepatocarcinoma cells in vitro. *Oncol Rep* 2011; **26**: 1465-1470 [PMID: 21822542 DOI: 10.3892/or.2011.1414]
39 **Chen T**, Hu W, Cui BP, Li JH. Panaxjaplcusvar inhibition of proliferation of H22 cells in mice and its mechanism of action. *World Chin J Digest* 2007; **15**: 2597-2601
40 **Wang Z**, Jiang C, Chen W, Zhang G, Luo D, Cao Y, Wu J, Ding Y, Liu B. Baicalein induces apoptosis and autophagy via endoplasmic reticulum stress in hepatocellular carcinoma cells. *Biomed Res Int* 2014; **2014**: 732516 [PMID: 24995326 DOI: 10.1155/2014/732516]
41 **Zhang DM**, Liu JS, Deng LJ, Chen MF, Yiu A, Cao HH, Tian HY, Fung KP, Kurihara H, Pan JX, Ye WC. Arenobufagin, a natural bufadienolide from toad venom, induces apoptosis and autophagy in human hepatocellular carcinoma cells through inhibition of PI3K/Akt/mTOR pathway. *Carcinogenesis* 2013; **34**: 1331-1342 [PMID: 23393227 DOI: 10.1093/carcin/bgt060]
42 **Lin HJ**, Kao ST, Siao YM, Yeh CC. The Chinese medicine Sini-San inhibits HBx-induced migration and invasiveness of human hepatocellular carcinoma cells. *BMC Complement Altern Med* 2015;

15: 348 [PMID: 26446078 DOI: 10.1186/s12906-015-0870-6]
43 **Wen B**, Sun H, He S, Cheng Y, Jia W, Fan E, Pang J. [Effects of Biejiajian Pills on Wnt signal pathway signal molecules β-catenin/TCF4 complex activities and downstream proteins cyclin D1 and MMP-2 in hepatocellular carcinoma cells]. *Nan Fang Yi Ke Da Xue Xue Bao* 2014; **34**: 1758-1762 [PMID: 25537897]
44 **Li LX**, Ye SL, Wang YH, Li JS, Sun RX, Xue Q, Chen J, Gao DM, Zhao Y. Inhibiting effect of Jinlong Capsule on high-metastatic human hepatocellular carcinoma cell lines. *Chin Hepatol* 2011; **16**: 240-241 [DOI: 10.14000/j.cnki.issn.1008-1704.2011.03.001]
45 **Hua HQ**, Shen XK, Qin SK, Chen HY. Anti-metastatic and anti-invasive ability of ginsenoside Rg3 on homotopic transplantation nude mouse model of human hepatocellular carcinoma cells. *Chin Clin Oncol* 2007; **12**: 897-901
46 **Sun J**, Zhou X, Liu Y. [Study on preventive and therapeutic effect of radix salviae miltiorrhizae on recurrence and metastasis of liver cancer]. *Zhongguo Zhong Xi Yi Jie He Za Zhi* 1999; **19**: 292-295 [PMID: 11783245]
47 **Tsang CM**, Cheung KC, Cheung YC, Man K, Lui VW, Tsao SW, Feng Y. Berberine suppresses Id-1 expression and inhibits the growth and development of lung metastases in hepatocellular carcinoma. *Biochim Biophys Acta* 2015; **1852**: 541-551 [PMID: 25496992 DOI: 10.1016/j.bbadis.2014.12.004]
48 **Zhao J**, Lin W, Cao Z, Zhuang Q, Zheng L, Peng J, Hong Z. Total alkaloids of Rubus alceifolius Poir inhibit tumor angiogenesis through suppression of the Notch signaling pathway in a mouse model of hepatocellular carcinoma. *Mol Med Rep* 2015; **11**: 357-361 [PMID: 25333354 DOI: 10.3892/mmr.2014.2702]
49 **Lin W**, Zhao J, Cao Z, Zhuang Q, Zheng L, Zeng J, Hong Z, Peng J. Livistona chinensis seeds inhibit hepatocellular carcinoma angiogenesis in vivo via suppression of the Notch pathway. *Oncol Rep* 2014; **31**: 1723-1728 [PMID: 24573440 DOI: 10.3892/or.2014.3051]
50 **Sun ZJ**, Yu HB, Zhang Y, Liu XG, Du LX. The effects of Resveratrol on growth and angiogenesis of HepG2 tumor model in vivo. *Shaanxi Med J* 2010; **39**: 279-281
51 **Wang M**, Zhang X, Xiong X, Yang Z, Sun Y, Yang Z, Hoffman RM, Liu Y. Efficacy of the Chinese traditional medicinal herb Celastrus orbiculatus Thunb on human hepatocellular carcinoma in an orthothopic fluorescent nude mouse model. *Anticancer Res* 2012; **32**: 1213-1220 [PMID: 22493351]
52 **Yin F**, Yao SK, Wu XM, Gao HS. Effect of Qinggan Huayu Decoction (QHD) on angiogenesis of hepatocellular carcinoma in rats. *Pharmacol Clin Chin Mater Medica* 2005; **21**: 29-32 [DOI: 10.13412/j.cnki.zyyl.2005.01.014]
53 **Li A**, Shuai X, Jia Z, Li H, Liang X, Su D, Guo W. Ganoderma lucidum polysaccharide extract inhibits hepatocellular carcinoma growth by downregulating regulatory T cells accumulation and function by inducing microRNA-125b. *J Transl Med* 2015; **13**: 100 [PMID: 25889022 DOI: 10.1186/s12967-015-0465-5]
54 **Xu DJ**, Chen MZ. Antitumor activity of APS and its mechanism of action. Chin *Hosp Pharm J* 2005; **25**: 923-925
55 **Chen XZ**, Cao ZY, Yang JL, Du J. Effects of Chinese medicine compound recipe on apoptosis and immune function of subcutaneous transplanted tumor with H22hepatic carcinoma. *Fujian J Tradit Chin Med* 2009; **40**: 52-54 [DOI: 10.13260/j.cnki.jfjtcm.009689]
56 **Sun Y**, Zhang AQ, Gao FY. Immune effect of Shaoyao Ruangan Recipe on hepatocarcinoma in tumor-bearing H22 mice and its effect on VEGF and PCNA expression. *J Emerg Tradit Chin Med* 2015; **24**: 590-592 [DOI: 10.3969/j.issn.1004-745X.2015.04.008]
57 **Luo QD**, Wang YH, Zhao HY, Wang B, Du FX, Deng FC, Jiang DY, Wang LQ. Interventional action of Biejiajian Pill on cellular immune function of tumor-bearing mice with hepatic carcinoma. *Acta Chin Med Pharm* 2012; **40**: 21-23 [DOI: 10.19664/j.cnki.1002-2392.2012.03.007]
58 **Wang X**, He YM. Experimental investigation on the antioxidation and immunity in mice with H22 liver cancer by time-selected administration of ginsenodise. *J Wannan Med College* 2012; **31**: 106-108 [DOI: 10.3969/j.issn.1002-0217.2012.02.006]
59 **Xiao PY**, Wang ZL, Huang JW. Effects of Lyciumchinensis Polysaccharides on tumor suppression and immune function of liver cancer model mice. *Chin Pharm* 2014; **25**: 4046-4048 [DOI: 10.6039/j.issn.1001-0408.2014.43.05]
60 **Gan L**. Regulating effect of fructusschisandrae polysaccharide on tumor growth and immune function of H22 vaccination mice. *Immunol J* 2013; **29**: 867-870 [DOI: 10.13431/j.cnki.immunol.j.20130189]
61 **Wang XB**, Wang SS, Zhang QF, Liu M, Li HL, Liu Y, Wang JN, Zheng F, Guo LY, Xiang JZ. Inhibition of tetramethylpyrazine on P-gp, MRP2, MRP3 and MRP5 in multidrug resistant human hepatocellular carcinoma cells. *Oncol Rep* 2010; **23**: 211-215 [PMID: 19956884]
62 **Gu W**, Liu L, Fang FF, Huang F, Cheng BB, Li B. Reversal effect of bufalin on multidrug resistance in human hepatocellular carcinoma BEL-7402/5-FU cells. *Oncol Rep* 2014; **31**: 216-222 [PMID: 24173654 DOI: 10.3892/or.2013.2817]
63 **Liao ZZ**, Wei LM, Xu LY, Liang G. Effect of Hedyoticdiffusa injection in reversing multi-drug resistance of human hepatoma BEL-7402/5-FU cells. *J Xi'an Jiaotong Univ* (Med Sci) 2015; **36**: 554-557
64 **Huang XD**, Guo YL, Huang LZ, Zhou Q, Tian XF. Study on the effects and mechanism of hirudo extract on the sensitivity of chemotherapeutic drugs and apoptosis inducing in human hepatoma HepG2 cells. *Chin J Tradit Chin Med Pharm* 2015; **30:** 2094-2096
65 **Zhou SF**, Li YF, Liu C, Li M, Yang W. The reverse effect of Qizhu decoction on multidrug-resistant human colorectal carcinoma cell line HCT-8/V and hepatocarcinoma cell line Bel/FU. *Chin J Integr Trad West Med Dig* 2014; **22**: 126-128 [DOI: 10.3969/j.issn.1671-038X.2014.03.04]
66 **Zhang XL**, Huang T, Yang XF, Huang L, Li Y. Experimental studies on reversion effects of Shehuang Xiaoliu decoction on multidrug resistance of human hepatoma cells. *Chin J Tradit Med Sci Tech* 2014; **21**: 25-27, 32
67 **Ling BF**, Wang RP, Zou X, Hou Q. Impacts of Jianpi Huayu Formula on Bel-7402/5-FU cell surface drug resistance protein in liver cancer. *World J Integr Tradit West Med* 2013; **8**: 120-123 [DOI: 10.13935/j.cnki.sjzx.2013.02.020]
68 **Wei Y**, Zhang HY, Liang G. The reverse effect of Quercetin on multidrug resistance of human hepatocellular carcinoma. *Tianjin Med J* 2012; **40**: 1022-1025 [DOI: 10.3969/j.issn.0253-9896.2012.10.018]

P- Reviewer: Dumitraşcu T, Niu ZS **S- Editor**: Ji FF
L- Editor: Filipodia **E- Editor**: Tan WW

World Journal of Hepatology

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

World J Hepatol 2018 November 27; 10(11): 807-821

DOI: 10.4254/wjh.v10.i11.807

ISSN 1948-5182 (online)

REVIEW

Genetic diversity of hepatitis viruses in West-African countries from 1996 to 2018

Maléki Assih, Abdoul Karim Ouattara, Birama Diarra, Albert Theophane Yonli, Tegwindé Rebeca Compaore, Dorcas Obiri-Yeboah, Florencia Wendkuuni Djigma, Simplice Karou, Jacques Simpore

Maléki Assih, Abdoul Karim Ouattara, Birama Diarra, Albert Theophane Yonli, Tegwindé Rebeca Compaore, Florencia Wendkuuni Djigma, Jacques Simpore, Biochemistry-Microbiology, CERBA/LABIOGENE, Ouagadougou 02006, Burkina Faso

Maléki Assih, Abdoul Karim Ouattara, Birama Diarra, Albert Theophane Yonli, Tegwindé Rebeca Compaore, Florencia Wendkuuni Djigma, Jacques Simpore, Laboratory of Molecular Biology and Molecular Genetics (LABIOGENE) UFR/SVT, University Ouaga I Prof Joseph KI-ZERBO, Ouagadougou 00226, Burkina Faso

Dorcas Obiri-Yeboah, Department of Microbiology and Immunology, School of Medical Sciences, University of Cape Coast, Cape Coast 00233, Ghana

Simplice Karou, Ecole Supérieure des Techniques Biologiques et Alimentaires (ESTBA-UL), Universite de Lome, Lome 00229, Togo

ORCID number: Maleki Assih (0000-0003-1280-0040); Abdoul Karim Ouattara (0000-0002-9767-0646); Birama Diarra (0000-0002-3562-6535); Albert Theophane Yonli (0000-0002-4796-0426); Tegwinde Rebeca Compaore (0000-0002-5956-3444); Dorcas Obiri-Yeboah (0000-0003-4562-9294); Florencia Wendkuuni Djigma (0000-0002-6895-6725); Simplice Karou (0000-0002-6172-7487); Jacques Simpore (0000-0002-0415-9161).

Author contributions: Assih M, Ouattara AK, Diarra B, Yonli AT and Simpore J conceived and designed the study; Assih M, Ouattara AK and Diarra B were involved in independent research of relevant articles; Assih M, Ouattara AK, Diarra B and Yonli AT were involved in full text review of relevant articles; Assih M, Ouattara AK and Diarra B were involved in data extraction, analysis and interpretation; Assih M, Ouattara AK, Diarra B, Yonli AT, Compaore TB, Obiri-Yeboah D, Djigma FW, Karou S and Simpore J were involved with drafting or revising the manuscript; Djigma FW, Karou S and Simpore J provided administrative, technical and material support; supervision of the study was made by Karou S and Simpore J; all authors critically revised and approved the final version of this publication.

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

Manuscript source: Unsolicited manuscript

Correspondence to: Abdoul Karim Ouattara, PhD, Research Associate, Biochemistry-Microbiology, CERBA/LABIOGENE, 01 BP 364, Ouagadougou 00226, Burkina Faso. ak.ouattara02@gmail.com
Telephone: +22-6-70010147

Received: August 2, 2018
Peer-review started: August 3, 2018
First decision: August 20, 2018
Revised: September 10, 2018
Accepted: October 23, 2018
Article in press: October 23, 2018
Published online: November 27, 2018

Abstract

The severity of hepatic pathology and the response to treatment depend on the hepatitis virus genotype in the infected host. The objective of this review was to determine the distribution of hepatitis virus genotypes in West African countries. A systematic review of the literature in PubMed, Google Scholar and Science Direct was performed to identify 52 relevant articles reporting hepatitis A, B, C, D, E and G viruses genotypes.

Hepatitis B virus (HBV) genotype E with a prevalence of 90.6% (95%CI: 0.891-0.920) found in this review, is characterized by low genetic diversity. Hepatitis C virus (HCV) genotypes 1 and 2 represented 96.4% of HCV infections in West African countries, while hepatitis delta virus, hepatitis A virus, hepatitis G virus genotypes 1 and HEV genotype 3 were reported in some studies in Ghana and Nigeria. HBV genotype E is characterized by high prevalence, low genetic diversity and wide geographical distribution. Further studies on the clinical implications of HBV genotype E and HCV genotypes 1 and 2 are needed for the development of an effective treatment against this viral hepatitis in West African countries. Surveillance of the distribution of different genotypes is also needed to reduce recombination rates and prevent the emergence of more virulent viral strains.

Key words: Hepatitis virus; Mutations; Genotypes; Recombination; West African Economic and Monetary Union

Core tip: The determination of hepatitis viruses genotypes is very important for the management and treatment of infected patients. Indeed, mutation development, disease progression and antiretroviral response are all dependent on the genotype of the infecting virus. Genotype determination is therefore very important to identify patients who are at increased risk of disease progression and to optimize treatment. The objective of this review was to determine the prevalence and distribution of different hepatitis viruses genotypes in 10 West African countries.

Assih M, Ouattara AK, Diarra B, Yonli AT, Compaore TR, Obiri-Yeboah D, Djigma FW, Karou S, Simpore J. Genetic diversity of hepatitis viruses in West-African countries from 1996 to 2018. *World J Hepatol* 2018; 10(11): 807-821 Available from: URL: http://www.wjgnet.com/1948-5182/full/v10/i11/807.htm DOI: http://dx.doi.org/10.4254/wjh.v10.i11.807

INTRODUCTION

Viral hepatitis is an inflammation of the liver that may progress spontaneously to healing or lead to cirrhosis or hepatocellular carcinoma (HCC). Viral hepatitis caused 1.34 million deaths in 2015, a figure comparable to deaths from tuberculosis (TB) and human immunodeficiency virus (HIV). However, mortality attributable to TB and HIV is decreasing, while that due to hepatitis is constantly increasing[1]. There are five types of hepatitis viruses designated by the letters A, B, C, D and E. The most common forms of the disease are hepatitis A, B and C. Another human lymphotropic virus belonging to the Flaviviridae family and closely related to hepatitis C virus (HCV) was identified as hepatitis G virus (HGV) or GB virus C (GBV-C). However, several studies have shown that GBV-C/HGV infection is not clearly associated with any disease and may play a role in modulating HIV disease[2,3]. In a study conducted in Burkina Faso, a prevalence of 7.4% of HGV was reported in blood donors[4]. Viral hepatitis usually occurs as a result of parenteral contact with infected body fluids: Blood transfusions or contaminated blood products.

Hepatitis B virus (HBV) is ubiquitous, but the prevalence of infection varies across different regions of the world. According to the World Health Organization, about two billion people have been in contact with HBV worldwide, with more than 240 million cases of chronic infections[5]. About 80 to 120 million cases of chronic HBV infection occur in sub-Saharan Africa[6,7]. According to the carriage of hepatitis B surface antigen (HBsAg), there are zones of low endemicity (< 2%) such as Western Europe or North America; areas of average prevalence (2%-7%) such as North Africa or Eastern Europe and finally high endemic areas (> 8%) such as West Africa or Southeast Asia[8]. Indeed, hepatitis B is highly endemic in West Africa, with the highest prevalence in the world (> 8%). In sub-Saharan Africa, about 47% of HCC have been attributed to HBV[9]. Despite the availability of a vaccine, HBV remains a major public health problem, with approximately 686 thousand deaths per year worldwide due to the consequences (cirrhosis and HCC) of this infection[5].

More than 71 million people worldwide are chronically infected with HCV and may develop liver cirrhosis and/or HCC[10]. There is currently no effective vaccine against HCV; and about 399000 people die each year from hepatitis C, mainly cirrhosis and HCC. In North and West Africa, the prevalence of HCV infection ranges from 0.5% to 1.0%[10]. Hepatitis delta virus (HDV) is a small defective ribonucleic acid (RNA) virus that depends on HBV for the assembly of new virions and proliferation of infection to hepatocytes. HDV infection can be therefore prevented through vaccination or any strategy to eliminate HBV infection. Approximately 15 to 20 million people worldwide are co-infected with these two viruses, with a high risk of severe liver disease[11]. In a study of pregnant women in Benin, the prevalence of HDV was 11.4% in 15.5% of HBsAg-positive individuals[12].

There are several genotypes of hepatitis viruses with different clinical implications and distinct geographic distributions. HBV is classified into 10 genotypes (A-J) and about 40 subgenotypes with a correlation between genotypes and their modes of transmission[13]. In fact, HBV genotype A is found in North America, Europe, South-East Africa and India; genotypes B and C in Asia and Oceania while genotype D is the most common in North America, North Africa, Europe, the Middle-East and Oceania. HBV genotype E is hyperendemic in West Africa; genotype F is found in

South America; and genotypes G and H are in Central and South America[13]. HCV has a high genetic diversity with a predominance of genotype 1 and 3 worldwide. The endemic strains of genotypes 1 and 2 are mainly found in West Africa, genotype 3 in Asia, genotypes 4 in Central Africa and the Middle East, while genotypes 5 and 6 are predominant in South Africa and South East Asia, respectively[14]. HCV genotype 2 is the most common genotype in West African countries, followed by genotype 1 and genotype 3[15]. HDV genotype Ⅰ is more common in Europe and North America, genotype Ⅱ is predominant in the Far East and Japan while genotype Ⅲ is predominant in the Amazonian area and northern South America[16].

The severity of hepatic pathology and the response to treatment depend on the virus genotype in the infected host. For example, HBV genotype A infection tends to chronicity, whereas genotype D has a high frequency of mutation influencing response to treatment. Liver cirrhosis and progression to HCC are strongly associated with HBV genotypes C and D compared to other genotypes[17,18]. Furthermore, superinfection of chronic HBV patients by HDV leads to increased liver damage and more rapid progression of cirrhosis in 90% of cases[16]. HDV genotype Ⅲ is thought to be associated with severe forms of liver disease, while a more moderate clinical evolution and a wide variety of clinical conditions are observed with genotypes Ⅱ and Ⅰ, respectively. The response to interferon treatments is more effective against HBV genotypes A and B compared to genotypes C, D and Ⅰ. HBV genotype E seems to have the worst response to treatment[18]. Rapid progression of hepatic disease and HCC has also been associated with HBV genotype A1[13]. HCV subtype 1b is associated with a high risk of developing HCC compared to other genotypes[19]. Early generations of vaccines protected against subtype 1b, while genotype 3, which accounts for 30.1% of HCV global infections, is less likely to respond to first and second generation direct-acting antivirals currently used for HCV treatment[14,20].

Determination of the viral genotype is an essential element of the pre-therapeutic assessment, because it is one of the predictors of the response to treatment and determines the choice of molecules used with the new anti-HCV treatments. It has also been shown that HBV genotypes differ according to disease course, mutation development and response to antiviral therapy[21]. Indeed, genotype determination is important to identify patients who are at increased risk of disease progression and to optimize treatment[22]. Here, we review various publications on the genotypes of hepatitis viruses in West African countries [West African Economic and Monetary Union (WAEMU) countries, Ghana and Nigeria] in order to map the genotypes distribution and discuss the infections associated with the different viruses identified.

HEPATITIS VIRUSES' INFECTION IN WEST AFRICA

HBV infection

HBV belongs to the *Hepadnaviridae* family and the *Orthohepadnavirus* genus[23]. It is a double-stranded circular DNA enveloped virus of small circumference (1.6 million Dalton) associated with a DNA-dependent DNA polymerase that acts as a reverse transcriptase during replication. HBV is highly contagious, 100 times more contagious than HIV and can remain stable at 25 ℃ for seven days in dried blood. Sexual, parenteral (through the blood), mother-to-child or even close intrafamily non-sexual contact over a long period of time are the different modes of infection. The most common modes of spread of hepatitis B in endemic areas are mother-to-child transmission and exposure to infected blood. The appearance of a chronic infection is very common for infants infected by their mother before the age of 5 years.

Markers, such as the HBsAg, the HBs antibody (anti-HBs), the core antigen (HBcAg), the HBe antigen (HBeAg) and the HBe antibody (anti-HBe) make it possible to monitor the evolution of this virus. Despite the small size of the genome and the constraints imposed by its organization, HBV is highly variable.

HBV strains are divided into several genotypes. These genotypes are defined by a divergence of at least 8.0% of the whole genome nucleotide sequence and at least 4.1% in the S gene[24]. The main genotypes were divided into subgenotypes based on the divergence between 4.1% and 8.0% of their complete nucleotide sequence[25]. In the last decade, phylogenetic studies of sequences of different viral genomes have tentatively classified HBV into 10 genotypes (A-J)[26]. Genotypes and subgenotypes have a distinct geographic distribution[27]. Genotype A is the only predominant genotype in East Africa, where the prevalence of other genotypes is less than 5%[24,28]. The subgenotype A1 is predominant in Africa, while subgenotypes A3-A6 are found in Central and West Africa[29].

Subgenotype D1 is highly prevalent in East Africa[30]; D7 has been isolated in Tunisia[31]; and D8 has been characterized in Niger[32]. West Africa is the main focus of genotype E. Vaccination is the safest way to prevent HBV infection[33]. Major advances in the treatment of chronic HBV have been made with the development of nucleoside reverse transcriptase inhibitors with anti-HBV activity, such as L-nucleosides (lamivudine 3TC) or alkylphosphates (tenofovir disoproxil fumarate)[34].

HCV infection

HCV belongs to the family Flaviviridae and the genus *hepacivirus*[35]. HCV mainly infects hepatocytes but may also be present in blood mononuclear cells and dendritic cells[36]. HCV is a small single-stranded RNA virus of positive polarity, enveloped 55-65 nm in diameter.

Parenteral route is the major mode of transmission of HCV. Transfusion and intravenous drug addiction are also routes of transmission. To this day, the main cause of HCV transmission in developed countries is drug abuse[37]. The HCV genome shows a high rate of mutations with considerable genetic heterogeneity of the virus in infected people worldwide. Phylogenetic approaches made it possible to classify HCV into 11 major genotypes (designated by the Arabic numerals from 1 to 11), with many subtypes (indicated by lower case letters a, b, c, *etc*.)[38].

Subgenotypes 1a, 1b, 2a, 2b and 3a are widely distributed worldwide[39], while 5a and 6a are common in South Africa and Southeast Asia[40,41]. Genotype 4 is predominant in Central Africa[42,43] and in North Africa[44]. In Africa, divergent HCV genotype 1 and 2 strains were found endemic in the West African subregion[45-47]. Recently, analysis of the epidemic history of HCV infections has traced modern HCV lines in West Africa to the 17th and 20th centuries[46]. The current standard treatment for chronic infection is the combination of pegylated interferon alpha (pegIFNα) and ribavirin (RBV)[48]. Currently, new therapeutic approaches using direct-acting antivirals have been developed for the treatment of chronic hepatitis C. These molecules inhibit certain stages of the viral cycle and prevent the production of viral particles by infected hepatocytes.

Others hepatitis virus infection (HDV and HGV)

HDV infection: HDV is an infectious agent that can only infect patients previously or simultaneously infected with the HBV[49]. It is a single-stranded RNA negative polarity virus, 1700 nucleotides in size, which encodes a single structural protein, the hepatitis delta antigen (HDAg), and requires HBV to replicate. HDV infection can only occur with simultaneous coinfection with HBV or superinfection[1]. HDV transmission is predominantly parenteral, and the sexual route is less effective than HBV. Mother-to-child transmission is rare. Hepatitis D is a liver disease that can take the acute form and chronic form. There can be no hepatitis D in the absence of HBV. Co-infection with HBV or HDV superinfection results in more severe disease than HBV mono-infection[1]. HDV infection is diagnosed by high titers of immunoglobulin G (IgG) and immunoglobulin M (IgM) anti-HDV and confirmed by serum detection of HDV RNA by polymerase chain reaction[1]. HDV isolates in the world are divided into at least eight phylogenetically distinct genotypes[50].

Genotype 1 is the predominant form of HDV with worldwide distribution, while genotypes 2 and 4 are present in Japan and Taiwan and are often associated with a milder form of disease[50]. Genotype 3 has been reported in the Amazonian region[51]. Genotypes 5-8 were detected in the sera of patients of African origin[52]. In addition, genotype 8 infection was also detected in the state of Maranhão in northeastern Brazil[53]. Some studies have shown that genotypes 3 and 4 can be associated with particularly severe clinical forms of hepatitis (fulminant hepatitis)[51]. HDV infection is rarely studied in West Africa despite the high prevalence of HBV. There is currently no effective antiviral therapy for hepatitis D. The prevention of hepatitis D involves vaccination against hepatitis B. pegIFNα is the only effective anti-HDV drug; nucleoside analogues active against HBV have little or no effect on HDV replication[1].

HGV infection: The HGV, called GBV-C or HGV, is a flavivirus, such as HCV, that causes spontaneously resolving acute hepatitis or fulminant hepatitis. It can cause chronic infections. HGV is a single-stranded positive-strand RNA virus[54]. Its transmission is mainly parenteral. Maternal-fetal and sexual transmissions are higher than those seen with HCV. IFN is effective in normalizing hypertransaminasemia in infected patients, but relapse appears to be common when treatment is discontinued.

METHODOLOGY

Research strategy and selection criteria

A systematic review of the literature was conducted to identify relevant articles reporting genotypes of hepatitis viruses in WAEMU countries including Ghana and Nigeria from 1996 to 2018. The research was conducted in French and/or English in three databases: PubMed, Google Scholar and Science Direct. The keywords used were "HBV and/or HBV and other viruses "+" the name of each of the 10 countries included in the study". A filter limiting the search for keywords in the title and/or abstract of articles was used [PubMed: (tiab); Google Scholar: Allintitle and Science Direct: TITLE-ABSTR-KEY]. Searches with similar terms such as "hepatitis virus", "hepatitis virus", "hepatitis virus genotypes" or "hepatitis virus genotype" were also conducted.

The studies were then selected on the basis of the following criteria: (1) data published in a peer-reviewed scientific journal; (2) only patients residing in one of the WAEMU countries, Ghana or Nigeria; and (3) patients from these countries infected with hepatitis viruses whose genotypes have been identified. All scientific publications (52) that reported data on genotypes of hepatitis viruses in populations from WAEMU countries, Ghana and Nigeria, between 1996 and 2018 and met the selection criteria were included in this systematic review (Figure 1). Eligible studies had to report the genotype of viral hepatitis in populations from included countries regardless of method used for viremia detection. Both risk groups or general population were eligible for inclusion.

HBV and HCV viremia detection were based on

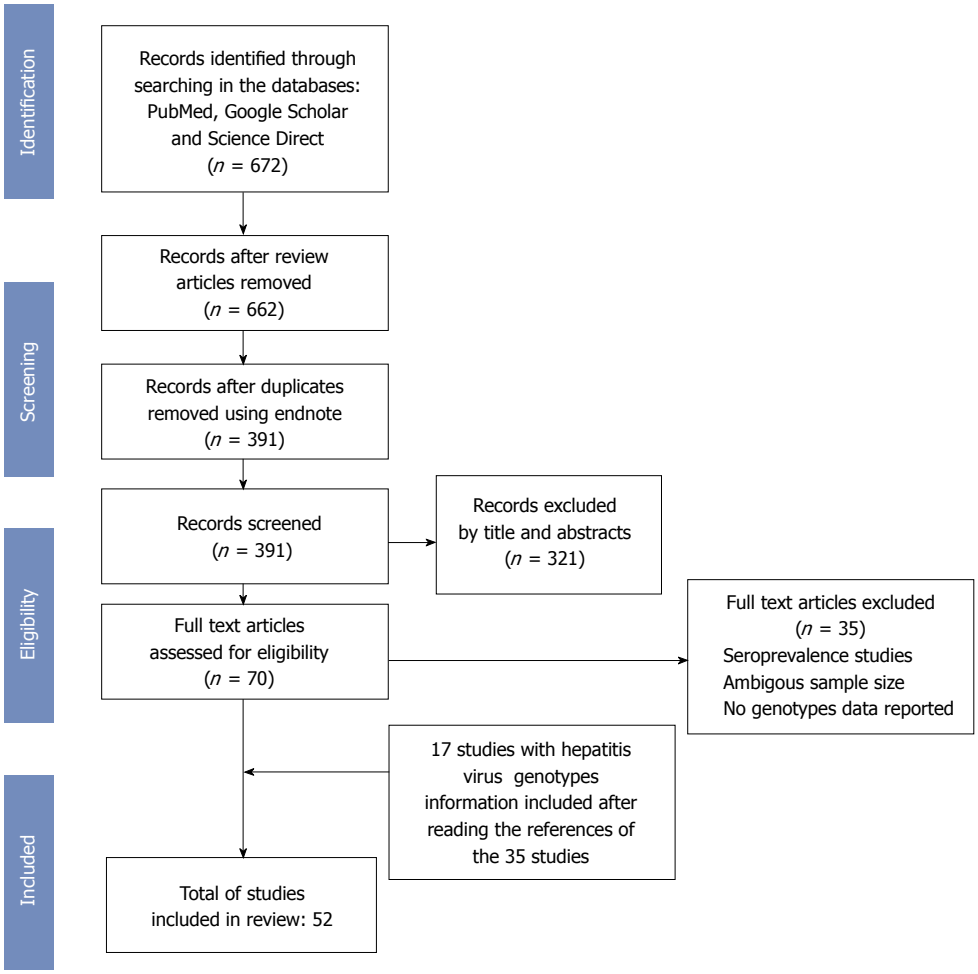

Figure 1 Flow diagram showing the method for the study selection. The database search for the search strategy described in the section was cleaned up to exclude review articles and duplicates. Titles and abstracts were included in the literature review. Seroprevalence articles, articles with ambiguous data that did not meet the inclusion criteria were then excluded during the full-text review. Fifty-two (52) relevant articles were finally included for this review.

DNA/RNA amplification. Genotypes detection was performed using polymerase chain reaction or direct sequencing. HCV genotype classification was considered because in many studies, HCV cases were classified at the genotype level but not at the subtype level. Journal articles, publisher correspondence, news, letters, book chapters and studies whose data were ambiguous or could not be extracted were systematically excluded. The search and selection of the relevant articles in the three databases were carried out by two independent reviewers. The inclusion of a study by both reviewers was a requirement. In case of disagreement on the eligibility of a study, the problem was solved through a discussion and/or consensus with a third reviewer.

Extractions of data and analysis

The genotyping data were extracted from the different studies carried out in Ghana, Nigeria and WAEMU countries. The data extracted from the various studies included in this review are: The first author, the year of the data publication, the study population, the type of study or data collection (prospective or retrospective), the country, the number of samples successfully genotyped and the results of identified genotypes.

In multi-center studies, only data from the countries included in this mini-review were considered. Prevalence was determined by making the ratio of the genotype considered to the total number of samples tested for that genotype. Confidence intervals were calculated using the R software. Phylogenetic analysis was performed with 53 HBV sequences using the neighbor-joining algorithm based on the Kimura two-parameter distance estimation method. Only bootstrap values of > 80% are shown (1.000 replicates). The maps were made using genotyping data from each country (source: Dr. Ouattara AK).

SEARCH RESULTS

Selection of studies

The initial search in the three databases, according to the search strategy described in the methodology, found 391 articles after elimination of reviews and duplicates. Examination of titles and abstracts led

to the elimination of 321 studies that did not meet the inclusion criteria of this review. Only 70 studies were considered eligible after full text review. This step allowed the exclusion of 35 articles presenting data reporting only seroprevalences or presenting ambiguous genotyping data. Finally, 52 studies, 35 of which were obtained after the full text examination and 17 after references examination of the 35 articles selected, were included in this systematic review.

Characteristics of included studies

Tables 1 and 2 present the characteristics of the different studies included in this systematic review. The majority of included studies used a prospective method of sample collection. A case-control study, two Cas reports, two multi-center studies and four cohort studies were included in the review, while the rest were cross-sectional or prospective studies. Twelve studies were performed in HIV-infected individuals compared to 11 in blood donors and seven in pregnant women, while populations and age groups were variable for the rest of the studies.

HBV genotypes

The frequency of the different genotypes was determined by dividing the number of samples presenting the genotype considered by the total number of successfully sequenced samples. In this systematic review, the largest number of successfully sequenced samples were recorded in Ghana (457/1620), followed by Nigeria (269/1620) and Côte d'Ivoire (251/1620). Genotyping studies of hepatitis viruses were rare in Guinea-Bissau and almost non-existent in Togo. The HBV genotype E was the predominantly isolated genotype in the various studies conducted in the WAEMU countries, Ghana and Nigeria (Table 1).

Indeed, out of a total of 1620 successfully sequenced HBV samples, E genotypes were individually isolated in 90.6% (1468/1620, 95%CI: 0.891-0.920) of HBV infection cases. In addition, its prevalence of recombination or coinfection with genotypes A and D was estimated at 0.86% (14/1620, 95%CI: 0.005-0.014) in our study area. HBV genotype E is characterized by low genetic diversity compared to other genotypes, including genotype A (Figure 2). The second HBV genotype reported in terms of frequency in the countries included in this review was genotype A with an individual prevalence of 7.8% (126/1620, 95%CI: 0.065-0.092), while a prevalence of 0.74% (12/1620, 95%CI: 0.004-0.013) of genotypes D was observed in the study area. A slight decrease in the overall frequency of HBV genotype E in West African countries was found between 2003 and 2010 (94.4%) compared to 2011-2018 (90.0%) with emergence of genotypes A and D. Some studies have focused on the genotyping of other hepatitis viruses with a predominance of HCV infections (528/570, Table 2). Figure 3 shows the geographical distribution of the different HBV genotypes in the countries included in this review.

Genotypes of other hepatitis viruses (HCV, HDV, HAV)

Of the 535 strains of HCV isolated and sequenced successfully, genotype 1 was found in the majority of cases of infections (56.4% or 298/528) against 40.0% (211/528) for genotype 2 while 3.6% (19/528) of the samples had genotypes 3 (9/19), 4 (7/19) and 5 (3/19) of HCV. HCV genotype 2 was most common in Benin, Burkina Faso, Ghana Guinea Bissau and Mali, while genotype 1 was predominant in Côte d'Ivoire, Senegal and Nigeria (Figure 4), with a high number of sequenced samples. Genotypes 1 of other hepatitis viruses such as HDV, a satellite virus still found in coinfection with HBV, HAV, HGV, and HEV genotype 3, have been reported in some studies in Ghana and Nigeria (Table 2).

DISCUSSION

Selection of studies

The aim of this review was to map the genotypes of the different hepatitis viruses identified in WAEMU countries, Ghana and Nigeria. The systematic review in the PubMed, Google Scholar and Sciences Direct databases included 52 studies reporting genotypes of hepatitis A, B, C, D, E and G. The availability of genetic data varied across country due to the prevalence or clinical relevance of the virus or the difficulty of sequencing in a context of limited resources. Indeed, most of the genotyping studies (29/52) focused on HBV because of its endemicity in sub-Saharan Africa and its clinical implications[1]. In West Africa, chronic carriage of HBV in the general unvaccinated population is estimated to be between 10% and 18%[55]. Several studies (18/52) have also provided HCV genotype data, which is the second virus of clinical interest in this West African sub-region after HBV, while very little genetic data is available on HDV, a satellite of HBV. The genetic data on HAV come only from Nigeria where it is endemic[56], while the genotypes of hepatitis E and G viruses, very scarce in WAEMU countries[57], were reported respectively in Nigeria and Ghana.

HBV genotypes

Knowledge of hepatitis viruses genotypes is of great epidemiological and clinical interest. Indeed, genotypes are responsible for variable clinical manifestations with differences depending on the stage of the disease, mutations and response to treatments[58,59]. They are also an invaluable tool for mapping the molecular evolution and dynamics of infection transmission because the different genotypes have a distinct geographic distribution. The study of Archampong *et al*[59]

Table 1 Distribution of hepatitis B virus genotypes in West African Economic and Monetary Union countries, Ghana and Nigeria

Ref.	Year	Countries	Patients	Type of study	Samples	HBV genotypes (*n*)
Diarra *et al*[80]	2018	Burkina Faso	Occult HBV	Cross-sectional	21	E (17) and A3 (4)
Archampong *et al*[59]	2017	Ghana	HBV-HIV coinfected	Cross-sectional	63	E (58), A (4) and D (1)
Boyce *et al*[58]	2017	Ghana	HBV-HIV coinfected	Case reports	3	D/E (3)
Cella *et al*[63]	2017	Mali	Malian refugees	Cross-sectional	16	E (16)
Lawson-Ananissoh *et al*[81]	2017	Côte d'Ivoire	Chronic HBV	Prospective	33	E (27), A (6)
Dongdem *et al*[73]	2016	Ghana	Chronic Hepatitis B	Cross-sectional	58	E (47), A (8) and D (3)
Opaleye *et al*[82]	2016	Nigeria	HBV+	Cross-sectional	17	E (17)
Compaore *et al*[62]	2016	Burkina Faso	HIV-1+ and HIV-1-	Case-Control	120	E (120)
Candotti *et al*[83]	2016	Burkina Faso	Blood donors	Prospective	99	E (71) A3QS (28)
Brah *et al*[84]	2016	Niger	HBV infected	Prospective	23	E (21), A3E (1) and D/E (1)
Boyd *et al*[85]	2016	Côte d'Ivoire	HBV-HIV coinfected	Prospective	100	E (98) and A (2)
Ampah *et al*[61]	2016	Ghana	Randomized volunteers	Prospective	52	E (52)
Traore *et al*[55]	2015	Mali	Adults volunteers	Cohort study	90	E (82), D/E (5), D (1) and A (2)
Faleye *et al*[86]	2015	Nigeria	Pregnant women	Cross-sectional	6	E (6)
Faleye *et al*[87]	2015	Nigeria	Asymptomatic individuals	Cross-sectional	13	E (13)
Maylin *et al*[88]	2015	Senegal	Chronic HBV	Cohort study	87	E (65), A (22)
Honge *et al*[76]	2014	Guinea-Bissau	HIV+	Cross-sectional	26	E (25) and D (1)
De Paschale *et al*[12]	2014	Benin	Pregnant women	Prospective	19	E (19)
Forbi *et al*[56]	2013	West Africa[1]	Pregnant women and HIV+	Multicenter	83	E (74) and A (9)
Hübschen *et al*[89]	2011	Nigeria	Cohorts samples	Cohorts study	163	E (154) and A (9)
Geretti *et al*[90]	2010	Ghana	HIV+	Cross-sectional	86	E (82) and A (4)
Forbi *et al*[69]	2010	Nigeria	Asymptomatic volunteers	Cross-sectional	55	E (53) and A3 (2)
Chekaraou *et al*[32]	2010	Niger	Blood donors	Cross-sectional	24	E (20), D/E (4)
Candotti *et al*[91]	2007	Ghana	Pregnant women	Cross-sectional	70	E (69) and A (1)
Vray *et al*[92]	2006	Senegal	Blood donors	Cross-sectional	32	E (23) and A (9)
Huy *et al*[60]	2006	Ghana	Blood donors	Cross-sectional	12	E (12)
Candotti *et al*[66]	2006	Ghana	Blood donors	Cross-sectional	100	E (87), A (10) and D (3)
Fujiwara *et al*[65]	2005	Benin	Blood donors	Cross-sectional	21	E (20) and A (1)
Mulders *et al*[64]	2004	West Africa[2]	Measles or HIV+	Multicenter	79	E (78), and A (1)
Suzuki *et al*[70]	2003	Côte d'Ivoire	HBV carriers	Cross-sectional	48	E (42), A (3) and D (3)

[1]Ghana 13 (E = 100%), Côte d'Ivoire 70 (E = 87%); [2]Benin 13 strains, Burkina Faso 11 with 1 case of HBV-A genotypes (BFA-S121), Mali 18 strains, 15 strains from Nigeria and Togo 22 strains. WAEMU: West African Economic and Monetary Union; HBV: Hepatitis B virus; HIV: Human immunodeficiency virus.

demonstrated that the majority of HBV-positive and patients co-infected with lamivudine 3TC resistance were infected with HBV genotype E. This review confirms the endemicity of HBV genotype E, with a prevalence of 90.6% (1468/1620, 95%CI: 0.891-0.920) and a predominance of serotype awy4. Indeed, some studies conducted in Ghana[60,61], Burkina Faso[62] and Mali[63] exclusively reported the HBV genotype E in

Table 2 Distribution of non-hepatitis B virus genotypes in West African Economic and Monetary Union countries, Ghana and Nigeria

Ref.	Year	Countries	Patients	Type of study	Samples	Others hepatitis genotypes (*n*)
Abubakar *et al*[93]	2017	Nigeria	HCV+	Prospective	173	HCV G1 (159) and G2 (14)
Ndiaye *et al*[94]	2015	Senegal	Drug users	Cohort study	25	HCV G1 (21), G2 (1), G3 (1) and G4 (2)
Henquell *et al*[95]	2016	Burkina Faso	woman	Case report	1	HCV G5 (1)
Opaleye *et al*[82]	2016	Nigeria	HBV+	Cross-sectional	14	HDV G1 (14)
De Paschale *et al*[12]	2014	Benin	Pregnant women	Prospective	6	HCV G1 (1), G2 (5)
Honge *et al*[76]	2014	Guinea Bissau	HIV+	Cross-sectional	8	HCV G2 (8)
Zeba *et al*[96]	2014	Burkina Faso	Blood donors	Cross-sectional	36	HCV G1 (4), G2 (22), G3 (8), G4 (2)
Forbi *et al*[56]	2013	Nigeria	Apparently healthy adult	Cross-sectional	12	HAV sub-G1A (12)
Diarra *et al*[97]	2013	Mali	Diabetic	Prospective	25	HCV G1 (7) and G2 (18)
Bouare *et al*[98]	2013	Mali	Old women	Prospective	14	HCV G1 (2) and G2 (12)
Forbi *et al*[47]	2012	Nigeria	Asymptomatic indigenes	Prospective	60	HCV G1 (51) and G2 (9)
Sombie *et al*[99]	2011	Burkina Faso	HCV+	Prospective	38	HCV G1 (10), G2 (27) and G5 (1)
Bengue *et al*[100]	2008	Côte d'Ivoire	Blood donors	Prospective	27	HCV G1 (21), G2 (5) and G5 (1)
Plamondon *et al*[101]	2007	Guinea Bissau	Adult volunteers	Cross-sectional	57	HCV G1 (1) and G2 (56)
Simpore *et al*[102]	2005	Burkina Faso	Pregnant women	Prospective	5	HCV G1 (2) and G2 (3)
Rouet *et al*[103]	2004	Côte d'Ivoire	HIV+/Pregnant women	Cross-sectional	6	HCV G1 (3) and G2 (3)
Agwale *et al*[104]	2004	Nigeria	HIV+ under ART	Prospective	12	HCV G1 (9) and G2 (3)
Candotti *et al*[45]	2003	Ghana	Blood donors	Cross-sectional	23	HCV G1 (3) and G2 (20)
Buisson *et al*[105]	2000	Nigeria	Acute hepatitis	Cross-sectional	7	HEV G3 (7)
Saito *et al*[79]	1999	Ghana	HIV+ and HIV-	Cross-sectional	9	HGV G1 (9)
Wansbrough-Jones *et al*[106]	1998	Ghana	Blood donors	Cross-sectional	7	HCV G1 (2) and G2 (5)
Oni *et al*[107]	1996	Nigeria	blood donors	Cross-sectional	5	HCV G1 (2) and G4 (3)

WAEMU: West African Economic and Monetary Union; HBV: Hepatitis B virus; HCV: Hepatitis C virus; HDV: Hepatitis D virus; HEV: Hepatitis E virus; HGV: Hepatitis G virus; HIV: Human immunodeficiency virus; ART: Antiretroviral treatment.

their study populations.

In addition to the presence of other genotypes, including HBV genotypes A and D, studies have reported a strong predominance of genotype E[64-66]. Similar observations have led several authors to support further the common presence of HBV genotype E in West African populations[24]. Indeed, the predominance and almost exclusive circulation of genotype E in sub-Saharan Africa certainly indicates its West African origin[67-69]. Its distribution is limited to West Africa, unlike other HBV genotypes, despite the migration of slaves from West Africa to North America[66]. This review also reports low genetic diversity of HBV genotype E in West Africa (Figure 2)[64,66]. The low genome diversity and large distribution of genotype E in West Africa suggests a recent introduction of this genotype in the human host[64,70]. It is possible that it has been introduced relatively recently into an animal reservoir (the chimpanzee) as well as for HIV or that variant of genotype D (the closest to genotype E) has acquired an evolutionary advantage[66]. HBV genotypes A and D were also reported in this review with respective prevalence of 7.8% (126/1620, 95%CI: 0.065-0.092) and 0.74% (12/1620, 95%CI: 0.004-0.013). HBV genotype A, which is also found in sub-Saharan Africa, has been reported in eight of the 10 countries included in this review. Indeed, genotype E is predominant in West Africa, while genotype A has a relatively high prevalence in East Africa[71].

In 2006, Candotti *et al*[66] reported a prevalence of 10% and 3%, respectively, for HBV genotypes A and D in blood donors in Ghana. Similar results have also highlighted the cocirculation of genotypes A and D in Ghana, Mali, Côte d'Ivoire and Nigeria[59]. The majority of genotypes A identified in Burkina Faso are quasi-A3 genotypes (A3Q) documented in West African populations[72]. Indeed, data from previous studies suggest a predominance of the A1 genotype in East Africa and the A3 genotype in West and Central Africa, while the A2 subgenotype has a high frequency in North Africa where the genotype D is predominant. Africa has a high diversity of HBV genotypes and subgenotypes displaying distinct geographical distributions. Genotype A is found mainly in south-eastern Africa, genotype E in western and central Africa and genotype D prevails in northern Africa. Genotype E is rarely found outside Africa, except in individuals of African descent.

Characterization of HBV genotypes allows clinicians to determine patients' response to treatment and potential risks of complications[58,73]. Flink *et al*[74] have indeed reported that genotype A responds better to interferon alpha and pegIFNα than genotype D. Genotype recombination occurs in areas where multiple genotypes are in co-circulation, thus facilitating diversification between individuals within the general population. Our review reports a recombination prevalence of HBV genotype E with genotypes A and D of 0.87% (14/1599, 95%CI: 0.005-0.015). A/B, A/C, A/E, C/E, D/E and D/E/A recombination have been reported in West Africa[58]. Recombination requires co-infection with more than one genotype in the same patient. Appropriate treatment and elimination of risky

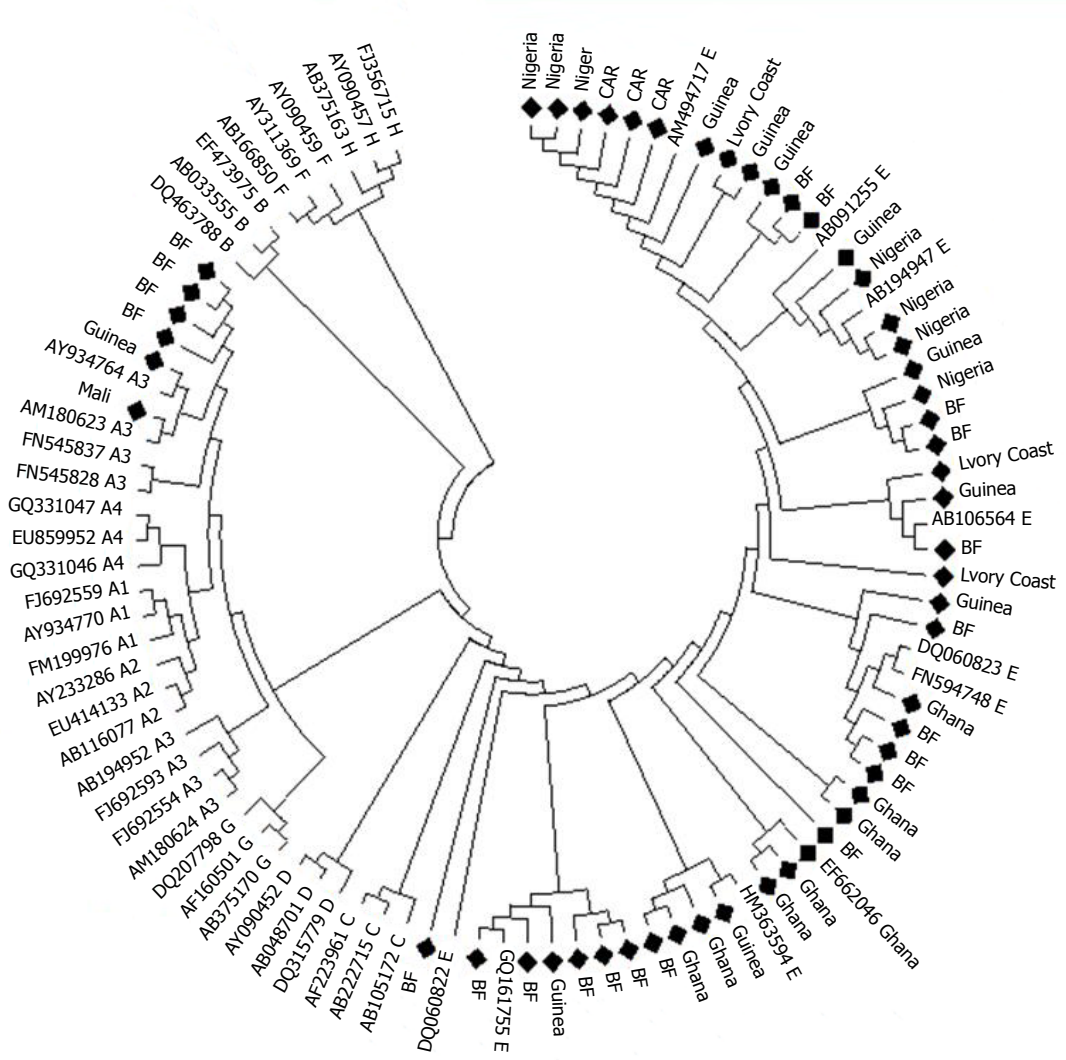

Figure 2 Phylogenetic tree of 53 hepatitis B virus genotype E and A sequences identified in West African Economic and Monetary Union countries including Ghana and Nigeria (indicated ♦). Phylogenetic analysis was performed with the neighbor-joining algorithm based on the Kimura two-parameter distance estimation method. Only bootstrap values of > 80% are shown (1.000 replicates). Reference hepatitis B virus sequences (44) recovered from GenBank are denoted with their accession numbers and genotypes/sub-genotypes are indicated.

behavior in people infected with the virus are therefore necessary for a considerable reduction in the spread of recombinant viruses.

Genotypes of HCV and other hepatitis viruses

The data in this review report five circulating HCV genotypes in the WAEMU countries, Ghana and Nigeria, with an overall predominance of genotype 1 (56.4%, 298/528). The prevalence of HCV genotype 1 has been reported by several authors in Nigeria, Senegal and Côte d'Ivoire, who record the most data presented in this review. Genotype 2 with a general frequency of 40.0% (211/528) was the main genotype found in Benin, Burkina Faso, Ghana, Guinea-Bissau and Mali (Figure 2). Indeed, several previous studies have reported a predominance of HCV genotype 2 in West Africa[75]. Most authors suggest a West African genotype 2 origin of HCV in the region, including The Gambia and Guinea-Bissau[46]. Indeed, HCV genotyping data in Guinea-Bissau report an almost exclusive predominance of genotype 2[76]. Candotti *et al*[45] reported 87.0% of genotype 2 was associated with chronic HCV infection with 13% of cases for genotype 1. In a study in Ghana in HCV/HIV coinfected patients, HCV sequences were phylogenetically assigned genotype 2 and subtypes 21 and 2r[77]. Although no published data on HCV genotypes were found in Togo, genotypes 2 and 1 were the most frequently isolated, with respective prevalence 73.2% and 17.1%, in a study conducted in 2014 in the Togolese general population.

Genotype 2 is, therefore, predominant in Togo, as it is in most parts of West Africa (unpublished data). In Martinique, where three quarters of the slaves sent in the 17th and 18th centuries came from West Africa, there is a great diversity of genotype 2[78]. The majority of molecular and epidemiological studies suggest that HCV genotype 2 has been present in West Africa for several centuries.

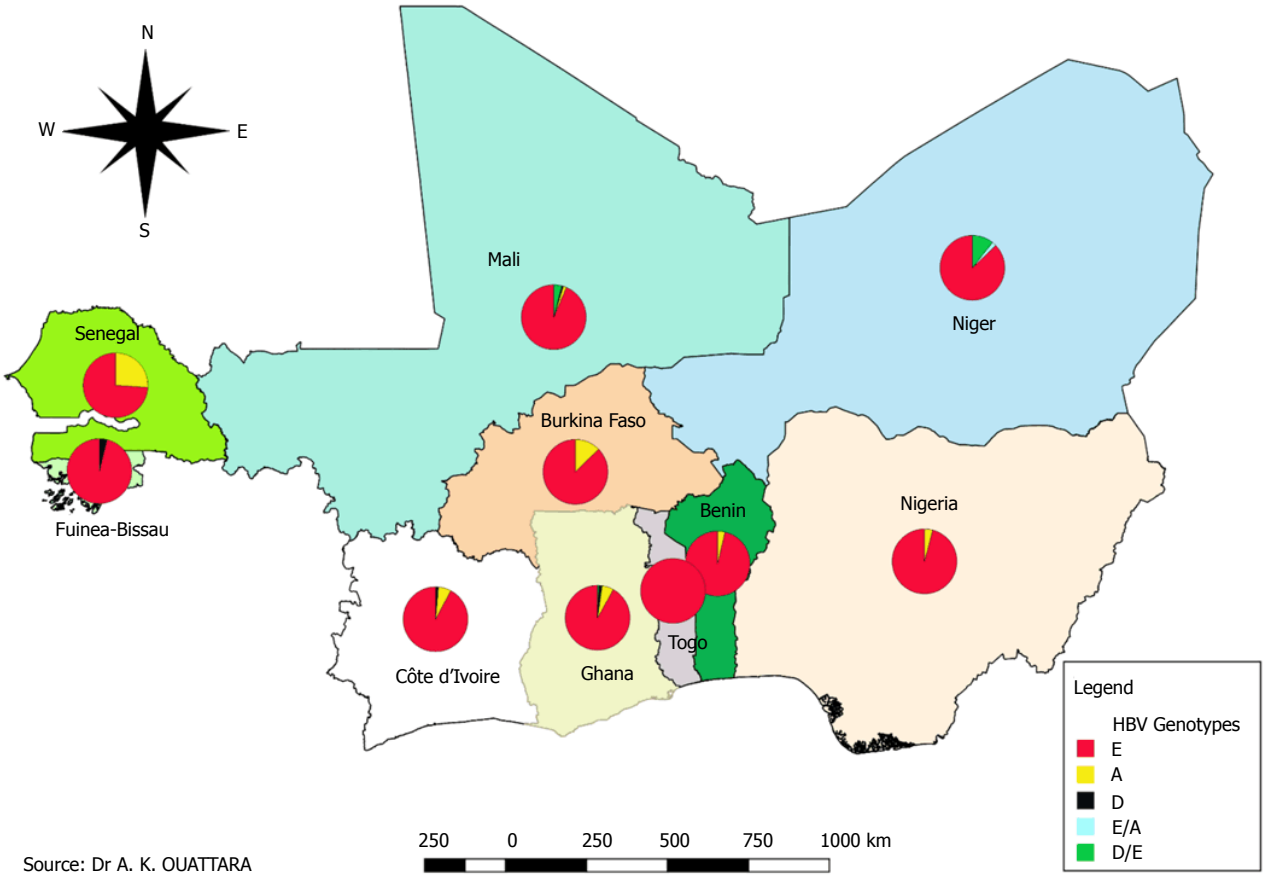

Figure 3 Hepatitis B virus genotypes reported in West African Economic and Monetary Union countries, Ghana and Nigeria. Pie charts show the proportion of different hepatitis B virus genotypes in West African countries according to the data in Table 1.

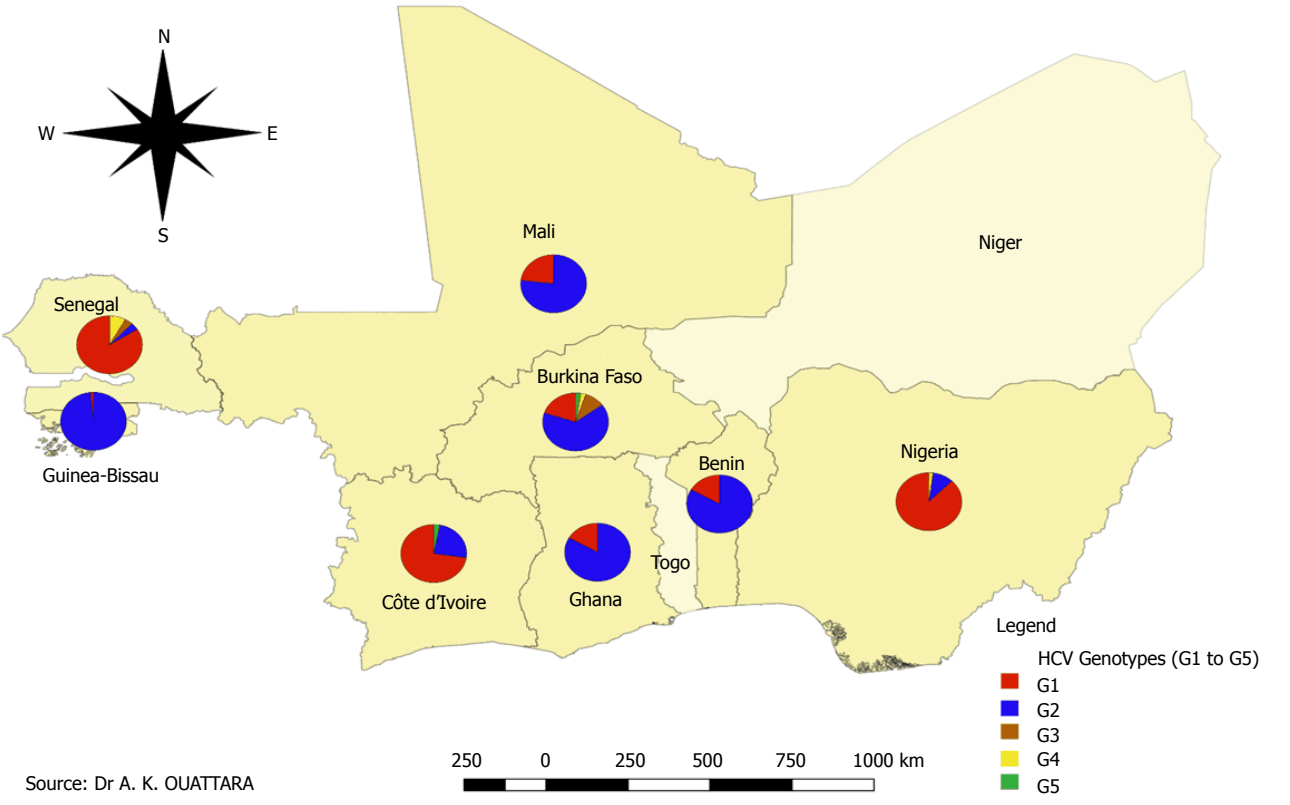

Figure 4 Hepatitis C virus genotypes reported in West African Economic and Monetary Union countries, Ghana and Nigeria. Pie charts show the proportion of different hepatitis B virus genotypes in West African countries according to the data in Table 2.

Data on the genotypes of other hepatitis viruses that are very infrequent or with relatively high frequencies in some areas have also been reported in this review. HAV genotype 1 and HEV and HDV were reported in Nigeria, while genotype 1 of HGV was found in Ghana. HAV, whose transmission is closely associated with lack of clean drinking water, unsuitable food, inadequate sanitation and poor personal hygiene, is prevalent in parts of Nigeria (World Health Organization). HDV is a satellite virus of HBV, because HDV only infects people with HBV. Limited data is available on circulating HDV genotypes. In a study in Togo, it was reported that 94.3% of the general population was infected with genotype 1, and 5.7% was infected with genotype 5. Studies on HGV are very limited[57,79]. The analysis of the 5% untranslated region nucleotide sequence of the genome of the HGV shows that the nine Ghanaian isolates of the HGV belong to genotype 1, the West-African type of the HGV[79].

CONCLUSION

The complexity of hepatitis virus genotypes often leads to a specificity of treatment associated with the genotype. The present review reporting a mapping of genotypes of hepatitis viruses A, B, C, D, E and G in the WAEMU, Ghana and Nigeria, reveals that the majority of studies conducted in Ghana and Nigeria have very little information on hepatitis D and G. In the WAEMU area including Ghana and Nigeria, HBV strains were classified as genotypes E, A, D with a predominance of genotype E and serotype ayw4. Genotype E is characterized by a high prevalence, low genetic diversity and wide geographical distribution.

The majority of HCV genotype data came from Nigeria, Senegal and Côte d'Ivoire were characterized by a predominance of genotype 1, while a high prevalence of genotype 2 was found in Benin, Burkina Faso, Ghana, in Guinea-Bissau and Mali. Further studies on the clinical implications of HBV genotype E are needed for the development of an effective treatment for HBV in West Africa. Monitoring the distribution of the different genotypes is also needed to reduce recombination levels and prevent the emergence of other viral strains. There is a diversity of genotypes and subtypes of hepatitis viruses with risks of recombination and emergence of even more virulent forms. Hepatitis viruses do not need a passport or visa to move from one country to another, and they have preceded us in WAEMU or ECOWAS. It is therefore appropriate for us to develop the adequate means to prevent, treat and even eradicate these viral infections using a vaccine covering all variants.

REFERENCES

1 **World Health Organization**. Global Hepatitis Report, 2017. Available from: URL: http://www.who.int/hepatitis/publications/global-hepatitis-report2017/en/

2 **Halasz R**, Weiland O, Sällberg M. GB virus C/hepatitis G virus. *Scand J Infect Dis* 2001; **33**: 572-580 [PMID: 11525349 DOI: 10.1080/00365540110027123]

3 **Blackard JT**, Ma G, Polen C, DuBois JC, Gast J, Radens CM, Sterling RK, Sherman KE. Recombination among GB virus C (GBV-C) isolates in the United States. *J Gen Virol* 2016; **97**: 1537-1544 [PMID: 27072634 DOI: 10.1099/jgv.0.000477]

4 **Tao I**, Bisseye C, Nagalo BM, Sanou M, Kiba A, Surat G, Compaoré TR, Traoré L, Nikiema JB, Pietra V, Zongo JD, Simpore J. Screening of Hepatitis G and Epstein-Barr Viruses Among Voluntary non Remunerated Blood Donors (VNRBD) in Burkina Faso, West Africa. *Mediterr J Hematol Infect Dis* 2013; **5**: e2013053 [PMID: 24106603 DOI: 10.4084/MJHID.2013.053]

5 **World Health Organization**. Global health sector strategy on viral hepatitis 2016-2021. Available from: URL: http://www.who.int/hepatitis/strategy2016-2021/ghss-hep/en/

6 **Livinec B**. Estimating the burden of hepatitis. *Lancet* 2016; **388**: 2738-2739 [PMID: 27924771 DOI: 10.1016/S0140-6736(16)31649-X]

7 **Stanaway JD**, Flaxman AD, Naghavi M, Fitzmaurice C, Vos T, Abubakar I, Abu-Raddad LJ, Assadi R, Bhala N, Cowie B, Forouzanfour MH, Groeger J, Hanafiah KM, Jacobsen KH, James SL, MacLachlan J, Malekzadeh R, Martin NK, Mokdad AA, Mokdad AH, Murray CJL, Plass D, Rana S, Rein DB, Richardus JH, Sanabria J, Saylan M, Shahraz S, So S, Vlassov VV, Weiderpass E, Wiersma ST, Younis M, Yu C, El Sayed Zaki M, Cooke GS. The global burden of viral hepatitis from 1990 to 2013: findings from the Global Burden of Disease Study 2013. *Lancet* 2016; **388**: 1081-1088 [PMID: 27394647 DOI: 10.1016/S0140-6736(16)30579-7]

8 **Alter MJ**. Epidemiology of hepatitis B in Europe and worldwide. *J Hepatol* 2003; **39** Suppl 1: S64-S69 [PMID: 14708680 DOI: 10.1016/S0168-8278(03)00141-7]

9 **Perz JF**, Armstrong GL, Farrington LA, Hutin YJ, Bell BP. The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide. *J Hepatol* 2006; **45**: 529-538 [PMID: 16879891 DOI: 10.1016/j.jhep.2006.05.013]

10 **World Health Organization**. Principaux repères sur l'hépatite C. Available from: URL: http://www.who.int/fr/news-room/fact-sheets/detail/hepatitis-c

11 **Alfaiate D**, Dény P, Durantel D. Hepatitis delta virus: From biological and medical aspects to current and investigational therapeutic options. *Antiviral Res* 2015; **122**: 112-129 [PMID: 26275800 DOI: 10.1016/j.antiviral.2015.08.009]

12 **De Paschale M**, Ceriani C, Cerulli T, Cagnin D, Cavallari S, Ndayaké J, Zaongo D, Priuli G, Viganò P, Clerici P. Prevalence of HBV, HDV, HCV, and HIV infection during pregnancy in northern Benin. *J Med Virol* 2014; **86**: 1281-1287 [PMID: 24777580 DOI: 10.1002/jmv.23951]

13 **Rajoriya N**, Combet C, Zoulim F, Janssen HLA. How viral genetic variants and genotypes influence disease and treatment outcome of chronic hepatitis B. Time for an individualised approach? *J Hepatol* 2017; **67**: 1281-1297 [PMID: 28736138 DOI: 10.1016/j.jhep.2017.07.011]

14 **Messina JP**, Humphreys I, Flaxman A, Brown A, Cooke GS, Pybus OG, Barnes E. Global distribution and prevalence of hepatitis C virus genotypes. *Hepatology* 2015; **61**: 77-87 [PMID: 25069599 DOI: 10.1002/hep.27259]

15 **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]

16 **Romeo R**, Petruzziello A, Pecheur EI, Facchetti F, Perbellini R, Galmozzi E, Khan NU, Di Capua L, Sabatino R, Botti G, Loquercio G. Hepatitis delta virus and hepatocellular carcinoma: an update. *Epidemiol Infect* 2018; **146**: 1612-1618 [PMID: 29991359 DOI: 10.1017/S0950268818001942]

17 **Petruzziello A**. Epidemiology of Hepatitis B Virus (HBV) and

Hepatitis C Virus (HCV) Related Hepatocellular Carcinoma. *Open Virol J* 2018; **12**: 26-32 [PMID: 29541276 DOI: 10.2174/1874357901812010026]
18 **Sunbul M**. Hepatitis B virus genotypes: global distribution and clinical importance. *World J Gastroenterol* 2014; **20**: 5427-5434 [PMID: 24833873 DOI: 10.3748/wjg.v20.i18.5427]
19 **Petruzziello A**, Marigliano S, Loquercio G, Coppola N, Piccirillo M, Leongito M, Azzaro R, Izzo F, Botti GJIA, Cancer. Hepatitis C Virus (HCV) genotypes distribution among hepatocellular carcinoma patients in Southern Italy: a three year retrospective study. *Infect Agents Cancer* 2017; **12**: 52 [DOI: 10.1186/s13027-017-0162-5]
20 **Schietroma I**, Scheri GC, Pinacchio C, Statzu M, Petruzziello A, Vullo V. Hepatitis C Virus and Hepatocellular Carcinoma: Pathogenetic Mechanisms and Impact of Direct-Acting Antivirals. *Open Virol J* 2018; **12**: 16-25 [PMID: 29541275 DOI: 10.2174/1874357901812010016]
21 **Shi YH**. Correlation between hepatitis B virus genotypes and clinical outcomes. *Jpn J Infect Dis* 2012; **65**: 476-482 [PMID: 23183198 DOI: 10.7883/yoken.65.476]
22 **Tanwar S**, Dusheiko G. Is there any value to hepatitis B virus genotype analysis? *Curr Gastroenterol Rep* 2012; **14**: 37-46 [PMID: 22105466 DOI: 10.1007/s11894-011-0233-5]
23 **Lazar C**, Macovei A, Petrescu S, Branza-Nichita N. Activation of ERAD pathway by human hepatitis B virus modulates viral and subviral particle production. *PLoS One* 2012; **7**: e34169 [PMID: 22461906 DOI: 10.1371/journal.pone.0034169]
24 **Norder H**, Couroucé AM, Coursaget P, Echevarria JM, Lee SD, Mushahwar IK, Robertson BH, Locarnini S, Magnius LO. Genetic diversity of hepatitis B virus strains derived worldwide: genotypes, subgenotypes, and HBsAg subtypes. *Intervirology* 2004; **47**: 289-309 [PMID: 15564741 DOI: 10.1159/000080872]
25 **Schaefer S**. Hepatitis B virus: significance of genotypes. *J Viral Hepat* 2005; **12**: 111-124 [PMID: 15720525 DOI: 10.1111/j.1365-2893.2005.00584.x]
26 **Tatematsu K**, Tanaka Y, Kurbanov F, Sugauchi F, Mano S, Maeshiro T, Nakayoshi T, Wakuta M, Miyakawa Y, Mizokami M. A genetic variant of hepatitis B virus divergent from known human and ape genotypes isolated from a Japanese patient and provisionally assigned to new genotype J. *J Virol* 2009; **83**: 10538-10547 [PMID: 19640977 DOI: 10.1128/JVI.00462-09]
27 **Schaefer S**. Hepatitis B virus taxonomy and hepatitis B virus genotypes. *World J Gastroenterol* 2007; **13**: 14-21 [PMID: 17206751 DOI: 10.3748/wjg.v13.i1.14]
28 **Kurbanov F**, Tanaka Y, Mizokami M. Geographical and genetic diversity of the human hepatitis B virus. *Hepatol Res* 2010; **40**: 14-30 [PMID: 20156297 DOI: 10.1111/j.1872-034X.2009.00601.x]
29 **Kramvis A**, Kew MC. Epidemiology of hepatitis B virus in Africa, its genotypes and clinical associations of genotypes. *Hepatol Res* 2007; **37**: S9-S19 [PMID: 17627641 DOI: 10.1111/j.1872-034X.2007.00098.x]
30 **Banerjee A**, Kurbanov F, Datta S, Chandra PK, Tanaka Y, Mizokami M, Chakravarty R. Phylogenetic relatedness and genetic diversity of hepatitis B virus isolates in Eastern India. *J Med Virol* 2006; **78**: 1164-1174 [PMID: 16847957 DOI: 10.1002/jmv.20677]
31 **Meldal BH**, Moula NM, Barnes IH, Boukef K, Allain JP. A novel hepatitis B virus subgenotype, D7, in Tunisian blood donors. *J Gen Virol* 2009; **90**: 1622-1628 [PMID: 19339480 DOI: 10.1099/vir.0.009738-0]
32 **Abdou Chekaraou M**, Brichler S, Mansour W, Le Gal F, Garba A, Dény P, Gordien E. A novel hepatitis B virus (HBV) subgenotype D (D8) strain, resulting from recombination between genotypes D and E, is circulating in Niger along with HBV/E strains. *J Gen Virol* 2010; **91**: 1609-1620 [PMID: 20147517 DOI: 10.1099/vir.0.018127-0]
33 **World Health Organization**. Guidelines for the prevention, care and treatment of persons with chronic hepatitis B infection. Available from: URL: http://www.who.int/hiv/pub/hepatitis/hepatitis-b-guidelines/en/
34 **Zoulim F**. Hepatitis B virus resistance to antiviral drugs: where are we going? *Liver Int* 2011; **31** Suppl 1: 111-116 [PMID: 21205147 DOI: 10.1111/j.1478-3231.2010.02399.x]
35 **Lundin M**, Monné M, Widell A, Von Heijne G, Persson MA. Topology of the membrane-associated hepatitis C virus protein NS4B. *J Virol* 2003; **77**: 5428-5438 [PMID: 12692244 DOI: 10.1128/JVI.77.9.5428-5438.2003]
36 **Navas MC**, Fuchs A, Schvoerer E, Bohbot A, Aubertin AM, Stoll-Keller F. Dendritic cell susceptibility to hepatitis C virus genotype 1 infection. *J Med Virol* 2002; **67**: 152-161 [PMID: 11992576 DOI: 10.1002/jmv.2204]
37 **Murray KF**, Richardson LP, Morishima C, Owens JW, Gretch DR. Prevalence of hepatitis C virus infection and risk factors in an incarcerated juvenile population: a pilot study. *Pediatrics* 2003; **111**: 153-157 [PMID: 12509569 DOI: 10.1542/peds.111.1.153]
38 **Smith DB**, Bukh J, Kuiken C, Muerhoff AS, Rice CM, Stapleton JT, Simmonds P. Expanded classification of hepatitis C virus into 7 genotypes and 67 subtypes: updated criteria and genotype assignment web resource. *Hepatology* 2014; **59**: 318-327 [PMID: 24115039 DOI: 10.1002/hep.26744]
39 **Ramia S**, Eid-Fares J. Distribution of hepatitis C virus genotypes in the Middle East. *Int J Infect Dis* 2006; **10**: 272-277 [PMID: 16564719 DOI: 10.1016/j.ijid.2005.07.008]
40 **Pybus OG**, Barnes E, Taggart R, Lemey P, Markov PV, Rasachak B, Syhavong B, Phetsouvanah R, Sheridan I, Humphreys IS, Lu L, Newton PN, Klenerman P. Genetic history of hepatitis C virus in East Asia. *J Virol* 2009; **83**: 1071-1082 [PMID: 18971279 DOI: 10.1128/JVI.01501-08]
41 **Prabdial-Sing N**, Puren AJ, Bowyer SM. Sequence-based in silico analysis of well studied hepatitis C virus epitopes and their variants in other genotypes (particularly genotype 5a) against South African human leukocyte antigen backgrounds. *BMC Immunol* 2012; **13**: 67 [PMID: 23227878 DOI: 10.1186/1471-2172-13-67]
42 **Ndong-Atome GR**, Makuwa M, Ouwe-Missi-Oukem-Boyer O, Pybus OG, Branger M, Le Hello S, Boye-Cheik SB, Brun-Vezinet F, Kazanji M, Roques P, Bisser S. High prevalence of hepatitis C virus infection and predominance of genotype 4 in rural Gabon. *J Med Virol* 2008; **80**: 1581-1587 [PMID: 18649323 DOI: 10.1002/jmv.21252]
43 **Cantaloube JF**, Gallian P, Laperche S, Elghouzzi MH, Piquet Y, Bouchardeau F, Jordier F, Biagini P, Attoui H, de Micco P. Molecular characterization of genotype 2 and 4 hepatitis C virus isolates in French blood donors. *J Med Virol* 2008; **80**: 1732-1739 [PMID: 18712846 DOI: 10.1002/jmv.21285]
44 **Antaki N**, Craxi A, Kamal S, Moucari R, Van der Merwe S, Haffar S, Gadano A, Zein N, Lai CL, Pawlotsky JM, Heathcote EJ, Dusheiko G, Marcellin P. The neglected hepatitis C virus genotypes 4, 5 and 6: an international consensus report. *Liver Int* 2010; **30**: 342-355 [PMID: 20015149 DOI: 10.1111/j.1478-3231.2009.02188.x]
45 **Candotti D**, Temple J, Sarkodie F, Allain JP. Frequent recovery and broad genotype 2 diversity characterize hepatitis C virus infection in Ghana, West Africa. *J Virol* 2003; **77**: 7914-7923 [PMID: 12829831 DOI: 10.1128/JVI.77.14.7914-7923.2003]
46 **Markov PV**, Pepin J, Frost E, Deslandes S, Labbé AC, Pybus OG. Phylogeography and molecular epidemiology of hepatitis C virus genotype 2 in Africa. *J Gen Virol* 2009; **90**: 2086-2096 [PMID: 19474244 DOI: 10.1099/vir.0.011569-0]
47 **Forbi JC**, Purdy MA, Campo DS, Vaughan G, Dimitrova ZE, Ganova-Raeva LM, Xia GL, Khudyakov YE. Epidemic history of hepatitis C virus infection in two remote communities in Nigeria, West Africa. *J Gen Virol* 2012; **93**: 1410-1421 [PMID: 22456613 DOI: 10.1099/vir.0.042184-0]
48 NIH Consensus Statement on Management of Hepatitis C: 2002. *NIH Consens State Sci Statements* 2002; **19**: 1-46 [PMID: 14768714]
49 **Madejón A**, Romero M, Hernández Á, García-Sánchez A, Sánchez-Carrillo M, Olveira A, García-Samaniego J. Hepatitis B and D viruses replication interference: Influence of hepatitis B genotype. *World J Gastroenterol* 2016; **22**: 3165-3174 [PMID: 27003993 DOI: 10.3748/wjg.v22.i11.3165]

50 **Alvarado-Mora MV**, Locarnini S, Rizzetto M, Pinho JR. An update on HDV: virology, pathogenesis and treatment. *Antivir Ther* 2013; **18**: 541-548 [PMID: 23792471 DOI: 10.3851/IMP2598]

51 **Gomes-Gouvêa MS**, Soares MC, Bensabath G, de Carvalho-Mello IM, Brito EM, Souza OS, Queiroz AT, Carrilho FJ, Pinho JR. Hepatitis B virus and hepatitis delta virus genotypes in outbreaks of fulminant hepatitis (Labrea black fever) in the western Brazilian Amazon region. *J Gen Virol* 2009; **90**: 2638-2643 [PMID: 19605587 DOI: 10.1099/vir.0.013615-0]

52 **Dény P**. Hepatitis delta virus genetic variability: from genotypes I, II, III to eight major clades? *Curr Top Microbiol Immunol* 2006; **307**: 151-171 [PMID: 16903225 DOI: 10.1007/3-540-29802-9_8]

53 **Barros LM**, Gomes-Gouvêa MS, Pinho JR, Alvarado-Mora MV, Dos Santos A, Mendes-Corrêa MC, Caldas AJ, Sousa MT, Santos MD, Ferreira AS. Hepatitis Delta virus genotype 8 infection in Northeast Brazil: inheritance from African slaves? *Virus Res* 2011; **160**: 333-339 [PMID: 21798297 DOI: 10.1016/j.virusres.2011.07.006]

54 **Stapleton JT**, Foung S, Muerhoff AS, Bukh J, Simmonds P. The GB viruses: a review and proposed classification of GBV-A, GBV-C (HGV), and GBV-D in genus Pegivirus within the family Flaviviridae. *J Gen Virol* 2011; **92**: 233-246 [PMID: 21084497 DOI: 10.1099/vir.0.027490-0]

55 **Traoré F**, Gormally E, Villar S, Friesen MD, Groopman JD, Vernet G, Diallo S, Hainaut P, Maiga MY. Molecular characteristics of Hepatitis B and chronic liver disease in a cohort of HB carriers from Bamako, Mali. *BMC Infect Dis* 2015; **15**: 180 [PMID: 25886382 DOI: 10.1186/s12879-015-0916-x]

56 **Forbi JC**, Esona MD, Agwale SM. Molecular characterization of hepatitis A virus isolates from Nigeria. *Intervirology* 2013; **56**: 22-26 [PMID: 23052106 DOI: 10.1159/000341612]

57 **Tao I**, Compaoré TR, Diarra B, Djigma F, Zohoncon TM, Assih M, Ouermi D, Pietra V, Karou SD, Simpore J. Seroepidemiology of hepatitis B and C viruses in the general population of burkina faso. *Hepat Res Treat* 2014; **2014**: 781843 [PMID: 25161770 DOI: 10.1155/2014/781843]

58 **Boyce CL**, Ganova-Raeva L, Archampong TNA, Lartey M, Sagoe KW, Obo-Akwa A, Kenu E, Kwara A, Blackard JT. Identification and comparative analysis of hepatitis B virus genotype D/E recombinants in Africa. *Virus Genes* 2017; **53**: 538-547 [PMID: 28567562 DOI: 10.1007/s11262-017-1469-4]

59 **Archampong TN**, Boyce CL, Lartey M, Sagoe KW, Obo-Akwa A, Kenu E, Blackard JT, Kwara A. HBV genotypes and drug resistance mutations in antiretroviral treatment-naive and treatment-experienced HBV-HIV-coinfected patients. *Antivir Ther* 2017; **22**: 13-20 [PMID: 27167598 DOI: 10.3851/IMP3055]

60 **Huy TT**, Ishikawa K, Ampofo W, Izumi T, Nakajima A, Ansah J, Tetteh JO, Nii-Trebi N, Aidoo S, Ofori-Adjei D, Sata T, Ushijima H, Abe K. Characteristics of hepatitis B virus in Ghana: full length genome sequences indicate the endemicity of genotype E in West Africa. *J Med Virol* 2006; **78**: 178-184 [PMID: 16372296 DOI: 10.1002/jmv.20525]

61 **Ampah KA**, Pinho-Nascimento CA, Kerber S, Asare P, De-Graft D, Adu-Nti F, Paixão IC, Niel C, Yeboah-Manu D, Pluschke G, Röltgen K. Limited Genetic Diversity of Hepatitis B Virus in the General Population of the Offin River Valley in Ghana. *PLoS One* 2016; **11**: e0156864 [PMID: 27271290 DOI: 10.1371/journal.pone.0156864]

62 **Compaore TR**, Diarra B, Assih M, Obiri-Yeboah D, Soubeiga ST, Ouattara AK, Tchelougou D, Bisseye C, Bakouan DR, Compaore IP, Dembele A, Djigma WF, Simpore J. HBV/HIV co-infection and APOBEC3G polymorphisms in a population from Burkina Faso. *BMC Infect Dis* 2016; **16**: 336 [PMID: 27449138 DOI: 10.1186/s12879-016-1672-2]

63 **Cella E**, Ceccarelli G, Vita S, Lai A, Presti AL, Blasi A, Palco ML, Guarino MP, Zehender G, Angeletti S, Ciccozzi M; Sanitary Bureau of Asylum Seekers Center of Castelnuovo di Porto. First epidemiological and phylogenetic analysis of Hepatitis B virus infection in migrants from Mali. *J Med Virol* 2017; **89**: 639-646 [PMID: 27576107 DOI: 10.1002/jmv.24671]

64 **Mulders MN**, Venard V, Njayou M, Edorh AP, Bola Oyefolu AO, Kehinde MO, Muyembe Tamfum JJ, Nebie YK, Maiga I, Ammerlaan W, Fack F, Omilabu SA, Le Faou A, Muller CP. Low genetic diversity despite hyperendemicity of hepatitis B virus genotype E throughout West Africa. *J Infect Dis* 2004; **190**: 400-408 [PMID: 15216479 DOI: 10.1086/421502]

65 **Fujiwara K**, Tanaka Y, Orito E, Ohno T, Kato T, Sugihara K, Hasegawa I, Sakurai M, Ito K, Ozasa A, Sakamoto Y, Arita I, El-Gohary A, Benoit A, Ogoundele-Akplogan SI, Yoshihara N, Ueda R, Mizokami M. Distribution of HBV genotypes among HBV carriers in Benin:phylogenetic analysis and virological characteristics of HBV genotype E. *World J Gastroenterol* 2005; **11**: 6410-6415 [PMID: 16425408 DOI: 10.3748/wjg.v11.i41.6410]

66 **Candotti D**, Opare-Sem O, Rezvan H, Sarkodie F, Allain JP. Molecular and serological characterization of hepatitis B virus in deferred Ghanaian blood donors with and without elevated alanine aminotransferase. *J Viral Hepat* 2006; **13**: 715-724 [PMID: 17052270 DOI: 10.1111/j.1365-2893.2006.00741.x]

67 **Hübschen JM**, Andernach IE, Muller CP. Hepatitis B virus genotype E variability in Africa. *J Clin Virol* 2008; **43**: 376-380 [PMID: 18922739 DOI: 10.1016/j.jcv.2008.08.018]

68 **Andernach IE**, Nolte C, Pape JW, Muller CP. Slave trade and hepatitis B virus genotypes and subgenotypes in Haiti and Africa. *Emerg Infect Dis* 2009; **15**: 1222-1228 [PMID: 19751583 DOI: 10.3201/eid1508.081642]

69 **Forbi JC**, Vaughan G, Purdy MA, Campo DS, Xia GL, Ganova-Raeva LM, Ramachandran S, Thai H, Khudyakov YE. Epidemic history and evolutionary dynamics of hepatitis B virus infection in two remote communities in rural Nigeria. *PLoS One* 2010; **5**: e11615 [PMID: 20657838 DOI: 10.1371/journal.pone.0011615]

70 **Suzuki S**, Sugauchi F, Orito E, Kato H, Usuda S, Siransy L, Arita I, Sakamoto Y, Yoshihara N, El-Gohary A, Ueda R, Mizokami M. Distribution of hepatitis B virus (HBV) genotypes among HBV carriers in the Cote d'Ivoire: complete genome sequence and phylogenetic relatedness of HBV genotype E. *J Med Virol* 2003; **69**: 459-465 [PMID: 12601751 DOI: 10.1002/jmv.10331]

71 **Bekondi C**, Olinger CM, Boua N, Talarmin A, Venard V, Muller CP, Le Faou A. [Characterization of hepatitis B virus strains from the Central African Republic: preliminary results]. *Pathol Biol* (Paris) 2008; **56**: 310-313 [PMID: 18321662 DOI: 10.1016/j.patbio.2007.12.007]

72 **Olinger CM**, Venard V, Njayou M, Oyefolu AO, Maïga I, Kemp AJ, Omilabu SA, le Faou A, Muller CP. Phylogenetic analysis of the precore/core gene of hepatitis B virus genotypes E and A in West Africa: new subtypes, mixed infections and recombinations. *J Gen Virol* 2006; **87**: 1163-1173 [PMID: 16603517 DOI: 10.1099/vir.0.81614-0]

73 **Dongdem AZ**, Dzodzomenyo M, Asmah RH, Nyarko KM, Nortey P, Agyei A, Adjei DN, Kenu E, Adjei AA. Hepatitis B virus genotypes among chronic hepatitis B patients reporting at Korle-Bu teaching hospital, Accra, Ghana. *Pan Afr Med J* 2016; **25**: 5 [PMID: 28210373 DOI: 10.11604/pamj.supp.2016.25.1.6170]

74 **Flink HJ**, van Zonneveld M, Hansen BE, de Man RA, Schalm SW, Janssen HL; HBV 99-01 Study Group. Treatment with Peg-interferon alpha-2b for HBeAg-positive chronic hepatitis B: HBsAg loss is associated with HBV genotype. *Am J Gastroenterol* 2006; **101**: 297-303 [PMID: 16454834 DOI: 10.1111/j.1572-0241.2006.00418.x]

75 **Purdy MA**, Forbi JC, Sue A, Layden JE, Switzer WM, Opare-Sem OK, Phillips RO, Khudyakov YE. A re-evaluation of the origin of hepatitis C virus genotype 2 in West Africa. *J Gen Virol* 2015; **96**: 2157-2164 [PMID: 25888623 DOI: 10.1099/vir.0.000153]

76 **Hønge BL**, Jespersen S, Medina C, Té Dda S, da Silva ZJ, Lewin S, Østergaard L, Erikstrup C, Wejse C, Laursen AL, Krarup H; Bissau HIV cohort study group. Hepatitis B and Delta virus are prevalent but often subclinical co-infections among HIV infected patients in Guinea-Bissau, West Africa: a cross-sectional study. *PLoS One* 2014; **9**: e99971 [PMID: 24915064 DOI: 10.1371/journal.pone.0099971]

77 **Geretti AM**, King S, Adjei-Asante K, Appiah LT, Owusu DO, Sarfo FS, Chadwick D, Phillips RO, Beloukas A. Hepatitis C Virus (HCV) RNA screening and sequencing using dry plasma spots. *J Clin Virol* 2017; **97**: 18-21 [PMID: 29080433 DOI: 10.1016/j.jcv.2017.10.012]

78 **Martial J**, Morice Y, Abel S, Cabié A, Rat C, Lombard F, Edouard A, Pierre-Louis S, Garsaud P, Béra O, Chout R, Gordien E, Deny P, Césaire R. Hepatitis C virus (HCV) genotypes in the Caribbean island of Martinique: evidence for a large radiation of HCV-2 and for a recent introduction from Europe of HCV-4. *J Clin Microbiol* 2004; **42**: 784-791 [PMID: 14766854 DOI: 10.1128/JCM.42.2.784-791.2004]

79 **Saito T**, Ishikawa K-i, Osei-Kwasi M, Kaneko T, Brandful JA, Nuvor V, Aidoo S, Ampofo W, Apeagyei FA, Ansah JE, Adu-Sarkodie Y, Nkrumah FK, Abea K. Prevalence of hepatitis G virus and characterization of viral genome in Ghana. *Hepatol Res* 1999; **13**: 221-231 [DOI: 10.1016/S1386-6346(98)00095-3]

80 **Diarra B**, Yonli AT, Sorgho PA, Compaore TR, Ouattara AK, Zongo WA, Tao I, Traore L, Soubeiga ST, Djigma FW, Obiri-Yeboah D, Nagalo BM, Pietra V, Sanogo R, Simpore J. Occult Hepatitis B Virus Infection and Associated Genotypes among HBsAg-negative Subjects in Burkina Faso. *Mediterr J Hematol Infect Dis* 2018; **10**: e2018007 [PMID: 29326804 DOI: 10.4084/MJHID.2018.007]

81 **Lawson-Ananissoh L**, Attia K, Diallo D, Doffou S, Kissi Y, Bangoura D, Kouame D, Mahassadi K, Yao-Bathaix F, Yoman T. Distribution and Clinical Implications of the Genotypes of the Hepatitis B Virus in 33 Chronic Carriers of Hepatitis B Virus in Cote-d'Ivoire. *J Afr Hepato Gastroentero* 2017; **11**: 1-5 [DOI: 10.1007/s12157-017-0726-4]

82 **Opaleye OO**, Japhet OM, Adewumi OM, Omoruyi EC, Akanbi OA, Oluremi AS, Wang B, Tong Hv, Velavan TP, Bock CT. Molecular epidemiology of hepatitis D virus circulating in Southwestern Nigeria. *Virol J* 2016; **13**: 61 [PMID: 27044424 DOI: 10.1186/s12985-016-0514-6]

83 **Candotti D**, Diarra B, Bisseye C, Tao I, Pham Quang K, Sanou M, Laperche S, Sanogo R, Allain JP, Simpore J. Molecular characterization of hepatitis B virus in blood donors from Burkina Faso: Prevalence of quasi-subgenotype A3, genotype E, and mixed infections. *J Med Virol* 2016; **88**: 2145-2156 [PMID: 27253483 DOI: 10.1002/jmv.24589]

84 **Brah S**, Moussa S, Inoua A, Alhousseini DM, Daou M, Madougou B, Romera MH, Hamadou A, Adehossi E, Parola P, Colson P. Molecular characterization of hepatitis B virus from chronically-infected patients in Niamey, Niger. *Int J Infect Dis* 2016; **45**: 18-23 [PMID: 26899956 DOI: 10.1016/j.ijid.2016.02.009]

85 **Boyd A**, Maylin S, Moh R, Mahjoub N, Gabillard D, Eholié SP, Danel C, Anglaret X, Zoulim F, Girard PM, Delaugerre C, Lacombefor K; ANRS 12240 VarBVA study. Hepatitis B surface antigen quantification as a predictor of seroclearance during treatment in HIV-hepatitis B virus coinfected patients from Sub-Saharan Africa. *J Gastroenterol Hepatol* 2016; **31**: 634-644 [PMID: 26313291 DOI: 10.1111/jgh.13156]

86 **Faleye TO**, Adewumi MO, Ifeorah IM, Omoruyi EC, Bakarey SA, Akere A, Awokunle F, Ajibola HO, Makanjuola DO, Adeniji JA. Detection of hepatitis B virus isolates with mutations associated with immune escape mutants among pregnant women in Ibadan, southwestern Nigeria. *Springerplus* 2015; **4**: 43 [PMID: 25674500 DOI: 10.1186/s40064-015-0813-1]

87 **Faleye TO**, Adewumi OM, Ifeorah IM, Akere A, Bakarey AS, Omoruyi EC, Oketunde K, Awonusi OB, Ajayi MR, Adeniji JA. Detection and circulation of hepatitis B virus immune escape mutants among asymptomatic community dwellers in Ibadan, southwestern Nigeria. *Int J Infect Dis* 2015; **39**: 102-109 [PMID: 26283552 DOI: 10.1016/j.ijid.2015.08.008]

88 **Maylin S**, Sire JM, Mbaye PS, Simon F, Sarr A, Evra ML, Fall F, Daveiga J, Diallo A, Debonne JM, Chartier L, Vray M. Short-term spontaneous fluctuations of HBV DNA levels in a Senegalese population with chronic hepatitis B. *BMC Infect Dis* 2015; **15**: 154 [PMID: 25887383 DOI: 10.1186/s12879-015-0881-4]

89 **Hübschen JM**, Mbah PO, Forbi JC, Otegbayo JA, Olinger CM, Charpentier E, Muller CP. Detection of a new subgenotype of hepatitis B virus genotype A in Cameroon but not in neighbouring Nigeria. *Clin Microbiol Infect* 2011; **17**: 88-94 [PMID: 20219082 DOI: 10.1111/j.1469-0691.2010.03205.x]

90 **Geretti AM**, Patel M, Sarfo FS, Chadwick D, Verheyen J, Fraune M, Garcia A, Phillips RO. Detection of highly prevalent hepatitis B virus coinfection among HIV-seropositive persons in Ghana. *J Clin Microbiol* 2010; **48**: 3223-3230 [PMID: 20631103 DOI: 10.1128/JCM.02231-09]

91 **Candotti D**, Danso K, Allain JP. Maternofetal transmission of hepatitis B virus genotype E in Ghana, west Africa. *J Gen Virol* 2007; **88**: 2686-2695 [PMID: 17872520 DOI: 10.1099/vir.0.83102-0]

92 **Vray M**, Debonne JM, Sire JM, Tran N, Chevalier B, Plantier JC, Fall F, Vernet G, Simon F, Mb PS. Molecular epidemiology of hepatitis B virus in Dakar, Sénégal. *J Med Virol* 2006; **78**: 329-334 [PMID: 16419106 DOI: 10.1002/jmv.20544]

93 **Abubakar UM**, Yahaya M, Maishanu SH, Ibrahim I, Ishaq AR, Nnaemeka AM, Ahmad AS, Yahaya M. Molecular Epidemiology of HCV Genotype in Relation to Viral Load of Infected Individuals in Northwestern Nigeria. *GJMS* 2017 [DOI: 10.15580/GJMS.2017.2.021317025]

94 **Ndiaye O**, Gozlan J, Diop-Ndiaye H, Sall AS, Chapelain S, Leprêtre A, Maynart M, Gueye M, Lo G, Thiam M, Ba I, Lacombe K, Girard PM, Mboup S, Kane CT. Usefulness of Dried Blood Spots (DBS) to perform hepatitis C virus genotyping in drug users in Senegal. *J Med Virol* 2017; **89**: 484-488 [PMID: 26705258 DOI: 10.1002/jmv.24460]

95 **Henquell C**, Yameogo S, Sangaré L. First genome characterization of a novel hepatitis C virus genotype 5 variant. *Infect Genet Evol* 2016; **39**: 173-175 [PMID: 26807921 DOI: 10.1016/j.meegid.2016.01.016]

96 **Zeba MT**, Sanou M, Bisseye C, Kiba A, Nagalo BM, Djigma FW, Compaoré TR, Nebié YK, Kienou K, Sagna T, Pietra V, Moret R, Simporé J. Characterisation of hepatitis C virus genotype among blood donors at the regional blood transfusion centre of Ouagadougou, Burkina Faso. *Blood Transfus* 2014; **12** Suppl 1: s54-s57 [PMID: 24599906]

97 **Diarra M**, Konaté A, Diakité Y, Samaké KD, Coulibaly HS, Kassambra Y, Tounkara M, Kaya AS, Kallé A, Sidibé AT. Hepatitis C virus infection among diabetics in CHU Gabriel Touré and Bamako Center of diabetes control (Mali). *J Afr Hepato Gastroenterol* 2013: 188 [DOI: 10.1007/s12157-013-0487-7]

98 **Bouare N**, Gothot A, Delwaide J, Bontems S, Vaira D, Seidel L, Gerard P, Gerard C. Epidemiological profiles of human immunodeficiency virus and hepatitis C virus infections in Malian women: Risk factors and relevance of disparities. *World J Hepatol* 2013; **5**: 196-205 [PMID: 23671724 DOI: 10.4254/wjh.v5.i4.196]

99 **Sombie R**, Bougouma A, Somda S, Sangare L, Lompo O, Kabore Z, Tieno H, Drabo J, Ilboudo D. Chronic hepatitis C: epidemiology, diagnosis and treatment in Yalgado-Ouedraogo teaching hospital in Ouagadougou. *J Afr Hepato Gastroenterol* 2011; **1**: 6-13 [DOI: 10.1007/s12157-010-0213-7]

100 **Bengue AK-M**, Kouacou MJL, Ekaza E, Siransy-Bogui L, Nrsquo DC, Labonté P, Dosso M. Hepatitis C virus infection in Abidjan Cote d Ivoire: heterogeneity of genotypes. *Sci Res Essays* 2008: 139

101 **Plamondon M**, Labbé AC, Frost E, Deslandes S, Alves AC, Bastien N, Pepin J. Hepatitis C virus infection in Guinea-Bissau: a sexually transmitted genotype 2 with parenteral amplification? *PLoS One* 2007; **2**: e372 [PMID: 17440608 DOI: 10.1371/journal.pone.0000372]

102 **Simpore J**, Ilboudo D, Samandoulougou A, Guardo P, Castronovo P, Musumeci S. HCV and HIV co-infection in pregnant women attending St. Camille Medical Centre in Ouagadougou (Burkina Faso). *J Med Virol* 2005; **75**: 209-212 [PMID: 15602740 DOI: 10.1002/jmv.20258]

103 **Rouet F**, Chaix ML, Inwoley A, Msellati P, Viho I, Combe P, Leroy V, Dabis F, Rouzioux C; ANRS 1236 DITRAME-B&C Study

Group. HBV and HCV prevalence and viraemia in HIV-positive and HIV-negative pregnant women in Abidjan, Côte d'Ivoire: the ANRS 1236 study. *J Med Virol* 2004; **74**: 34-40 [PMID: 15258966 DOI: 10.1002/jmv.20143]

104 **Agwale SM**, Tanimoto L, Womack C, Odama L, Leung K, Duey D, Negedu-Momoh R, Audu I, Mohammed SB, Inyang U, Graham B, Ziermann R. Prevalence of HCV coinfection in HIV-infected individuals in Nigeria and characterization of HCV genotypes. *J Clin Virol* 2004; **31** Suppl 1: S3-S6 [PMID: 15567088 DOI: 10.1016/j.jcv.2004.09.001]

105 **Buisson Y**, Grandadam M, Nicand E, Cheval P, van Cuyck-Gandre H, Innis B, Rehel P, Coursaget P, Teyssou R, Tsarev S. Identification of a novel hepatitis E virus in Nigeria. *J Gen Virol* 2000; **81**: 903-909 [PMID: 10725415 DOI: 10.1099/0022-1317-81-4-903]

106 **Wansbrough-Jones MH**, Frimpong E, Cant B, Harris K, Evans MR, Teo CG. Prevalence and genotype of hepatitis C virus infection in pregnant women and blood donors in Ghana. *Trans R Soc Trop Med Hyg* 1998; **92**: 496-499 [PMID: 9861360 DOI: 10.1016/S0035-9203(98)90887-2]

107 **Oni AO**, Harrison TJ. Genotypes of hepatitis C virus in Nigeria. *J Med Virol* 1996; **49**: 178-186 [DOI: 10.1002/(SICI)1096-9071(199607)49:3<178::AID-JMV4>3.0.CO;2-1]

P- Reviewer: Arriagada GL, Chen CJ, Petruzziello A
S- Editor: Ji FF **L- Editor**: Filipodia **E- Editor**: Tan WW

World Journal of Hepatology

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

World J Hepatol 2018 November 27; 10(11): 822-836

DOI: 10.4254/wjh.v10.i11.822

ISSN 1948-5182 (online)

REVIEW

Bioengineered functional humanized livers: An emerging supportive modality to bridge the gap of organ transplantation for management of end-stage liver diseases

Sandeep Kumar Vishwakarma, Chandrakala Lakkireddy, Avinash Bardia, Syed Ameer Basha Paspala, Chaturvedula Tripura, Md Aejaz Habeeb, Aleem Ahmed Khan

Sandeep Kumar Vishwakarma, Chandrakala Lakkireddy, Avinash Bardia, Syed Ameer Basha Paspala, Md Aejaz Habeeb, Aleem Ahmed Khan, Central Laboratory for Stem Cell Research and Translational Medicine, Centre for Liver Research and Diagnostics, Deccan College of Medical Sciences, Kanchanbagh, Hyderabad 500058, Telangana, India

Sandeep Kumar Vishwakarma, Chandrakala Lakkireddy, Avinash Bardia, Syed Ameer Basha Paspala, Md Aejaz Habeeb, Aleem Ahmed Khan, Dr Habeebullah Life Sciences, Attapur, Hyderabad 500058, Telangana, India

Chaturvedula Tripura, CSIR-Centre for Cellular and Molecular Biology, Habsiguda, Hyderabad 500007, Telangana, India

ORCID number: Sandeep Kumar Vishwakarma (0000-0001-5731-8210); Chandrakala Lakkireddy (0000-0003-3994-6269); Avinash Bardia (0000-0002-0657-4588); Syed Ameer Basha Paspala (0000-0001-5780-274X); Chaturvedula Tripura (0000-0003-2766-0861); Md Aejaz Habeeb (0000-0003-1519-3186); Aleem Ahmed Khan (0000-0001-7075-9037).

Author contributions: Vishwakarma SK and Khan AA conceptualized the study; Vishwakarma SK, Lakkireddy C, Bardia A, Tripura C and Khan AA wrote the manuscript; Vishwakarma SK, Lakkireddy C and Bardia A performed literature survey; Tripura C, Paspala SAB, Habeeb MA and Khan AA gave his basic and clinical inputs during manuscript draft preparation; Vishwakarma SK, Paspala SAB, Habeeb MA and Khan AA edited the manuscript; Vishwakarma SK and Lakkireddy C formatted the manuscript.

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

Manuscript source: Unsolicited manuscript

Correspondence to: Aleem Ahmed Khan, PhD, Associate Professor, Central Laboratory for Stem Cell Research and Translational Medicine, Centre for Liver Research and Diagnostics, Deccan College of Medical Sciences, Kanchanbagh, Hyderabad 500058, Telangana, India. aleem_a_khan@rediffmail.com
Telephone: +91-40-24342954
Fax: +91-40-24342954

Received: July 10, 2018
Peer-review started: July 10, 2018
First decision: August 20, 2018
Revised: August 24, 2018
Accepted: October 11, 2018
Article in press: October 11, 2018
Published online: November 27, 2018

Abstract

End stage liver diseases (ESLD) represent a major, neglected global public health crisis which requires an urgent action towards finding a proper cure. Orthotropic liver transplantation has been the only definitive treatment modality for ESLD. However, shortage of donor organs, timely unavailability, post-surgery related complications and financial burden on the patients limits the number of patients receiving the transplants. Since last two decades cell-based therapies have revolutionized the field of organ/tissue regeneration. However providing an alternative organ source to address the donor liver shortage still poses potential challenges. The developments made in this direction provide useful futuristic approaches, which could be translated into preclinical and clinical settings targeting appropriate app-

lications in specific disease conditions. Earlier studies have demonstrated the applicability of this particular approach to generate functional organ in rodent system by connecting them with portal and hepatic circulatory networks. However, such strategy requires very high level of surgical expertise and also poses the technical and financial questions towards its future applicability. Hence, alternative sites for generating secondary organs are being tested in several types of disease conditions. Among different sites, omentum has been proved to be more appropriate site for implanting several kinds of functional tissue constructs without eliciting much immunological response. Hence, omentum may be considered as better site for transplanting humanized bioengineered *ex vivo* generated livers, thereby creating a secondary organ at intra-omental site. However, the expertise for generating such bioengineered organs are limited and only very few centres are involved for investigating the potential use of such implants in clinical practice due to gap between the clinical transplant surgeons and basic scientists working on the concept evolution. Herein we discuss the recent advances and challenges to create functional secondary organs through intra-omental transplantation of *ex vivo* generated bioengineered humanized livers and their further application in the management of ESLD as a supportive bridge for organ transplantation.

Key words: Bioengineered liver; Omentum; Secondary organ; Transplantation; End stage liver diseases

Core tip: The concept of bioengineering functional humanized neo-organs relies on finding more appropriate immunologically tolerable transplantation site. We have experienced omentum as more appropriate ectopic site with excellent properties of angiogenesis, regeneration, fibrotic reconstruction, and immunological compatibility which together endorse vascularisation, promote tissue healing, and minimize rejection of foreign body. However, regeneration of liver tissue in omentum is still unknown. Despite the amazing breakthroughs in the bioengineered organs, there is much work left to do. The approach described herein harbours enormous potential to overcome the limitations of organ transplantation and may support failing liver through ectopic transplantation as secondary organ.

Vishwakarma SK, Lakkireddy C, Bardia A, Paspala SAB, Tripura C, Habeeb MA, Khan AA. Bioengineered functional humanized livers: An emerging supportive modality to bridge the gap of organ transplantation for management of end-stage liver diseases. *World J Hepatol* 2018; 10(11): 822-836 Available from: URL: http://www.wjgnet.com/1948-5182/full/v10/i11/822.htm DOI: http://dx.doi.org/10.4254/wjh.v10.i11.822

INTRODUCTION

End stage liver diseases (ESLD) have become the major reason for the increasing deaths worldwide. According to the World Health Organisation, the total deaths caused by cirrhosis and liver cancer have increased by 50 million/year since 1990[1]. Liver transplantation is the only standard treatment available so far. However, more than 20% patients die on the waiting list due to a shortage of organ donors[2]. In order to expand the supply of livers available for transplantation, transplant surgeons and physicians have explored several new approaches including split liver transplants, living-related partial donor procedures[3] and the increasing use of "marginal" organs such as older donors, steatotic livers, non-heart-beating donors, donors with viral hepatitis, and donors with non-metastatic malignancy[4]. Despite these medical and surgical developments, it is unlikely that the availability of good liver grafts will ever be sufficient to meet the increasing demand of patients with end stage liver disease.

In order to overcome these limitations, various other treatment options are being explored among which hepatocytes transplantation has been described as the first supportive modality in regenerative medicine. But major challenges with such treatment is its limited availability of therapeutic dose from surgical samples, liver grafts or biopsies and their maintenance *in vitro* which requires cell-to-cell and cell-to-matrix interactions for proper functioning of anchorage dependent hepatocytes[5]. Usage of hepatocytes from xenogenic sources such as rabbit, porcine or canine, pose the risk of immunogenicity and transmission of zoonosis. This limitation can be addressed to certain extent by the usage of cell lines which can be maintained for longer time with higher growth rates under *in vitro* culture conditions but modification of gene expression under culture conditions might lead to problems and has issues related to its clinical applicability[6].

The first land mark study to bring hepatocyte transplantation into clinics was by Mito *et al*[7] in cirrhotic patients. In line with this study, our centre has treated seven acute liver failure patients by intra-peritoneal transplantation of human primary hepatocytes extracted from human foetus's which showed clinical improvement and support to the failing liver[8]. Following this, various other studies have reported successful transplantation of primary hepatocytes in treating various metabolic diseases[9,10]. Although higher successful rate has been reported using hepatocyte transplantation, yet use of fetal hepatocytes poses major hurdle of ethical issues for its wider clinical applicability. Other potential treatment alternatives discovered in recent years included induced pluripotent stem cells, Mesenchymal stromal cells (MSC) which have the ability to differentiate into hepatocytes but still they couldn't completely mimic the fully functional hepatocytes pointing towards a need to identify better niche for functional utilization of these cells[11-14].

Other alternative of direct cellular transplantation includes the use of extracorporeal liver support devices which can support a failing liver for a short period of time before organ transplantation[15]. But all these above mentioned treatment strategies may not fulfil the requirements to treat ESLD and may not provide immediate support for a failing liver to maintain normal functions. Hence, there is a need to develop bioengineered transplantable liver grafts which can retain the natural three-dimensional extra cellular matrix (3D-ECM) components and intact vascular networks similar to the native liver with repopulated functional hepatocytes or human hepatic progenitor cells. Rapid progress in the area of stem cell research and organ bioengineering paved a way in generating alternatives to liver transplantation.

After addressing all these limitations next comes the question of choosing an exact transplantable site where in these bioengineered organs can be easily acceptable and can able to perform the function. Recently omentum has been discovered as a wonderful ectopic site for transplantation with excellent properties like remarkable angiogenic[16], stem cell[17,18], fibrotic[19], and immune activities[20], which together endorse vascularization, promote wound healing, and minimize infection. Several studies have already demonstrated the importance of intra-omental transplantation in diabetic animal models[21,22]. However, the regeneration of liver tissue in ectopic sites is still unknown. Few studies have shown the omentum as a reservoir for proliferating renal, pancreatic, splenic[23-25] cells and as a site for hepatocytes engraftment which can be used in tissue engineering[26]. Hence, opting omental transplantation of bioengineered liver may offer development of secondary liver for the treatment of ESLD. This particular approach should offer promising treatment strategy in future and may rule out above mentioned limitations to answer for shortage of organ donors for ESLD.

CURRENT STATE OF REGENERATIVE STRATEGIES IN ESLD

Since last two decades, significant developments have been made to overcome the limitations of liver transplantation in ESLD. Among these strategies cell transplantation, use of extra-corporeal devices and transplantable bioengineered organs have been explored extensively.

CELL TRANSPLANTATION

In cell transplantation strategies, hepatocytes transplantation has been the most preferred cell types for infusion into liver due to their ability to perform major liver specific functions. However, getting therapeutic dose of human hepatocytes represents major limitation towards its wider clinical application[27,28]. Although several studies have reported use of 10% liver tissues to isolate enough number of hepatocytes post-*in vitro* expansion which can provide required clinical response in both animal models and human[5,29]. The *in vitro* enrichment of hepatocytes is challenging due to their contact-dependent growth, long-term survival and function and maintenance of normal phenotype without de-differentiation[5,30]. Therefore alternative strategies are highly desirable to overcome these limitations.

Recent studies have reported use of embryonic and adult pluripotent stem cells to generate desired number of functional hepatocytes for therapeutic applications. However, use of embryonic stem cells (ESCs) represents ethical hurdles and immune incompatibility for the transplant recipients[31-33]. Moreover, use of induced pluripotent stem cells (iPSCs) has been reported for effective differentiation into functional hepatocytes, however poses potential issues related to genetic instability and lack of functional transplantation studies[11,12]. Mesenchymal stromal cells (MSCs) represents another alternative type of pluripotent cells to generate functional hepatocytes and support liver regeneration[13,14]. However, multi-lineage differentiation of MSCs represents major challenge to control the effective trans-differentiation into desired number of functional hepatocytes while restricting other default lineage cells. Although, stem cell transplantation strategies have showed potential in liver regeneration through various mechanisms, still it has not been considered as durable solution to completely support the lost liver functions[15]. Hence, alternative strategies are highly desirable to generate therapeutic number of functional heaptocytes under controlled conditions.

MAJOR SOURCES OF REGENERATIVE CELLS FOR THE TREATMENT OF ESLD

Liver-derived stem sells

These are the stem cells that are derived from adult or fetal livers. Adult stem cells are known as oval cells which play an important role in liver regeneration when replication capacity of hepatocytes is impaired[34]. Fetal liver stem cells are known as bipotent hepatoblasts that has ability to differentiate into bile duct cells or hepatocytes[35-37]. Fetal liver stem cells have been used to repopulate liver in animal models[38,39] and cultured hepatoblasts transplanted into immunodeficient mice showed greater *in vivo* engraftment and differentiation[40]. But the limitation in use of liver derived stem cells is their low number around 0.3% to 0.7% of oval cells in adult liver[41], whereas fetal liver mass comprises only 0.1% of hepatoblasts[42] and has associated ethical issues. Thus isolation and expansion of these cells and usage for transplantation is challenging.

Bone marrow-derived stem cells

Stem cells derived from bone marrow comprise hematopoietic and MSCs[43]. Among these, mesenchymal stem cells consists greater potential in liver

regeneration[44] with immunosuppressive and immunomodulatory properties[45]. But they always pose problem with low rates of liver repopulation[46] and have low trans-differentiation ability to hepatoblasts which limits to restore normal liver function[47].

Annex group of stem cells

Stem cells derived from human umbilical cord, human placental tissue, amniotic fluid and human umbilical cord blood constitutes Annex group of stem cells. These are pluripotent stem cells with higher proliferation and differentiation rates than adult stem cells[46,48,49] and are not known to cause teratomas or teratocarcinomas formation in humans. Di Campli *et al*[50] study on diabetic severe combined immunodeficient mice after acute toxic liver injury when treated with intraperitoneal administration of human umbilical cord stem cells showed rapid liver engraftment, differentiation into hepatocytes, improved liver regeneration, and reduced mortality rates[50].

IPSCs

These are similar to ESCs and the limitation of ethical issue can be overruled by *in vitro* generation of iPSCs from somatic cells avoiding the usage of embryonic tissue or oocytes[51]. However, the use of these cells in clinical practice is limited due to major hurdles with the genomic instability of these cells.

EXTRA-CORPOREAL LIVER SUPPORT SYSTEMS

Extracorporeal liver support devices have been designed with a goal to carry out normal liver function in patients with end-stage chronic, acute-on-chronic and acute liver failure for a short period of time until donor organ gets available. Two types of liver support systems have been designed: (1) Non-biological; and (2) Bio-artificial liver support devices.

Non-biological liver support devices

Designed to filter and adsorb accumulated toxins that are not cleared by non-functional liver[52]. Three major types of such devices have been explored as follows:

Molecular adsorbent recirculating system: Molecular adsorbent recirculating system (MARS) has been well explored device which is a hollow fiber membrane hemodialyzer which removes soluble and protein-bound substances against albumin-rich dialysate. This device was approved by FDA in 2012 for the treatment of hepatic encephalopathy. However, the major limitation of such devices represents: (1) Short-term detoxification function; (2) Chance of getting sepsis; (3) Cost issues; (4) Can remove only albumin-bound toxins or drugs which are excreted in circulation; (5) Safety and efficacy of MARS has not been demonstrated in controlled, randomized trials; and (6) The effectiveness of MARS in patients that are sedative could not be established in clinical studies and therefore can't be predicted in sedated patients.

Promethus fractionated plasma separator and adsorption system: Other type of devices includes, promethus fractionated plasma separator and adsorption system (FPSA) which is an artificial device which removes both albumin-bound and water soluble toxins from blood more effectively than MARS. However, its wide applicability has been limited due to following reasons: (1) Direct contact between fractionated plasma and the Prometh anion exchanger causes significant adsorption of procoagulant and anti-coagulant factors, associated with clinically relevant adverse events; (2) Broad disturbances of the coagulation system have been confirmed in FPSA treated liver failure patients; and (3) An *ex vivo* recirculation model demonstrated nonspecific adsorption of coagulation factors protein S and protein C on the anion exchange cartridge.

Single-pass albumin dialysis: Moreover, to overcome on the limitations of above mentioned extracorporeal liver assist device, single-pass albumin dialysis (SPAD) system was evolved which functions as one-pass dialysis against albumin solution to remove albumin-bound toxins and water-soluble substances. Detoxification system in SPAD is similar to or greater than MARS and is less expensive than MARS and FPSA. However, again the suitability and wide clinical applicability of SPAD is limited due to following limitations: (1) Only albumin bound or water soluble toxins can be removed; (2) Lipid soluble toxins can't be removed by SPAD; (3) Bleeding risk from acquired coagulopathy; (4) Albumin solution is discarded after a single passage of membrane without being recycled; and (5) Absence of clinical data.

Bioartificial liver support systems

These are the bioreactors containing viable hepatocytes in a 3D network of hollow fibers. These are designed to achieve plasma perfusion and enhance the activities of living liver cells. Conversely, the membranes separating cells from plasma are not capable of achieving enough *in vivo* perfusion rates, and lack sources of safe, reliable, strongly proliferating and functionally active human cells. Still following major challenges remain to resolve: (1) Bio-artificial livers should be able to provide at least 10% of liver functioning; (2) Very difficult acquiring this many hepatocyte cells; (3) Controversy over the use of porcine cells due to possible transmission of infections; (4) Hepatocytes and plasma have very different physio-chemical properties; (5) Hepatocytes do not perform well when in contact with plasma; (6) Have a very high oxygen uptake rate; (7) Hepatocytes undergo a lot of stress inside of bio-artificial liver; (8) Any stress above 5 dyn/cm^2 renders cells useless; (9) Limited volume of the bioreactor;

(10) Maximum blood/plasma that can be safely drawn out of liver failure patient is one liter; (11) Difficult to achieve 10% of liver functioning within one liter; and (12) Makes Bio-artificial liver designing very difficult.

TRANSPLANTABLE BIOENGINEERED ORGANS

Owing to the hurdles in above mentioned devices, there is need to develop transplantable biological systems to provide: (1) Suitable three-dimensional organ architecture; (2) Organ specific intact vasculature for homogeneous supply of oxygen and nutrients; (3) Long-term cell survival and function within the natural organ specific niche; and (4) Metabolic, synthetic and detoxification functions similar to native liver.

MAJOR COMPONENTS OF HUMAN LIVER FOR BIOENGINEERING

Major components of human liver for bioengineering includes (1) Organ specific 3D-bioscaffolds; (2) Organ capsule; (3) Organ vasculature; (4) Cellular distribution in spatial anatomical organization of liver; (5) Biomolecules and growth factors for enhanced survival and function to transplanted cells; (6) Types of cells required for long-term support; and (7) Long-term functional response.

To provide these crucial components recently two major technological advancements have been made: (1) Organ bio-printing; and (2) Humanized neo-organ development.

Organ bio-printing

With the advancements in tissue engineering it is possible to construct complex parenchymal organ structures along with intact vascular network by 3D bio-printing[53]. 3D bio-printing is one of the prevalent examples of bioengineered organs in the science world today, and it is growing and advancing quickly. This jaw dropping technology is one of the hot topics in bioengineering. It still fascinates that we have the potential to build organs from the push of a button. 3D bio-printing is a form of tissue engineering which utilizes inkjet printers and builds the scaffolding of a particular organ, layer by layer[54]. These inkjet printers allow the use of multiple cell types for printing. Robbins *et al*[55] developed a metabolically active 3D hepatic tissue where they identified increased liver specific function lasting for up to 135 h, and compartment-specific organization, along with a primitive hepatocyte microanatomy of hepatic stellate cells and endothelial cells. Researchers have also build bone repair constructs by coating the 3D printed scaffold with stem cells, which can grow into tissues over time[54]. The mild conditions used for bio-printing and material sintering have allowed viable cells and active therapeutic proteins to be incorporated into the construct production process. Today, this particular technology has been emerged only for *ex vivo* and its application *in vivo* has not been experienced which needs to be validated further.

Humanized neo-organ development

The recent concept of bioengineering functional humanized neo-organs has given a hope towards finding permanent cure as an alternative support to the failing organ. This concept of artificial organs was first originated in the radiation field post-World War Ⅱ, and was executed in the first bone marrow transplant in the 1970s[56]. According the Llames S tissue engineering has three main constituents: The *ex vivo* expansion of cells, seeding of these expanded cells in three dimensional structures that mimic physiological conditions and grafting the prototype. The technology relies on the development of whole intact organ scaffolds through whole organ perfusion acellularization procedure which retains extra-cellular matrix and circulatory networks of the native organ post-acellularization[57]. This important phenomenon allows three-dimensional intact acellular organ specific scaffold for efficient repopulation of desired cell population further to generate functional neo-organ system.

With advancement in regenerative medicine it has been possible to create bioengineered functional tissues or organs that can be used clinically[58,59]. So far several successful studies have been published in generating various organs and tissues based on these acellularization and stem cell repopulation[59-61] that can be used for treating patients. Significant progress in generating several types of complex organ biological scaffolds has led to development of an efficient acellularization protocols for whole organs through perfusion based techniques[62-66] (Tables 1 and 2). These acellularized whole organs combined with an efficient recellularization process[67-70] have made it possible to use these bioengineered organs for *in vivo* preclinical studies in small animal models[71-73].

Our centre has well expertise in generating various types of acellularized whole organ bioscaffolds including xenogeneic liver through detergent-based perfusion. So far, we have successfully generated acellularized and repopulated humanized whole liver and demonstrated its applicability as better natural 3D-drug testing model system[74]. Apart from liver, we have also generated acellularized kidney[75], heart[76], spleen, meninges, and many more. Still various other studies are in pipeline in generating humanized bioengineered organs from our centre.

WHOLE LIVER BIOENGINEERING

Highly specialized thick and complex organs like liver can be subjected to acellularization technology to obtain intact 3D-ECM. Due to delayed co-morbidity beyond marginal criteria or because of delayed ischemic time,

Table 1 Method adopted for whole complex organ acellularization techniques for different organisms

Organ	Acellularization agent	Perfusion method	Animal model	Reference
Heart	SDS, PEG, Triton X-100, and enzyme-based protocols deoxycholic acid	Antegrade coronary perfusion	Rat	[98]
	Trypsin, EDTA, NaN3, Triton X-100, and deoxycholic acid	Retrograde aortic perfusion	Pig	[65]
Lung	0.1% and 0.5% SDS	Antegrade pulmonary arterial perfusion	Rat	[63]
	CHAPS	Pulmonary artery and tracheal perfusion	Rat	[66]
	Triton X-100 and sodium deoxycholate	Right ventricle and tracheal perfusion	Mouse	[99]
Liver	Triton X-100 plus 0.1% SDS	Portal vein perfusion	Rat	[100]
	SDS		Rat	[70]
	1% Triton X-100 and 0.1% ammonium hydroxide		Mouse, rat, ferret, rabbit and pig	[69]
	0.25% and 0.5% SDS		Pig	[101]
	Sodium citrate + SDS + Triton-X-100	Hepatic artery perfusion	Rat	[74]
Kidney	0.5, 3, 6, 10% Triton X-100, 5 mM	Renal artery perfusion	Rat	[71]
	calcium chloride, 5 mM magnesium sulfate, 1 M sodium chloride, DNase, and 4% sodium deoxycholate		Rat	[72]
	3% Triton X-100, DNase, and 4% SDS			
	1% SDS and 1% Triton X-100			
	1% Triton X-100 and 0.1% ammonium hydroxide		Pig	[68]
	Heparin and antibiotic-containing physiological saline, 0.1-1.0% SDS, 0.1% Triton-X-100 and 0.0025% deoxyribonuclease 1		Goat	[75]

Table 2 Study outcome and major limitations of different types of acellularization techniques adopted for different types of whole organ scaffold development

Organ	Acellularization Method	Study out come	Limitation	References
Rat liver	Perfusion with detergents (SDS, Triton X-100)	Perfusion with SDS removes most of cells, damages the ECM when treated with Triton X-100 and removes 97 % of DNA	SDS damages the ECM	[69,74]
Porcine liver	Mechanical perfusion (electroporation)	Most of the cells are removed, preserves the blood vessels	Disruption of microfilament and microtubule	[102]
Mouse heart	Enzymatic, detergents, Acids	Cells are removed	Damages the ECM proteins, poorly maintains the 3D architecture	[103]
Porcine trachea	Enzymatic (trypsin) non-enzymatic (EDTA), detergent (Triton X-100) and deionized Water	Cells are removed, clear the cell debris	Disruption of glycosaminoglycan, reduce the laminin and fibronectin	[104]
Rat kidney	Perfuse with SDS, deionized water, dTriton X-100 and PBS along with antibiotics	Twice filtration is observed	Loss of cell-mediated functions like transport of solutes	[105]
Rat heart	Perfused with detergents		Long-term cell survival, oxygen tension and continuous rhythmic beating	[63,98]
Goat kidney	Perfused with Trypsin-EDTA in PBS, perfuse antibiotics and then with SDS in PBS	Cells are removed, pore to pore interconnection in the scaffold		[75,106]

ECM: Extra cellular matrix.

in United Kingdom livers offered for transplantation are usually discarded[77]. This act offers a way to use this kind of livers for acellularization. The liver is the largest gland in the body and carries out numerous essential functions such as metabolism, maintaining homeostasis, and the synthesis of amino acids[57]. Therefore, acellularization is extremely beneficial to the liver because it not only maintains the microstructure but also its bio signals such as extracellular matrix proteins and adhesion peptides[57].

Since extracellular matrices are similar from species to species, whole organ scaffolds have become possible for livers. Several recent studies have been reported for efficient acellularization of livers obtained from various xenogeneic sources[78-81] and the resulting 3D-ECM structure has become an outstanding source for generating highly functionalized liver cells *in vitro*[82,83]. As these extracellular matrices are conserved between species, the process of recellularization with human cells into an animal scaffold is easier[57] and this kind of approach does not elicit any kind of immune rejection, cross contamination and zoonosis. In our recent study, we have demonstrated development of humanized whole liver using human hepatic progenitor cells repopulation through hepatic artery infusion into acellularized liver scaffolds[74]. These humanized livers perform detoxification and metabolic functions similar to the native liver. However, the complete recellularization of a fully function human liver has not yet been accomplished[57]. Recent advances in isolating and culturing both native cells and stem cells, as well as the development of acellularized organ scaffolds and biocompatible synthetic biomaterials, suggest that we are making rapid progress towards providing new alternatives to donor livers for transplantation[56].

CHALLENGES NEED TO BE ADDRESSED IN GENERATING COMPLETE BIOENGINEERED FUNCTIONAL LIVER

Despite the amazing breakthroughs in the bioengineered organs, there is much work left to do. Simply reconstructing the whole organ will not be sufficient to replace organ transplantation. The approaches described above are fairly new and are still in the developmental stages. There has been only handful of successful transplantation of bioengineered organs into actual humans. Scientists are still working on ways to engineer more complex organs such as the liver. There are also long-term issues to resolve, such as the preservation of the overall function of these bioengineered organs. However, little is known about the mechanisms by which these grafts may integrate and maintain function. When more complex organs are involved, the scenario is completely different, as investigations are still in very early stages and clinical translation is not foreseeable on the basis of current knowledge and available data[84]. The following major critical issues are yet to be resolved to make these approaches a clinical reality: (1) Liver is a complex organ with various cell types, hence rebuilding liver micro architectures with these cells is yet to be addressed; (2) The optimal cell source that can meet the criteria for recellularization of acellularized liver scaffolds still remains unclear; (3) The first and foremost challenge is the need to address the reconstruction of complete and functional uniform endothelial cell layer throughout acellularized liver scaffolds; (4) It is necessary to reconstruct biliary system which is needed for bile acid excretion to develop a fully functional bioengineered liver; (5) Assessing the functionality of these bioengineered livers after *in vivo* transplantation for long term needs to be studied clearly; (6) For organ functionality, maintaining its vascular structure is much more important. As hepatocytes require higher amounts of oxygen for their functionality, it is necessary to maintain hierarchical vascular network structure in acellularized liver scaffolds[85]. Critical step in engineering a transplantable liver is the creation of a functional vasculature capable of long-term perfusion following anastomosis. Without an appropriate endothelial lining of the vessels, continuous blood perfusion of the graft in the absence of anticoagulation quickly results in thrombosis; and (7) Finding an appropriate site for providing enough support to the failing liver has been one of the most challenging issue to use the bioengineered organs as secondary liver.

OMENTUM AS BETTER ECTOPIC SITE FOR TRANSPLANTATION TO GENERATE SECONDARY ORGAN *IN VIVO*

The major question for applying these humanized bioengineered livers relies on finding an exact and more appropriate transplantable site where in these bioengineered organs can be easily acceptable and are able to perform the function. Recently omentum has been discovered as a potential ectopic site for transplantation with excellent properties like remarkable angiogenic[16], stem cell[17,18], fibrotic[19], and immune[20] activities, which together endorse vascularization, promote wound healing, and minimize infection (Table 3). Several studies have already demonstrated the importance of intra-omental transplantation in diabetic animal models[22,86].

The omentum is a visceral adipose tissue derived from mesothelial cells[87] connected to the spleen, stomach, pancreas, and colon[88,89]. Although well known as a visceral fat depot, the role of the omentum in peritoneal immunity was not recognized until the early 1900s, when a British surgeon referred to it as 'the police man of the abdomen' due to its ability to attenuate peritonitis and promote surgical wound healing[90]. In fact, omentum was noted to move about the peritoneal cavity and occlude sites of inflammation, such as ruptured ovaries, inflamed

Table 3 List of recent studies reporting use of omentum as transplantation site to support the lost organ function from ectopic transplantation of engineered tissues or grafts

Animal model	Site of transplantation	Mode of graft used	Results	Reference
Femoral bone of New Zealand rabbit was	Greater omentum on the left side	Free transplant of the greater omentum	Process of the callus formation and its mineralisation are much quicker and thicker on the defect that was covered with the free transplant of the greater omentum.	[107]
Pancreatectomized dogs	Spleen or Omentum	Islet auto-transplantation	Beta cell response to mild non-insulin induced hypoglycemia was normal, whereas the alpha cell response was not.	[108]
Murine carotid artery injury model	Omentum was applied to the injured vessel	Omentum + Omental progenitor cells	Omentum can directly contribute reparative progenitor cells to injured tissues upon treatment with Tβ4.	[109]
Nondiabetic nude rats	Omentum/kidney capsule	Perinatal porcine islet cell grafts	In both sites, the A-cell volume increased fourfold between weeks 1 and 10 reflecting a rise in A-cell number. In the omental implants, however, the cellular insulin reserves and the percent of proliferating cells were twofold higher than in kidney implants. In parallel, the blood vessel density in omental implants increased twofold, reaching a density comparable with islets in adult pig pancreas.	[110]
Diabetic rat and nonhuman primate (NHP) models	Intra-omental	*In situ*-generated adherent, resorbable plasma thrombin biologic scaffold	Improved metabolic function and preservation of islet cytoarchitecture, with reconstitution of rich intrainsular vascular networks in both species.	[21]
Adult male Sprague Dawley rats	Omental transposition	Hepatic tissue sutured into the omentum mobilization of the omentum and transposition onto the left hepatic lobe	Omental transposition provided adequate microcirculation for proliferation of ectopic hepatic cells after liver resection.	[111]

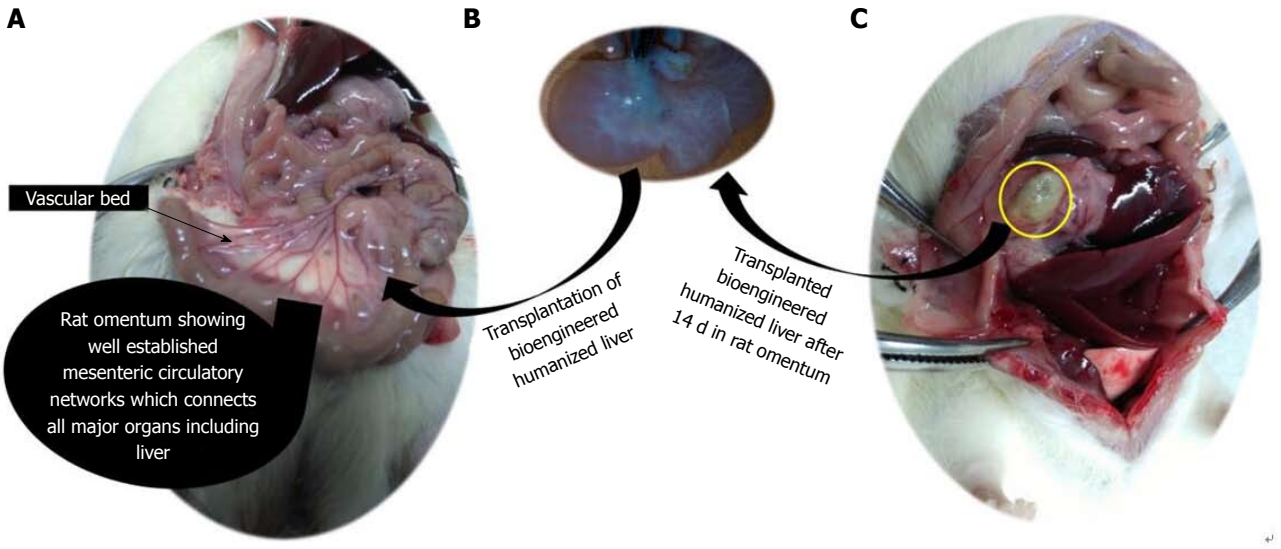

Figure 1 Intra-omental transplantation of bioengineered humanized livers showing development of secondary liver after 14 d. A: Anatomy of rat omentum showing well-established web of circulatory networks which connected with major organs; B: Developed bioengineered humanized liver in our lab *ex vivo*; C: Intra-omentally transplanted bioengineered humanized liver showing well engraftment with the surrounding tissue.

appendices, ulcerated intestines, or wounds due to trauma or surgery[90]. Consistent with this observation, the omentum has remarkable angiogenic[16], fibrotic[19], regenerative[17,18] and immune[20] activities, which together promote vascularization, accelerate wound healing, and limit infection. However, these same activities are also likely involved in pathological responses, such as the rapid growth of omental tumour metastases[91].

Once thought of as just a large amount of redundant fat overlying the intestines, surgeons' attitudes towards the omentum have changed. It is recognized as an organ in its own right, with many diverse functions ranging from its ability to attenuate the

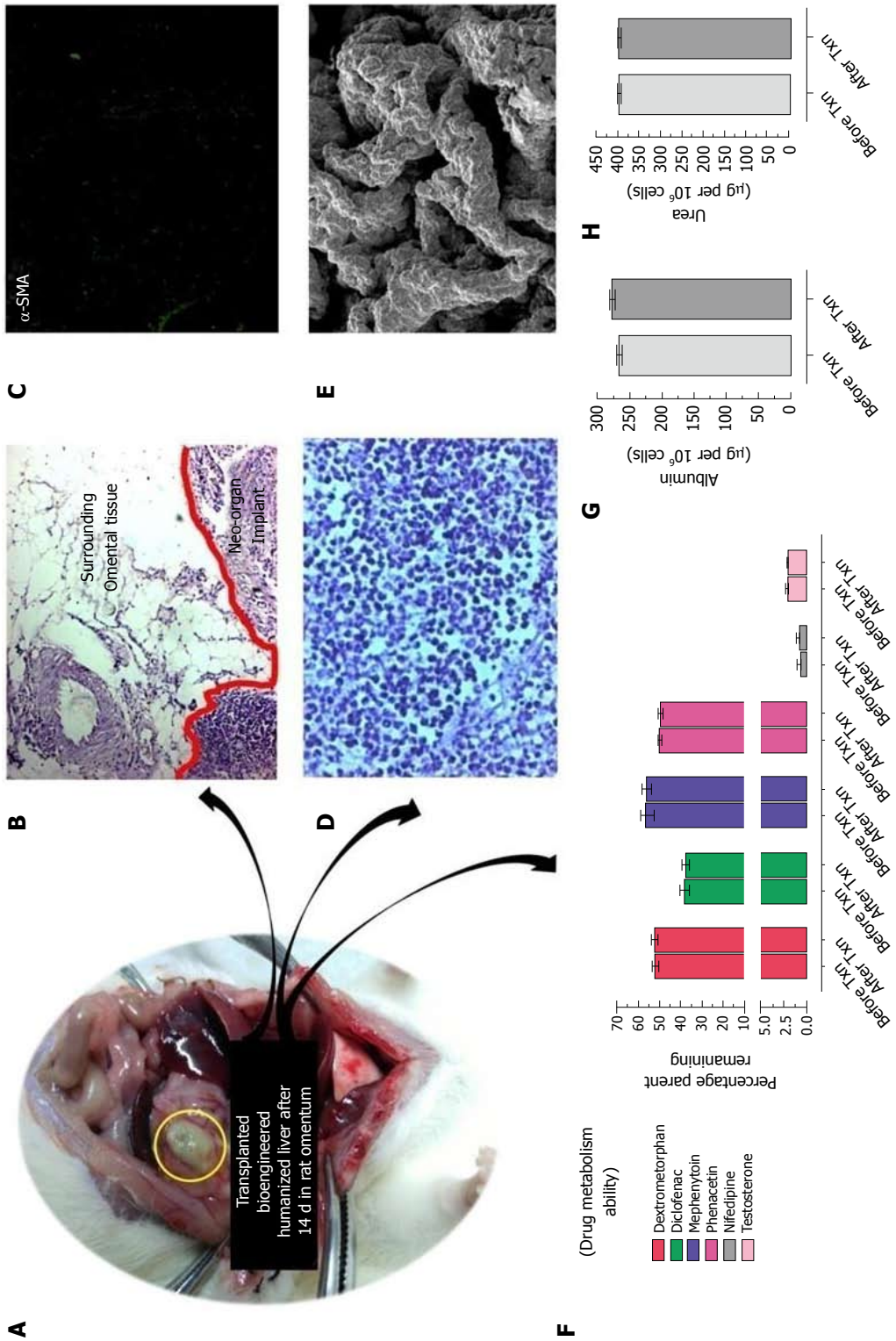

Figure 2 Intra-omental transplantation of bioengineered humanized livers showing no sign of fibrosis or immunological response at transplantation site. A: Optical image of transplanted implant at intra-omental site; B: Hematoxyeleine and eosin (HE) staining of the transplanted implant along with surrounding tissues showing no sign of immunological cells infiltration or tissue damage. Moreover, neo-vascularization was seen into nearby surrounding tissues which connects with the implant; C: Immunocytochemical staining using α-SMA showed no sign of fibrotic reactions to implant; D: HE staining showed well organized distribution and proliferation of hepatic cells into the implant post-transplantation; E: Scanning electron microscopy (SEM) image of retrieved graft at day 15 post-transplantation showing almost similar anatomy of bioengineered livers with natural liver; F: Retrieved livers at day 15 post intra-omental transplantation showed almost similar metabolic activity to before transplantation ($P > 0.05$). The other two important liver cell functions such as G: Albumin synthesis and H: Ammonia detoxification (*i.e.* urea production) is almost similar to the bioengineered humanized livers prior to transplantation ($P > 0.05$).

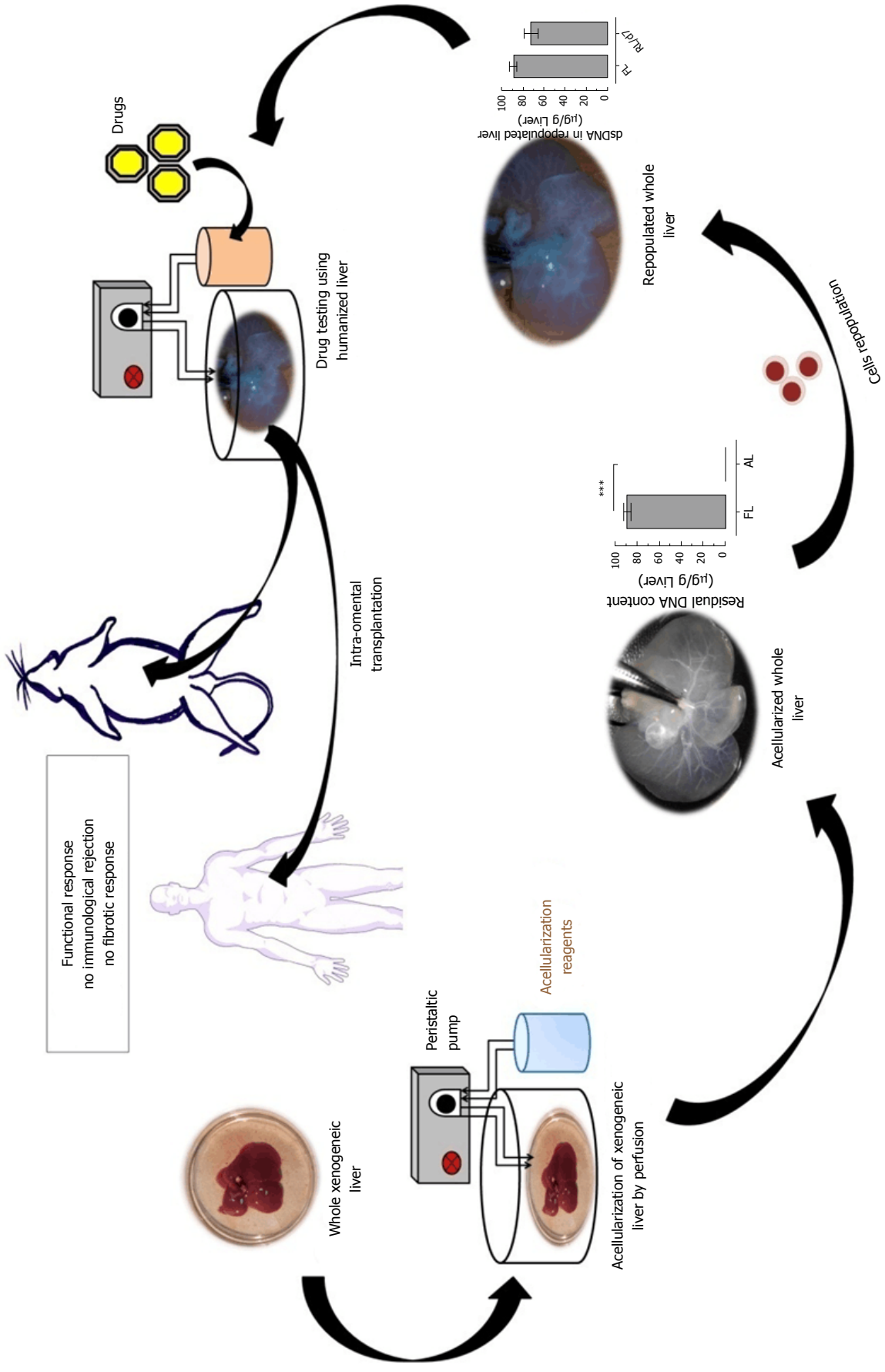

Figure 3 Brief overview of strategy for the development of immune-competent bioengineered humanized liver using acellularization and repopulation technology for future biomedical applications.

spread of sepsis in peritonitis to acting as a source of angiogenic and hemostatic factors involved in tissue healing and repair. The omentum has been identified as a source of adult stem cells which may have future prospects in the fields of tissue engineering and the synthesis of vascular grafts. Its regenerative properties have been exploited in virtually every field of surgery from the reconstruction of complex wounds to the protection of gastrointestinal anastomosis.

The regenerative properties of the omentum have been exploited by surgeons for over a century, ranging from the protection of anastomosis in gastrointestinal surgery, revascularization of arterial ulcers, to the reconstruction of head and neck deformities[92]. The advantage of the omentum is that it is an accessible and versatile source of growth factors, angiogenic factors, and leukocytes. It can be lengthened considerably by careful dissection to produce a mobile organ[93].

The regeneration of liver tissue in ectopic sites is still unknown. It has been discovered that the omnetum is a reservoir for proliferating renal, pancreatic, splenic tissues[23-25] and as a site for hepatocytes engraftment which can be used in tissue engineering[26]. Hepatocyte transplantation has been done in various tissues like spleen, pancreas and omentum[26,72,94-96]. With advancements in tissue engineering hepatocytes seeded onto polymer scaffolds and have been transplanted into omentum wherein engraftment of hepatocytes occurred due to elevated rates of angiogenesis into cell-polymer constructs within the omentum[96].

Thus intra-omental transplantation of bioengineered livers may provide adequate microcirculation for proliferation of ectopic hepatic cells repopulated within the bio-artificial liver. It has been observed that portal vein ligation does not affect the ectopic liver regeneration[97]. In our preliminary experiences, we have observed that intra-omental transplantation of bioengineered liver lobes gets easily accommodated into the site without eliciting immunological responses while maintain their biological functions and communicates blood borne growth factors for survival and function of the graft (Figure 1). We also observed that these bioengineered liver grafts survive at omental site in long-term and functions as secondary liver (Figure 2). These findings are well supported by earlier studies wherein other types of grafts have been transplanted into the omentum[21]. Future efforts at understanding mechanisms to regulate ectopic liver regeneration may assist the pursuit for liver tissue/organ bioengineering to support the failing liver functions in long-term.

CONCLUSION

Engineers and researches have been making monumental breakthroughs in the area of bioengineered organs. These bio-artificial organs may redefine transplants for human applications in future with more critical advancements. The introduction of cells into the human body is designed to stimulate regeneration, promote vascularization and/or supplement the production of hormones and growth factors[56]. Consequently, bioengineered biological substitutes present a new way to restore damaged tissue and maintain their functions. Not only does this provide a new source of organs, but probably even more reliable organs at that. Not only would people not need an organ donation, but their body will more readily accept a bioengineered organ through intra-omental transplantation, most likely reducing recovery time as well (Figure 3). In near future these potential strategies can overcome the limitation of organ donors and these bioengineered organs can even serve as a best natural 3D-drug testing models[74] and investigating precise molecular mechanisms in biomimetic natural organ system[112] and could support failing liver through ectopic transplantation as secondary organ in ESLD.

REFERENCES

1 **Murray CJ**, Lopez AD. Measuring the global burden of disease. *N Engl J Med* 2013; **369**: 448-457 [PMID: 23902484 DOI: 10.1056/NEJMra1201534]

2 **Dutkowski P**, Oberkofler CE, Béchir M, Müllhaupt B, Geier A, Raptis DA, Clavien PA. The model for end-stage liver disease allocation system for liver transplantation saves lives, but increases morbidity and cost: a prospective outcome analysis. *Liver Transpl* 2011; **17**: 674-684 [PMID: 21618688 DOI: 10.1002/lt.22228]

3 **Brown KA**, Moonka DK. Liver transplantation. *Curr Opin Gastroenterol* 2001; **17**: 299-303 [PMID: 17031172 DOI: 10.1097/00001574-200105000-00014]

4 **Busuttil RW**, Tanaka K. The utility of marginal donors in liver transplantation. *Liver Transpl* 2003; **9**: 651-663 [PMID: 12827549 DOI: 10.1053/jlts.2003.50105]

5 **Dhawan A**, Puppi J, Hughes RD, Mitry RR. Human hepatocyte transplantation: current experience and future challenges. *Nat Rev Gastroenterol Hepatol* 2010; **7**: 288-298 [PMID: 20368738 DOI: 10.1038/nrgastro.2010.44]

6 **Nagaki M**, Miki K, Kim YI, Ishiyama H, Hirahara I, Takahashi H, Sugiyama A, Muto Y, Moriwaki H. Development and characterization of a hybrid bioartificial liver using primary hepatocytes entrapped in a basement membrane matrix. *Dig Dis Sci* 2001; **46**: 1046-1056 [PMID: 11341648 DOI: 10.1023/A:1010714112675]

7 **Mito M,** Kusano M. Hepatocyte transplantation in man. *Cell Transplant* 1993; **2**: 65-74 [DOI: 10.1177/096368979300200109]

8 **Habibullah CM**, Syed IH, Qamar A, Taher-Uz Z. Human fetal hepatocyte transplantation in patients with fulminant hepatic failure. *Transplantation* 1994; **58**: 951-952 [PMID: 7940741 DOI: 10.1097/00007890-199410270-00016]

9 **Fox IJ**, Chowdhury JR, Kaufman SS, Goertzen TC, Chowdhury NR, Warkentin PI, Dorko K, Sauter BV, Strom SC. Treatment of the Crigler-Najjar syndrome type I with hepatocyte transplantation. *N Engl J Med* 1998; **338**: 1422-1426 [PMID: 9580649 DOI: 10.1056/NEJM199805143382004]

10 **Sokal EM**, Smets F, Bourgois A, Van Maldergem L, Buts JP, Reding R, Bernard Otte J, Evrard V, Latinne D, Vincent MF, Moser A, Soriano HE. Hepatocyte transplantation in a 4-year-old girl with peroxisomal biogenesis disease: technique, safety, and metabolic follow-up. *Transplantation* 2003; **76**: 735-738 [PMID: 12973120 DOI: 10.1097/01.TP.0000077420.81365.53]

11 **Roy-Chowdhury N**, Wang X, Guha C, Roy-Chowdhury J. Hepatocyte-like cells derived from induced pluripotent stem cells. *Hepatol Int* 2017; **11**: 54-69 [PMID: 27530815 DOI: 10.1007/s12072-016-9757-y]

12 **Zeilinger K**, Freyer N, Damm G, Seehofer D, Knöspel F. Cell

sources for in vitro human liver cell culture models. *Exp Biol Med (Maywood)* 2016; **241**: 1684-1698 [PMID: 27385595 DOI: 10.1177/1535370216657448]
13 **Zhou X**, Cui L, Zhou X, Yang Q, Wang L, Guo G, Hou Y, Cai W, Han Z, Shi Y, Han Y. Induction of hepatocyte-like cells from human umbilical cord-derived mesenchymal stem cells by defined microRNAs. *J Cell Mol Med* 2017; **21**: 881-893 [PMID: 27874233 DOI: 10.1111/jcmm.13027]
14 **Meyer U**, Wiesmann HP. Tissue engineering: a challenge of today's medicine. *Head Face Med* 2005; **1**: 2 [PMID: 16270925 DOI: 10.1186/1746-160X-1-2]
15 **Struecker B**, Raschzok N, Sauer IM. Liver support strategies: cutting-edge technologies. *Nat Rev Gastroenterol Hepatol* 2014; **11**: 166-176 [PMID: 24166083 DOI: 10.1038/nrgastro.2013.204]
16 **García-Gómez I**, Goldsmith HS, Angulo J, Prados A, López-Hervás P, Cuevas B, Dujovny M, Cuevas P. Angiogenic capacity of human omental stem cells. *Neurol Res* 2005; **27**: 807-811 [PMID: 16354540 DOI: 10.1179/016164105X63674]
17 **Shah S**, Lowery E, Braun RK, Martin A, Huang N, Medina M, Sethupathi P, Seki Y, Takami M, Byrne K, Wigfield C, Love RB, Iwashima M. Cellular basis of tissue regeneration by omentum. *PLoS One* 2012; **7**: e38368 [PMID: 22701632 DOI: 10.1371/journal.pone.0038368]
18 **Russo V**, Yu C, Belliveau P, Hamilton A, Flynn LE. Comparison of human adipose-derived stem cells isolated from subcutaneous, omental, and intrathoracic adipose tissue depots for regenerative applications. *Stem Cells Transl Med* 2014; **3**: 206-217 [PMID: 24361924 DOI: 10.5966/sctm.2013-0125]
19 **Divoux A**, Tordjman J, Lacasa D, Veyrie N, Hugol D, Aissat A, Basdevant A, Guerre-Millo M, Poitou C, Zucker JD, Bedossa P, Clément K. Fibrosis in human adipose tissue: composition, distribution, and link with lipid metabolism and fat mass loss. *Diabetes* 2010; **59**: 2817-2825 [PMID: 20713683 DOI: 10.2337/db10-0585]
20 **Rangel-Moreno J**, Moyron-Quiroz JE, Carragher DM, Kusser K, Hartson L, Moquin A, Randall TD. Omental milky spots develop in the absence of lymphoid tissue-inducer cells and support B and T cell responses to peritoneal antigens. *Immunity* 2009; **30**: 731-743 [PMID: 19427241 DOI: 10.1016/j.immuni.2009.03.014]
21 **Berman DM**, Molano RD, Fotino C, Ulissi U, Gimeno J, Mendez AJ, Kenyon NM, Kenyon NS, Andrews DM, Ricordi C, Pileggi A. Bioengineering the Endocrine Pancreas: Intraomental Islet Transplantation Within a Biologic Resorbable Scaffold. *Diabetes* 2016; **65**: 1350-1361 [PMID: 26916086 DOI: 10.2337/db15-1525]
22 **Kim HI**, Yu JE, Park CG, Kim SJ. Comparison of four pancreatic islet implantation sites. *J Korean Med Sci* 2010; **25**: 203-210 [PMID: 20119571 DOI: 10.3346/jkms.2010.25.2.203]
23 **Yokoo T**, Fukui A, Ohashi T, Miyazaki Y, Utsunomiya Y, Kawamura T, Hosoya T, Okabe M, Kobayashi E. Xenobiotic kidney organogenesis from human mesenchymal stem cells using a growing rodent embryo. *J Am Soc Nephrol* 2006; **17**: 1026-1034 [PMID: 16524947 DOI: 10.1681/ASN.2005101043]
24 **Cuervas-Mons V**, Cienfuegos JA, Maganto P, Rodriguez V, Eroles G, Pinedo I, Santamaria L, Ramos J, Ortiz JL, Castillo-Olivares JL. Long-term evaluation of isolated syngeneic hepatocytes transplanted into the normal rat spleen by TC-99M-HIDA scintigraphy. *Transplantation* 1985; **39**: 87-90 [PMID: 3880971 DOI: 10.1097/00007890-198501000-00014]
25 **Mazzoni G**, Di Martino C, Scarpelli F, Cristini F, Citarella G, Martini ME. Liver autotransplantation into the pancreas. *Transplantation* 1982; **34**: 108-109 [PMID: 6753266 DOI: 10.1097/00007890-198208000-00012]
26 **Lee H**, Cusick RA, Utsunomiya H, Ma PX, Langer R, Vacanti JP. Effect of implantation site on hepatocytes heterotopically transplanted on biodegradable polymer scaffolds. *Tissue Eng* 2003; **9**: 1227-1232 [PMID: 14670110 DOI: 10.1089/10763270360728134]
27 **Bianconi E**, Piovesan A, Facchin F, Beraudi A, Casadei R, Frabetti F, Vitale L, Pelleri MC, Tassani S, Piva F, Perez-Amodio S, Strippoli P, Canaider S. An estimation of the number of cells in the human body. *Ann Hum Biol* 2013; **40**: 463-471 [PMID: 23829164 DOI: 10.3109/03014460.2013.807878]
28 **Sussman NL**, Kelly JH. Artificial liver: a forthcoming attraction. *Hepatology* 1993; **17**: 1163-1164 [PMID: 8514267 DOI: 10.1002/hep.1840170632]
29 **Bilir BM**, Guinette D, Karrer F, Kumpe DA, Krysl J, Stephens J, McGavran L, Ostrowska A, Durham J. Hepatocyte transplantation in acute liver failure. *Liver Transpl* 2000; **6**: 32-40 [PMID: 10648575 DOI: 10.1002/lt.500060113]
30 **Treyer A**, Müsch A. Hepatocyte polarity. *Compr Physiol* 2013; **3**: 243-287 [PMID: 23720287 DOI: 10.1002/cphy.c120009]
31 **Zaret KS**, Grompe M. Generation and regeneration of cells of the liver and pancreas. *Science* 2008; **322**: 1490-1494 [PMID: 19056973 DOI: 10.1126/science.1161431]
32 **McLaren A**. Ethical and social considerations of stem cell research. *Nature* 2001; **414**: 129-131 [PMID: 11689959 DOI: 10.1038/35102194]
33 **Swijnenburg RJ**, Schrepfer S, Govaert JA, Cao F, Ransohoff K, Sheikh AY, Haddad M, Connolly AJ, Davis MM, Robbins RC, Wu JC. Immunosuppressive therapy mitigates immunological rejection of human embryonic stem cell xenografts. *Proc Natl Acad Sci U S A* 2008; **105**: 12991-12996 [PMID: 18728188 DOI: 10.1073/pnas.0805802105]
34 **Fausto N**, Campbell JS. The role of hepatocytes and oval cells in liver regeneration and repopulation. *Mech Dev* 2003; **120**: 117-130 [PMID: 12490302 DOI: 10.1016/S0925-4773(02)00338-6]
35 **Evarts RP**, Nagy P, Marsden E, Thorgeirsson SS. A precursor-product relationship exists between oval cells and hepatocytes in rat liver. *Carcinogenesis* 1987; **8**: 1737-1740 [PMID: 3664968 DOI: 10.1093/carcin/8.11.1737]
36 **Lázaro CA**, Rhim JA, Yamada Y, Fausto N. Generation of hepatocytes from oval cell precursors in culture. *Cancer Res* 1998; **58**: 5514-5522 [PMID: 9850088]
37 **Kubota H**, Reid LM. Clonogenic hepatoblasts, common precursors for hepatocytic and biliary lineages, are lacking classical major histocompatibility complex class I antigen. *Proc Natl Acad Sci U S A* 2000; **97**: 12132-12137 [PMID: 11050242 DOI: 10.1073/pnas.97.22.12132]
38 **Oertel M**, Rosencrantz R, Chen YQ, Thota PN, Sandhu JS, Dabeva MD, Pacchia AL, Adelson ME, Dougherty JP, Shafritz DA. Repopulation of rat liver by fetal hepatoblasts and adult hepatocytes transduced ex vivo with lentiviral vectors. *Hepatology* 2003; **37**: 994-1005 [PMID: 12717380 DOI: 10.1053/jhep.2003.50183]
39 **Haridass D**, Yuan Q, Becker PD, Cantz T, Iken M, Rothe M, Narain N, Bock M, Nörder M, Legrand N, Wedemeyer H, Weijer K, Spits H, Manns MP, Cai J, Deng H, Di Santo JP, Guzman CA, Ott M. Repopulation efficiencies of adult hepatocytes, fetal liver progenitor cells, and embryonic stem cell-derived hepatic cells in albumin-promoter-enhancer urokinase-type plasminogen activator mice. *Am J Pathol* 2009; **175**: 1483-1492 [PMID: 19717639 DOI: 10.2353/ajpath.2009.090117]
40 **Mahieu-Caputo D**, Allain JE, Branger J, Coulomb A, Delgado JP, Andreoletti M, Mainot S, Frydman R, Leboulch P, Di Santo JP, Capron F, Weber A. Repopulation of athymic mouse liver by cryopreserved early human fetal hepatoblasts. *Hum Gene Ther* 2004; **15**: 1219-1228 [PMID: 15684698 DOI: 10.1089/hum.2004.15.1219]
41 **Hayner NT**, Braun L, Yaswen P, Brooks M, Fausto N. Isozyme profiles of oval cells, parenchymal cells, and biliary cells isolated by centrifugal elutriation from normal and preneoplastic livers. *Cancer Res* 1984; **44**: 332-338 [PMID: 6690044]
42 **Schmelzer E**, Wauthier E, Reid LM. The phenotypes of pluripotent human hepatic progenitors. *Stem Cells* 2006; **24**: 1852-1858 [PMID: 16627685 DOI: 10.1634/stemcells.2006-0036]
43 **Xu YQ**, Liu ZC. Therapeutic potential of adult bone marrow stem cells in liver disease and delivery approaches. *Stem Cell Rev* 2008; **4**: 101-112 [PMID: 18481229 DOI: 10.1007/s12015-008-9019-z]
44 **Cho KA**, Ju SY, Cho SJ, Jung YJ, Woo SY, Seoh JY, Han HS, Ryu KH. Mesenchymal stem cells showed the highest potential for the regeneration of injured liver tissue compared with other subpopulations of the bone marrow. *Cell Biol Int* 2009; **33**: 772-777 [PMID: 19427913 DOI: 10.1016/j.cellbi.2009.04.023]

45 **Shi M**, Liu ZW, Wang FS. Immunomodulatory properties and therapeutic application of mesenchymal stem cells. *Clin Exp Immunol* 2011; **164**: 1-8 [PMID: 21352202 DOI: 10.1111/j.1365-2249.2011.04327.x]

46 **Lee OK**, Kuo TK, Chen WM, Lee KD, Hsieh SL, Chen TH. Isolation of multipotent mesenchymal stem cells from umbilical cord blood. *Blood* 2004; **103**: 1669-1675 [PMID: 14576065 DOI: 10.1182/blood-2003-05-1670]

47 **di Bonzo LV**, Ferrero I, Cravanzola C, Mareschi K, Rustichell D, Novo E, Sanavio F, Cannito S, Zamara E, Bertero M, Davit A, Francica S, Novelli F, Colombatto S, Fagioli F, Parola M. Human mesenchymal stem cells as a two-edged sword in hepatic regenerative medicine: engraftment and hepatocyte differentiation versus profibrogenic potential. *Gut* 2008; **57**: 223-231 [PMID: 17639088 DOI: 10.1136/gut.2006.111617]

48 **Kestendjieva S**, Kyurkchiev D, Tsvetkova G, Mehandjiev T, Dimitrov A, Nikolov A, Kyurkchiev S. Characterization of mesenchymal stem cells isolated from the human umbilical cord. *Cell Biol Int* 2008; **32**: 724-732 [PMID: 18396423 DOI: 10.1016/j.cellbi.2008.02.002]

49 **Yen BL**, Huang HI, Chien CC, Jui HY, Ko BS, Yao M, Shun CT, Yen ML, Lee MC, Chen YC. Isolation of multipotent cells from human term placenta. *Stem Cells* 2005; **23**: 3-9 [PMID: 15625118 DOI: 10.1634/stemcells.2004-0098]

50 **Di Campli C**, Piscaglia AC, Pierelli L, Rutella S, Bonanno G, Alison MR, Mariotti A, Vecchio FM, Nestola M, Monego G, Michetti F, Mancuso S, Pola P, Leone G, Gasbarrini G, Gasbarrini A. A human umbilical cord stem cell rescue therapy in a murine model of toxic liver injury. *Dig Liver Dis* 2004; **36**: 603-613 [PMID: 15460845 DOI: 10.1016/j.dld.2004.03.017]

51 **Zacharias DG**, Nelson TJ, Mueller PS, Hook CC. The science and ethics of induced pluripotency: what will become of embryonic stem cells? *Mayo Clin Proc* 2011; **86**: 634-640 [PMID: 21719620 DOI: 10.4065/mcp.2011.0054]

52 **Jalan R**, Williams R. Acute-on-chronic liver failure: pathophysiological basis of therapeutic options. *Blood Purif* 2002; **20**: 252-261 [PMID: 11867872 DOI: 10.1159/000047017]

53 **Guillemot F**, Mironov V, Nakamura M. Bioprinting is coming of age: Report from the International Conference on Bioprinting and Biofabrication in Bordeaux (3B'09). *Biofabrication* 2010; **2**: 010201 [PMID: 20811115 DOI: 10.1088/1758-5082/2/1/010201]

54 **Sawkins MJ**, Mistry P, Brown BN, Shakesheff KM, Bonassar LJ, Yang J. Cell and protein compatible 3D bioprinting of mechanically strong constructs for bone repair. *Biofabrication* 2015; **7**: 035004 [PMID: 26133398 DOI: 10.1088/1758-5090/7/3/035004]

55 **Robbins JB,** Gorgen V, Min P, Shepherd BR, Presnell SC. A novel in vitro three-dimensional bioprinted liver tissue system for drug development. *FASEB J* 2013; **27**: Abstract 7979

56 **Murphy SV**, Atala A. Organ engineering--combining stem cells, biomaterials, and bioreactors to produce bioengineered organs for transplantation. *Bioessays* 2013; **35**: 163-172 [PMID: 22996568 DOI: 10.1002/bies.201200062]

57 **Peloso A**, Dhal A, Zambon JP, Li P, Orlando G, Atala A, Soker S. Current achievements and future perspectives in whole-organ bioengineering. *Stem Cell Res Ther* 2015; **6**: 107 [PMID: 26028404 DOI: 10.1186/s13287-015-0089-y]

58 **Atala A**, Bauer SB, Soker S, Yoo JJ, Retik AB. Tissue-engineered autologous bladders for patients needing cystoplasty. *Lancet* 2006; **367**: 1241-1246 [PMID: 16631879 DOI: 10.1016/S0140-6736(06)68438-9]

59 **Atala A**. Engineering tissues, organs and cells. *J Tissue Eng Regen Med* 2007; **1**: 83-96 [PMID: 18038397 DOI: 10.1002/term.18]

60 **Shin'oka T**, Imai Y, Ikada Y. Transplantation of a tissue-engineered pulmonary artery. *N Engl J Med* 2001; **344**: 532-533 [PMID: 11221621 DOI: 10.1056/NEJM200102153440717]

61 **L'Heureux N**, McAllister TN, de la Fuente LM. Tissue-engineered blood vessel for adult arterial revascularization. *N Engl J Med* 2007; **357**: 1451-1453 [PMID: 17914054 DOI: 10.1056/NEJMc071536]

62 **Badylak SF**, Taylor D, Uygun K. Whole-organ tissue engineering: decellularization and recellularization of three-dimensional matrix scaffolds. *Annu Rev Biomed Eng* 2011; **13**: 27-53 [PMID: 21417722 DOI: 10.1146/annurev-bioeng-071910-124743]

63 **Ott HC**, Clippinger B, Conrad C, Schuetz C, Pomerantseva I, Ikonomou L, Kotton D, Vacanti JP. Regeneration and orthotopic transplantation of a bioartificial lung. *Nat Med* 2010; **16**: 927-933 [PMID: 20628374 DOI: 10.1038/nm.2193]

64 **Taylor DA**. From stem cells and cadaveric matrix to engineered organs. *Curr Opin Biotechnol* 2009; **20**: 598-605 [PMID: 19914057 DOI: 10.1016/j.copbio.2009.10.016]

65 **Wainwright JM**, Czajka CA, Patel UB, Freytes DO, Tobita K, Gilbert TW, Badylak SF. Preparation of cardiac extracellular matrix from an intact porcine heart. *Tissue Eng Part C Methods* 2010; **16**: 525-532 [PMID: 19702513 DOI: 10.1089/ten.TEC.2009.0392]

66 **Petersen TH**, Calle EA, Zhao L, Lee EJ, Gui L, Raredon MB, Gavrilov K, Yi T, Zhuang ZW, Breuer C, Herzog E, Niklason LE. Tissue-engineered lungs for in vivo implantation. *Science* 2010; **329**: 538-541 [PMID: 20576850 DOI: 10.1126/science.1189345]

67 **Cortiella J**, Niles J, Cantu A, Brettler A, Pham A, Vargas G, Winston S, Wang J, Walls S, Nichols JE. Influence of acellular natural lung matrix on murine embryonic stem cell differentiation and tissue formation. *Tissue Eng Part A* 2010; **16**: 2565-2580 [PMID: 20408765 DOI: 10.1089/ten.tea.2009.0730]

68 **Baptista PM**, Orlando G, Mirmalek-Sani SH, Siddiqui M, Atala A, Soker S. Whole organ decellularization - a tool for bioscaffold fabrication and organ bioengineering. *Conf Proc IEEE Eng Med Biol Soc* 2009; **2009**: 6526-6529 [PMID: 19964173 DOI: 10.1109/IEMBS.2009.5333145]

69 **Baptista PM**, Siddiqui MM, Lozier G, Rodriguez SR, Atala A, Soker S. The use of whole organ decellularization for the generation of a vascularized liver organoid. *Hepatology* 2011; **53**: 604-617 [PMID: 21274881 DOI: 10.1002/hep.24067]

70 **Uygun BE**, Soto-Gutierrez A, Yagi H, Izamis ML, Guzzardi MA, Shulman C, Milwid J, Kobayashi N, Tilles A, Berthiaume F, Hertl M, Nahmias Y, Yarmush ML, Uygun K. Organ reengineering through development of a transplantable recellularized liver graft using decellularized liver matrix. *Nat Med* 2010; **16**: 814-820 [PMID: 20543851 DOI: 10.1038/nm.2170]

71 **Ross EA**, Williams MJ, Hamazaki T, Terada N, Clapp WL, Adin C, Ellison GW, Jorgensen M, Batich CD. Embryonic stem cells proliferate and differentiate when seeded into kidney scaffolds. *J Am Soc Nephrol* 2009; **20**: 2338-2347 [PMID: 19729441 DOI: 10.1681/ASN.2008111196]

72 **Liu CX**, Liu SR, Xu AB, Kang YZ, Zheng SB, Li HL. [Preparation of whole-kidney acellular matrix in rats by perfusion]. *Nan Fang Yi Ke Da Xue Xue Bao* 2009; **29**: 979-982 [PMID: 19460725]

73 **Nakayama KH**, Batchelder CA, Lee CI, Tarantal AF. Decellularized rhesus monkey kidney as a three-dimensional scaffold for renal tissue engineering. *Tissue Eng Part A* 2010; **16**: 2207-2216 [PMID: 20156112 DOI: 10.1089/ten.tea.2009.0602]

74 **Vishwakarma SK**, Bardia A, Lakkireddy C, Nagarapu R, Habeeb MA, Khan AA. Bioengineered humanized livers as better three-dimensional drug testing model system. *World J Hepatol* 2018; **10**: 22-33 [PMID: 29399275 DOI: 10.4254/wjh.v10.i1.22]

75 **Vishwakarma SK**, Bhavani PG, Bardia A, Abkari A, Murthy GS, Venkateshwarulu J, Khan AA. Preparation of natural three-dimensional goat kidney scaffold for the development of bioartificial organ. *Indian J Nephrol* 2014; **24**: 372-375 [PMID: 25484531 DOI: 10.4103/0971-4065.133008]

76 **Rout S,** Vishwakarma SK, Khan AA. Decellularized heart: a step towards creating personalized bioengineered organs. *Current Sci* 2014**; 107**: 10

77 **NHS Blood and Transplant**. Organ Donation and Transplantation - Activity Report 2013/14. Available from: URL: https://nhsbtmediaservices.blob.core.windows.net/organ-donation-assets/pdfs/activity_report_2013_14.pdf

78 **Ren H**, Shi X, Tao L, Xiao J, Han B, Zhang Y, Yuan X, Ding Y. Evaluation of two decellularization methods in the development of a whole-organ decellularized rat liver scaffold. *Liver Int* 2013; **33**:

448-458 [PMID: 23301992 DOI: 10.1111/liv.12088]
79 **Pan MX**, Hu PY, Cheng Y, Cai LQ, Rao XH, Wang Y, Gao Y. An efficient method for decellularization of the rat liver. *J Formos Med Assoc* 2014; **113**: 680-687 [PMID: 23849456 DOI: 10.1016/j.jfma.2013.05.003]
80 **Nari GA**, Cid M, Comín R, Reyna L, Juri G, Taborda R, Salvatierra NA. Preparation of a three-dimensional extracellular matrix by decellularization of rabbit livers. *Rev Esp Enferm Dig* 2013; **105**: 138-143 [PMID: 23735020 DOI: 10.4321/S1130-01082013000300004]
81 **Kajbafzadeh AM**, Javan-Farazmand N, Monajemzadeh M, Baghayee A. Determining the optimal decellularization and sterilization protocol for preparing a tissue scaffold of a human-sized liver tissue. *Tissue Eng Part C Methods* 2013; **19**: 642-651 [PMID: 23270591 DOI: 10.1089/ten.TEC.2012.0334]
82 **Wang Y**, Cui CB, Yamauchi M, Miguez P, Roach M, Malavarca R, Costello MJ, Cardinale V, Wauthier E, Barbier C, Gerber DA, Alvaro D, Reid LM. Lineage restriction of human hepatic stem cells to mature fates is made efficient by tissue-specific biomatrix scaffolds. *Hepatology* 2011; **53**: 293-305 [PMID: 21254177 DOI: 10.1002/hep.24012]
83 **Soto-Gutierrez A**, Zhang L, Medberry C, Fukumitsu K, Faulk D, Jiang H, Reing J, Gramignoli R, Komori J, Ross M, Nagaya M, Lagasse E, Stolz D, Strom SC, Fox IJ, Badylak SF. A whole-organ regenerative medicine approach for liver replacement. *Tissue Eng Part C Methods* 2011; **17**: 677-686 [PMID: 21375407 DOI: 10.1089/ten.tec.2010.0698]
84 **Orlando G**, Soker S, Stratta RJ. Organ bioengineering and regeneration as the new Holy Grail for organ transplantation. *Ann Surg* 2013; **258**: 221-232 [PMID: 23782908 DOI: 10.1097/SLA.0b013e31829c79cf]
85 **Sasagawa T**, Shimizu T, Sekiya S, Haraguchi Y, Yamato M, Sawa Y, Okano T. Design of prevascularized three-dimensional cell-dense tissues using a cell sheet stacking manipulation technology. *Biomaterials* 2010; **31**: 1646-1654 [PMID: 19962187 DOI: 10.1016/j.biomaterials.2009.11.036]
86 **Berman DM**, O'Neil JJ, Coffey LC, Chaffanjon PC, Kenyon NM, Ruiz P Jr, Pileggi A, Ricordi C, Kenyon NS. Long-term survival of nonhuman primate islets implanted in an omental pouch on a biodegradable scaffold. *Am J Transplant* 2009; **9**: 91-104 [PMID: 19133931 DOI: 10.1111/j.1600-6143.2008.02489.x]
87 **Chau YY**, Bandiera R, Serrels A, Martínez-Estrada OM, Qing W, Lee M, Slight J, Thornburn A, Berry R, McHaffie S, Stimson RH, Walker BR, Chapuli RM, Schedl A, Hastie N. Visceral and subcutaneous fat have different origins and evidence supports a mesothelial source. *Nat Cell Biol* 2014; **16**: 367-375 [PMID: 24609269 DOI: 10.1038/ncb2922]
88 **Williams R**, White H. The greater omentum: its applicability to cancer surgery and cancer therapy. *Curr Probl Surg* 1986; **23**: 789-865 [PMID: 3780294 DOI: 10.1016/0011-3840(86)90007-9]
89 **Wilkosz S**, Ireland G, Khwaja N, Walker M, Butt R, de Giorgio-Miller A, Herrick SE. A comparative study of the structure of human and murine greater omentum. *Anat Embryol (Berl)* 2005; **209**: 251-261 [PMID: 15662530 DOI: 10.1007/s00429-004-0446-6]
90 **Morrison R**. On the functional aspects of the greater omentum. *Br Med J* 1906; **1**: 76-78
91 **Koppe MJ**, Nagtegaal ID, de Wilt JH, Ceelen WP. Recent insights into the pathophysiology of omental metastases. *J Surg Oncol* 2014; **110**: 670-675 [PMID: 24962271 DOI: 10.1002/jso.23681]
92 **Losken A**, Carlson GW, Culbertson JH, Scott Hultman C, Kumar AV, Jones GE, Bostwick J 3rd, Jurkiewicz MJ. Omental free flap reconstruction in complex head and neck deformities. *Head Neck* 2002; **24**: 326-331 [PMID: 11933173 DOI: 10.1002/hed.10082]
93 **Ross WE**, Pardo AD. Evaluation of an omental pedicle extension technique in the dog. *Vet Surg* 1993; **22**: 37-43 [PMID: 8488673 DOI: 10.1111/j.1532-950X.1993.tb00366.x]
94 **Pancholi N**, Patel J, Gudehithlu KP, Kraus MA, Dunea G, Arruda JA, Singh AK. Culture of omentum-induced regenerating liver yielded hepatocyte-committed stem cells. *Transl Res* 2010; **156**: 358-368 [PMID: 21078497 DOI: 10.1016/j.trsl.2010.09.002]
95 **Panis Y**, Puts JP, Ballet F, Penin E, Delelo R, Verthier N, Nordlinger B. The isolated perfused rat spleen. An original method for studying the function of hepatocytes transplanted into the spleen. *Transplantation* 1990; **49**: 756-759 [PMID: 2326870 DOI: 10.1097/00007890-199004000-00020]
96 **Dave S**, Bal CS, Mathur M, Bhatnagar V. Evaluation of transplanted hepatocytes using HIDA scintigraphy. *Indian J Gastroenterol* 2001; **20**: 177-179 [PMID: 11676327]
97 **Chen AA**, Thomas DK, Ong LL, Schwartz RE, Golub TR, Bhatia SN. Humanized mice with ectopic artificial liver tissues. *Proc Natl Acad Sci U S A* 2011; **108**: 11842-11847 [PMID: 21746904 DOI: 10.1073/pnas.1101791108]
98 **Ott HC**, Matthiesen TS, Goh SK, Black LD, Kren SM, Netoff TI, Taylor DA. Perfusion-decellularized matrix: using nature's platform to engineer a bioartificial heart. *Nat Med* 2008; **14**: 213-221 [PMID: 18193059 DOI: 10.1038/nm1684]
99 **Price AP**, England KA, Matson AM, Blazar BR, Panoskaltsis-Mortari A. Development of a decellularized lung bioreactor system for bioengineering the lung: the matrix reloaded. *Tissue Eng Part A* 2010; **16**: 2581-2591 [PMID: 20297903 DOI: 10.1089/ten.TEA.2009.0659]
100 **Shupe T**, Williams M, Brown A, Willenberg B, Petersen BE. Method for the decellularization of intact rat liver. *Organogenesis* 2010; **6**: 134-136 [PMID: 20885860 DOI: 10.4161/org.6.2.11546]
101 **Barakat O**, Abbasi S, Rodriguez G, Rios J, Wood RP, Ozaki C, Holley LS, Gauthier PK. Use of decellularized porcine liver for engineering humanized liver organ. *J Surg Res* 2012; **173**: e11-e25 [PMID: 22099595 DOI: 10.1016/j.jss.2011.09.033]
102 **Sano MB**, Neal RE 2nd, Garcia PA, Gerber D, Robertson J, Davalos RV. Towards the creation of decellularized organ constructs using irreversible electroporation and active mechanical perfusion. *Biomed Eng Online* 2010; **9**: 83 [PMID: 21143979 DOI: 10.1186/1475-925X-9-83]
103 **Lu TY**, Lin B, Kim J, Sullivan M, Tobita K, Salama G, Yang L. Repopulation of decellularized mouse heart with human induced pluripotent stem cell-derived cardiovascular progenitor cells. *Nat Commun* 2013; **4**: 2307 [PMID: 23942048 DOI: 10.1038/ncomms3307]
104 **Gilbert TW**, Sellaro TL, Badylak SF. Decellularization of tissues and organs. *Biomaterials* 2006; **27**: 3675-3683 [PMID: 16519932 DOI: 10.1016/j.biomaterials.2006.02.014]
105 **Khan AA**, Capoor AK, Parveen N, Naseem S, Venkatesan V 5th, Habibullah CM. In vitro studies on a bioreactor module containing encapsulated goat hepatocytes for the development of bioartificial liver. *Indian J Gastroenterol* 2002; **21**: 55-58 [PMID: 11995635]
106 **Gupta SK**, Dinda AK, Potdar PD, Mishra NC. Modification of decellularized goat-lung scaffold with chitosan/nanohydroxyapatite composite for bone tissue engineering applications. *Biomed Res Int* 2013; **2013**: 651945 [PMID: 23841083 DOI: 10.1155/2013/651945]
107 **Josip K,** Vjenceslav N, Dubravko H, Irena N, Josip T, Dragutin K, Tomislav A, Tomislav B, Dražen V, Mario Kr, Ozren S. Healing of bone defect by application of free transplant of greater omentum. *Veterinarski Arhiv* 2006; **76**: 367-379
108 **Gustavson SM**, Rajotte RV, Hunkeler D, Lakey JR, Edgerton DS, Neal DW, Snead WL, Penaloza AR, Cherrington AD. Islet autotransplantation into an omental or splenic site results in a normal beta cell but abnormal alpha cell response to mild non-insulin-induced hypoglycemia. *Am J Transplant* 2005; **5**: 2368-2377 [PMID: 16162184 DOI: 10.1111/j.1600-6143.2005.01041.x]
109 **Shelton EL**, Poole SD, Reese J, Bader DM. Omental grafting: a cell-based therapy for blood vessel repair. *J Tissue Eng Regen Med* 2013; **7**: 421-433 [PMID: 22318999 DOI: 10.1002/term.528]
110 **Bartholomeus K**, Jacobs-Tulleneers-Thevissen D, Shouyue S, Suenens K, In't Veld PA, Pipeleers-Marichal M, Pipeleers DG, Hellemans K. Omentum is better site than kidney capsule for growth, differentiation, and vascularization of immature porcine β-cell implants in immunodeficient rats. *Transplantation* 2013; **96**: 1026-1033 [PMID: 24056625 DOI: 10.1097/TP.0b013e3182a6ee41]
111 **Macedo FI**, Eid JJ, Decker M, Herschman B, Negussie E, Mittal

VK. Autogenous hepatic tissue transplantation into the omentum in a novel ectopic liver regeneration murine model. *J Surg Res* 2018; **223**: 215-223 [PMID: 29433876 DOI: 10.1016/j.jss.2017.11.032]

112 **Vishwakarma SK**, Lakkireddy C, Bardia A, Raju N, Paspala SAB, Habeeb MA, Khan AA. Molecular dynamics of pancreatic transcription factors in bioengineered humanized insulin producing neoorgan. *Gene* 2018; **675**: 165-175 [PMID: 30180963 DOI: 10.1016/j.gene.2018.07.006]

P- Reviewer: Bredt LC, Preda C **S- Editor**: Wang JL
L- Editor: A **E- Editor**: Tan WW

World Journal of Hepatology

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

World J Hepatol 2018 November 27; 10(11): 849-855

DOI: 10.4254/wjh.v10.i11.849

ISSN 1948-5182 (online)

ORIGINAL ARTICLE

Retrospective Study

African Americans are less likely to receive curative treatment for hepatocellular carcinoma

Lindsay A Sobotka, Alice Hinton, Lanla F Conteh

Lindsay A Sobotka, Department of Internal Medicine, The Ohio State University Wexner Medical Center, Columbus, OH 43210, United States

Alice Hinton, Division of Biostatistics, College of Public Health, The Ohio State University, OH 43210, United States

Lanla F Conteh, Department of Gastroenterology and Hepatology, The Ohio State Wexner Medical Center, Columbus, OH 43210, United States

ORCID number: Lindsay A Sobotka (0000-0003-1052-2067); Alice Hinton (0000-0003-4505-4021); Lanla F Conteh (0000-0002-4372-993X).

Author contributions: All authors helped to perform the research; Sobotka LA conceived and designed the study, interpreted the data, drafted the article, and approved the final version of the article to be published; Hinton A acquired data, analyzed data, made critical revisions related to important intellectual content of the manuscript, and approved the final article to be published; Conteh LF conceived and designed the study, interpreted data, drafted the article, made critical revisions related to important intellectual content of the manuscript, and approved the final version of the article to be published.

Institutional review board statement: This research is not a clinical trial and did not require institutional review board approval through The Ohio State University given the de-identified nature of this database.

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

Manuscript source: Unsolicited manuscript

Corresponding author to: Lanla F Conteh, Doctor, MD, MPH, Department of Gastroenterology and Hepatology, The Ohio State Wexner Medical Center, 410 W. 10th Street, Columbus, OH 43210, United States. Lanla.Conteh@osumc.edu
Telephone: +1-614-2936255
Fax: +1-614-2931456

Received: June 2, 2018
Peer-review started: June 2, 2018
First decision: July 9, 2018
Revised: July 23, 2018
Accepted: August 21, 2018
Article in press: August 21, 2018
Published online: November 27, 2018

Abstract

AIM

To determine if racial disparities continue to exist in the treatment of hepatocellular carcinoma (HCC).

METHODS

A retrospective database analysis using the Nationwide Inpatient Sample was performed including patients with a primary diagnosis of HCC. Univariate and multivariate analyses were utilized to determine racial disparities in liver decompensation, treatment, inpatient mortality, and metastatic disease.

RESULTS

A total of 62604 patients with HCC were included consisting of 32428 Caucasian, 9726 African-American, 8988 Hispanic, and 11462 patients of other races. Caucasian patients were more likely to undergo curative therapies of liver transplant (OR: 2.66, 95%CI: 1.92-3.68), resection (OR: 1.82, 95%CI: 1.48-2.23), and ablation (OR: 1.77, 95%CI: 1.36-2.30) than African-American patients. Hispanic patients were more likely to undergo transplant (OR: 2.18, 95%CI: 1.40-3.39) and ablation (OR: 1.46, 95%CI:

1.05-2.03) than African-American patients. Patients of other races were more likely to receive a liver transplant (OR: 2.41, 95%CI: 1.62-3.61), resection (OR: 1.79 95%CI: 1.39-2.32), and ablation (OR: 2.03, 95%CI: 1.47-2.80) than African-American patients. There are no differences in the rates of transarterial chemoembolization between races.

CONCLUSION

Racial disparities in HCC treatment exist despite emphasis to support equality in healthcare. African-American patients are less likely to undergo curative treatments for HCC.

Key words: Racial disparity; Hepatocellular carcinoma; Liver transplantation; Resection; Ablation

Core tip: Racial disparities in the treatment of hepatocellular carcinoma (HCC) have been noted previously. This study investigated continued disparities in healthcare utilizing the Nationwide Inpatient Sample. African-American patients were less likely to undergo curative treatments, such as liver transplantation, liver resection, ablation, and transarterial chemoablation for HCC despite having less features of liver decompensation.

Sobotka LA, Hinton A, Conteh LF. African Americans are less likely to receive curative treatment for hepatocellular carcinoma. *World J Hepatol* 2018; 10(11): 849-855 Available from: URL: http://www.wjgnet.com/1948-5182/full/v10/i11/849.htm DOI: http://dx.doi.org/10.4254/wjh.v10.i11.849

INTRODUCTION

The incidence of hepatocellular carcinoma (HCC) has increased about 50% since 2003[1,2]. An increasing incidence across all races and ethnic groups has been noted, however the incidence in African-American and Hispanic patients has had the largest increase over the past ten years[1]. The incidence of HCC is two times higher in African-American, American-Indian, Alaskan-Native, and Hispanic patients compared to Caucasian patients[2]. Unlike other malignancies such as prostate cancer, where despite increases in incidence, mortality has actually declined, we have seen a concurrent increase in the mortality of HCC as the incidence increases. The mortality rates are twice as high in African-American patients compared to Caucasian patients[2,3]. A recent study completed using the Surveillance, Epidemiology, and End Results (SEER) database showed the median overall survival of all patients with HCC was 11 mo. However, African-American patients had a significantly worse prognosis compared to Caucasian patients with only a nine-month survival rate[4].

Racial disparities in the treatment of HCC have been highlighted in previous studies. African-American patients who presented with localized disease were less likely to undergo curative therapy with liver transplantation, surgical resection, and ablation compared to Caucasian patients with the same tumor burden[5,6]. Previous studies have also noted that African-American patients were more likely to present with metastatic HCC at the time of diagnosis and were therefore no longer candidates for specific curative treatments[7].

This study aims to investigate continued disparities in the treatment of HCC. We hypothesize that racial disparities will continue to be present despite recent emphasis for equal treatment in healthcare.

MATERIALS AND METHODS

Data source

Utilizing the (Nationwide) Inpatient Sample, which is part of the Healthcare Cost and Utilization Project (HCUP), we performed a retrospective database analysis. The HCUP is one of the largest publically available inpatient databases. Information obtained included primary and secondary diagnoses and procedures, patient demographics, expected payment source, total charges, discharge status, and length of stay[8]. This study is exempt from review from The Ohio State University Institutional Review Board because patient information is de-identified.

Study sample

Utilizing International Classification of Diseases, Ninth Revision, Clinical Modification codes, patient with a primary diagnosis of HCC (ICD-9 155.0) were included in this study. Patients were excluded if they were under the age of 18, or if they had a malignancy in the liver that was not hepatic in origin.

Outcomes of interest

Primary outcomes of interest included treatment disparities in HCC based on race, which was defined as Caucasian, African-American, Hispanic, or other. Specific treatments for HCC that were evaluated included liver transplantation, liver resection, ablation, and transarterial chemoablation (TACE). Secondary outcomes included differences in inpatient mortality, liver decompensation, and metastatic disease.

Covariates

Other variables evaluated included gender, age, insurance provider, region where treatment was received, etiology of cirrhosis, features of liver decompensation, metastatic disease, and comorbidities, defined by the Elixhauser Comorbidity (Table 1)[9] . Modification of the Elixhauser Comorbidity score was performed to exclude liver disease. Features of liver decompensation included ascites, jaundice, and hepatic encephalopathy as previously defined in other studies[10]. These variables

were determined by the appropriate ICD-9 code.

Statistical analysis

Association between race and factors of interest were evaluated using chi square tests. Multivariate regression models were fit for the presence of metastatic HCC, liver decompensation, mortality, and treatment. Terms in each module were determined through backwards selection where hepatitis C, hepatitis B, alcohol, non-alcoholic steatohepatitis (NASH), primary sclerosing cholangitis, primary biliary cirrhosis, autoimmune liver disease, metastasis, Elixhauser comorbidity score, and treatment were eligible for inclusion where appropriate. Data was analyzed using SAS software (version 9.4).

RESULTS

Demographics

There were 62604 patients with a primary diagnosis of HCC included in this study. The majority of the patients were Caucasian (32428, 52%) followed by African American (9726, 16%), Hispanic (8988, 14%), and patients of other races (11462, 18%).

Liver severity, metastatic HCC, and inpatient mortality

Upon univariate analysis, features of liver decompensation were significantly different between races ($P < 0.001$) (Table 1). Multivariate analysis demonstrated that Caucasian and Hispanic patients were more likely to have decompensated liver disease than African-American patients [(OR: 1.16, 95%CI: 1.03-1.30), (OR: 1.28, 95%CI: 1.10-1.30)] (Table 2).

Univariate analysis concluded the presence of metastatic disease was significantly different between races ($P = 0.007$) (Table 1). Upon multivariate analysis, Caucasian patients were less likely to have metastatic disease then African-American patients with HCC (OR: 0.82, 95%CI: 0.71-0.94). There was no statistical difference between other races (Table 2).

Inpatient mortality was significantly different between races upon univariate analysis ($P = 0.017$) (Table 1). Upon multivariate analysis, Caucasian patients were less likely to have inpatient mortality compared to African-American patients (OR: 0.78, 95%CI: 0.65-0.93). There was no statistical difference between other races (Table 2).

Inpatient treatment of HCC

There was a significant difference in treatment between races in the univariate analysis ($P < 0.001$) (Table 1). Upon stepwise multivariate analysis, Caucasian, Hispanic, and patients of other races were more likely to undergo liver transplantation compared to African-American patients [(OR: 2.66, 95%CI: 1.92-3.68), (OR: 2.18, 95%CI: 1.40-3.39), (OR: 2.41, 95%CI: 1.62-3.61)]. Caucasian patients and patients of other races were also more likely to undergo surgical resection than African-American patients (OR: 1.82, 95%CI: 1.48-2.23), (OR: 1.79, 95%CI: 1.39-2.32). Caucasian, Hispanic, and patients of other races were more likely to undergo ablation compared to African-American patients (OR: 1.77, 95%CI: 1.36-2.30), (OR 1.46, 95%CI: 1.05-2.03), (OR: 2.03, 95%CI: 1.47, 2.80)]. There was no significant difference in the rates of TACE between races (Table 3).

DISCUSSION

African-American patients are less likely to undergo curative treatments for HCC, and this study confirms that treatment disparities continue to exist despite efforts to reduce healthcare disparities. TACE was the only treatment without a disparity in utilization between races. TACE, however, is not considered to be curative for HCC. It is a means of controlling the malignancy and is often used to downstage tumor burden for liver transplantation or to keep tumor burden within transplant criteria. Differences in treatment exist despite African-American patients being less likely to present with decompensated disease compared to Caucasian patients. We know that patients whose disease is better compensated have a better tolerance of liver directed therapies for HCC. African-American patients have increased rates of metastatic disease and higher inpatient mortality. There are multiple factors that contribute to racial disparities in the management of HCC, including disease progression at time of diagnosis and social determinants of health. It is crucial to recognize these factors and their associations in order to formulate interventions to reduce racial disparities in treatment given the direct effect on patient survival and quality of life.

African-American patients are less likely to undergo curative treatments for HCC and many factors influence this disparity with the presence of metastatic disease being one major limitation to treatment[11]. African-American patients were more likely to have metastatic disease at the time of initial diagnosis[7]. This may be influenced by decreased HCC screening exams in African-American patients[12] and also by genetic differences between races. The presence of metastatic disease is not the only contributor to treatment discrepancies, however. Previous studies have found that African-American patients were less likely to undergo surgical treatment for HCC than Caucasian patients even when they presented with the same tumor burden and both groups were within Milan criteria[13].

Social factors, specifically the location in which patients receive therapy play a crucial role when considering treatment for patients with HCC. This study showed that the majority of African-American patients receive care in the Southern region of the United States, and patients living in the Southern United States are less likely to undergo curative therapies of liver transplantation and surgical resection[14,15]. Decreased access to providers who are able to provide timely diagnosis and treatment contributes to this disparity because of the increased rates of physician and hospital bed inequality in the South compared to the North. This makes it more challenging for patients in these areas

Table 1 Demographics and clinical parameters in patients with hepatocellular carcinoma grouped by race

	Caucasian (*n* = 32428)		African-American (*n* = 9726)		Hispanic (*n* = 8988)		Other (*n* = 11462)		*P* value
	n	%	*n*	%	*n*	%	*n*	%	
Age (yr)									< 0.001
⩽ 64	17695	54.6	6980	71.8	5357	59.6	6609	57.7	
65-79	10982	33.9	2287	23.5	2888	32.1	3762	32.8	
⩾ 80	3751	11.6	458	4.7	743	8.3	1090	9.5	
Sex									0.880
Male	23845	73.5	7172	73.7	6572	73.1	8319	72.7	
Female	8583	26.5	2554	26.3	2416	26.9	3122	27.3	
Primary payer									< 0.001
Medicare	15765	48.9	3471	35.9	3645	40.6	4520	39.5	
Medicaid	4155	12.9	2707	27.9	2206	24.5	2573	22.5	
Private insurance	9497	29.4	2236	23.1	2020	22.5	3143	27.5	
Other	2844	8.8	1267	13.1	1118	12.4	1202	10.5	
Geographic region									< 0.001
Northeast	7726	23.8	2603	26.8	1829	20.4	2395	20.9	
Midwest	5904	18.2	1785	18.4	536	5.9	3016	26.3	
South	13086	40.4	4454	45.8	3126	34.8	2102	18.3	
West	5712	17.6	883	9.1	3497	38.9	3950	34.5	
Discharge year									0.917
2010	8180	25.2	2194	22.6	2223	24.7	3162	27.6	
2011	8118	25.0	2457	25.3	2386	26.5	2980	26.0	
2012	7910	24.4	2490	25.6	2170	24.1	2660	23.2	
2013	8220	25.4	2585	26.6	2210	24.6	2660	23.2	
Hepatitis C	5056	15.6	2497	25.7	1518	16.9	1737	15.2	< 0.001
Hepatitis B	610	1.9	598	6.2	236	2.6	1975	17.2	< 0.001
Alcohol	5566	17.2	1278	13.2	2023	22.5	1157	10.1	< 0.001
NASH	10802	33.3	3436	35.3	3601	40.1	4140	36.1	< 0.001
Primary sclerosing cholangitis	326	1.0	81	0.8	35	0.4	140	1.2	0.046
Primary biliary cirrhosis	131	0.4	0	0.0	10	0.1	34	0.3	-
Autoimmune	91	0.3	25	0.3	60	0.7	⩽ 10	0.1	0.007
Other	14336	44.2	3818	39.3	2967	33.0	4566	39.9	< 0.001
Liver decompensation features									< .0010
Zero	18388	56.7	5690	58.5	4475	49.8	6827	59.6	
One	8968	27.7	2756	28.3	2846	31.7	3092	27.0	
Two	4074	12.6	1010	10.4	1340	14.9	1242	10.8	
Three or greater	998	3.1	270	2.8	328	3.6	301	2.6	
Metastasis									0.007
None	27328	84.3	7841	80.6	7497	83.4	9567	83.5	
Single site	3945	12.2	1453	14.9	1092	12.2	1516	13.2	
Two or more site	1155	3.6	433	4.5	399	4.4	379	3.3	
Elixhauser comorbidity score									< 0.001
< 3	15593	48.1	4177	43.0	4255	47.3	6642	57.9	
⩾ 3	16835	51.9	5549	57.1	4733	52.7	4821	42.1	
Treatment options									< 0.001
Transplant	1254	3.9	190	1.9	252	2.8	349	3.1	
Resection	4306	13.3	823	8.5	728	8.1	1644	14.3	
Ablation	2031	6.3	425	4.4	517	5.8	833	7.3	
TACE	2419	7.5	754	7.8	836	9.3	939	8.2	
Noninvasive treatment	22418	69.1	7534	77.5	6656	74.1	7697	67.2	
In hospital mortality	2924	9.0	1120	11.5	928	10.3	1151	10.1	0.017

TACE: Transarterial chemoablation; NASH: Non-alcoholic steatohepatitis.

to access a Hepatologist and a hospital that is better equipped to meet their needs[16].

Racial disparities in the utilization of TACE was not noted in this study, though previous studies have determined a discrepancy in Native-American patients and Hispanic patients compared to Caucasian patients[17]. This intervention may be considered more frequently in African-American patients because of increased frequency of the disease burden outside of Milan criteria. TACE is considered to be a first line treatment for large or multifocal HCC[18] and may be the ideal treatment for many African-American patients. However, it should be noted that TACE is not considered curative therapy for HCC. The question of whether it is being offered as the sole treatment option for patients who would otherwise be candidates for curative therapy should be raised.

The cost of intervention also influences treatment options. This study shows that African-American patients are the largest percentage of patients with Medicaid insurance compared to other races. Previous analysis on

Table 2 Multivariate logistic regressions comparing outcomes of hepatocellular carcinoma by race

Outcome	Race	Adjusted odds ratio	95%CI
Metastatic Hepatocellular Carcinoma[1]	African-American	Reference	
	Caucasian	0.82	0.71-0.94
	Hispanic	0.87	0.73-1.05
	Other/unknown	0.84	0.70-1.001
Liver Decompensation[2]	African-American	Reference	
	Caucasian	1.16	1.03-1.30
	Hispanic	1.28	1.10-1.51
	Other/unknown	1.14	0.995-1.31
Inpatient Mortality[3]	African-American	Reference	
	Caucasian	0.78	0.65-0.93
	Hispanic	0.82	0.66-1.03
	Other/unknown	0.86	0.69-1.07

[1]Model is adjusted for age, sex, race, geographic region, hepatitis C, alcohol, non-alcoholic steatohepatitis (NASH), liver decompensation features, and Elixhauser comorbidity score; [2]Model is adjusted for age, sex, race, geographic region, hepatitis C, alcohol, NASH, primary biliary cirrhosis, metastasis, and Elixhauser comorbidity score; [3]Model is adjusted for age, sex, race, geographic region hepatitis C, hepatitis B, alcohol, NASH, liver decompensation features, metastasis, and treatment.

Table 3 Multinomial logistic regression to evaluate disparities in treatment for hepatocellular carcinoma based on payer[1, 2]

Intervention	Race	Odds ratio	Confidence interval
Liver Transplant	African-American	Reference	
	Caucasian	2.66	1.92-3.68
	Hispanic	2.18	1.40-3.39
	Other/unknown	2.41	1.62-3.61
Resection	African-American	Reference	
	Caucasian	1.82	1.48-2.23
	Hispanic	1.24	0.94-1.64
	Other/unknown	1.79	1.39-2.32
Ablation	African-American	Reference	
	Caucasian	1.77	1.36-2.30
	Hispanic	1.46	1.05-2.03
	Other/unknown	2.03	1.47-2.80
TACE	African-American	Reference	
	Caucasian	1.15	0.93-1.41
	Hispanic	1.29	0.97-1.72
	Other/unknown	1.19	0.90-1.58

[1]Noninvasive treatment is treated as the reference category; [2]Model adjusts for age, gender, race, geographic region, hepatitis C, hepatitis B, alcohol, non-alcoholic steatohepatitis, liver decompensation features, and Elixhauser comorbidity score. TACE: Transarterial chemoablation.

cost of intervention for HCC has shown TACE to be one of the least costly interventions[19], and therefore may be the intervention most likely to be reimbursed from government funded insurance.

While it is important to recognize racial disparities in the treatment of patients with HCC, it is crucial to recognize the effect this has on an underrepresented patient's quality of life and life expectancy. Studies regarding quality of life in patients with chronic liver disease show decreased functional status and increased chronic, debilitating symptoms such as pain, edema, weakness, anorexia, and vomiting compared to patients without any liver disease[20]. These symptoms, specifically bodily pain and fatigue, are worse in patients with liver disease and HCC[21]. Patients with HCC are noted to have higher rates of depression compared to many other malignancies[22], therefore African-American patients with HCC that fail to undergo treatment are subject to increased complications and diminished quality of life compared to patients that undergo treatment. Life expectancy is also different between patients that undergo treatment for HCC compared to patients that are not treated[23]. If a patient were to undergo treatment, life expectancy and quality of life are improved[24,25].

Multiple interventions could be utilized to reduce disparities in the treatment of HCC. For example, early recognition of liver disease and risk factors for HCC are key to initiate and continue HCC screening in all patients, but specifically in minority patients that may have reduced access to care. This could potentially lead to earlier diagnosis in patients, and therefore the patient would be a candidate for curative treatment.

Limitations in this study must be noted. This data was obtained through the NIS database through ICD-9 coding. Verification of ICD-9 codes could not be obtained for each patient included in the study given patient privacy restrictions. However, these codes have been verified in previous studies. Other factors, specifically

size and number of lesions that affect the treatment for HCC were not included in this study. We were not able to obtain laboratory values and were unable to determine MELD score. Therefore, disease severity was defined by features of liver decompensation. Despite limitations, this study has several strengths. The primary strength was the number of patients that were enrolled in the study over a wide geographic area. Using the NIS database allowed for the collection of a large number of patients that otherwise would not have been obtained in a single institution study.

Disparities in the treatment of HCC based on patient race continue to exist despite emphasis to decrease disparities in healthcare. Despite having decreased rates of liver decompensation, African-American patient have higher rates of inpatient mortality and are less likely to undergo curative treatments, such as liver transplantation, surgical resection, or ablation. Because these patients are less likely to undergo these interventions, African-American patients with HCC are prone to a decreased quality of life and increased mortality rates. Further research needs to be conducted to find ways to decrease this disparity.

ARTICLE HIGHLIGHTS

Research background

Rates of hepatocellular carcinoma (HCC) continue to increase. Despite new treatment options, mortality rates are also increasing specifically in minority patients.

Research motivation

Given recent emphasis to minimize health care disparities, we aimed to determine if racial disparities in the treatment of HCC were decreasing.

Research methods

We performed a retrospective database analysis utilizing The Nationwide Inpatient Sample including patients with a diagnosis of HCC. Univariate and multivariate analyses were utilized to determine racial disparities in liver decompensation, treatment, inpatient mortality, and metastatic disease.

Research results

This large database analysis included 62604 patients with HCC, including 32428 Caucasian, 9726 African-American, 8988 Hispanic, and 11462 patients of other races. Despite having decreased rates of liver decompensation, African-American patient have higher rates of inpatient mortality and are less likely to undergo curative treatments, such as liver transplantation, surgical resection, or ablation than Caucasian patients.

Research conclusions

Racial disparities in HCC treatment exist despite emphasis to support equality in healthcare. African-American patients are less likely to undergo curative treatments for HCC.

Research perspectives

Further emphasis should be placed on determining why disparities continue to exist and hypothesize ways to reduce them in order to facilitate equality in healthcare.

REFERENCES

1 **Mittal S**, El-Serag HB. Epidemiology of hepatocellular carcinoma: consider the population. *J Clin Gastroenterol* 2013; **47** Suppl: S2-S6 [PMID: 23632345 DOI: 10.1097/MCG.0b013e3182872f29]

2 **American Cancer Society**. Atlanta: American Cancer Society, 2016

3 **Nguyen GC**, Thuluvath PJ. Racial disparity in liver disease: Biological, cultural, or socioeconomic factors. *Hepatology* 2008; **47**: 1058-1066 [PMID: 18302296 DOI: 10.1002/hep.22223]

4 **Xu L**, Kim Y, Spolverato G, Gani F, Pawlik TM. Racial disparities in treatment and survival of patients with hepatocellular carcinoma in the United States. *Hepatobiliary Surg Nutr* 2016; **5**: 43-52 [PMID: 26904556 DOI: 10.3978/j.issn.2304-3881.2015.08.05]

5 **Crissien AM**, Frenette C. Current management of hepatocellular carcinoma. *Gastroenterol Hepatol* (NY) 2014; **10**: 153-161 [PMID: 24829542]

6 **Zak Y**, Rhoads KF, Visser BC. Predictors of surgical intervention for hepatocellular carcinoma: race, socioeconomic status, and hospital type. *Arch Surg* 2011; **146**: 778-784 [PMID: 21422327 DOI: 10.1001/archsurg.2011.37]

7 **Sloane D**, Chen H, Howell C. Racial disparity in primary hepatocellular carcinoma: tumor stage at presentation, surgical treatment and survival. *J Natl Med Assoc* 2006; **98**: 1934-1939 [PMID: 17225837]

8 **Overview of the National (Nationwide) Inpatient Sample (NIS).** [Accessed 2016 March 31]. Available from: URL: https://www.hcup-us.ahrq.gov/nisoverview.jsp

9 **Elixhauser A**, Steiner C, Harris DR, Coffey RM. Comorbidity measures for use with administrative data. *Med Care* 1998; **36**: 8-27 [PMID: 9431328 DOI: 10.1097/00005650-199801000-00004]

10 **Cauble S**, Abbas A, Balart L, Bazzano L, Medvedev S, Shores N. United States women receive more curative treatment for hepatocellular carcinoma than men. *Dig Dis Sci* 2013; **58**: 2817-2825 [PMID: 23812858 DOI: 10.1007/s10620-013-2731-9]

11 **Soriano A**, Varona A, Gianchandani R, Moneva ME, Arranz J, Gonzalez A, Barrera M. Selection of patients with hepatocellular carcinoma for liver transplantation: Past and future. *World J Hepatol* 2016; **8**: 58-68 [PMID: 26783421 DOI: 10.4254/wjh.v8.i1.58]

12 **Harlan LC**, Parsons HM, Wiggins CL, Stevens JL, Patt YZ. Treatment of hepatocellular carcinoma in the community: disparities in standard therapy. *Liver Cancer* 2015; **4**: 70-83 [PMID: 26020030 DOI: 10.1159/000367729]

13 **Artinyan A**, Mailey B, Sanchez-Luege N, Khalili J, Sun CL, Bhatia S, Wagman LD, Nissen N, Colquhoun SD, Kim J. Race, ethnicity, and socioeconomic status influence the survival of patients with hepatocellular carcinoma in the United States. *Cancer* 2010; **116**: 1367-1377 [PMID: 20101732 DOI: 10.1002/cncr.24817]

14 **Artiga S,** Damico A. Health and health coverage in the south: A data update. [accessed 2016 February 10]. Available from: URL: http://kff.org/disparities-policy/issue-brief/health-and-health-coverage-in-the-south-a-data-update/

15 **Rana A**, Kaplan B, Riaz IB, Porubsky M, Habib S, Rilo H, Gruessner AC, Gruessner RW. Geographic inequities in liver allograft supply and demand: does it affect patient outcomes? *Transplantation* 2015; **99**: 515-520 [PMID: 25700168 DOI: 10.1097/TP.0000000000000372]

16 **Horev T**, Pesis-Katz I, Mukamel DB. Trends in geographic disparities in allocation of health care resources in the US. *Health Policy* 2004; **68**: 223-232 [PMID: 15063021 DOI: 10.1016/j.healthpol.2003.09.011]

17 **Alkhalili E**, Greenbaum A, Luo L, Rodriguez R, Munoz OE, O'Neill J, Nir I, Morris KT. Racial disparities in treatment and survival of hepatocellular carcinoma in native Americans and Hispanics. *Am J Surg* 2017; **214**: 100-104 [PMID: 28624027 DOI: 10.1016/j.amjsurg.2016.09.033]

18 **Bruix J**, Sherman M; American Association for the Study of Liver Diseases. Management of hepatocellular carcinoma: an update. *Hepatology* 2011; **53**: 1020-1022 [PMID: 21374666 DOI: 10.1002/hep.24199]

19 **Ray CE Jr**, Battaglia C, Libby AM, Prochazka A, Xu S, Funaki B.

Interventional radiologic treatment of hepatocellular carcinoma-a cost analysis from the payer perspective. *J Vasc Interv Radiol* 2012; **23**: 306-314 [PMID: 22277271 DOI: 10.1016/j.jvir.2011.11.016]
20 **Sun VC**, Sarna L. Symptom management in hepatocellular carcinoma. *Clin J Oncol Nurs* 2008; **12**: 759-766 [PMID: 18842532 DOI: 10.1188/08.CJON.759-766]
21 **Bianchi G**, Loguercio C, Sgarbi D, Abbiati R, Brunetti N, De Simone T, Zoli M, Marchesini G. Reduced quality of life of patients with hepatocellular carcinoma. *Dig Liver Dis* 2003; **35**: 46-54 [PMID: 12725608 DOI: 10.1016/S1590-8658(02)00011-7]
22 **Zabora J**, BrintzenhofeSzoc K, Curbow B, Hooker C, Piantadosi S. The prevalence of psychological distress by cancer site. *Psychooncology* 2001; **10**: 19-28 [PMID: 11180574 DOI: 10.1002/1099-1611(200101/02)10:1<19::AID-PON501>3.0.CO;2-6]
23 **Schneider H**. Special problems in the use of respirators in the anesthesia of newborn infants and small children. *Anaesthesist* 1966; **15**: 118-120 [PMID: 5234419 DOI: 10.1002/hep.27443]
24 **Poon RT**, Fan ST, Yu WC, Lam BK, Chan FY, Wong J. A prospective longitudinal study of quality of life after resection of hepatocellular carcinoma. *Arch Surg* 2001; **136**: 693-699 [PMID: 11387012 DOI: 10.1001/archsurg.136.6.693]
25 **Eltawil KM**, Berry R, Abdolell M, Molinari M. Quality of life and survival analysis of patients undergoing transarterial chemoembolization for primary hepatic malignancies: a prospective cohort study. *HPB* (Oxford) 2012; **14**: 341-350 [PMID: 22487072 DOI: 10.1111/j.1477-2574.2012.00455.x]

P- Reviewer: Bramhall S, Chiu KW, Kinoshita A, Niu ZS, Tomizawa M
S- Editor: Cui LJ **L- Editor**: Filipodia **E- Editor**: Bian YN

World Journal of Hepatology

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

World J Hepatol 2018 November 27; 10(11): 856-866

DOI: 10.4254/wjh.v10.i11.856

ISSN 1948-5182 (online)

ORIGINAL ARTICLE

Observational Study

Factors associated with DAA virological treatment failure and resistance-associated substitutions description in HIV/HCV coinfected patients

Dominique Salmon, Pascale Trimoulet, Camille Gilbert, Caroline Solas, Eva Lafourcade, Julie Chas, Lionel Piroth, Karine Lacombe, Christine Katlama, Gilles Peytavin, Hugues Aumaitre, Laurent Alric, François Boué, Philippe Morlat, Isabelle Poizot-Martin, Eric Billaud, Eric Rosenthal, Alissa Naqvi, Patrick Miailhes, Firouzé Bani-Sadr, Laure Esterle, Patrizia Carrieri, François Dabis, Philippe Sogni, Linda Wittkop; ANRS CO13 Hepavih study group

Dominique Salmon, Assistance Publique des Hôpitaux de Paris, Hôpitaux Universitaires Paris Centre, Hôpital Hôtel Dieu, Unité des Maladies infectieuses et tropicales, Paris 75004, France

Dominique Salmon, Université Paris Descartes, Sorbonne Paris Cité, Paris 75006, France

Pascale Trimoulet, CHU de Bordeaux, Hôpital Pellegrin, Laboratoire de Virologie, Bordeaux 33000, France

Pascale Trimoulet, CNRS-UMR 5234, Microbiologie fondamentale et Pathogénicité, Université de Bordeaux, Bordeaux 3000, France

Camille Gilbert, Eva Lafourcade, Philippe Morlat, Laure Esterle, François Dabis, Linda Wittkop, Univ. Bordeaux, ISPED, Inserm, Bordeaux Population Health Research Center, team MORPH3EUS, UMR 1219, CIC-EC 1401, Bordeaux F-33000, France

Caroline Solas, APHM, Hôpital La Timone, Laboratoire de Pharmacocinétique et Toxicologie, Marseille 13005, France

Julie Chas, Assistance Publique des Hôpitaux de Paris, Hôpital Tenon, Service Maladies infectieuses et tropicales, Paris 75020, France

Lionel Piroth, Centre Hospitalier Universitaire de Dijon, Département d'Infectiologie, Dijon cedex 21079, France

Lionel Piroth, INSERM-CIC 1342 Université de Bourgogne, Dijon 21000, France

Karine Lacombe, Assistance Publique des Hôpitaux de Paris, GHUEP site Saint-Antoine, Services Maladies infectieuses et tropicales, Paris 75011, France

Karine Lacombe, Université Pierre et Marie Curie, UMR S1136, Institut Pierre Louis d'Epidémiologie et de Santé Publique, Paris 75646, France

Christine Katlama, Université Paris-Sorbonne, Paris 75005, France

Christine Katlama, Assistance Publique des Hôpitaux de Paris Hôpital Pitié Salpêtrière, Services Maladies infectieuses et tropicales, Paris 75013, France

Gilles Peytavin, Assistance Publique des Hôpitaux de Paris, Hôpital Bichat-Claude Bernard, Laboratoire de Pharmacologie, Paris 75877, France

Gilles Peytavin, IAME, UMR 1137, Sorbonne Paris Cité, INSERM, Université Paris Diderot, Paris 75890, France

Hugues Aumaitre, Centre Hospitalier de Perpignan, Service Maladies infectieuses et tropicales, Perpignan 66000, France

Laurent Alric, Centre Hospitalier Universitaire de Toulouse, Hôpital Purpan, Service Médecine interne-Pôle Digestif, Toulouse 31300, France

Laurent Alric, UMR 152 IRD Université Toulouse Ⅲ, Paul Sabatier, Toulouse 31330, France

François Boué, Hôpital Antoine-Béclère, Assistance Publique des Hôpitaux de Paris, Université Paris Sud, Service Médecine interne et immunologie, Clamart 92140, France

Philippe Morlat, Centre Hospitalier Universitaire de Bordeaux, Service de médecine interne, Hôpital Saint-André, Bordeaux 33000, France

Isabelle Poizot-Martin, Aix-Marseille Univ, APHM Sainte-

Marguerite, Service d'Immuno-hématologie clinique, Marseille 13274, France

Isabelle Poizot-Martin, Patrizia Carrieri, Sciences Economiques and Sociales de la Santéand Traitement de l'Information Médicale, UMR912 INSERM, Aix-Marseille Université, IRD, Marseille 13009, France

Eric Billaud, Department of Infectious Diseases, CHU de Nantes and CIC 1413, Inserm, Nantes 44000, France

Eric Rosenthal, Centre Hospitalier Universitaire de Nice, Service de Médecine Interne, Hôpital l'Archet, Nice 06202, France

Eric Rosenthal, Université de Nice-Sophia Antipolis, Nice 06100, France

Alissa Naqvi, Centre Hospitalier Universitaire de Nice, Service d' Infectiologie, Hôpital l'Archet, Nice 06100, France

Patrick Miailhes, Service des Maladies Infectieuses et Tropicales, Hospices Civils de Lyon, Hôpital de la Croix Rousse, Lyon 69004, France

Firouzé Bani-Sadr, Centre Hospitalier Universitaire de Reims, Service de Médecine Interne, Maladies Infectieuses et Immunologie Clinique, Reims 51100, France

Firouzé Bani-Sadr, Faculté de Médecine EA-4684/SFR CAP-SANTE, Université de Reims, Champagne-Ardenne, Reims 51100, France

Philippe Sogni, Assistance Publique des Hôpitaux de Paris, Hôpital Cochin, Service d'Hépatologie, Paris 75014, France

Philippe Sogni, Inserm U-1223 - Institut Pasteur, Paris 75015, France

Linda Wittkop, CHU de Bordeaux, Pôle de santé Publique, Service d'information médicale, Bordeaux F-33000, France

ORCID number: Dominique Salmon (0000-0002-6817-8951); Pascale Trimoulet (0000-0002-8371-381X); Camille Gilbert (0000-0003-3959-6174); Caroline Solas (0000-0002-0943-9648); Eva Lafourcade (0000-0001-8537-4201); Julie Chas (0000-0002-1001-9229); Lionel Piroth (0000-0003-4478-1032); Karine Lacombe (0000-0001-8772-9029); Christine Katlama (0000-0002-5862-3863); Gilles Peytavin (0000-0002-4359-537X); Hugues Aumaitre (0000-0002-0023-7652); Laurent Alric (0000-0003-0676-7539); François Boué (0000-0003-0161-4533); Philippe Morlat (0000-0001-6474-383X); Isabelle Poizot-Martin (0000-0002-5676-5411); Eric Billaud (0000-0002-3420-1228); Eric Rosenthal (0000-0003-1010-0964); Alissa Naqvi (0000-0001-6474-383X); Patrick Miailhes (0000-0002-7979-3829); Firouzé Bani-Sadr (0000-0001-8268-866X); Laure Esterle (0000-0002-1017-1327); Patrizia Carrieri (0000-0002-6794-4837); François Dabis (0000-0002-1614-8857); Philippe Sogni (0000-0003-3316-8785); Linda Wittkop (0000-0003-2403-0960).

Author contributions: All the authors contributed to this work

Supported by Inserm-ANRS (French National Institute for Health and Medical Research - ANRS/France REcherche Nord and Sud Sida-hiv Hépatites).

Institutional review board statement: The study was approved by the Institutional Review Board Ile de France Ⅲ, Paris, France

Informed consent statement: A written informed consent was obtained from each participant to the study.

Conflict-of-interest statement: Dominique Salmon has been speaker and received invitation to conferences by Gilead, Abott, and MSD. Laurent Alric received grant and personal fees from MSD, Gilead, Abbvie, Janssen and BMS outside the submitted work. Christine Katlama received consultancy fees and/or travel grants from MSD, Janssen, ViiV outside the submitted work. Karine Lacombe personal fees from Gilead, personal fees from Janssen, personal fees from Abbvie, personal fees from Merck outside the submitted work. Philippe Morlat received personal fees and non-financial support from GILEAD, Janssen, MSD and ViiV Health Care outside the submitted work. Gilles Peytavin received travel grants, consultancy fees or study grants from pharmaceutical companies including Abbvie, Bristol-Myers Squibb, Gilead sciences, Janssen, Merck and ViiV Healthcare outside the submitted work. Eric Rosenthal received personal fees from Gilead and Abbvie and travel grants, consultancy fees from Gilead, Abbvie, MSD and BMS outside the submitted work. Philippe Sogni received personal fees and non-financial support from Gilead, BMS, MSD Abvie outside the submitted work. Caroline Solas received personal fees from Gilead, Abbvie, Janssen, MSD and ViiV Healthcare outside the submitted work. Linda Wittkop reports grants from ANRS during the conduct of the study; personal fees from Janssen, Gilead, MSD, outside the submitted work. Other authors had nothing to declare.

STROBE statement: The guidelines of the STROBE Statement have been adopted. The authors have read the STROBE Statement-checklist of items, and the manuscript was prepared and revised according to the STROBE Statement-checklist of items.

Manuscript source: Unsolicited manuscript

Corresponding author to: Dominique Salmon, MD, PhD, Professor, Assistance Publique des Hôpitaux de Paris, Hôpitaux Universitaires Paris Centre, Hôpital Hôtel Dieu, Unité des Maladies infectieuses et tropicales, Sorbonne Paris Cité, 1 place du Parvis Notre-Dame, Paris 75004, France. dominique.salmon@aphp.fr
Telephone: +33-1-42347956
Fax: +33-1-42348852

Received: June 15, 2018
Peer-review started: June 15, 2018
First decision: July 9, 2018
Revised: September 10, 2018
Accepted: October 10, 2018
Article in press: October 10, 2018
Published online: November 27, 2018

Abstract

AIM

To describe factors associated with treatment failure and frequency of resistance-associated substitutions (RAS).

METHODS

Human immunodeficiency virus (HIV)/hepatitis C virus (HCV) coinfected patients starting a first direct-acting antiviral (DAA) regimen before February 2016 and included in the French ANRS CO13 HEPAVIH cohort were eligible. Failure was defined as: (1) non-response [HCV-RNA remained detectable during treatment, at end of treatment (EOT)]; and (2) relapse (HCV-RNA suppressed at EOT but detectable thereafter). Sequencing analysis was performed to describe prevalence of drug class-specific RAS. Factors associated with failure were determined using logistic regression models.

RESULTS

Among 559 patients, 77% had suppressed plasma HIV-RNA < 50 copies/mL at DAA treatment initiation, 41% were cirrhotic, and 68% were HCV treatment-experienced. Virological treatment failures occurred in 22 patients and were mainly relapses (17, 77%) then undefined failures (3, 14%) and non-responses (2, 9%). Mean treatment duration was 16 wk overall. Post-treatment NS3, NS5A or NS5B RAS were detected in 10/14 patients with samples available for sequencing analysis. After adjustment for age, sex, ribavirin use, HCV genotype and treatment duration, low platelet count was the only factor significantly associated with a higher risk of failure (OR: 6.5; 95%CI: 1.8-22.6).

CONCLUSION

Only 3.9% HIV-HCV coinfected patients failed DAA regimens and RAS were found in 70% of those failing. Low platelet count was independently associated with virological failure.

Key words: Human immunodeficiency virus; Hepatitis C virus; Direct-acting antiviral; Treatment virological failure; Resistant associated mutations

Core tip: In co-infected human immunodeficiency virus-hepatitis C virus (HCV) patients, after adjustment for age, sex, ribavirin use, HCV genotype and treatment duration, low platelets count was the only factor significantly associated with a higher risk of failure.

Salmon D, Trimoulet P, Gilbert C, Solas C, Lafourcade E, Chas J, Piroth L, Lacombe K, Katlama C, Peytavin G, Aumaitre H, Alric L, Boué F, Morlat P, Poizot-Martin I, Billaud E, Rosenthal E, Naqvi A, Miailhes P, Bani-Sadr F, Esterle L, Carrieri P, Dabis F, Sogni P, Wittkop L; ANRS CO13 Hepavih study group. Factors associated with DAA virological treatment failure and resistance-associated substitutions description in HIV/HCV coinfected patients. *World J Hepatol* 2018; 10(11): 856-866 Available from: URL: http://www.wjgnet.com/1948-5182/full/v10/i11/856.htm DOI: http://dx.doi.org/10.4254/wjh.v10.i11.856

INTRODUCTION

The treatment of hepatitis C virus (HCV) infection had been revolutionized with the recent development of direct-acting antiviral (DAA) combinations. Cure rates of over 90%, similar to those in HCV monoinfected patients, can now be achieved in human immunodeficiency virus (HIV)/HCV coinfected patients. This has been documented in clinical trials[1-5] as well as in real-life cohorts[6-9]. For the few patients failing treatment, resistance-associated substitutions (RAS) can emerge and emerging resistant strains appearing at viral rebound are a consequence rather than a cause of failure[10,11].

The real causes of failure to all-oral DAA regimens can be multiple. Several social and medical factors can jeopardize treatment adherence. Some first-generation regimens may not be optimal to treat difficult cases of hepatitis C, such as decompensated cirrhosis or genotype 3 HCV infection. In rare circumstances, especially for genotype 1a viruses, baseline mutations in the non-structural-5A (*NS5A*) gene can preexist in the viral species before treatment introduction and may have a potentially deleterious impact on sustained virological response (SVR)[12]. Drug-drug interactions between DAA and ARV therapy or other commonly prescribed medications in HIV/HCV coinfected patients are frequent and can decrease drug levels, thereby reducing the efficacy of therapy. Finally, adverse events, although rare with new DAA combinations, can occur and lead to treatment interruption and thus to treatment failure.

We aimed to describe the characteristics of patients failing first-line DAA treatment in the real-life French nationwide ANRS CO13 HEPAVIH cohort of HIV/HCV coinfected patients. Furthermore, we described the emergence of clinically relevant RAS to DAA classes upon DAA treatment failure, and report pharmacological drug monitoring results. Finally, we identified factors associated with the occurrence of virological treatment failure.

MATERIALS AND METHODS

Study population

The ANRS CO13 HEPAVIH cohort (ClinicalTrials.gov Identifier: NCT03324633) is a national multi-centre prospective hospital-based observational study of patients coinfected with HIV and viral hepatitis C that received approval by an Institutional Review board [Comité de Protection des Personnes (CPP) Ile de France Ⅲ, Paris, France].

All patients included in the cohort gave their consent

for study participation. In addition, patients from the 29 centers participating in the ANRS CO13 HEPAVIH cohort, who weren't included in the cohort but who gave their consent for specific follow-up during and after DAA treatment, were also eligible. For this sub-study, patients were included if they had started an all-oral DAA-based regimen before January 2016 (3 mo treatment), February 2016 (2 mo treatment) or October 2015 (6 mo treatment). Patients who participated in completed and published clinical trials were included in the analysis. We did not include patients who were participating in an ongoing clinical trial (including those completed but not yet published), patients who were treated with combinations including Peg-interferon (PegIFN), or with the sofosbuvir (SOF) + ribavirin (RBV) combination. Patients with premature treatment interruption for intolerance or death were also excluded because we were specifically interested in a virological outcome. The DAA regimen was at the discretion of the patient's physician[13-15].

Data collection and definitions

The following data were collected prospectively by each participating center, using an eCRF: Age, sex, risk factors for both HIV and HCV infections, HCV genotype, previous anti-HCV treatment, HIV-related characteristics, start and end dates of DAA treatment, initial doses of anti-HCV and anti-HIV drugs, any changes during follow-up, and HCV-RNA at each time point [baseline, week (W)2, W4, W8, W12 if treatment duration was 24 wk, EOT, follow-up W4 (FU-W4) and FU-W12]. Virological treatment failures were categorized as: (1) Non-response: HCV-RNA never undetectable during treatment; (2) Relapse: HCV-RNA undetectable at EOT and then detectable within the following 12 wk; and (3) Undefined failure: HCV-RNA unknown at end of treatment (EOT) and positive thereafter, without premature discontinuation of treatment. Cirrhotic status was based on liver biopsy (METAVIR fibrosis stage F4), liver stiffness $\geqslant$ 12.5 kPa (FibroScan®; Echosens, France), a FibroTest® value $\geqslant$ 0.75 (Biopredictive, France) or physical and biological signs of end-stage liver disease, as previously published[16,17].

Sequencing analysis

Patients with virological treatment failure, who provided specific consent for HCV genotype testing and who had HCV-RNA > 1000 IU/mL at the sequencing time point were included for HCV testing. Prevalence of drug class-specific RAS was evaluated at failure. The HCV NS3, NS5A and/or NS5B domains were amplified by reverse transcriptase nested polymerase chain reaction (PCR) using genotype and subtype-specific PCR primers to ensure successful amplification of the target gene(s). PCR products were purified and analyzed by population sequencing using an automated sequencer (ABI-3500xL Dx). The cutoff frequency for detecting variants with Sanger sequencing was approximately 15%. Sanger-derived sequences were aligned with Clustal_W, version 1.74 (Conway Institute UCD, Dublin, Ireland). NS3, NS5A and NS5B RAS were defined as clinically relevant when inducing > 10-fold resistance to DAA[13,18-20].

Drug concentrations

Plasma drug concentrations for DAA and RBV were collected, when available, for patients included in the cohort as part of routine therapeutic drug monitoring performed in several centers. Drug concentrations were measured using liquid chromatography coupled with the tandem mass spectrometry method[21]. Data were considered interpretable if concentrations were determined at steady-state and information regarding the time of the last drug intake was available.

A suboptimal concentration was defined as below the 2 μg/mL threshold for RBV[22,23], and when concentrations were below the reported expected range for DAA[24-27].

Statistical analysis

We included all patients who met the inclusion criteria, as described in the study population section. Variables are described as number and percentages, or median and IQR [or mean (SD)], as appropriate. Patient characteristics are reported upon initiation of DAA treatment. The Wilcoxon-Mann-Whitney test and Fisher's exact test were used to compare quantitative and qualitative variables between groups, respectively. Factors associated with virological treatment failure were determined using logistic regression models. In order to identify new independent predictors of virological treatment failure, we systematically adjusted for a fixed set of potential confounders based on literature reports. The following variables were thus forced in all models: age, sex, RBV use, and prescribed treatment duration[28]. We then tested the following variables in the model containing the forced variables: HCV genotype (3 *vs* others), cirrhosis (Yes *vs* No), severe cirrhosis (Yes *vs* No, and defined by a B or C or an elastometry value $\geqslant$ 20 kPa), plasma HIV-RNA (detectable *vs* undetectable), and platelet count (< 100 Giga/L *vs* $\geqslant$ 100 Giga/L). The effect of RBV on virological treatment failure and other potential factors was assessed by a marginal structural model (MSM) in order to consider a potential indication bias for the prescription of RBV. Sensitivity analyses, including patients with premature treatment discontinuations for intolerance/death, were also performed. The statistical methods of this study were reviewed by Linda Wittkop from Bordeaux Population Health Research Center, Bordeaux. SAS software version 9.4 (SAS Institute Inc., Cary, North Carolina) was used for all analyses.

RESULTS

General characteristics at DAA initiation

Among 877 patients treated with DAA-combination, 559 met the inclusion criteria and were included in the analysis (318 were not included for the following reasons: Treatment with PegIFN (n = 30), inclusion

Table 1 Patient characteristics at treatment initiation according to virological response

	Overall (n = 559)	SVR (n = 537)	Virological treatment failure (n = 22)	P value
Male sex	431 (77)	414 (77)	17 (77)	0.985
Age (yr)	52 (49-56)	52 (49-56)	53 (51-57)	0.586
CD4 (/mm^3) (n = 557)	618 (426-850)	619 (429-861)	527 (346-704)	0.040
Undetectable HIV-RNA (n = 558)	486 (87)	469 (88)	17 (77)	0.186
ARV treatment	549 (98)	527 (98)	22 (100)	1.000
PI[1]	127 (23)	122 (23)	5 (23)	
NNRTI[2]	98 (18)	95 (18)	3 (14)	
II[3]	204 (37)	197 (37)	7 (32)	
Others	120 (22)	113 (21)	7 (32)	
Active tobacco consumption (n = 263)	153 (58)	148 (58)	5 (71)	0.703
Active alcohol consumption (n = 266)	135 (51)	132 (51)	3 (43)	0.719
Active drug consumption (n = 257)	7 (3)	7 (3)	0 (0)	1.000
HCV genotype (n = 558)				0.475
1 without precision	26 (5)	24 (5)	2 (9)	
1a	232 (42)	221 (41)	11 (50)	
1b	64 (12)	64 (12)	0 (0)	
2	6 (1)	6 (1)	0 (0)	
3	62 (11)	60 (11)	2 (9)	
4	165 (30)	158 (30)	7 (32)	
5	1 (0)	1 (0)	0 (0)	
6	2 (0)	2 (0)	0 (0)	
Cirrhosis (n = 555)	209 (38)	200 (38)	9 (41)	0.748
Child Pugh, if cirrhosis (n = 189)				0.537
A	172 (91)	165 (91)	7 (88)	
B/C	17 (9)	16 (9)	1 (12)	
FIB-4 (n = 405)	2.1 (1.4-3.7)	2.1 (1.4-3.7)	3.3 (1.9-7.3)	0.313
FIB-4 > 3.25 (n = 405)	120 (30)	113 (29)	7 (50)	0.132
Elastometry (kPa) (n = 115)	9 (6-14)	9 (6-14)	10 (6-17)	0.942
Elastometry ⩾ 12.5 kPa (n = 115)	32 (28)	30 (27)	2 (50)	0.309
Elastometry ⩾ 20 kPa (n = 115)	17 (15)	16 (14)	1 (25)	0.478
HCV treatment history				0.570
Naïve	210 (38)	203 (38)	7 (32)	
Pretreated	349 (62)	334 (62)	15 (68)	
HCV viral load (log_{10} IU/mL) (n = 558)	6.09 (5.59-6.51)	6.09 (5.59-6.51)	6.04 (5.72-6.49)	0.886
Prothrombin rate (n = 298)	99 (89-100)	99 (89-100)	92 (82-100)	0.116
Prothrombin rate < 85% (n = 298)	54 (18)	50 (17)	4 (40)	0.087
Platelets (Giga/L) (n = 408)	171 (131-219)	171 (133-219)	148 (97-184)	0.168
Platelets < 100 Giga/L (n = 408)	57 (14)	51 (13)	6 (43)	0.007
Albumin (g/L) (n = 301)	41 (38-44)	41 (38-44)	42 (37-45)	0.939
Albumin < 35 g/L (n = 301)	26 (9)	24 (8)	2 (25)	0.146
DAA-combination				NA[5]
SOF + DCV ± RBV[4]	240 (43)	231 (43)	9 (41)	
SOF/LDV ± RBV	271 (49)	261 (49)	10 (46)	
SOF + SMV ± RBV	26 (4)	23 (4)	3 (14)	
Others[4]	22 (4)	22 (4)	0 (0)	
Mean (SD) DAA treatment durationa	16 (6)	15 (5)	16 (6)	

Results are presented as number (as percentages in brackets) or median (IQR in brackets) unless stated otherwise. [1]PI was boosted in 98 patients with SVR and in five patients with treatment failure; [2]NNRTI molecule was rilpivirine in 60 patients with SVR and three with failure, and was efavirenz in 25 patients with SVR; [3]II molecule was raltegravir in 153 patients with SVR and four patients with failure, and was dolutegravir in 38 patients with SVR and two with treatment failure; [4]Initial doses of DCV were 30, 60, 90 mg/d in respectively 57, 159 and 21 patients. The dose was unknown for the five other patients; [5]NA: not applicable, no formal statistical comparison was performed as the prescription of the DAA regimen was chosen by each patient's physician. SVR: Sustained virological response; ARV: Antiretroviral; PI: Protease inhibitor; NNRTI: Non-nucleoside reverse-transcriptase inhibitor; II: Integrase inhibitor; DAA: All-oral direct-acting antiviral; SOF: Sofosbuvir; RBV: Ribavirin; DCV: Daclatasvir; LDV: Ledipasvir; SMV: Simeprevir.

in an ongoing clinical trial (n = 2), treatment after the period of analysis (n = 190), no available treatment result (n = 32), treatment with SOF + RBV (n = 60), premature treatment interruption for intolerance (n = 3), and one patient died while on treatment). Mean treatment duration was 16 wk overall (15 wk in patients who failed DAA therapy and 16 wk in those with SVR). The characteristics of the 559 patients are summarized in Table 1.

Virological treatment failure

The virological treatment failure rate was 3.9% (95%CI: 2.5-5.9). Overall, 22 virological treatment failures were observed: Two non-responses, 17 relapses and three undefined virological treatment failures (HCV-RNA unknown at EOT). By univariate analysis (Table

Table 2 Adjusted logistic regression for factors associated with virological treatment failure

Covariables	Model 1		Model 2		Model 3		Model 4	
	OR (95%CI)	*P* value	OR (95% CI)	*P* value	OR (95%CI)	*P* value	OR (95%CI)	*P* value
	n = 538		*n* = 538		*n* = 526		*n* = 395	
Age at treatment initiation (per 10 yr)	1.2 (0.6-2.4)	0.58	1.3 (0.7-2.5)	0.48	1.2 (0.6-2.4)	0.53	1.6 (0.7-4.0)	0.29
Ribavirin *vs* no ribavirin	1.0 (0.3-3.0)	0.97	1.1 (0.3-3.2)	0.93	1.0 (0.3-3.0)	0.97	1.4 (0.4-5.5)	0.61
Male sex *vs* female	1.0 (0.4-2.8)	0.98	0.9 (0.3-2.7)	0.92	1.0 (0.3-2.8)	0.97	0.8 (0.2-2.7)	0.69
Treatment duration 24 wk *vs* 12 wk	0.4 (0.1-1.4)	0.15	0.5 (0.2-1.5)	0.21	0.4 (0.1-1.4)	0.16	0.2 (0.0-1.0)	0.05
Platelet count < 100 Giga/L *vs* ⩾ 100							6.5 (1.8-22.6)	0.004
Cirrhosis *vs* no cirrhosis	1.4 (0.5-3.9)	0.51						
HIV-RNA detectable *vs* undetectable			2.1 (0.7-5.9)	0.17				
Severe cirrhosis *vs* no severe cirrhosis					2.1 (0.4-10.3)	0.35		
HCV genotype 3 *vs* others							0.9 (0.1-7.5)	0.91

HCV: Hepatitis C virus; HIV: Human immunodeficiency virus.

1), patients with virological treatment failure had a significantly lower CD4 cell count (median 527 cells/mm^3) compared to patients with SVR (619 cells/mm^3; P = 0.040). They also more frequently had a platelet count below 100 Giga/L (P = 0.007) and a trend for more frequently having a prothrombin time < 85% (40% *vs* 17%, P = 0.087) and albumin < 35 g/L (25% *vs* 8%, P = 0.146). They also had a non-significant trend for less frequent HIV-RNA suppression (77% *vs* 88%, P = 0.186).

Factors associated with treatment failure

In adjusted models (Table 2), platelet count < 100 Giga/L was significantly associated with a higher probability of virological treatment failure (Model 4). However, clinical cirrhosis status (Model 1), severe cirrhosis status (Model 3) or blood albumin (data not shown) were not associated with a higher probability of failure. Neither HIV-RNA (Model 2) nor CD4 cell count (data not shown) were associated with virological treatment failure. In addition, in the model containing platelet count, a prescribed treatment duration of 24 wk was associated with a lower risk of virological treatment failure (Model 4). RBV use was not associated with outcome in adjusted logistic regression models, and this result was confirmed by an analysis using MSMs (data not shown).

Sensitivity analyses, including patients with premature treatment discontinuations for intolerance/death, showed similar results (data not shown).

HCV resistance at virological treatment failure

The results of RAS analysis in the 14 patients with virological treatment failure, in whom either mutation NS3, NS5A or NS5B could be sequenced, are presented in Table 3. Almost three quarters of patients with available data (10/14; 71%) had at least one detectable RAS at the time of virological treatment failure. In patients receiving an NS5A inhibitor-based regimen, 55% (6/11) had NS5A RAS upon virological treatment failure. Common substitutions detected at failure included Q30R/H, 30E, 58D and/or Y93C/N, all found in patients with HCV genotype 1. In patients treated with daclatasvir (DCV) and an available NS5A RAS result (n = 5), four patients developed resistance to DCV. Furthermore, among six treated with ledipasvir (LDV) with available NS5A RAS result, two developed resistance to LDV. Overall, in all patients with available genotype (n = 14), six (43%) presented at least one NS5A RAS, leading to a high level of resistance to NS5A inhibitors (> 10-fold resistance). In patients receiving NS3 protease inhibitors, 2/2 patients with available data had NS3 RAS upon virological treatment failure. The substitutions detected at failure were 80K, 170T, 174N and 168V, leading to a high level of resistance to most protease inhibitors.

Multiple RAS conferring a higher level of resistance were detected in three (21%) patients, including two with NS3 + NS5A RAS and one with NS3 + NS5A + NS5B RAS. These three patients were previously treated with PegIFN + RBV and were exposed to NS5A inhibitors but not NS3 inhibitors.

Pharmacological data

Nine of the 22 (41%) patients who had DAA therapy failure had measurements of DAA and/or RBV concentration at W2 or W4 of treatment, seven of which were interpretable. Among these seven patients, suboptimal concentrations were reported in two (29%). These low concentrations concerned either DCV (in a patient treated with SOF + DCV, whose ARV treatment was rilpivirine + raltegravir), or RBV (in a patient treated with SOF/LDV + RBV who was taking rilpivirine + dolutegravir).

DISCUSSION

In this cohort of HIV/HCV coinfected patients, who were treated with an interferon-free DAA regimen with or without RBV, we report a low virological treatment failure rate of 3.9%. Our results are similar to those observed in clinical trials[28] or previous real-world studies of HIV/HCV coinfection[6,7]. Most of these virological treatment failures were due to relapse (77%) followed by non-response (9%), while 14% were due to undefined virological treatment failures (HCV-RNA unknown at EOT).

Due to very high rates of SVR, it has been difficult to identify factors associated with virological treatment failure of DAA in real-world studies, and no study to date has focused on HIV coinfection. In studies of HCV

Table 3 Resistance-associated substitution results in 14 patients with virological treatment failure for whom sequencing was performed in routine care

Pat	HCV treatment history	Treatment received	HCV genotype		Cirrhosis	ARV treatment	RAS		
			Before treatment	After treatment			NS3	NS5A	NS5B
A	Pretreated	SOF + SMV 12 wk	1a	1a	Yes	II	Q80K, I170T, S174N	Abs	Abs
B	Pretreated	SOF + SMV 12 wk	1a	1a	Yes	II	D168V	Abs	Abs
C	Pretreated	SOF/LDV 12 wk	4	4a	No	PI	Abs	Abs	Abs
G	Pretreated	SOF/LDV + RBV 12 wk	4	4d	No	Others	Abs	Abs	Abs
H	Pretreated	SOF + DCV 10 wk[3]	4	4	No	Others	Abs	ND	Abs
I	Naive	SOF/LDV 12 wk	1a	1a	No	PI	Abs	Abs	Abs
J	Pretreated	SOF/LDV 12 wk	1a	1a	No	Others	ND	Y93C	Abs
L	Naive	SOF + DCV 13 wk[2]	1a	1a	Yes	NNRTI	Q80K	Abs	Abs
M	Pretreated	SOF/LDV 12 wk	4	4a	No	PI	ND	Abs	A421V, M414L
N	Pretreated	SOF/LDV + RBV 12 wk	1a	1a	Yes	II	A168V	30E, 58D	Abs
P	Pretreated	SOF + DCV + RBV 12 wk[1]	1a	1a	No	PI	Abs	Y93N	Abs
Q	Pretreated	SOF + DCV + RBV 24 wk[1]	1a	1a	No	Others	T54S	Q30R	Abs
R	Pretreated	SOF + DCV 24 wk[2]	1a	1a	Yes	II	Q80K	Y93C	Y448H
W	Pretreated	SOF + DCV 24 wk[1]	1	1a	Yes	II	Abs	Q30H	Abs

[1]Initial dose of DCV: 30 mg/d; [2]Initial dose of DCV: 60 mg/d; [3]Initial dose of DCV: 90 mg/d. Pat: Patient; ARV: Antiretroviral; RAS: Resistance-associated substitution; NS3: Non-structural-3; NS5A: Non-structural-5A; NS5B: Non-structural-5B; SOF: Sofosbuvir; RBV: Ribavirin; DCV: Daclatasvir; LDV: Ledipasvir; SMV: Simeprevir; ND: Not done; PI: Protease inhibitor; NNRTI: Non-nucleoside reverse-transcriptase inhibitor; II: Integrase inhibitor; Abs: No RAS found.

monoinfected patients, however, several factors have been found to be associated with virological treatment failure: severity of cirrhosis (assessed by presence of ascites), low albumin, low platelet count/high total bilirubin[29-35], male sex[30,31], and the preexistence of baseline RAS[34,36].

In our study, we found that low platelet count was significantly associated with a higher rate of virological treatment failure. It is likely that low platelet count is a surrogate marker of cirrhosis, since we found an association between low albumin levels and low PT time by univariate analysis. However, we failed to observe a significant relationship between severe cirrhosis and failure. This might be due to the fact that in cases of severe cirrhosis, physicians adapted the treatment to each complex situation by extending the duration or by adding RBV (76% of the patients with Child Pugh B or C cirrhosis received treatment of 24 wk duration *vs* 29% of the other patients in our study), and this might be explained by unreported events of decompensation.

In the first randomized phase 3 clinical trials, which assessed the efficacy and safety of DAA, decompensated cirrhosis was an exclusion criterion, which precluded the possibility of assessing this factor as a potential predictor of failure. More recently, several trials have clearly demonstrated that patients with Child Pugh B or C cirrhosis and those with genotype 3 infection have a lower rate of SVR with DAA alone and need the addition of RBV. This was the case for the SOF/LDV combination and for a combination[37,38] of velpatasvir/SOF[39].

We observed a trend (by univariate analysis only) toward a higher rate of detectable HIV-RNA in patients with virological treatment failure *vs* in those with SVR (P = 0.19). This might reflect suboptimal adherence, with patients who are non-compliant for their HIV treatment while possibly also non-adherent to their HCV treatment. Nonetheless, this result did not remain significant by multivariable analysis and thus may also simply reflect a biased estimate.

Moreover, among seven patients with failure and interpretable pharmacological data, suboptimal blood concentrations of DAA were measured in two of them. These results could reflect different situations (drug interactions, suboptimal dosing errors, suboptimal adherence) and warrant both further investigation and wider-scale assessment of pharmacological data. Regarding RAS in our study, we did not determine pretreatment RAS and we cannot exclude the possibility that some failures may be due to pre-existing RAS. However, at a population level, the effects of baseline RAS in *NS5A*, although not rare, are minimal[10,36,40,41]. This prompted EASL experts[18,20] to recommend that genotyping should not be performed for naïve patients but instead considered when retreatment is anticipated with a *NS5A* inhibitor regimen in patients who have previously failed NS5A treatment.

In most of our patients who failed DAA-treatment, RAS was investigated. We found RAS in 50% of those failing NS5A-based therapy and in the two patients failing NS3, but no major RAS S282T to NS5B. This high prevalence of *NS5A* and -3 RAS failure in our study confirms the EASL recommendation to evaluate HCV resistance to NS5A inhibitors (spanning amino acids 24 to 93) if resistance testing is available, as these analyses can guide decisions for further treatment[18,20].

There are several limitations to this study. Firstly, since the study was an observational cohort, our results must be interpreted with caution, since treatment

prescriptions were dependent on drug availability (with variations over time) and known efficacy with regards to HCV genotypes. Those results were obtained with second generation DAA (LDV, DCV, elbasvir/grazoprevir), and those results may not be entirely applicable to the newer, pangenotypic regimens such as velpatasvir/SOF or pibentasvir/glecaprevir. Our analysis is limited by the small number of subjects with virological treatment failure, and thus likely has limited power to identify all potential risk factors. All patients with virological treatment failure could not be explored by genotyping to investigate the emergence of RAS due to the need to obtain patient consent. Furthermore, baseline genotyping was not available routinely, since this test is not recommended in France for treatment-naïve patients. Finally, Sanger sequencing was used for the detection of RAS, which may not be sensitive enough to detect minor populations of RAS (< 15%). The strengths of our study include prospective data collection with regular monitoring and high quality data.

In conclusion, our study identified that low platelet count is associated with a higher probability of DAA failure. This parameter likely reflects hepatic insufficiency, and our results are concordant with previously published findings on HCV monoinfected patients. We also speculate that some degree of low adherence could explain some cases of failure, since suboptimal drugs levels were observed in 29% of the cases that could be explored, and HIV viral load was often detectable in patients with virological treatment failure to DAA. This study confirms the very low rate of treatment failure with all-oral DAA in HIV/HCV coinfected patients, as well as the high risk of the emergence of non-structural NS3 or NS5A RAS in patients with virological DAA failure.

ARTICLE HIGHLIGHTS

Research background

In human immunodeficiency virus (HIV)/hepatitis C virus (HCV) coinfection, all-oral direct-acting antiviral (DAA) regimens achieve virological cures in > 95% of patients.

Research motivation

Risk factors for failure are mainly related to severity of cirrhosis in HCV monoinfected patients, but are unknown in the population of HIV HCV coinfected patients. We wanted to know whether additional factors related to non-adherence or HIV status could be involved in the occurrence of failures. We believed that identifying the risk factors for failure would allow for the adaptation of treatment to patients with higher risk of failure.

Research objectives

The main objectives were to determine the risk factors for virological treatment failure to DAA in HIV/HCV coinfected patients and to describe the frequency of RAS.

Research methods

HIV/HCV coinfected patients who started the first DAA regimen before February 2016 and who were included in the French ANRS CO13 HEPAVIH cohort were eligible. Failure was defined as: i) Non-response (HCV-RNA remained detectable during treatment, at end of treatment (EOT)), ii) relapse (HCV-RNA suppressed at EOT but detectable thereafter). Sequencing analysis was performed to describe prevalence of drug class specific RAS. Factors associated with failure were determined using logistic regression models.

Research results

Research findings: Among 559 patients, 77% had suppressed plasma HIV-RNA < 50 copies/mL at DAA treatment initiation, 41% were cirrhotic, and 68% were HCV treatment-experienced. Virological treatment failures occurred in 22 patients and were mainly relapses (17, 77%) then undefined failure (3, 14%) and non-responses (2, 9%). Mean treatment duration was 16 wk overall. Post-treatment NS3, NS5A or NS5B RAS were detected in 10/14 patients with samples available for sequencing analysis. After adjustment for age, sex, RBV use, HCV genotype and treatment duration, low platelet count was the only factor significantly associated with a higher risk of failure (OR: 6.5; 95%CI: 1.8-22.6); Contributions to the field: In HIV/HCV coinfected patients, the risk factors of failure were more related to the severity of cirrhosis than to HIV immunovirological status or non-adherence issues. Problems that remain to be solved: It remains to be determined whether the low platelet count associated with a higher probability of failure reflects the severity of cirrhosis.

Research conclusions

In our study of HIV/HCV patients receiving all-oral DAA, only 3.9% HIV-HCV coinfected patients failed DAA regimens. RAS were found in 70% of those failing. Low platelet count was independently associated with virological failure. We think that this low platelet count reflects the severity of cirrhosis.

Research perspectives

As the treatment failure number is low, it would be useful to build international collaborations and gather data for several cohorts in order to gain significance power. The results obtained with first generation all-oral DAA could be compared with the newer, pangenotypic drug regimen.

ACKNOWLEDGMENTS

Patients of the ANRS CO13 HEPAVIH Cohort. Scientific Committee: Salmon D (co-Principal Investigator), Wittkop L (co-Principal Investigator), Sogni P (co-Principal Investigator), Esterle L (project manager), Trimoulet V, Izopet J, Serfaty L, Paradis V, Spire B, Carrieri P, Valantin MA, Pialoux G, Chas J, Poizot-Martin I, Barangue K, Naqvi A, Rosenthal E, Bicart-See A, Bouchaud O, Gervais A, Lascoux-Combe C, Goujard V, Lacombe K, Duvivier C, Vittecoq D, Neau D, Morlat P, Bani-Sadr F, Meyer L, Boufassa F, Dominguez S, Autran B, Roque AM, Solas C, Fontaine H, Costagliola D, Piroth L, Simon A, Zucman D, Boué F, Miailhes P, Billaud E, Aumaître H, Rey D, Peytavin G, Petrov-Sanchez V, Pailhe A. Clinical Centres: APHP Cochin, Paris (Médecine Interne et Maladies Infectieuses: Salmon D, Usubillaga R; Hépato-gastro-entérologie: Sogni P; Anatomo-pathologie: Terris B; Virologie: Tremeaux P); APHP Pitié-Salpétrière, Paris (Maladies Infectieuses et Tropicales: Katlama C, Valantin MA, Stitou H; Hépato-gastro-entérologie: Benhamou Y; Anatomo-pathologie: Charlotte F; Virologie: Fourati S); APHP Pitié-Salpétrière, Paris (Médecine Interne: Simon A, Cacoub P, Nafissa S); APHM Sainte-Marguerite, Marseille (Service d'Immuno-Hématologie Clinique: Poizot-Martin I, Zaegel O, Laroche H; Virologie: Tamalet C); APHP Tenon, Paris (Maladies Infectieuses et Tropicales: Pialoux G, Chas J; Anatomo-pathologie: Callard P, Bendjaballah F; Virologie: Le Pendeven C); CHU Purpan, Toulouse (Maladies Infectieuses et

Tropicales: Marchou B; Hépato-gastro-entérologie: Alric L, Barange K, Metivier S; Anatomo-pathologie: Selves J; Virologie: Larroquette F); CHU Archet, Nice (Médecine Interne: Rosenthal E; Infectiologie: Naqvi A, Rio V; Anatomo-pathologie: Haudebourg J, Saint-Paul MC; Virologie: Partouche C); APHP Avicenne, Bobigny (Médecine Interne - Unité VIH: Bouchaud O; Anatomo-pathologie: Ziol M; Virologie: Baazia Y); Hôpital Joseph Ducuing, Toulouse (Médecine Interne: Uzan M, Bicart-See A, Garipuy D, Ferro-Collados MJ; Anatomo-pathologie: Selves J; Virologie: Nicot F); APHP Bichat - Claude-Bernard, Paris (Maladies Infectieuses: Gervais A, Yazdanpanah Y; Anatomo-pathologie: Adle-Biassette H; Virologie: Alexandre G); APHP Saint-Louis, Paris (Maladies infectieuses: Lascoux-Combe C, Molina JM; Anatomo-pathologie: Bertheau P; Virologie: Chaix ML, Delaugerre C, Maylin S); APHP Saint-Antoine (Maladies Infectieuses et Tropicales: Lacombe K, Bottero J, Krause J, Girard PM, Anatomo-pathologie: Wendum D, Cervera P, Adam J; Virologie: Viala C); APHP Bicêtre, Paris (Médecine Interne: Goujard C, Quertainmont Y, Teicher E; Virologie: Pallier C; Maladies Infectieuses: Vittecoq D); APHP Necker, Paris (Maladies Infectieuses et Tropicales: Lortholary O, Duvivier C, Rouzaud C, Lourenco J, Touam F, Louisin C; Virologie: Avettand-Fenoel V, Mélard A); CHU Pellegrin, Bordeaux (Maladies Infectieuses et Tropicales: Neau D, Ochoa A, Blanchard E, Castet-Lafarie S, Cazanave C, Malvy D, Dupon M, Dutronc H, Dauchy F, Lacaze-Buzy L; Anatomo-pathologie: Bioulac-Sage P; Virologie: Trimoulet P, Reigadas S); Hôpital Saint-André, Bordeaux (Médecine Interne et Maladies Infectieuses: Médecine Interne et Maladies Infectieuses: Morlat P, Lacoste D, Bonnet F, Bernard N, Hessamfar M, Paccalin JF, Martell C, Pertusa MC, Vandenhende M, Merciéer P, Malvy D, Pistone T, Receveur MC,. Méchain M, Duffau P, Rivoisy C, Faure I, Caldato S; Anatomo-pathologie: Bioulac-Sage P; Virologie: Trimoulet P, Reigadas S); Hôpital Haut-Levêque, Bordeaux (Médecine Interne: Pellegrin JL, Viallard JF, Lazzaro E, Greib C; Anatomo-pathologie: Bioulac-Sage P; Virologie: Trimoulet P, Reigadas S); Hôpital FOCH, Suresnes (Médecine Interne: Zucman D, Majerholc C; Virologie: Farfour E); APHP Antoine Béclère, Clamart (Médecine Interne: Boué F, Polo Devoto P, Kansau I, Chambrin C, Pignon C, Berroukeche L, Fior R, Martinez V; Virologie: Deback C); CHU Henri Mondor, Créteil (Immunologie Clinique: Lévy Y, Dominguez S, Lelièvre JD, Lascaux AS, Melica G); CHU Hôtel Dieu, Nantes (Maladies Infectieuses et Tropicales: Billaud E, Raffi F, Allavena C, Reliquet V, Boutoille D, Biron C; Virologie: Rodallec A, Le Guen L); Hôpital de la Croix Rousse, Lyon (Maladies Infectieuses et Tropicales: Miailhes P, Peyramond D, Chidiac C, Ader F, Biron F, Boibieux A, Cotte L, Ferry T, Perpoint T, Koffi J, Zoulim F, Bailly F, Lack P, Maynard M, Radenne S, Amiri M; Virologie: Scholtes C, Le-Thi TT); CHU Dijon, Dijon (Département d'infectiologie: Piroth L, Chavanet P, Duong Van Huyen M, Buisson M, Waldner-Combernoux A, Mahy S, Binois R, Simonet-Lann AL, Croisier-Bertin D); CH Perpignan, Perpignan (Maladies infectieuses et tropicales: Aumaître H); CHU Robert Debré, Reims (Médecine interne, maladies infectieuses et immunologie clinique: Bani-Sadr F, Lambert D, Nguyen Y, Berger JL); CHRU Strasbourg (Le Trait d'Union: Rey D, Partisani M, Batard ML, Cheneau C, Priester M, Bernard-Henry C, de Mautort E; Virologie: Gantner et P, Fafi-Kremer S), APHP Bichat-Claude Bernard (Pharmacologie: Peytavin G). Data collection: Roustant F, Kmiec I, Traore L, Lepuil S, Parlier S, Sicart-Payssan V, Bedel E, Touam F, Louisin C, Mole M, Bolliot C, Mebarki M, Adda-Lievin A, Makhoukhi F-Z, Braik O, Bayoud R, Pietri M-P, Le Baut V, Bornarel D, Chesnel C, Beniken D, Pauchard M, Akel S, Caldato S, Lions C, Chalal L, Julia Z, Hue H, Soria A, Cavellec M, Breau S, Joulie A, Fisher P, Ondo Eyene C, Ogoudjobi S, Brochier C, Thoirain-Galvan V. Management, statistical analyses: Boerg E, Carrieri P, Conte V, Dequae-Merchadou L, Desvallees M, Douiri N, Esterle L, Gilbert C, Gillet S, Knight R, Marcellin F, Michel L, Mora M, Nordmann S, Protopopescu C, Roux P, Spire B, Tezkratt S, Vilotitch A, Yaya I, Wittkop L.

REFERENCES

1 **Osinusi A**, Townsend K, Kohli A, Nelson A, Seamon C, Meissner EG, Bon D, Silk R, Gross C, Price A, Sajadi M, Sidharthan S, Sims Z, Herrmann E, Hogan J, Teferi G, Talwani R, Proschan M, Jenkins V, Kleiner DE, Wood BJ, Subramanian GM, Pang PS, McHutchison JG, Polis MA, Fauci AS, Masur H, Kottilil S. Virologic response following combined ledipasvir and sofosbuvir administration in patients with HCV genotype 1 and HIV co-infection. *JAMA* 2015; **313**: 1232-1239 [PMID: 25706232 DOI: 10.1001/jama.2015.1373]

2 **Rockstroh JK**, Nelson M, Katlama C, Lalezari J, Mallolas J, Bloch M, Matthews GV, Saag MS, Zamor PJ, Orkin C, Gress J, Klopfer S, Shaughnessy M, Wahl J, Nguyen BY, Barr E, Platt HL, Robertson MN, Sulkowski M. Efficacy and safety of grazoprevir (MK-5172) and elbasvir (MK-8742) in patients with hepatitis C virus and HIV co-infection (C-EDGE CO-INFECTION): a non-randomised, open-label trial. *Lancet HIV* 2015; **2**: e319-e327 [PMID: 26423374 DOI: 10.1016/S2352-3018(15)00114-9]

3 **Sulkowski MS**, Eron JJ, Wyles D, Trinh R, Lalezari J, Wang C, Slim J, Bhatti L, Gathe J, Ruane PJ, Elion R, Bredeek F, Brennan R, Blick G, Khatri A, Gibbons K, Hu YB, Fredrick L, Schnell G, Pilot-Matias T, Tripathi R, Da Silva-Tillmann B, McGovern B, Campbell AL, Podsadecki T. Ombitasvir, paritaprevir co-dosed with ritonavir, dasabuvir, and ribavirin for hepatitis C in patients co-infected with HIV-1: a randomized trial. *JAMA* 2015; **313**: 1223-1231 [PMID: 25706092 DOI: 10.1001/jama.2015.1328]

4 **Wyles D**, Bräu N, Kottilil S, Daar ES, Ruane P, Workowski K, Luetkemeyer A, Adeyemi O, Kim AY, Doehle B, Huang KC, Mogalian E, Osinusi A, McNally J, Brainard DM, McHutchison JG, Naggie S, Sulkowski M; ASTRAL-5 Investigators. Sofosbuvir and Velpatasvir for the Treatment of Hepatitis C Virus in Patients Coinfected With Human Immunodeficiency Virus Type 1: An Open-Label, Phase 3 Study. *Clin Infect Dis* 2017; **65**: 6-12 [PMID: 28369210 DOI: 10.1093/cid/cix260]

5 **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]
6 **d'Arminio Monforte A**, Cozzi-Lepri A, Ceccherini-Silberstein F, De Luca A, Lo Caputo S, Castagna A, Mussini C, Cingolani A, Tavelli A, Shanyinde M, Gori A, Girardi E, Andreoni M, Antinori A, Puoti M; Icona Foundation and HepaIcona Study Group. Access and response to direct antiviral agents (DAA) in HIV-HCV co-infected patients in Italy: Data from the Icona cohort. *PLoS One* 2017; **12**: e0177402 [PMID: 28520749 DOI: 10.1371/journal.pone.0177402]
7 **Ingiliz P**, Christensen S, Kimhofer T, Hueppe D, Lutz T, Schewe K, Busch H, Schmutz G, Wehmeyer MH, Boesecke C, Simon KG, Berger F, Rockstroh JK, Schulze zur Wiesch J, Baumgarten A, Mauss S. Sofosbuvir and Ledipasvir for 8 Weeks for the Treatment of Chronic Hepatitis C Virus (HCV) Infection in HCV-Monoinfected and HIV-HCV-Coinfected Individuals: Results From the German Hepatitis C Cohort (GECCO-01). *Clin Infect Dis* 2016; **63**: 1320-1324 [PMID: 27535952 DOI: 10.1093/cid/ciw567]
8 **Piroth L**, Wittkop L, Lacombe K, Rosenthal E, Gilbert C, Miailhes P, Carrieri P, Chas J, Poizot-Martin I, Gervais A, Dominguez S, Neau D, Zucman D, Billaud E, Morlat P, Aumaitre H, Lascoux-Combe C, Simon A, Bouchaud O, Teicher E, Bani-Sadr F, Alric L, Vittecoq D, Boué F, Duvivier C, Valantin MA, Esterle L, Dabis F, Sogni P, Salmon D; ANRS CO13 HEPAVIH study group. Efficacy and safety of direct-acting antiviral regimens in HIV/HCV-co-infected patients - French ANRS CO13 HEPAVIH cohort. *J Hepatol* 2017; **67**: 23-31 [PMID: 28235612 DOI: 10.1016/j.jhep.2017.02.012]
9 **Sogni P**, Gilbert C, Lacombe K, Piroth L, Rosenthal E, Miailhes P, Gervais A, Esterle L, Chas J, Poizot-Martin I, Dominguez S, Simon A, Morlat P, Neau D, Zucman D, Bouchaud O, Lascoux-Combe C, Bani-Sadr F, Alric L, Goujard C, Vittecoq D, Billaud E, Aumaître H, Boué F, Valantin MA, Dabis F, Salmon D, Wittkop L. All-oral Direct-acting Antiviral Regimens in HIV/Hepatitis C Virus-coinfected Patients With Cirrhosis Are Efficient and Safe: Real-life Results From the Prospective ANRS CO13-HEPAVIH Cohort. *Clin Infect Dis* 2016; **63**: 763-770 [PMID: 27317796 DOI: 10.1093/cid/ciw379]
10 **Di Maio VC**, Cento V, Lenci I, Aragri M, Rossi P, Barbaliscia S, Melis M, Verucchi G, Magni CF, Teti E, Bertoli A, Antonucci F, Bellocchi MC, Micheli V, Masetti C, Landonio S, Francioso S, Santopaolo F, Pellicelli AM, Calvaruso V, Gianserra L, Siciliano M, Romagnoli D, Cozzolongo R, Grieco A, Vecchiet J, Morisco F, Merli M, Brancaccio G, Di Biagio A, Loggi E, Mastroianni CM, Pace Palitti V, Tarquini P, Puoti M, Taliani G, Sarmati L, Picciotto A, Vullo V, Caporaso N, Paoloni M, Pasquazzi C, Rizzardini G, Parruti G, Craxì A, Babudieri S, Andreoni M, Angelico M, Perno CF, Ceccherini-Silberstein F; HCV Italian Resistance Network Study Group. Multiclass HCV resistance to direct-acting antiviral failure in real-life patients advocates for tailored second-line therapies. *Liver Int* 2017; **37**: 514-528 [PMID: 28105744 DOI: 10.1111/liv.13327]
11 **Wyles D**, Dvory-Sobol H, Svarovskaia ES, Doehle BP, Martin R, Afdhal NH, Kowdley KV, Lawitz E, Brainard DM, Miller MD, Mo H, Gane EJ. Post-treatment resistance analysis of hepatitis C virus from phase II and III clinical trials of ledipasvir/sofosbuvir. *J Hepatol* 2017; **66**: 703-710 [PMID: 27923693 DOI: 10.1016/j.jhep.2016.11.022]
12 **Zeuzem S**, Mizokami M, Pianko S, Mangia A, Han KH, Martin R, Svarovskaia E, Dvory-Sobol H, Doehle B, Hedskog C, Yun C, Brainard DM, Knox S, McHutchison JG, Miller MD, Mo H, Chuang WL, Jacobson I, Dore GJ, Sulkowski M. NS5A resistance-associated substitutions in patients with genotype 1 hepatitis C virus: Prevalence and effect on treatment outcome. *J Hepatol* 2017; **66**: 910-918 [PMID: 28108232 DOI: 10.1016/j.jhep.2017.01.007]
13 **American Association for the Study of Liver Diseases**. HCV Guidance: Recommendations for Testing, Managing, and Treating Hepatitis C. 2016. Available from: URL: https://www.hcvguidelines.org/
14 **AFEF**. Recommandations AFEF sur la prise en charge des hépatites virales C. 2017. Available from: URL: http://www.afef.asso.fr/ckfinder/userfiles/files/recommandations-textes-officiels/recommandations/RecommandationsAFEFMars2017.pdf
15 **European AIDS Clinical Society**. Guidelines version 8.0, 2015. Available from: URL: http://www.eacsociety.org/files/guidelines_8_0-english_web.pdf
16 **Loko MA**, Salmon D, Carrieri P, Winnock M, Mora M, Merchadou L, Gillet S, Pambrun E, Delaune J, Valantin MA, Poizot-Martin I, Neau D, Bonnard P, Rosenthal E, Barange K, Morlat P, Lacombe K, Gervais A, Rouges F, See AB, Lascoux-Combe C, Vittecoq D, Goujard C, Duvivier C, Spire B, Izopet J, Sogni P, Serfaty L, Benhamou Y, Bani-Sadr F, Dabis F; ANRS CO 13 HEPAVIH Study Group. The French national prospective cohort of patients co-infected with HIV and HCV (ANRS CO13 HEPAVIH): early findings, 2006-2010. *BMC Infect Dis* 2010; **10**: 303 [PMID: 20969743 DOI: 10.1186/1471-2334-10-303]
17 **Miailhes P**, Gilbert C, Lacombe K, Arends JE, Puoti M, Rockstroh JK, Sogni P, Fontaine H, Rosenthal E, Winnock M, Loko MA, Wittkop L, Dabis F, Salmon D; ESCMID European Study Group on Viral Hepatitis. Triple therapy with boceprevir or telaprevir in a European cohort of cirrhotic HIV/HCV genotype 1-coinfected patients. *Liver Int* 2015; **35**: 2090-2099 [PMID: 25650873 DOI: 10.1111/liv.12799]
18 **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]
19 **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]
20 **European Association for the Study of the Liver**. EASL Recommendations on Treatment of Hepatitis C 2018. *J Hepatol* 2018; **69**: 461-511 [PMID: 29650333 DOI: 10.1016/j.jhep.2018.03.026]
21 **Rezk MR**, Bendas ER, Basalious EB, Karim IA. Development and validation of sensitive and rapid UPLC-MS/MS method for quantitative determination of daclatasvir in human plasma: Application to a bioequivalence study. *J Pharm Biomed Anal* 2016; **128**: 61-66 [PMID: 27232152 DOI: 10.1016/j.jpba.2016.05.016]
22 **Solas C**, Paré M, Quaranta S, Stanke-Labesque F; pour le groupe Suivi Thérapeutique Pharmacologique de la Société Francaise de Pharmacologie et de Thérapeutique. [Not Available]. *Therapie* 2011; **66**: 221-230 [PMID: 27393202 DOI: 10.2515/therapie/2011036]
23 **Dominguez S**, Ghosn J, Cassard B, Melica G, Poizot-Martin I, Solas C, Lascaux AS, Bouvier-Alias M, Katlama C, Lévy Y, Peytavin G. Erythrocyte and plasma ribavirin concentrations in the assessment of early and sustained virological responses to pegylated interferon-alpha 2a and ribavirin in patients coinfected with hepatitis C virus and HIV. *J Antimicrob Chemother* 2012; **67**: 1449-1452 [PMID: 22396433 DOI: 10.1093/jac/dks045]
24 **Food and Drug Administration**. Clinical Pharmacology and Biopharmaceutics Reviews. Copegus, 2002: 21-511. Available from: URL: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2002/21-411_Strattera_biopharmr_P3.pdf
25 **Food and Drug Administration**. Clinical Pharmacology and Biopharmaceutics Reviews. Daklinza 206843 Orig1s000, 2014. Available from: URL: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2015/206843Orig1s000ClinPharmR.pdf
26 **Food and Drug Administration**. Clinical Pharmacology and Biopharmaceutics Review(s). Harvoni 205834 Orig1s000, 2014. Available from: URL: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2014/205834Orig1s000MedR.pdf
27 **Food and Drug Administration**. Clinical Pharmacology and Biopharmaceutics Reviews. Sofosbuvir (GS-7977) 204671Orig1s000, 2013. Available from: URL: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2013/204671Orig1s000ClinPharmR.pdf
28 **Welzel TM**, Petersen J, Herzer K, Ferenci P, Gschwantler M, Wedemeyer H, Berg T, Spengler U, Weiland O, van der Valk M, Rockstroh J, Peck-Radosavljevic M, Zhao Y, Jimenez-Exposito

MJ, Zeuzem S. Daclatasvir plus sofosbuvir, with or without ribavirin, achieved high sustained virological response rates in patients with HCV infection and advanced liver disease in a real-world cohort. *Gut* 2016; **65**: 1861-1870 [PMID: 27605539 DOI: 10.1136/gutjnl-2016-312444]

29 **Chang CY**, Nguyen P, Le A, Zhao C, Ahmed A, Daugherty T, Garcia G, Lutchman G, Kumari R, Nguyen MH. Real-world experience with interferon-free, direct acting antiviral therapies in Asian Americans with chronic hepatitis C and advanced liver disease. *Medicine* (Baltimore) 2017; **96**: e6128 [PMID: 28178174 DOI: 10.1097/MD.0000000000006128]

30 **Dalgard O**, Weiland O, Noraberg G, Karlsen L, Heggelund L, Färkkilâ M, Balslev U, Belard E, Øvrehus A, Skalshøi Kjær M, Krarup H, Thorup Røge B, Hallager S, Madsen LG, Lund Laursen A, Lagging M, Weis N. Sofosbuvir based treatment of chronic hepatitis C genotype 3 infections-A Scandinavian real-life study. *PLoS One* 2017; **12**: e0179764 [PMID: 28704381 DOI: 10.1371/journal.pone.0179764]

31 **Ioannou GN**, Beste LA, Chang MF, Green PK, Lowy E, Tsui JI, Su F, Berry K. Effectiveness of Sofosbuvir, Ledipasvir/Sofosbuvir, or Paritaprevir/Ritonavir/Ombitasvir and Dasabuvir Regimens for Treatment of Patients With Hepatitis C in the Veterans Affairs National Health Care System. *Gastroenterology* 2016; **151**: 457-471.e5 [PMID: 27267053 DOI: 10.1053/j.gastro.2016.05.049]

32 **Ippolito AM**, Milella M, Messina V, Conti F, Cozzolongo R, Morisco F, Brancaccio G, Barone M, Santantonio T, Masetti C, Tundo P, Smedile A, Carretta V, Gatti P, Termite AP, Valvano MR, Bruno G, Fabrizio C, Andreone P, Zappimbulso M, Gaeta GB, Napoli N, Fontanella L, Lauletta G, Cuccorese G, Metrangolo A, Francavilla R, Ciracì E, Rizzo S, Andriulli A. HCV clearance after direct-acting antivirals in patients with cirrhosis by stages of liver impairment: The ITAL-C network study. *Dig Liver Dis* 2017; **49**: 1022-1028 [PMID: 28487083 DOI: 10.1016/j.dld.2017.03.025]

33 **Jiménez-Macías FM**, Cabanillas-Casafranca M, Maraver-Zamora M, Romero-Herrera G, García-García F, Correia-Varela-Almeida A, Cabello-Fernández A, Ramos-Lora M. Experience in real clinical practice with new direct acting antivirals in chronic hepatitis C. *Med Clin* (Barc) 2017; **149**: 375-382 [PMID: 28416232 DOI: 10.1016/j.medcli.2017.03.007]

34 **Kan H**, Imamura M, Kawakami Y, Daijo K, Teraoka Y, Honda F, Nakamura Y, Morio K, Kobayashi T, Nakahara T, Nagaoki Y, Kawaoka T, Tsuge M, Aikata H, Hayes CN, Miki D, Ochi H, Honda Y, Mori N, Takaki S, Tsuji K, Chayama K. Emergence of drug resistance-associated variants and changes in serum lipid profiles in sofosbuvir plus ledipasvir-treated chronic hepatitis C patients. *J Med Virol* 2017; **89**: 1963-1972 [PMID: 28657143 DOI: 10.1002/jmv.24885]

35 **Terrault NA**, Zeuzem S, Di Bisceglie AM, Lim JK, Pockros PJ, Frazier LM, Kuo A, Lok AS, Shiffman ML, Ben Ari Z, Akushevich L, Vainorius M, Sulkowski MS, Fried MW, Nelson DR; HCV-TARGET Study Group. Effectiveness of Ledipasvir-Sofosbuvir Combination in Patients With Hepatitis C Virus Infection and Factors Associated With Sustained Virologic Response. *Gastroenterology* 2016; **151**: 1131-1140.e5 [PMID: 27565882 DOI: 10.1053/j.gastro.2016.08.004]

36 **Sarrazin C**, Dvory-Sobol H, Svarovskaia ES, Doehle BP, Pang PS, Chuang SM, Ma J, Ding X, Afdhal NH, Kowdley KV, Gane EJ, Lawitz E, Brainard DM, McHutchison JG, Miller MD, Mo H. Prevalence of Resistance-Associated Substitutions in HCV NS5A, NS5B, or NS3 and Outcomes of Treatment With Ledipasvir and Sofosbuvir. *Gastroenterology* 2016; **151**: 501-512.e1 [PMID: 27296509 DOI: 10.1053/j.gastro.2016.06.002]

37 **Charlton M**, Gane E, Manns MP, Brown RS Jr, Curry MP, Kwo PY, Fontana RJ, Gilroy R, Teperman L, Muir AJ, McHutchison JG, Symonds WT, Brainard D, Kirby B, Dvory-Sobol H, Denning J, Arterburn S, Samuel D, Forns X, Terrault NA. Sofosbuvir and ribavirin for treatment of compensated recurrent hepatitis C virus infection after liver transplantation. *Gastroenterology* 2015; **148**: 108-117 [PMID: 25304641 DOI: 10.1053/j.gastro.2014.10.001]

38 **Rockstroh JK**, Peters L, Grint D, Soriano V, Reiss P, Monforte Ad, Beniowski M, Losso MH, Kirk O, Kupfer B, Mocroft A; EuroSIDA in EuroCoord. Does hepatitis C viremia or genotype predict the risk of mortality in individuals co-infected with HIV? *J Hepatol* 2013; **59**: 213-220 [PMID: 23583272 DOI: 10.1016/j.jhep.2013.04.005]

39 **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]

40 **Bartolini B**, Giombini E, Taibi C, Lionetti R, Montalbano M, Visco-Comandini U, D'Offizi G, Capobianchi MR, McPhee F, Garbuglia AR. Characterization of Naturally Occurring NS5A and NS5B Polymorphisms in Patients Infected with HCV Genotype 3a Treated with Direct-Acting Antiviral Agents. *Viruses* 2017; **9**: pii: E212 [PMID: 28783119 DOI: 10.3390/v9080212]

41 **Halfon P**, Scholtès C, Izopet J, Larrat S, Trimoulet P, Zoulim F, Alric L, Métivier S, Leroy V, Ouzan D, de Lédinghen V, Mohamed S, Pénaranda G, Khiri H, Thélu MA, Plauzolles A, Chiche L, Bourlière M, Abravanel F. Baseline and post-treatment hepatitis C NS5A resistance in relapsed patients from a multicentric real-life cohort. *Antivir Ther* 2018; **23**: 307-314 [PMID: 28730994 DOI: 10.3851/IMP3184]

P- Reviewer: Abushady EAE, Bouare N, Lee GH, Milovanovic T
S- Editor: Ji FF **L- Editor**: Filipodia **E- Editor**: Bian YN

World Journal of Hepatology

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

World J Hepatol 2018 November 27; 10(11): 867-876

DOI: 10.4254/wjh.v10.i11.867

ISSN 1948-5182 (online)

ORIGINAL ARTICLE

Observational Study

Cross-sectional study to determine viral hepatitis knowledge in different urban populations in Brazil

Helena Medina Cruz, Jakeline Ribeiro Barbosa, Jeová Keny Baima Colares, Antonio Henrique Almeida de Moraes Neto, Maria de Fátima Leal Alencar, Francisco Inácio Bastos, Jurema Corrêa da Mota, Filipe Aníbal Carvalho-Costa, Claudia Alexandra Pontes Ivantes, Lia Laura Lewis-Ximenez, Livia Melo Villar

Helena Medina Cruz, Jakeline Ribeiro Barbosa, Lia Laura Lewis-Ximenez, Livia Melo Villar, Laboratory of Viral Hepatitis, Oswaldo Cruz Institute, Fiocruz, Rio de Janeiro 21040360, Brazil

Jakeline Ribeiro Barbosa, Jeová Keny Baima Colares, Postgraduate Program in Pathology, Federal University of Ceará, Fortaleza, Ceará 60020181, Brazil

Jeová Keny Baima Colares, Postgraduate Program in Medical Sciences, University of Fortaleza, Ceará 60430160, Brazil

Antonio Henrique Almeida de Moraes Neto, Maria de Fátima Leal Alencar, Laboratory of Innovations in Therapies, Teaching and Bioproducts, Oswaldo Cruz Institute, Fiocruz, Rio de Janeiro 21040360, Brazil

Francisco Inácio Bastos, Institute of Communication and Scientific Information and Technology for Health, Oswaldo Cruz Foundation, Rio de Janeiro 21040900, Brazil

Jurema Corrêa da Mota, Institute of Communication and Scientific Information and Technology for Health, Oswaldo Cruz Foundation, Rio de Janeiro 21040900, Brazil

Filipe Aníbal Carvalho-Costa, Laboratory of Epidemiology and Molecular Systematics, Oswaldo Cruz Institute, Fiocruz, Rio de Janeiro 21040900, Brazil

Claudia Alexandra Pontes Ivantes, Orientation and Counselling Centre, Curitiba, Paraná 80810070, Brazil

ORCID number: Helena Medina Cruz (0000-0003-2088-7705); Jakeline Ribeiro Barbosa (0000-0001-6238-7173); Jeová Keny Baima Colares (0000-0003-1367-6272); Antonio Henrique Almeida de Moraes Neto (0000-0002-0095-503X); Maria de Fátima Leal Alencar (0000-0003-3029-8867); Francisco Inácio Bastos (0000-0001-5970-8896); Jurema Corrêa da Mota (0000-0002-5007-1590); Filipe Aníbal Carvalho-Costa (0000-0001-8083-2840); Claudia Alexandra Pontes Ivantes (0000-0001-5422-557X); Lia Laura Lewis-Ximenez (0000-0002-3447-4621); Livia Melo Villar (0000-0001-7644-8969).

Author contributions: Villar LM conceived the study; Cruz HM and Villar LM designed the research and study protocol; Baima Colares JK, de Moraes Neto AH, Carvalho-Costa FA, Ivantes CA and Lewis-Ximenez LL carried out the participant selection and recruitment; Cruz HM, Barbosa JR and Alencar MF performed the application of questionnaire; Cruz HM and Villar LM performed the analysis and interpretation of the data; Bastos FI and da Mota JC performed the statistical analysis; Cruz HM and Villar LM drafted the manuscript; Barbosa JR, de Moraes Neto AH and Carvalho-Costa FA critically revised the manuscript for intellectual content. All authors read and approved the final manuscript.

Institutional review board statement: The study was reviewed and approved by Fiocruz Ethics Committee.

Informed consent statement: All study participants provided informed written consent prior to study enrollment.

Conflict-of-interest statement: There are no conflicts of interest to report.

Data sharing statement: No additional data are available.

STROBE statement: The manuscript was revised according to the STROBE statement.

Manuscript source: Unsolicited manuscript

Corresponding author to: Livia Melo Villar, PhD, Research Scientist, Viral Hepatitis Laboratory, Oswaldo Cruz Institute, Helio and Peggy Pereira Pavillion - Ground Floor - Room B09, Fiocruz Av 4365 Manguinhos, Rio de Janeiro 210360040, Brazil. lvillar@ioc.fiocruz.br
Telephone: +55-21-25621918

Received: April 18, 2018
Peer-review started: April 18, 2018
First decision: May 11, 2018
Revised: August 22, 2018
Accepted: October 8, 2018
Article in press: October 8, 2018
Published online: November 27, 2018

Abstract

AIM

To evaluate viral hepatitis knowledge among individuals from different resource areas and health conditions to identify possible gaps.

METHODS

A cross-sectional, descriptive study was carried out among 447 individuals from five distinct populations in Brazil: Southeast Viral Hepatitis Ambulatory (n = 100), South (n = 89) and Northeast (n = 114) Health Center, Southeast (n = 77) and Northeast (n = 67) low resource areas. All individuals answered a questionnaire assessing sociodemographic characteristics and viral hepatitis awareness. The perception was scored based on the average number of correct answers of all participants and categorized as "low" (0-28 correct answers) or "desirable" (29-46 correct answers). Associations between sociodemographic characteristics and perception were also evaluated.

RESULTS

A low level of knowledge was observed in individuals from Northeast Health Center, Northeast and Southeast low resource areas while desirable knowledge was observed in individuals from Viral Hepatitis Ambulatory and South Health Center. According to sociodemographic characteristics, desirable scores were more common among those with secondary education (47.1%), those who declared themselves as white (46.3%), and those who lived in houses with three individuals (25.5%). Multivariate analysis showed an association between viral hepatitis perception and type of population.

CONCLUSION

The results demonstrated high level of knowledge among study participants from health clinics from the Southeast region of Brazil and the importance of education programs in increasing the level of knowledge in low resource areas.

Key words: Viral hepatitis; Knowledge; Perception; Urban population; Brazil

Core tip: This study evaluated viral hepatitis knowledge among individuals from five different resource areas and health conditions in Brazil. Participants responded to a questionnaire and the perception was scored as "low" or "desirable". Individuals from Northeast Health Center and Northeast and Southeast low resource areas exhibited low perception, while Southeast and South Health Center exhibited a desirable perception. A positive association was observed between perception and education level, race, number of individuals living in the same house and population type. The results showed the importance of prevention campaigns, especially among individuals living in low resource areas.

Cruz HM, Barbosa JR, Baima Colares JK, de Moraes Neto AH, Alencar MF, Bastos FI, da Mota JC, Carvalho-Costa FA, Ivantes CA, Lewis-Ximenez LL, Villar LM. Cross-sectional study to determine viral hepatitis knowledge in different urban populations in Brazil. *World J Hepatol* 2018; 10(11): 867-876 Available from: URL: http://www.wjgnet.com/1948-5182/full/v10/i11/867.htm DOI: http://dx.doi.org/10.4254/wjh.v10.i11.867

INTRODUCTION

Hepatitis is the name given to liver inflammation resulting from autoimmune disease, excessive consumption of alcohol or drugs, bacteria and viruses. Viral hepatitis is a group of viruses (hepatitis A, B, C, D, E, G known as HAV, HBV, HCV, HDV, HEV, HGV) that are etiologically and epidemiologically distinct[1-3].

Ingestion of contaminated food or water transmits HAV and HEV; in this fashion, washing food and hands and treating water are methods of prevention. On the other hand, HBV, HCV and HDV can be transmitted by contact with infected bodily fluids (transfusion of blood or blood products, or invasive medical procedures), unsafe sexual practices, or from transmission from mother to child. Prevention of HBV, HCV and HDV is made by blood and organ donor selection, using disposable or sterilized materials and the use of condoms in sexual intercourse[1,4-6].

There are vaccines to prevent HAV and HBV that are safe and effective; one vaccine for HEV is commercialized only in China, but there are no Federal Drug Administration-approved vaccines for HCV and HDV[7]. The clinical course of hepatitis viruses can be acute and chronic for HBV, HCV, HDV and HEV. The clinical manifestations of hepatitis can be absent or appear when the disease is advanced, with cirrhosis or liver cancer[1,2]. Viral hepatitis laboratory diagnosis is performed through the detection of specific antigens, antibodies and viral genome, mainly by enzyme immunoassays and molecular assays such as the polymerase chain reaction[8].

HBV and HCV occur chronically in 257 and 71 million people respectively, causing more than 1.2 million deaths annually[2]. Approximately 15 million people are infected with HDV[2]. Annually, there are an estimated 126 million new cases of HAV and 3.3 million new cases of HEV[2,9,10]. In 2016, 61297 deaths were related to viral hepatitis in Brazil. HEV prevalence in Brazil varies from 2% to 29%[11-15].

The evaluation of knowledge is assessed to verify how far community knowledge corresponds to biomedical concepts[5]. Some factors, such as education, health literacy, family income, age, and access to information, could be associated with gaps in knowledge[16,17].

Around the world studies have been conducted in order to evaluate viral hepatitis perception among health professionals and students, viral hepatitis patients or other risk groups[17-21]. There are still few reports regarding viral hepatitis knowledge in low resource areas[5,22-24]. In view of these gaps, the aim of the present study is to evaluate the viral hepatitis knowledge among individuals from different resource areas and health conditions in Brazil to identify possible gaps and help authorities in the development of prevention and education programs.

MATERIALS AND METHODS

Population studied

This was a cross-sectional study conducted from March 2015 to November 2015, wherein a minimum sample size of 50 participants per group was defined. A nonprobability sampling method with consecutive sampling was used in which every subject meeting the criteria of inclusion was selected until the required sample size was achieved in this setting.

Individuals were previously informed about the study and participant eligibility criteria were: Both genders, more than 18 years of age, free from psychoactive drug use, agreement to inclusion, and signed, informed consent. The local ethical committee approved the study (CAEE 38846914.5.0000.5248).

The final sample was made up of a total of 447 questionnaires about hepatitis knowledge obtained from five groups belonging to different geographic regions in Brazil, as follows: (1) Southeast Viral Hepatitis Ambulatory, comprising 100 individuals living in the Rio de Janeiro state, both in nearby cities and in different districts of the city of Rio de Janeiro, who were referred to the outpatient clinic. These individuals included not only those with acute, chronic or suspected cases of viral hepatitis but also those accompanying patients to the Brazilian Referral center for viral hepatitis diagnosis. The recruitment was performed prior to medical consultation. The Rio de Janeiro state is situated in the Southeast region of Brazil, with a human development Index (HDI) of 0.761[25]; (2) South Health Center, comprising 89 individuals residing in the city of Curitiba (Paraná State) that were recruited in the Guidance and Monitoring Center prior to medical consultation. This center performs anonymous testing for hepatitis, syphilis and human immunodeficiency virus. Curitiba is situated in the South region of Brazil with an estimated population of 1908359, an HDI of 0.823, and poverty rate of 31.71%[25]; (3) Northeast Health Center, comprising 114 individuals resident in the city of Fortaleza (Ceará State) and who were users of the Brazilian Unified Health System seeking care in Medical Care Center integrated to the University of Fortaleza. Recruitment was carried out prior to medical consultation. Fortaleza is situated in the Northeast region of Brazil, with an estimated population of 2627482, an HDI of 0.754, and a poverty rate of 43.17%[25]; (4) Southeast low resource areas, comprising 77 individuals living in low resource communities from the Southeast region of Brazil (Complex of Manguinhos district of Rio de Janeiro city). Interviewers visited residents in their homes and only applied the questionnaires to those who agreed to participate. Rio de Janeiro city is situated in the Southeast region of Brazil, with an estimated population of 6520266, an HDI of 0.799, and a poverty rate of 23.85%. Manguinhos complex exhibited the fifth worst HDI (0.726) among the 126 neighborhood groups in the city of Rio de Janeiro, and the average family income in population was below a minimum Brazilian income[25]; and (5) Northeast low resource areas, comprising 67 individuals resident in a low-resource community from the Northeast Region of Brazil (Nossa Senhora de Nazaré city, Piauí State). This city had approximately 5000 inhabitants and residents had a low income. Interviewers visited residents in their homes and only applied the questionnaires to those who agreed to participate. Nossa Senhora de Nazaré is situated in the Northeast region of Brazil, with an estimated population of 4786, an HDI of 0.586, and a poverty rate of 56.6%[25].

To assess knowledge scores, five populations were further aggregated into three groups, which were categorized as Southeast Viral Hepatitis Ambulatory (n = 100), medical centers (n =203, South and Northeast) and under-privileged communities (n = 144, Southeast and Northeast low resource areas).

Data collection instrument

The questionnaire was composed of two parts: (1) Social-demographic characteristics; and (2) viral hepatitis perception. Social-demographic characteristics included gender, age, education level, race, monthly family income, marital status and number of people in-house. Monthly family income was considered in relation to the Brazilian minimum salary and classified as "low" (< US $276.00 approximately), "intermediate" (US $276.00 to $828.00 approximately) or "high" (> US $828.00 approximately).

Viral hepatitis perception was assessed by the participants' understanding of the proposed questions. The questionnaire was composed of nine groups of questions covering aspects about viral hepatitis including general information (questions 1 to 4), transmission (question 5), prevention (question 6), clinical manifestations (question 7), risk factors (question 8), and

complications (questions 9). All questions except items 3 and 4 had subdivisions (*i.e.* 1a, 1b, 1c and 1d); thus, a total of 46 answers could be correctly pointed out. Additionally, in items 3, 4, 5 and 6, individuals were asked to report which type of hepatitis virus related to their response.

The initial version of the questionnaire was structured in the Brazilian Portuguese language and developed from a questionnaire applied in a previous study[24] and through literature review about knowledge in viral hepatitis[5,16,17]. The questionnaire was then piloted with 30 respondents for its acceptability and consistency, including 15 self-administered and 15 interviewed. From the self-administered questionnaire, three of them had many unfilled questions, and one of them entirely unfilled. The questionnaire was modified after the pilot study and the interview format was chosen for data collection. After this evaluation, the questionnaire was made available for data collection. Data from the pilot study was not included in the final analysis. Participants were interviewed face-to-face in a confidential setting. At the end of the interview, the correct answers were shown to each volunteer.

Score of knowledge

The viral hepatitis perception score was created based on the average of correct answers of all participants' responses (28.7). The perception was divided in two scores: "low" (0-28 correct answers) and "desirable" (29-46 correct answers). Associations between sociodemographic characteristics and perception were also evaluated.

Statistical analysis

Descriptive statistics were generated for the responses and the chi-squared test for independence or for trend and Kruskal-Wallis test were used to compare categorical and continuous variables respectively, among the perception score groups. The variables that were associated with perception score categories were inserted into the logistic regression model, using a forward stepwise method. The 95%CIs of the estimated odds ratios were also calculated, and a *P*-value was calculated using the Statistical Package for the Social Sciences (SPSS for Windows, release 20.0; IBM Corp., Armonk, NY, United States).

RESULTS

Demographic characteristics

Most of the participants were female (269/60.2%), aged over 40 years (254/56.8%), had secondary education (186/41.6%), received intermediate monthly family income (250/55.9%), and declared themselves as non-white (225/50.3%), married (224/50.1%) and living in houses with three individuals (128/28.6%). Only marital status was not significantly different between the five populations ($P = 0.909$) (Table 1).

Viral hepatitis perception

In the case of most categories, the majority of questions were correctly answered (varying from 56.4% to 77.3%), with the exception of the complications category where only 39.4% were answered correctly. Individuals from Southeast Viral Hepatitis Ambulatory showed the highest number of correct answers in general (66.4%), clinical manifestations (84.7%), complications (46.5%), transmission (81.4%) and prevention (80.6%). Participants from South Health Center showed the highest number of correctly answered questions regarding risk of acquiring hepatitis (68.1%) (data not shown).

Table 2 describes the correct responses towards viral hepatitis knowledge separated by populations. More than 70% of participants recognized that hepatitis is caused by viruses, the existence of HAV, HBV, HCV and the availability of vaccines for hepatitis. Additionally, more than 60% of individuals did not know that hepatitis can be caused by alcohol or medicines and that an individual cannot have the same type of hepatitis more than once, while more than 70% of participants were unaware of the existence of HDV and HEV.

Clinical manifestations and risk of acquiring hepatitis questions were correctly answered by most individuals. However, work in rural areas as a risk factor in the acquisition of hepatitis was incorrectly answered by more than 60% of participants. Less than 27% of interviewees were able to associate loss of body movements, blood through the mouth, loss of vision and blood in the stool as complications of hepatitis. In addition, more than 50% of participants incorrectly answered questions about transmission by seafood, the absence of transmission by mosquito bite, and modes of prevention, such as killing mosquitoes and using masks.

In general, correct answers were more common in Southeast Viral Hepatitis Ambulatory and less common in Northeast low resource areas. In questions such as "Does hepatitis D exist?", "Can hepatitis be transmitted by mosquito bite?", "Can killing mosquitoes prevent viral hepatitis?" and "Does using masks prevent hepatitis?" less than 50% were correctly answered by all participants but more than 50% of such questions were correctly answered in Southeast Viral Hepatitis Ambulatory (Table 2).

Less than 10% of correct answers were observed in questions such as "Do hepatitis D and E exist?" in Northeast Health Center, "Is loss of body movement a complication of hepatitis?" in Southeast and Northeast low resource areas, and "Is blood in the stool a complication of hepatitis?" in Northeast Health Center, Southeast and Northeast low resource areas (Table 2).

In bivariate analysis of answered questions, some were not significant, such as those informing whether hepatitis can be caused by medicines, whether jaundice, pale stools and dark urine are clinical manifestations of hepatitis, whether people working in laboratories are at risk of infection, and whether loss of blood through the

Table 1 Participants' sociodemographic characteristics of studies, *n* (%)

Item	Total, 447	Southeast viral hepatitis ambulatory, 100	South health center, 89	Northeast health center, 114	Southeast low resource areas, 77	Northeast low resource areas, 67	*P* value
Gender							
Female	269 (60.2)	55 (55.0)	33 (37.1)	76 (66.7)	51 (66.2)	54 (80.6)	0.000
Male	178 (39.8)	45 (45.0)	56 (62.9)	38 (33.3)	26 (33.8)	13 (19.4)	
Age groups by yr							
≤ 40	193 (43.2)	29 (29.0)	28 (31.5)	68 (59.6)	27 (35.1)	41 (61.2)	0.000
> 40	254 (56.8)	71 (71.0)	61 (68.5)	46 (40.4)	50 (64.9)	26 (38.8)	
Education							
Illiterate	136 (30.4)	28 (28.0)	11 (12.4)	27 (23.7)	38 (49.3)	32 (47.8)	0.000
Primary school	66 (14.8)	16 (16.0)	12 (13.5)	15 (13.2)	13 (16.9)	10 (14.9)	
Secondary school	186 (41.6)	42 (42.0)	48 (53.9)	51 (44.7)	25 (32.5)	20 (29.8)	
College	59 (13.2)	14 (14.0)	18 (20.2)	21 (18.4)	1 (1.3)	5 (7.5)	
Family income							
Low	38 (8.5)	3 (3.0)	1 (1.1)	5 (4.4)	7 (9.1)	17 (25.3)	0.000
Intermediate	250 (55.9)	62 (62.0)	25 (28.1)	72 (63.2)	55 (71.4)	41 (61.2)	
High	145 (32.5)	35 (35.0)	61 (68.5)	34 (29.8)	11 (14.3)	4 (6.0)	
Race							
White	211 (47.2)	47 (47.0)	67 (75.3)	33 (28.9)	42 (54.5)	22 (32.8)	< 0.0001
Non-white	225 (50.3)	51 (51.0)	20 (22.4)	74 (64.9)	35 (45.5)	45 (67.2)	
Marital status							
Married	222 (49.7)	46 (46.0)	44 (49.4)	59 (51.8)	40 (51.9)	33 (49.3)	0.909
Unmarried	224 (50.1)	54 (54.0)	45 (50.6)	55 (48.2)	36 (46.8)	34 (50.7)	
People in home							
1	39 (8.7)	14 (14.0)	9 (10,1)	8 (7.0)	7 (9.1)	1 (1.5)	0.000
2	97 (21.7)	23 (23.0)	28 (31.5)	16 (14.0)	24 (31.1)	6 (9.0)	
3	128 (28.6)	34 (34.0)	23 (25.8)	30 (26.3)	24 (31.2)	17 (25.4)	
4	94 (21.0)	14 (14.0)	17 (19.1)	32 (28.1)	8 (10.4)	23 (34.3)	
5	88 (19.7)	14 (14.0)	12 (13.5)	28 (24.6)	14 (18.2)	20 (29.8)	

mouth or blood in the stool are complications of infection (P = 0.110, P = 0.922, P = 0.054, P = 0.233 and P = 0.121, respectively) (Table 2).

Figure 1 shows the distribution of correct answers in each population; the highest number of correct answered were found in the Southeast Viral Hepatitis Ambulatory group and the lowest number in Northeast low resource areas. Also, it was possible to observe a larger dispersion of correct-answered in Northeast low resource areas.

In 19 questions, it was necessary to determine which hepatitis type was related to the participant's response; only in three of them were more than 50% of the participants able to correctly identify at least one of the related hepatitis types. The percentage of incorrect answers (*i.e.* did not know, did not respond, or did not associate the correct hepatitis type with the question) from these three questions were 14.5% for "Selecting uninfected donors is hepatitis prevention", 40.3% for "Can hepatitis be transmitted by air?" and 41.2% for "Which hepatitis types have a vaccine?". For the other questions, the percentage of wrong answers varies from 50.6% ("Can hepatitis be transmitted by hemodialysis?") to 88.1% ("Can hepatitis be transmitted by seafood?") (data not shown).

Perception about viral hepatitis

The average of correct answers from all individuals was 28.7 ± 6.1- which was considered as the cut off value in this analysis; in this way, scores from 0 to 28 were considered "low" and scores of 29 to 46 were considered "desirable". Only Southeast Viral Hepatitis Ambulatory and South Health Center demonstrated a desirable knowledge (30.5 ± 5.0 and 29.5 ± 5.6, respectively) (Table 3).

Regarding the rate of correct answers, 255 (57.0%) individuals scored above average, with 87 (87.0%) from Southeast Viral Hepatitis Ambulatory, 52 (58.4%) from South Health Center, 56 (49.1%) from Northeast Health Center, 34 (44.1%) from Southeast low resource areas, and 26 (39.4%) from Northeast low resource areas.

The caveats of gender, age, marital status and number of people in the home were associated with approximately the same average number of correct answers. The majority of the individuals with both low and desirable scores received an intermediate family income; however, a lower average number of correct answers was observed in individuals who received low family income.

Desirable perception was more common among females (58.4%), subjects aged over 40 years (60.0%), with a secondary education (47.1%), receiving intermediate family income (56.9%), declaring themselves white (51.8%), married (50.2%) and living in houses with three individuals (25.5%), and belonging to Southeast Viral Hepatitis Ambulatory (34.1%) (Table 3).

Perception was associated only with education level, race, individuals living in the same home and populations in bivariate analysis (Table 3). In multivariate analysis, population-type was found to be statistically significant (Table 4).

Table 2 Correct answers regarding viral hepatitis given by individuals from each group evaluated (*n* = 447) according to general aspects, clinical manifestations, risk of acquiring hepatitis, complications, transmission and prevention, *n* (%)

Sentence	Total, *n* = 447	Southeast viral hepatitis ambulatory, *n* = 100	South health center, *n* = 89	Northeast health center, *n* = 114	Southeast low resource areas, *n* = 77	Northeast low resource areas, *n* = 67	*P* value
General aspects							
Can hepatitis be caused by viruses	321 (71.8)	84 (84.0)	69 (77.5)	75 (65.8)	48 (62.3)	45 (67.2)	0.005
Can hepatitis be caused by bacteria	242 (54.1)	50 (50.0)	19 (21.3)	74 (64.9)	56 (72.7)	43 (64.2)	0.000
Can hepatitis be caused by alcohol	172 (38.5)	31 (31.0)	31 (34.8)	38 (33.3)	34 (44.2)	38 (56.7)	0.006
Can hepatitis be caused by medicines	154 (34.5)	45 (45.0)	29 (32.6)	32 (28.1)	24 (31.2)	24 (35.8)	0.110
Does hepatitis A exist	394 (88.1)	98 (98.0)	78 (87.6)	97 (85.1)	68 (88.3)	53 (79.1)	0.004
Does hepatitis B exist	410 (91.7)	99 (99.0)	88 (98.9)	95 (83.3)	73 (94.8)	55 (82.1)	0.000
Does hepatitis C exist	359 (80.3)	99 (99.0)	86 (96.6)	66 (57.9)	61 (79.2)	47 (70.1)	0.000
Does hepatitis D exist	121 (27.1)	56 (56.0)	18 (20.2)	10 (8.8)	18 (23.4)	19 (28.4)	0.000
Does hepatitis E exist	92 (20.6)	40 (40.0)	15 (16.9)	7 (6.1)	14 (18.2)	16 (23.9)	0.000
Does a vaccine for hepatitis exist	376 (84.1)	91 (91.0)	78 (87.6)	97 (85.1)	58 (75.3)	52 (77.6)	0.026
Can you have the same hepatitis more the once	132 (29.5)	37 (37.0)	23 (25.8)	37 (32.5)	13 (16.9)	22 (32.8)	0.040
Clinical manifestations							
No clinical manifestations	292 (65.3)	89 (89.0)	75 (84.3)	61 (53.5)	35 (45.5)	32 (47.8)	0.000
After years	311 (69.6)	81 (81.0)	75 (84.3)	61 (53.5)	47 (61.0)	47 (70.1)	0.000
Fever discomfort, nausea	369 (82.6)	76 (76.0)	67 (75.3)	99 (86.8)	64 (83.1)	63 (94.0)	0.008
Jaundice, pale stools and dark urine	410 (91.7)	93 (93.0)	81 (91.0)	103 (90.4)	72 (93.5)	61 (91.0)	0.922
People at risk of acquiring hepatitis							
People working in laboratory	235 (52.6)	61 (61.0)	39 (43.8)	67 (58.8)	38 (49.4)	30 (44.8)	0.054
People who work in hospitals	310 (69.4)	72 (72.0)	58 (65.2)	88 (77.2)	56 (72.7)	36 (53.7)	0.014
Not people who work in rural areas	157 (35.1)	36 (36.0)	41 (46.1)	46 (40.4)	18 (23.4)	16 (23.9)	0.006
People who work in the beauty areas	353 (79.0)	91 (91.0)	70 (78.7)	89 (78.1)	57 (74.0)	46 (68.7)	0.007
People who use drugs	393 (87.9)	98 (98.0)	85 (95.5)	96 (84.2)	64 (83.1)	50 (74.6)	0.000
People who receive tattoos or piercings	389 (87.0)	98 (98.0)	79 (88.8)	96 (84.2)	64 (83.1)	52 (77.6)	0.001
People who live indoors	253 (56.6)	46 (46.0)	45 (50.6)	66 (57.9)	57 (74.0)	39 (58.2)	0.004
Not people who work in offices	299 (66.9)	19 (19.0)	68 (76.4)	28 (24.6)	26 (33.8)	31 (46.3)	0.000
Complications							
Cirrhosis	361 (80.8)	91 (91.0)	82 (92.1)	79 (69.3)	62 (80.5)	47 (70.1)	0.000
Liver cancer	378 (84.6)	91 (91.0)	78 (87.6)	95 (83.3)	65 (84.4)	49 (73.1)	0.031
There is no loss of body movements	88 (19.7)	32 (32.0)	17 (19.1)	27 (23.7)	6 (7.8)	6 (9.0)	0.233
There is no loss of blood through the mouth	65 (14.5)	17 (17.0)	18 (20.2)	16 (14.0)	8 (10.4)	6 (9.0)	0.000
There is no vision loss	117 (26.2)	30 (30.0)	23 (25.8)	40 (35.1)	13 (16.9)	11 (16.4)	0.016
There is no blood in the stool	49 (11.0)	18 (18.0)	10 (11.2)	9 (7.9)	7 (9.1)	5 (7.5)	0.121
Transmission							
By transfusion and transplantation	386 (86.4)	94 (94.0)	85 (95.5)	91 (79.8)	67 (87.0)	49 (73.1)	0.000
By sex	310 (69.4)	96 (96.0)	76 (85.4)	64 (56.1)	42 (54.5)	32 (47.8)	0.000
By water and contaminated vegetables	318 (71.1)	88 (88.0)	49 (55.1)	79 (69.3)	60 (77.9)	42 (62.7)	0.000
By seafood	135 (30.2)	59 (59.0)	17 (19.1)	27 (23.7)	23 (29.9)	9 (13.4)	0.000
By tattoo and piercing	361 (80.8)	96 (96.0)	76 (85.4)	88 (77.2)	57 (74.0)	44 (65.7)	0.000
By cutting instruments	385 (86.1)	99 (99.0)	77 (86.5)	90 (78.9)	66 (85.7)	53 (79.1)	0.005
By hemodialysis	280 (62.6)	74 (74.0)	58 (65.2)	60 (52.6)	53 (68.8)	35 (52.2)	0.010
Cannot be by mosquito bite	221 (49.4)	58 (58.0)	49 (55.1)	60 (52.6)	31 (40.3)	23 (34.3)	0.000
Cannot be by air	268 (60.0)	69 (69.0)	69 (77.5)	68 (59.6)	34 (44.2)	28 (41.8)	0.000
Prevention							
Building cesspools	324 (72.5)	78 (78.0)	49 (55.1)	89 (78.1)	63 (81.8)	45 (67.2)	0.000
Channeling water	318 (71.1)	76 (76.0)	53 (59.6)	84 (73.7)	63 (81.8)	42 (62.7)	0.007
Selecting uninfected donors	363 (81.2)	90 (90.0)	71 (79.8)	105 (92.1)	58 (75.3)	39 (58.2)	0.000
Filtering water and treating drinks	372 (83.2)	88 (88.0)	57 (64.0)	101 (88.6)	71 (92.2)	55 (82.1)	0.000
Killing mosquitoes does not prevent hepatitis	189 (42.3)	53 (53.0)	41 (46.1)	41 (36.0)	33 (42.9)	21 (31.3)	0.029
Providing vaccine	405 (90.6)	94 (94.0)	80 (89.9)	107 (93.9)	70 (90.9)	54 (80.6)	0.030
Using masks does not prevent hepatitis	210 (47.0)	69 (69.0)	57 (64.0)	46 (40.4)	26 (33.8)	12 (17.9)	0.000
Using condoms	378 (84.6)	97 (97.0)	82 (92.1)	93 (81.6)	56 (72.7)	50 (74.6)	0.000

Southeast Viral Hepatitis Ambulatory showed a higher number of desirable scores while underprivileged communities showed a lower number of desirable scores compared to low scores in the same areas. Medical centers also present a larger proportion of desirable scores compared to low scores though this was less pronounced (Figure 2).

DISCUSSION

In the present study, knowledge level was scored according to the mean number of correct answers. Individuals from Southeast Viral Hepatitis Ambulatory and South Health Center showed a desirable knowledge in contrast to those recruited at Northeast Health Center,

Table 3 Sociodemographic characteristics according to knowledge scores for viral hepatitis, *n* (%)

Item	Mean of knowledge score (± SD)	Knowledge score		Bivariate analysis *P* value
		Low (0-28), *n* = 192	Desirable (29-46), *n* = 255	
Gender				
Female	28.49 ± 6.16	120 (62.5)	149 (58.4)	0.430
Male	29.04 ± 6.10	72 (37.5)	106 (41.6)	
Age in yr				
≤ 40	27.6 ± 6.6	91 (47.4)	102 (40.0)	0.120
> 40	27.5 ± 8.5	101 (52.6)	153 (60.0)	
Education level				
Illiterate	25.4 ± 8.3	74 (38.5)	62 (24.3)	0.002
Primary school	28.1 ± 5.1	32 (16.7)	34 (13.3)	
Secondary school	30.9 ± 5.7	66 (34.4)	120 (47.1)	
College	30.8 ± 5.4	20 (10.4)	39 (15.3)	
Family income				
Low	26.1 ± 6.9	21 (10.9)	17 (6.7)	0.200
Indeterminate	29.4 ± 7.0	105 (54.7)	145 (56.9)	
High	30.3 ± 5.5	57 (29.7)	88 (34.5)	
Race				
White	29.8 ± 7.1	79 (41.1)	132 (51.8)	0.030
Non-white	28.2 ± 6.3	107 (55.7)	118 (46.3)	
Marital status				
Married	28.4 ± 7.1	94 (48.9)	128 (50.2)	0.840
Unmarried	27.3 ± 8.0	97 (50.5)	127 (49.8)	
Individuals living in the same home				
1	30.5 ± 5.0	11 (5.7)	28 (11.0)	0.014
2	29.5 ± 5.6	41 (21.4)	56 (22.0)	
3	28.3 ± 6.3	63 (32.8)	65 (25.5)	
4	28.8 ± 6.6	31 (16.1)	63 (24.7)	
≥ 5	27.6 ± 6.4	46 (24.0)	42 (16.5)	
Population				
Southeast viral hepatitis ambulatory	33.1 ± 4.5	13 (6.8)	87 (34.1)	< 0.0001
South health center	29.1 ± 5.3	37 (19.3)	52 (20.4)	
Northeast health center	27.5 ± 5.0	58 (30.2)	56 (22.0)	
Southeast low resource areas	27.6 ± 4.7	43 (22.4)	34 (13.3)	
Northeast low resource areas	25.0 ± 8.5	41 (21.3)	26 (10.2)	

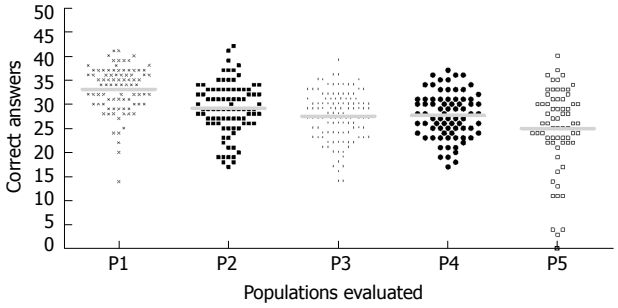

Figure 1 Distribution of correct answers plotted according to each population evaluated. The y-axis represents the number of correct answers. The solid lines represent the average for P1 (Southeast Viral Hepatitis Ambulatory), P2 (South Health Center), P3 (Northeast Health Center), P4 (Southeast low resource areas) and P5 (Northeast low resource areas), which were respectively: 33.1 ± 4.5; 29.1 ± 5.3; 27.5 ± 5.0; 27.6 ± 4.7; and, 25.0 ± 8.5.

Southeast and Northeast low resource areas. The findings of the current study are in line with previous findings[5,22,24]. However, the study in Egypt noted high baseline knowledge about HCV[23], likely due to the scale of the HCV epidemic in this country.

Complications arising from viral hepatitis was the worst set of questions evaluated in the current study. Although more than 80% of participants can correctly correlate cirrhosis and liver cancer with complications of viral hepatitis, most of them related complications that are not caused by hepatitis. In previous studies between health professionals, more than half of participants answered correctly to the questions about HCV complications[26]. However, an insufficient knowledge regarding HCV complications was observed in a study among health professionals[27]. Clinical manifestations of viral hepatitis were the best set of questions evaluated, contrary to previous observations[28].

In the present study, most individuals recognize the existence of HAV, HBV and HCV and do not recognize the existence of hepatitis D or E. The same finding has previously been observed in Brazil[24]. Another study[28] observed a very weak knowledge regarding the five hepatitis types among medical science students. Transmission and prevention modes were correctly answered in general; this data was also observed among medical and health science students in Ethiopia in the evaluation of HBV knowledge[21]. A large number of individuals do not know that viral hepatitis can be transmitted by seafood, as observed previously[24]. Since HAV and HEV can be transmitted in this way[29,30], the transmission may continue if preventive measures are not taken.

Viral hepatitis transmission by mosquito and forms to prevent it were incorrectly answered by most par-

Table 4 Final adjusted model of multivariate logistic regression for knowledge scores for viral hepatitis

Variable	Knowledge score			
	OR	95%CI		*P* value
		Lower	Upper	
Education level				
Illiterate	2.230	1.084	4.586	0.290
Primary school	1.799	0.807	4.009	0.151
Secondary school	1.028	0.528	2.002	0.936
College	1.000	-	-	-
Individuals living in the same home				
1	1.000	-	-	-
2	1.611	0.671	3.867	0.286
3	2.328	0.992	5.465	0.052
4	0.818	0.332	2.017	0.663
⩾ 5	1.832	0.748	4.486	0.185
Population				
Southeast viral hepatitis ambulatory	1.000	-	-	-
South health center	6.154	2.900	13.058	0.000
Northeast health center	8.617	4.177	17.777	0.000
Southeast low resource areas	7.491	3.508	15.994	0.000
Northeast low resource areas	11.262	5.007	25.327	0.000

CI: Confidence interval; OR: Odds ratio.

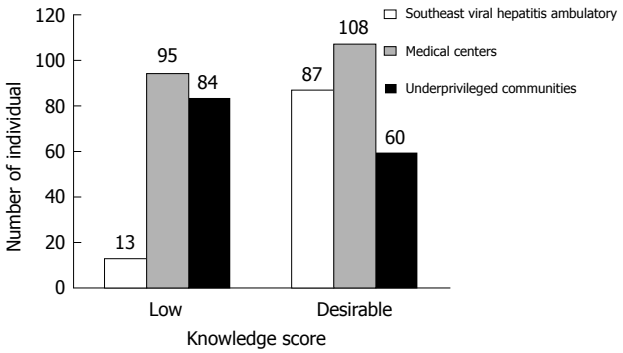

Figure 2 Number of individuals according to knowledge score in each group evaluated.

ticipants, probably due to the country-wide presence of Dengue virus, the transmission of which is widely understood by the public. Most individuals did not cite transmission by air but, curiously, masks to avoid airborne contamination were cited. These questions highlight the need for raising awareness among the public to reinforce knowledge related to the modes of transmission and prevention.

In present study, population type was the significant demographic factor associated with knowledge level in multivariate analysis, the same as found in other studies[5,24,31]. Contrary to a previous general population study in Brazil[24], monthly family income had no association with knowledge in the present study.

The results obtained in the present study can be used as a data source for the projection of intervention methods in health and public health policies, such as explanatory educational leaflet, educational booklets, lectures in schools, health campaigns, health fairs and others, in order to increase access to information of viral hepatitis and possibly to reduce the number of cases of these infections, especially among individuals from low-resource areas that showed a lower level of knowledge in the present study.

The present study has some limitations. The study did not assess the information regarding the neighborhood of each participant to observe the sociodemographic diversity. The study did not assess the occupation of participants to categorize and compare with studies in specific groups, such as health or beauty professionals. In Viral Hepatitis Ambulatory and in medical centers, it was not asked whether participants had previously consulted and whether they had any prior knowledge about hepatitis.

In conclusion, in general, desirable knowledge was observed among most participants. However, Northeast Health Center and under-privileged communities showed low knowledge. Knowledge levels were associated with education level, race and number of individuals living in the same home. The results of the present study should prove useful for information and prevention campaigns targeted at the general population, especially between neglected communities, in order to reduce the transmission of viral hepatitis.

ARTICLE HIGHLIGHTS

Research background

Viral hepatitis is an important public health problem in the world, causing more than 1 million deaths annually. It is important to evaluate viral hepatitis perception to identify the possible gaps and help public health authorities to create strategies to increase access to information about these infections.

Research motivation

Few studies have been done to evaluate viral hepatitis perception in uninfected individuals, particularly in Latin America.

Research objectives

The main aim of this study was to evaluate the viral hepatitis knowledge among

individuals from different resource areas and health conditions in Brazil to identify possible gaps and help authorities in the development of prevention and education programs.

Research methods

This was a cross-sectional study, wherein a questionnaire to evaluate viral hepatitis perception was applied among 447 individuals from five different populations in Brazil (Southeast low resource areas, Northeast low resource areas, South Health Center, Northeast Health Center, Southeast Viral Hepatitis Center). The viral hepatitis perception score was created based on the average of correct answers of all participants' responses (28.7), and associations between sociodemographic characteristics and perception were also evaluated.

Research results

High perception level about viral hepatitis was observed in Southeast Viral Hepatitis Ambulatory and South Health Center compared to Northeast Health Center, Southeast and Northeast low resource areas. According to sociodemographic characteristics, desirable scores were more common among those with secondary education (47.1%), those who declared themselves as white (46.3%), and those who lived in houses with three individuals (25.5%). Population type was associated with knowledge level in multivariate analysis.

Research conclusions

The study demonstrated a low level of perception about viral hepatitis among individuals from low resource areas. Identifying the knowledge gaps in this group could help to create strategies for increasing access to information and consequently reducing the transmission of these diseases.

Research perspectives

This study demonstrates that it is necessary to improve the access to health information about viral hepatitis, especially among residents of low-resource settings. It is important to conduct a random sampling evaluation of larger numbers of individuals to confirm the results observed. A questionnaire could help to conduct these studies, the same as was used in the present work.

ACKNOWLEDGMENTS

The authors would like to acknowledge the financial support of CAPES, CNPq and FAPERJ, and the volunteers who agreed to participate in this study.

REFERENCES

1 **Focaccia R**. Tratado de hepatites virais e doenças associadas. 3rd ed. São Paulo: Editora Atheneu, 2013
2 **World Health Organization**. Health topics Hepatitis, 2017. Available From URL: http://www.who.int/topics/hepatitis/en/
3 **Centers for Disease Control and Prevention**. Epidemiology and Prevention of Vaccine-Preventable Diseases. In: Atkinson W, Hamborsky J, Wolfe S, editors. 12th ed. Washington DC: Public Health Foundation, 2012
4 **Pereira LM**, Martelli CM, Merchán-Hamann E, Montarroyos UR, Braga MC, de Lima ML, Cardoso MR, Turchi MD, Costa MA, de Alencar LC, Moreira RC, Figueiredo GM, Ximenes RA; Hepatitis Study Group. Population-based multicentric survey of hepatitis B infection and risk factor differences among three regions in Brazil. *Am J Trop Med Hyg* 2009; **81**: 240-247 [PMID: 19635877 DOI: 10.4269/ajtmh.2009.81.240]
5 **ul Haq N**, Hassali MA, Shafie AA, Saleem F, Farooqui M, Aljadhey H. A cross sectional assessment of knowledge, attitude and practice towards Hepatitis B among healthy population of Quetta, Pakistan. *BMC Public Health* 2012; **12**: 692 [PMID: 22917489 DOI: 10.1186/1471-2458-12-692]
6 **Komatsu H**, Inui A, Sogo T, Tateno A, Shimokawa R, Fujisawa T. Tears from children with chronic hepatitis B virus (HBV) infection are infectious vehicles of HBV transmission: experimental transmission of HBV by tears, using mice with chimeric human livers. *J Infect Dis* 2012; **206**: 478-485 [PMID: 22508939 DOI: 10.1093/infdis/jis293]
7 **Ogholikhan S**, Schwarz KB. Hepatitis Vaccines. *Vaccines (Basel)* 2016; **4**: E6 [PMID: 26978406 DOI: 10.3390/vaccines4010006]
8 **Villar LM**, Cruz HM, Barbosa JR, Bezerra CS, Portilho MM, Scalioni Lde P. Update on hepatitis B and C virus diagnosis. *World J Virol* 2015; **4**: 323-342 [PMID: 26568915 DOI: 10.5501/wjv.v4.i4.323]
9 **Zhang X**, An J, Tu A, Liang X, Cui F, Zheng H, Tang Y, Liu J, Wang X, Zhang N, Li H. Comparison of immune persistence among inactivated and live attenuated hepatitis a vaccines 2 years after a single dose. *Hum Vaccin Immunother* 2016; **12**: 2322-2326 [PMID: 27494260 DOI: 10.1080/21645515.2015.1134069]
10 **Debing Y**, Moradpour D, Neyts J, Gouttenoire J. Update on hepatitis E virology: Implications for clinical practice. *J Hepatol* 2016; **65**: 200-212 [PMID: 26966047 DOI: 10.1016/j.jhep.2016.02.045]
11 **Pang L**, Alencar FE, Cerutti C Jr, Milhous WK, Andrade AL, Oliveira R, Kanesa-Thasan N, MaCarthy PO, Hoke CH Jr. Short report: hepatitis E infection in the Brazilian Amazon. *Am J Trop Med Hyg* 1995; **52**: 347-348 [PMID: 7741175 DOI: 10.4269/ajtmh.1995.52.347]
12 **Paraná R**, Vitvitski L, Andrade Z, Trepo C, Cotrim H, Bertillon P, Silva F, Silva L, de Oliveira IR, Lyra L. Acute sporadic non-A, non-B hepatitis in Northeastern Brazil: etiology and natural history. *Hepatology* 1999; **30**: 289-293 [PMID: 10385669 DOI: 10.1002/hep.510300143]
13 **Trinta KS**, Liberto MI, de Paula VS, Yoshida CF, Gaspar AM. Hepatitis E virus infection in selected Brazilian populations. *Mem Inst Oswaldo Cruz* 2001; **96**: 25-29 [PMID: 11285473 DOI: 10.1590/S0074-02762001000100004]
14 **Santos DC**, Souto FJ, Santos DR, Vitral CL, Gaspar AM. Seroepidemiological markers of enterically transmitted viral hepatitis A and E in individuals living in a community located in the North Area of Rio de Janeiro, RJ, Brazil. *Mem Inst Oswaldo Cruz* 2002; **97**: 637-640 [PMID: 12219125 DOI: 10.1590/S0074-02762002000500007]
15 **Lyra AC**, Pinho JR, Silva LK, Sousa L, Saraceni CP, Braga EL, Pereira JE, Zarife MA, Reis MG, Lyra LG, Silva LC, Carrilho FJ. HEV, TTV and GBV-C/HGV markers in patients with acute viral hepatitis. *Braz J Med Biol Res* 2005; **38**: 767-775 [PMID: 15917959 DOI: 10.1590/S0100-879X2005000500015]
16 **Ataei B**, Shirani K, Alavian SM, Ataie M. Evaluation of Knowledge and Practice of Hairdressers in Women's Beauty Salons in Isfahan About Hepatitis B, Hepatitis C, and AIDS in 2010 and 2011. *Hepat Mon* 2013; **13**: e6215 [PMID: 23658593 DOI: 10.5812/hepatmon.6215]
17 **Wu E**, Chen X, Guan Z, Cao C, Rao H, Feng B, Chan M, Fu S, Lin A, Wei L, Lok AS. A comparative study of patients' knowledge about hepatitis C in the United States and in urban and rural China. *Hepatol Int* 2015; **9**: 58-66 [PMID: 25788380 DOI: 10.1007/s12072-014-9559-z]
18 **Maniero VC,** Goldbach T, Marques APC, Cavaretto LSP, Santos AMO, Villar LM. Evaluation of the knowledge of nursing students about viral hepatitis. *J Nurs UFPE on line* 2012; **6**: 831-838
19 **Villar LM**, de Paula VS, de Almeida AJ, do Ó KM, Miguel JC, Lampe E. Knowledge and prevalence of viral hepatitis among beauticians. *J Med Virol* 2014; **86**: 1515-1521 [PMID: 24916521 DOI: 10.1002/jmv.23993]
20 **Ganczak M**, Dmytrzyk-Daniłów G, Korzeń M, Drozd-Dąbrowska M, Szych Z. Prevalence of HBV Infection and Knowledge of Hepatitis B Among Patients Attending Primary Care Clinics in Poland. *J Community Health* 2016; **41**: 635-644 [PMID: 26699149 DOI: 10.1007/s10900-015-0139-5]
21 **Abdela A**, Woldu B, Haile K, Mathewos B, Deressa T. Assessment of knowledge, attitudes and practices toward prevention of hepatitis B virus infection among students of medicine and health sciences in Northwest Ethiopia. *BMC Res Notes* 2016; **9**: 410 [PMID: 27543117 DOI: 10.1186/s13104-016-2216-y]
22 **Brouard C**, Gautier A, Saboni L, Jestin C, Semaille C, Beltzer N; KABP France group. Hepatitis B knowledge, perceptions

and practices in the French general population: the room for improvement. *BMC Public Health* 2013; **13**: 576 [PMID: 23764171 DOI: 10.1186/1471-2458-13-576]

23 **Chemaitelly H**, Abu-Raddad LJ, Miller FD. An apparent lack of epidemiologic association between hepatitis C virus knowledge and the prevalence of hepatitis C infection in a national survey in Egypt. *PLoS One* 2013; **8**: e69803 [PMID: 23922806 DOI: 10.1371/journal.pone.0069803]

24 **Cruz HM**, de Paula VS, Villar LM. A Cross-Sectional Study of Viral Hepatitis Perception among Residents from Southeast and North Regions of Brazil. *Int J Environ Res Public Health* 2018; **15**: E189 [PMID: 29364166 DOI: 10.3390/ijerph15020189]

25 **Instituto Brasileiro de Geografia e Etatística**. Portal do IBGE. Available from: URL: https://www.ibge.gov.br/index.php

26 **Sood A**, Midha V, Awasthi G. Hepatitis C--knowledge & practices among the family physicians. *Trop Gastroenterol* 2002; **23**: 198-201 [PMID: 12833713]

27 **Joukar F**, Mansour-Ghanaei F, Soati F, Meskinkhoda P. Knowledge levels and attitudes of health care professionals toward patients with hepatitis C infection. *World J Gastroenterol* 2012; **18**: 2238-2244 [PMID: 22611318 DOI: 10.3748/wjg.v18.i18.2238]

28 **Ghahramani F,** Mohammadbeigi A, Mohammadsalehi N. A survey of the students' knowledge about hepatitis in Shiraz University of Medical Sciences. *Hepat Mon* 2006; **6**: 59-62

29 **Polo D**, Varela MF, Romalde JL. Detection and quantification of hepatitis A virus and norovirus in Spanish authorized shellfish harvesting areas. *Int J Food Microbiol* 2015; **193**: 43-50 [PMID: 25462922 DOI: 10.1016/j.ijfoodmicro.2014.10.007]

30 **Cui W**, Sun Y, Xu A, Gao R, Gong L, Zhang L, Jiang M. Hepatitis E seroprevalence and related risk factors among seafood processing workers: a cross-sectional survey in Shandong Province, China. *Int J Infect Dis* 2016; **49**: 62-66 [PMID: 27265612 DOI: 10.1016/j.ijid.2016.05.028]

31 **ul Haq N**, Hassali MA, Shafie AA, Saleem F, Farooqui M, Haseeb A, Aljadhey H. A cross-sectional assessment of knowledge, attitude and practice among Hepatitis-B patients in Quetta, Pakistan. *BMC Public Health* 2013; **13**: 448 [PMID: 23641704 DOI: 10.1186/1471-2458-13-448]

P- Reviewer: Gigi E, Pokorska-Spiewak M, Shenoy SM, Nozic D
S- Editor: Wang JL **L- Editor**: Filipodia **E- Editor**: Bian YN

World Journal of Hepatology

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

World J Hepatol 2018 November 27; 10(11): 877-886

DOI: 10.4254/wjh.v10.i11.877

ISSN 1948-5182 (online)

META-ANALYSIS

Cardiac stress testing and coronary artery disease in liver transplantation candidates: Meta-analysis

Jonathan Soldera, Fábio Camazzola, Santiago Rodríguez, Ajacio Brandão

Jonathan Soldera, Fábio Camazzola, School of Medicine, Universidade de Caxias do Sul (UCS), Caxias do Sul 95070-560, Brazil

Jonathan Soldera, Santiago Rodríguez, Ajacio Brandão, School of Medicine, Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA), Porto Alegre 90050-170, Brazil

Ajacio Brandão, Liver Transplantation Group, Santa Casa de Misericórdia de Porto Alegre, Porto Alegre 90020-090, Brazil

ORCID number: Jonathan Soldera (0000-0001-6055-4783); Fábio Camazzola (0000-0002-2863-4158); Santiago Rodríguez (0000-0001-8610-3622); Ajacio Brandão (0000-0001-8411-5654).

Author contributions: Soldera J and Brandão A contributed to study concept and design, and drafting of the manuscript; Soldera J, Camazzola F and Rodríguez S contributed to acquisition of data, analysis and interpretation of data; Soldera J contributed to statistical analysis; Brandão A contributed to study supervision; all authors contributed to critical revision of the manuscript for important intellectual content.

Conflict-of-interest statement: Dr. Soldera has nothing to disclose.

Manuscript source: Unsolicited manuscript

Correspondence to: Jonathan Soldera, MSc, Associate Professor, School of Medicine, Universidade de Caxias do Sul (UCS), Av. Vereador Mário Pezzi, 699/601, Caxias do Sul 95070-560, Brazil. jonathansoldera@gmail.com
Telephone: +55-54-991028181

Received: July 17, 2018
Peer-review started: July 17, 2018
First decision: August 20, 2018
Revised: September 13, 2018
Accepted: October 11, 2018
Article in press: October 11, 2018
Published online: November 27, 2018

Abstract

AIM

To evaluate the diagnostic value of dobutamine stress echocardiography (DSE) and myocardial perfusion scintigraphy (MPS) in predicting coronary artery disease (CAD) in cirrhotic patients listed for liver transplantation (LT), using invasive coronary angiography (ICA) as gold-standard.

METHODS

Retrieval of studies was based on Medical Subject Headings and Health Sciences Descriptors, which were combined using Boolean operators. Searches were run on the electronic databases Scopus, Web of Science, EMBASE, MEDLINE (PubMed), BIREME (Biblioteca Regional de Medicina), LILACS (Latin American and Caribbean Health Sciences Literature), Cochrane Library for Systematic Reviews and Opengray.eu. There was no language or date of publication restrictions. The reference lists of the studies retrieved were searched manually.

RESULTS

The search strategy retrieved 322 references for DSE and 90 for MPS. In the final analysis, 10 references for DSE and 10 for MPS were included. Pooled sensitivity was 28% and 61% for DSE and MPS and specificity was 82% and 74%, for diagnosis of CAD using ICA as gold-standard, respectively.

CONCLUSION
DSE and MPS do not have adequate sensitivity for determination of whether CAD is present, despite having significant specificity.

Key words: Myocardial perfusion imaging; Coronary angiography; Liver transplantation; Echocardiography; Stress

Core tip: The concept of cardiac involvement in cirrhotic patients has been changing as patients listed for liver transplantation (LT) have become older and sicker. We aimed to evaluate the diagnostic value of dobutamine stress echocardiography (DSE) and myocardial perfusion scintigraphy (MPS) in predicting coronary artery disease (CAD) in cirrhotic patients listed for LT, using invasive coronary angiography as gold-standard. A systematic review and meta-analysis was performed, including 10 references for DSE and 10 for MPS. We concluded that DSE and MPS do not have adequate sensitivity for determination of whether CAD is present, despite having significant specificity.

Soldera J, Camazzola F, Rodríguez S, Brandão A. Cardiac stress testing and coronary artery disease in liver transplantation candidates: Meta-analysis. *World J Hepatol* 2018; 10(11): 877-886 Available from: URL: http://www.wjgnet.com/1948-5182/full/v10/i11/877.htm DOI: http://dx.doi.org/10.4254/wjh.v10.i11.877

INTRODUCTION

When liver transplantation (LT) programs were beginning three decades ago, it was believed that the systemic vasodilation that occurs in end-stage liver disease (ESLD) might be able to protect patients from coronary artery disease (CAD)[1]. Nevertheless, studies have shown that CAD is more prevalent in cirrhotic patients than previously suspected. In a cohort with high risk for CAD, 26% of the patients had previously unknown CAD on routine invasive coronary angiography (ICA)[2].

The cardiac profile for LT candidates has been changing, because they are now older and sicker[3]. Data from the United Network for Organ Sharing (UNOS) show that the proportion of LT recipients over the age of 65 years in the United States increased from 9.6% in 2003 to 16.3% in 2013[4]. This has been a cause for major concern regarding perioperative cardiac risk. For example, a publication from 1996 predicted that around 50% of patients with significant CAD would die from cardiac complications in the perioperative period[5]. However, in a more recent study, the presence of obstructive CAD did not significantly impact post-LT survival, when modern treatment of CAD pre-LT is taken into account[6]. Furthermore, patients with ESLD have a specific type of cardiovascular sickness, currently known as cirrhotic cardiomyopathy, whose role in LT survival is yet to be established[7].

These findings suggest a real need for protocols for cardiac evaluation of patients awaiting LT - particularly for cirrhotic patients. The American Association for the Study of Liver Diseases (AASLD) published a guideline in 2005 that recommends myocardial stress testing for every patient referred for LT[8]. Nevertheless, the guideline published in 2012 by the American Heart Association (AHA) and the American College of Cardiology (ACC)[9], suggested that myocardial stress testing should be reserved for patients with three or more CAD risk factors. A score has recently been published for evaluation of perioperative cardiac risk, but it has yet to be validated further[10].

The aim of this systematic review with meta-analysis is to summarize the evidence related to the diagnostic value of two non-invasive cardiac stress testing methods: Dobutamine stress echocardiography (DSE) and myocardial perfusion scintigraphy (MPS), for the diagnosis of CAD in cirrhotic pre-LT patients, using ICA as gold-standard.

MATERIALS AND METHODS

This study was carried out in accordance with the recommendations contained in the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA-P) guidelines[11]. Our systematic review was registered with the International Prospective Register of Systematic Reviews (PROSPERO), maintained by York University, on 17 August 2015 and was last updated on 5 April 2018 [registration No. 10.15124/CRD42015025391 (www.crd.york.ac.uk/prospero/)].

Data sources

Studies were retrieved using Medical Subject Headings (MeSH) and Health Sciences Descriptors (DeCS), which were combined with Boolean operators. Searches were run on the electronic databases Scopus, Web of Science, Embase, Medline (PubMed), BIREME (Biblioteca Regional de Medicina), LILACS (Latin American and Caribbean Health Sciences Literature), Cochrane Library for Systematic Reviews and Opengray.eu. There was no language or date of publication restrictions. The reference lists of the retrieved studies were submitted to manual search. The search strategies used for each test and each database are shown in Supplemental material. Databases were last searched between August and September of 2015.

Inclusion criteria and outcomes

Cohort or case-control studies were eligible for selection, hence it was analyzed the diagnostic accura-

cy of DSE and/or MPS in adult patients with cirrhosis submitted for pre-LT evaluation. The tests had to be performed as a part of cardiac evaluation before LT. Studies were excluded if they did not meet these inclusion criteria. If there was more than one study published using the same population, the most recent study was selected for the analysis. Studies published only as abstracts were included, as long as the data available made analysis possible. The outcome measured was a diagnosis of CAD using ICA as gold standard.

Study selection and data extraction

An initial screening of titles and abstracts was the first stage to select potentially relevant papers. The second step was the analysis of the full-length papers. Two independent reviewers (Jonathan Soldera, Fabio Camazzola) extracted data using a standardized data extraction form after assessing and reaching consensus on eligible studies. The same reviewers separately assessed each study and extracted data about the characteristics of the subjects, the diagnostic accuracy for DCE and MPS and the outcomes measured. A third party (Santiago Rodriguez) was responsible for divergences in data extraction, clearing them when required. Quality of evidence regarding diagnostic accuracy was evaluated according of the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2)[12].

Statistical analysis

In anticipation of possible heterogeneity between the populations of the studies, a random-effects DerSimonian and Laird model was used. Data regarding the tests' diagnostic accuracy was collected. The measures of diagnostic accuracy chosen were specificity, sensitivity, likelihood ratio and diagnostic odds ratio. Heterogeneity was assessed using the I^2 statistic. MetaDisc 1.4 was used for diagnostic accuracy. The small number of studies included made funnel plot analysis impossible.

RESULTS

Systematic review

The search strategy retrieved 322 references for DSE and 90 for MPS. After analyzing titles and abstracts, 111 references for DSE and 24 for MPS were excluded because they were duplicates and the full texts were retrieved for 60 references on DSE and 26 on MPS. In the final analysis, 10 references were included for DSE and 10 for MPS. Flowcharts illustrating the search strategies are shown in Supplemental Figures 1 and 2, respectively. Studies included were either a case-control study or a prospective or historical cohort study.

DSE

Data were collected after the conclusion of a systematic review of the 10 studies included in the diagnostic analysis that used ICA as the gold-standard. The data extracted are summarized in Table 1.

A minority of the patients included in these studies underwent ICA and they were generally higher risk patients with positive DSE findings or multiple risk factors. Data for risk factors specifically for the patients who underwent ICA were not available for most studies, therefore the data on risk factors described refer to the whole study population, as summarized in Supplemental Table 1.

The initial meta-analysis was performed including all studies. Global sensitivity was 28% [95% confidence interval (CI): 21.2%-35.6%] with high heterogeneity (I^2 = 69%) (Figure 1), specificity was 82.9% (95%CI: 78.5%-86.8%) with high heterogeneity (I^2 = 84.1%) (Figure 2) and the diagnostic odds ratio was 2.09 (95%CI: 0.96-4.58) with moderate heterogeneity (I^2 = 47.5%) (Supplemental Figure 3). The positive likelihood ratio was 1.7 (95%CI: 1.06-2.7) with moderate heterogeneity (I^2 = 51.4%) (Supplemental Figure 4) and the negative likelihood ratio was 0.92 (95%CI: 0.81-1.04) with little heterogeneity (I^2 = 18.8%) (Supplemental Figure 5). An asymmetrical Receiver Operating Characteristic (ROC) curve is provided in Supplemental Figure 6.

A meta-regression was performed using the subsets of patients from each of the study samples who had undergone ICA and no statistically significant association was detected between this variable and the diagnostic odds ratio (P = 0.0586).

In order to attempt to reduce heterogeneity between studies, a sub-analysis was performed of sensitivity and specificity according to the definition of a positive ICA result employed by each study. Studies that used a positive ICA defined as any number of lesions with at least one greater than 70%, had a sensitivity of 21% (95%CI: 13.4%-31.3%) with high heterogeneity (I^2 = 71%) and a specificity of 91.5% (95%CI: 86.8%-95%) with high heterogeneity (I^2 = 63.5%), while studies that defined positive ICA as any number of lesions, with at least one greater than 50%, had a sensitivity of 36.1% (95%CI: 25.1%-48.3%) with high heterogeneity (I^2 = 66.3%) and a specificity of 69.9% (95%CI: 61.4%-77.6%) with high heterogeneity (I^2 = 68%).

MPS

Data were collected after conclusion of a systematic review of the 10 studies included in the diagnostic analysis that used ICA as the gold-standard. The data extracted are summarized in Table 2.

As with DSE, a minority of the patients included in these studies underwent ICA, and they were generally

Table 1 Studies included in analysis - dobutamine stress echocardiography

Ref.	TP	FP	FN	TN	Total number of patients in the study	Proportion of patients who underwent ICA	Definition of patients included	Criteria for ICA indication	Lesion for definition of positive ICA	QUADAS-2 quality analysis criteria
Ibrahim *et al*[13]	5	8	5	22	366	10.9%	Cirrhotic patients in pre-LT evaluation	High risk patients/ positive DSE	NA	RB: P + I - R + F ? AC: P + I ? R +
Donovan *et al*[14]	3	6	1	8	190	9.5%	Cirrhotic patients in pre-LT evaluation	High risk patients/ positive DSE	> 50%	RB: P + I + R + F + AC: P + I + R +
Findlay *et al*[15]	1	6	0	0	117	6%	Cirrhotic patients in pre-LT evaluation	Transplanted patients	> 70%	RB: P + I + R + F + AC: P – I + R +
Harinstein *et al*[16]	2	7	14	41	105	61%	Cirrhotic patients in pre-LT evaluation	High risk patients/ positive DSE	> 70%	RB: P + I + R + F + AC: P + I + R +
Harinstein *et al*[16]	4	5	20	35	105	61%	Cirrhotic patients in pre-LT evaluation	High risk patients/ positive DSE	> 50%	RB: P + I + R + F + AC: P + I + R +
Plotkin *et al*[17]	2	0	0	19	40	52.5%	Cirrhotic patients in pre-LT evaluation	High risk patients/ positive DSE	> 70%	RB: P + I + R + F + AC: P + I + R +
Ramrakhiani *et al*[18]	4	10	0	0	201	7%	Cirrhotic patients in pre-LT evaluation	High risk patients/ positive DSE	> 70%	RB: P + I – R – F ? AC: P + I – R –
Tsutsui *et al*[19]	2	5	0	10	230	7.4%	Cirrhotic patients in pre-LT evaluation	High risk patients/ positive DSE	> 50%	RB: P + I + R + F + AC: P + I + R +
Umphrey *et al*[20]	0	0	0	9	157	5.7%	Cirrhotic patients in pre-LT evaluation	High risk patients	> 70%	RB: P + I + R + F + AC: P + I + R +
Snipelisky *et al*[21]	12	16	20	18	66	100%	Cirrhotic patients in pre-LT evaluation	High risk patients/ positive DSE	> 50%	RB: P + I + R + F + AC: P + I + R +
Patel *et al*[22]	15	10	56	124	420	48.8%	Cirrhotic patients in pre-LT evaluation	High risk patients/ positive DSE	> 70%	RB: P + I + R + F + AC: P + I + R +

TP: True positive; FP: False positive; FN: False negative; TN: True negative; ICA: Invasive coronary angiography; LT: Liver transplantation; DSE: Dobutamine stress echocardiography; NA: Not available; QUADAS-2: Quality assessment of diagnostic accuracy studies-2; RB: Risk of bias; P: Patient selection; I: Index text; R: Reference standard; F: Flow and timing; AC: Applicability concerns.

higher risk patients with a positive MPS result or multiple risk factors. As with DSE, data for risk factors specifically for the patients who underwent ICA were not available for most studies, therefore the data for risk factors described refer to the whole study population, as summarized in Supplemental Table 2.

The diagnostic data were used for meta-analysis. The initial meta-analysis was performed including all studies. Global sensitivity was 61.8% (95%CI: 50%-72.8%) with high heterogeneity (I^2 = 69.8%) (Figure 3), specificity was 74.3% (95%CI: 70.2%-78.2%) with high heterogeneity (I^2 = 77.1%) (Figure 4) and the diagnostic odds ratio was 4.74 (95%CI: 1.51-14.8) with high heterogeneity (I^2 = 61.9%) (Supplemental Figure 7). The positive likelihood ratio was 2.26 (95%CI: 1.47-3.48) with high heterogeneity (I^2 = 63.5%) (Supplemental Figure 8) and the negative likelihood ratio was 0.57 (95%CI: 0.32-1.02) with high heterogeneity (I^2 = 62.7%) (Supplemental Figure 9). An asymmetrical ROC curve is provided in Supplemental Figure 10.

A meta-regression was performed using the subsets of patients from each of the study samples who had undergone ICA and no statistically significant association was detected between this variable and the diagnostic odds ratio (P = 0.4984).

In order to attempt to reduce heterogeneity between studies, a sub-analysis was performed of sensitivity and specificity according to the definition of a positive ICA result employed by each study. Studies that used a positive ICA defined as any number of lesions with at least one greater than 70% had a sensitivity of 59.4% (95%CI: 46.4%-71.5%) with high heterogeneity (I^2 = 70.5%) and specificity of 76.3% (95%CI: 71.6%-80.5%) with high heterogeneity (I^2 = 80%). In another sub-analysis, including only the four studies in which ICA was performed for all patients, sensitivity was 57.1% (95%CI: 44%-69.5%) with high heterogeneity (I^2 = 71.1%) and specificity was 75.5% (95%CI: 71.4%-79.7%) with high heterogeneity (I^2 =

Table 2 Studies included for analysis - myocardial perfusion scintigraphy

Ref.	TP	FP	FN	TN	Total number of patients in the study	Proportion of patients who underwent ICA	Definition of patients included	Criteria for ICA indication	Lesion for definition of positive ICA	QUADAS-2 quality analysis criteria
Baker *et al*[23]	8	4	0	14	74	35.1%	Cirrhotic patients in pre-LT evaluation with cardiac risk factors	High risk patients/ positive MPS	> 70%	RB: P – I + R + F + AC: P – I + R +
Kryzhanovski *et al*[24]	0	1	0	0	63	1.6%	Cirrhotic patients in pre-LT evaluation with cardiac risk factors	High risk patients/ positive MPS	> 70%	RB: P – I + R + F + AC: P – I + R +
Senzolo *et al*[25]	0	2	0	0	24	8.3%	Cirrhotic patients in pre-LT evaluation	Positive MPS	> 70%	RB: P – I + R + F + AC: P – I + R +
Kandiah *et al*[26]	1	4	0	5	93	10.7%	Cirrhotic patients in pre-LT evaluation	High risk patients/ positive MPS	> 70%	RB: P – I + R + F + AC: P – I + R +
Oprea-Lager *et al*[27]	1	1	0	0	156	1.2%	Cirrhotic patients in pre-LT evaluation	High risk patients/ positive MPS	> 70%	RB: P – I + R + F + AC: P – I + R +
Davidson *et al*[28]	7	24	12	40	83	100%	Cirrhotic patients in pre-LT evaluation with cardiac risk factors	High risk patients/ positive MPS	> 70%	RB: P + I + R + F + AC: P + I + R +
Aydinalp *et al*[29]	6	34	0	64	389	26.7%	Cirrhotic patients in pre-LT evaluation	High risk patients/ positive MPS	> 50%	RB: P + I + R + F + AC: P + I + R +
Zoghbi *et al*[30]	2	11	2	12	87	31%	Cirrhotic patients in pre-LT evaluation	High risk patients/ positive MPS	> 70%	RB: P – I + R + F + AC: P – I + R +
Bezinover *et al*[31]	3	1	3	9	173	9.2%	Cirrhotic patients in pre-LT evaluation	High risk patients/ positive MPS	NA	RB: P + I + R + F + AC: P + I + R +
Bhutani *et al*[32]	20	46	12	215	414	70.7%	Cirrhotic patients in pre-LT evaluation	High risk patients/ positive MPS	> 70%	RB: P + I + R + F + AC: P + I + R +

TP: True positive; FP: False positive; FN: False negative; TN: True negative; ICA: Invasive coronary angiography; LT: Liver transplantation; MPS: Myocardial perfusion scintigraphy; NA: Not available; QUADAS-2: Quality assessment of diagnostic accuracy studies-2; RB: Risk of bias; P: Patient selection; I: Index text; R: Reference standard; F: Flow and timing; AC: Applicability concerns.

84.2%).

DISCUSSION

It is essential to understand the role of CAD in cirrhosis and LT patients. There is a need to improve pre-LT diagnostic tools because the age of LT candidates is rising and the proportion of NASH patients has been increasing. This systematic review is the largest current meta-analysis of diagnostic data for DSE and MPS in pre-LT patients. It increases the data available in a previous study of DSE as a diagnostic and prognostic tool for LT candidates, published by Nguyen *et al*[33], which found that DSE had a high negative predictive value for adverse outcomes post-LT.

Among the general population, a prior meta-analysis of five studies found that both DSE and MPS are accurate for detection of CAD, with sensitivity of 85% and specificity of 87%[34] for DSE and sensitivity of 83% and specificity of 77% for MPS[35]. However, this meta-analysis found much lower sensitivity values for diagnosis of CAD in patients awaiting LT, while specificity rates did not vary so much. This could have happened because results for stress testing might be false due to modifications in hemodynamics caused by ESLD, such as high-output cardiac failure, cirrhotic cardiomyopathy, anemia and the use of beta blockers[36,37].

Nevertheless, the most used method for pre-LT cardiac stress testing is DSE, since cirrhotic patients have a low tolerance of exercise[38]. When compared to ergometric cardiac stress testing, DSE has higher sensitivity (67% *vs* 88%) and specificity (71% *vs* 83%)[39-41]. The prognostic value of MPS has also been evaluated previously, with a hazard ratio of 3.17 for all-cause mortality for a group with reversible perfusion

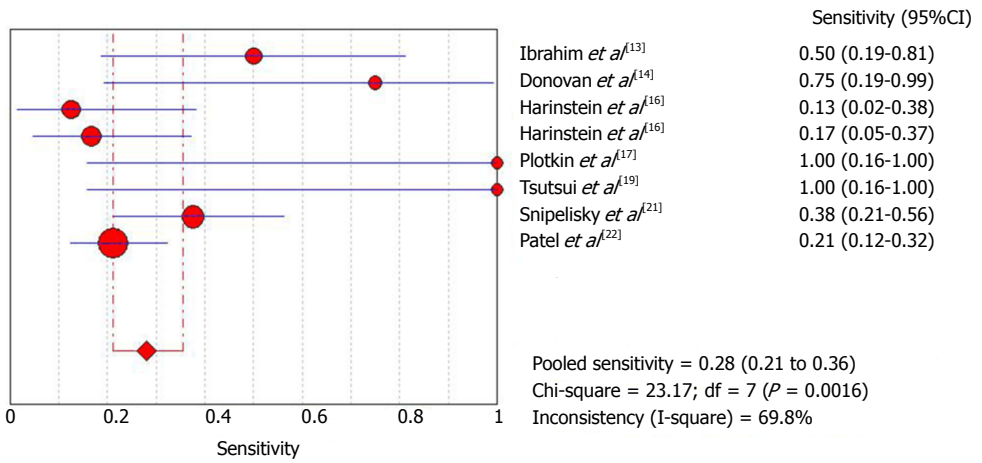

Figure 1 Forest plot for sensitivity meta-analysis - dobutamine stress echocardiography.

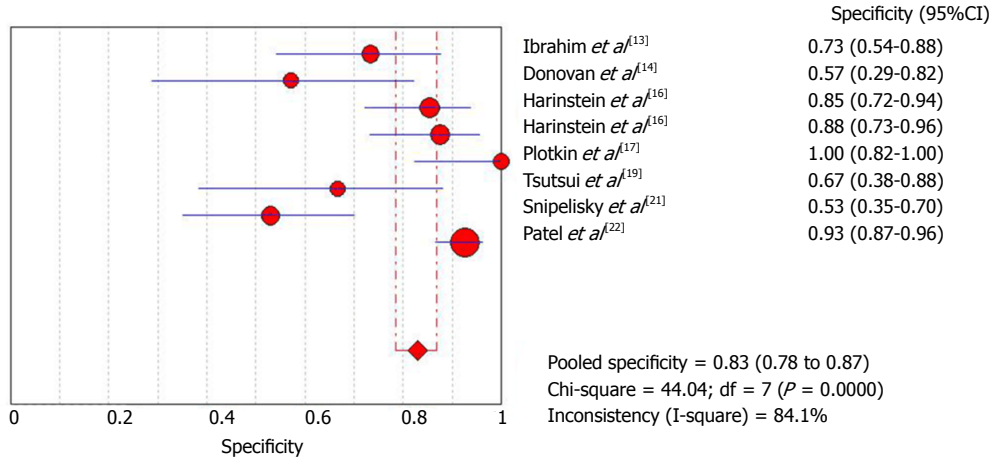

Figure 2 Forest plot for specificity meta-analysis - dobutamine stress echocardiography.

defect when compared to a group without perfusion defect[27].

The goal of both tests is to detect significant CAD prior to LT. In a high risk cohort in whom all patients underwent ICA and half had arterial systemic hypertension or diabetes, a 60% prevalence of CAD was found - one third with severe disease. Presence of moderate to severe CAD was associated with the presence of two or more cardiac risk factors[2]. If needed, ICA and stenting, seem to be safe in cirrhotic patients, taking precaution with the doubling of antiplatelet blockade in patients with esophageal varices[42]. The presence of CAD is associated with a poorer prognosis post-LT[43-45], although, Wray *et al*[6] did not detect a change in prognosis in the cohort they described. One must keep in mind also that pre-LT cardiac evaluation is costly and is not free from risks. In a previous study by Fili *et al*[46], the study protocol failed to demonstrate improvement in prognosis, but did raise costs.

One meta-analysis has found that DSE is superior to MPS among patients undergoing major vascular surgery - a positive DSE meant higher relative risk for perioperative MACE and all-cause mortality, when compared to MPS[47]. The prognostic role of DSE and MPS in patients undergoing kidney transplantation has been studied by two meta-analyses, which found these tests to be accurate in predicting outcomes, with DSE performing better than MPS in their analysis. Nevertheless, in this context, a normal non-invasive stress test did not necessarily exclude the possibility of adverse cardiac outcomes[48,49].

Analyzing the data collected and presented in this meta-analysis, it can be concluded that DSE and MPS offer limited accuracy for predicting CAD diagnoses. They both have low sensitivity and moderate specificity, which does not make them the ideal tests for pre-LT cardiac risk evaluation, as they also do

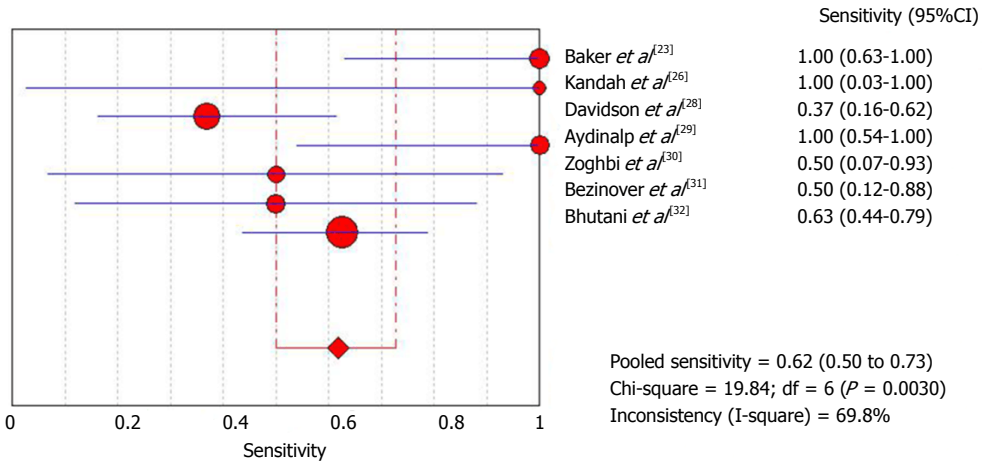

Figure 3 Forest plot for sensitivity meta-analysis - myocardial perfusion scintigraphy.

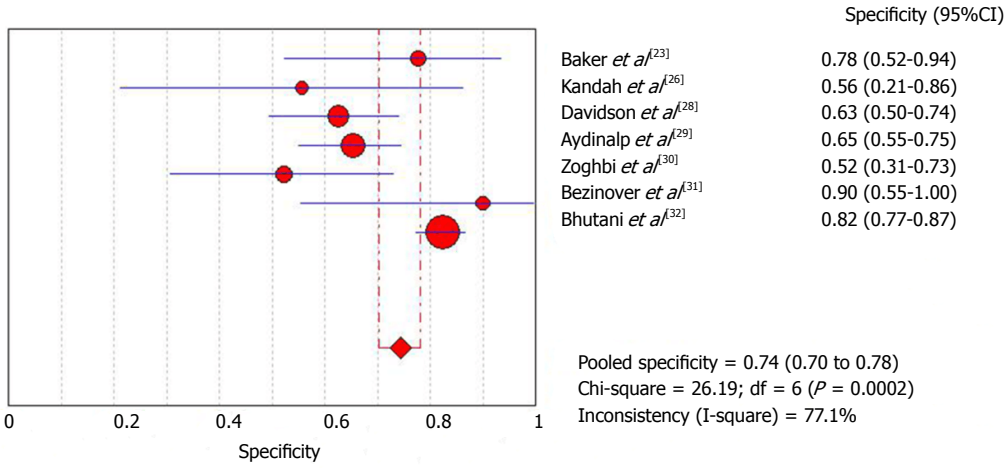

Figure 4 Forest plot for specificity meta-analysis - myocardial perfusion scintigraphy.

not predict adverse outcomes with accuracy[50]. This is consistent with the latest ACC/AHA guidelines, which describes non-invasive stress testing as of low sensitivity and specificity for detecting CAD in liver-transplant candidates[9]. Nevertheless, the high specificity found in this meta-analysis show that both DSE and MPS are useful for identifying patients with CAD. Notwithstanding, a negative stress test does not exclude the presence of CAD.

The element most likely to affect the results of this meta-analysis is selection of patients with indications for both LT and ICA. Generally, physicians happen to be more cautious in referring sicker and older patients for LT, which might mean that this group of patients is under-represented in this meta-analysis. Also, ICA is generally ordered only for high-risk patients with a positive DSE or MPS, and a positive ICA can lead to de-listing for LT, or even death before LT, due to advanced heart conditions.

This heterogeneity of indications for DSE and MPS as part of pre-LT evaluation is reflected in the heterogeneity found in this meta-analysis, which is high throughout. Sub-analyses and meta-regressions were attempted in order to minimize heterogeneity, but with no substantial success. A major limitation is that, in most studies, just a few patients were referred for ICA, generally those with higher risk or a positive non-invasive stress test, which might over represent the proportion of CAD in pre-LT patients.

The results of this meta-analysis call into question the AASLD rationale of recommending routine non-invasive stress testing in pre-LT cardiac evaluation, since DSE and MPS both have low sensitivity for detecting CAD and did not predict outcomes adequately. Nevertheless, further prospective studies with standardized and homogenous patient characteristics are necessary in order to arrive at a better understanding of the value of pre-LT cardiac evaluation and a better-grounded decision on whether it is more cost-effective to follow AASLD[8] or ACC/AHA reco-

mmendations[9]. Initiatives such as development of the CAR-OLT score might help clarify this problem[10]. This paper's strengths are its complete search strategy, performed in multiple databases. Nevertheless, results are just for pre-LT candidates; hence only patients referred for LT because of ESLD were reviewed.

The results of this systematic review and meta-analysis can also have been limited due to a post-referral bias, since patients with previously known serious cardiac conditions are generally not referred for LT. Early revascularization, in the general population, might lead to a significant change in the history of CAD and a better survival. This is somewhat unclear for ESLD patients. Because of the small number of studies and their limitations, the quality of evidence in the meta-analysis was low throughout, which might have negatively impacted this review.

In conclusion, this meta-analysis found that among few and limited studies, DSE and MPS are of limited value for predicting positive ICA. Their low sensitivity might make them inadequate for pre-LT cardiac evaluation. Prospective studies with larger samples are needed to better define an adequate test for predicting CAD in pre-LT patients.

ARTICLE HIGHLIGHTS

Research background

The concept of cardiac involvement with coronary artery disease (CAD) in cirrhotic patients has been changing as patients listed for liver transplantation (LT) have become older and sicker. A previous study of dobutamine stress echocardiography (DSE) as a diagnostic and prognostic tool for LT candidates, published by Nguyen *et al*, which found that DSE had a high negative predictive value for adverse outcomes post-LT. This study tries to elucidate the problem of CAD screening in pre-LT patients.

Research motivation

There is a real need for protocols for cardiac evaluation of patients awaiting LT - particularly for cirrhotic patients. The American Association for the Study of Liver Diseases (AASLD) published a guideline in 2005 that recommends myocardial stress testing for every patient referred for LT. Nevertheless, the guideline published in 2012 by the American Heart Association (AHA and the American College of Cardiology (ACC), suggested that myocardial stress testing should be reserved for patients with three or more CAD risk factors. Better understanding the use of these tools might lead to better choices for pre-LT patients and better prognosis post-LT.

Research objectives

To evaluate the diagnostic value of DSE and myocardial perfusion scintigraphy (MPS) in predicting CAD in cirrhotic patients listed for LT, using invasive coronary angiography (ICA) as gold-standard. This could help clinicians choose the best test for predicting adverse cardiac events post-LT.

Research methods

A systematic review and meta-analysis was performed. Searches were run on the electronic databases Scopus, Web of Science, EMBASE, MEDLINE (PubMed), BIREME (Biblioteca Regional de Medicina), LILACS (Latin American and Caribbean Health Sciences Literature), Cochrane Library for Systematic Reviews and Opengray.eu. There was no language or date of publication restrictions. The reference lists of the studies retrieved were searched manually.

Research results

The search strategy retrieved 322 references for DSE and 90 for MPS. In the final analysis, 10 references for DSE and 10 for MPS were included. Pooled sensitivity was 28% and 61% for DSE and MPS and specificity was 82% and 74%, for diagnosis of CAD using ICA as gold-standard, respectively.

Research conclusions

This study found that DSE and MPS do not have adequate sensitivity for determination of whether CAD is present, despite having significant specificity. There is a need for better tools in order to detect CAD in pre-LT patients. It is not feasible to determine whether AASLD or AHA/ACC is correct, hence both tests underperformed. It is proposed a hypothesis that new methods, tests or scores are need in order to clarify this question, which could impact pre-LT decisions in the future.

Research perspectives

It is possible to conclude that current evidence regarding pre-LT cardiac stress testing is lacking, and future research are bound to focus into solving this important clinical question. A comprehensive study, cohort or randomized, is necessary in order to gather more information on the utility and feasibility of the use of current and future tests in order to determine the presence of pre-LT CAD.

REFERENCES

1 **McCaughan GW**, Crawford M, Sandroussi C, Koorey DJ, Bowen DG, Shackel NA, Strasser SI. Assessment of adult patients with chronic liver failure for liver transplantation in 2015: who and when? *Intern Med J* 2016; **46**: 404-412 [PMID: 27062203 DOI: 10.1111/imj.13025]

2 **Tiukinhoy-Laing SD**, Rossi JS, Bayram M, De Luca L, Gafoor S, Blei A, Flamm S, Davidson CJ, Gheorghiade M. Cardiac hemodynamic and coronary angiographic characteristics of patients being evaluated for liver transplantation. *Am J Cardiol* 2006; **98**: 178-181 [PMID: 16828588 DOI: 10.1016/j.amjcard.2006.01.089]

3 **Xia VW**, Taniguchi M, Steadman RH. The changing face of patients presenting for liver transplantation. *Curr Opin Organ Transplant* 2008; **13**: 280-284 [PMID: 18685318 DOI: 10.1097/MOT.0b013e328300a070]

4 **Kim WR**, Lake JR, Smith JM, Skeans MA, Schladt DP, Edwards EB, Harper AM, Wainright JL, Snyder JJ, Israni AK, Kasiske BL. OPTN/SRTR 2013 Annual Data Report: liver. *Am J Transplant* 2015; **15** Suppl 2: 1-28 [PMID: 25626341 DOI: 10.1111/ajt.13197]

5 **Plotkin JS**, Scott VL, Pinna A, Dobsch BP, De Wolf AM, Kang Y. Morbidity and mortality in patients with coronary artery disease undergoing orthotopic liver transplantation. *Liver Transpl Surg* 1996; **2**: 426-430 [PMID: 9346688 DOI: 10.1002/lt.500020604]

6 **Wray C**, Scovotti JC, Tobis J, Niemann CU, Planinsic R, Walia A, Findlay J, Wagener G, Cywinski JB, Markovic D, Hughes C, Humar A, Olmos A, Sierra R, Busuttil R, Steadman RH. Liver transplantation outcome in patients with angiographically proven coronary artery disease: a multi-institutional study. *Am J Transplant* 2013; **13**: 184-191 [PMID: 23126562 DOI: 10.1111/j.1600-6143.2012.04293.x]

7 **Ruiz-del-Arbol L**, Serradilla R. Cirrhotic cardiomyopathy. *World J Gastroenterol* 2015; **21**: 11502-11521 [DOI: 10.3748/wjg.v21.i41.11502]

8 **Murray KF**, Carithers RL Jr; AASLD. AASLD practice guidelines: Evaluation of the patient for liver transplantation. *Hepatology* 2005; **41**: 1407-1432 [PMID: 15880505 DOI: 10.1002/hep.20704]

9 **Lentine KL**, Costa SP, Weir MR, Robb JF, Fleisher LA, Kasiske BL, Carithers RL, Ragosta M, Bolton K, Auerbach AD, Eagle KA; American Heart Association Council on the Kidney in Cardiovascular Disease and Council on Peripheral Vascular Disease; American Heart Association; American College of Cardiology

Foundation. Cardiac disease evaluation and management among kidney and liver transplantation candidates: a scientific statement from the American Heart Association and the American College of Cardiology Foundation: endorsed by the American Society of Transplant Surgeons, American Society of Transplantation, and National Kidney Foundation. *Circulation* 2012; **126**: 617-663 [PMID: 22753303 DOI: 10.1161/CIR.0b013e31823eb07a]

10 **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]

11 **Shamseer L**, Moher D, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P, Stewart LA; PRISMA-P Group. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. *BMJ* 2015; **350**: g7647 [PMID: 25555855 DOI: 10.1136/bmj.g7647]

12 **Whiting PF**, Rutjes AW, Westwood ME, Mallett S, Deeks JJ, Reitsma JB, Leeflang MM, Sterne JA, Bossuyt PM; QUADAS-2 Group. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. *Ann Intern Med* 2011; **155**: 529-536 [PMID: 22007046 DOI: 10.7326/0003-4819-155-8-201110180-00009]

13 **Ibrahim A**, Schuster A, Alraies MC, Sonny A, Cywinski JB, Jaber WA. Liver transplant candidates: to stress or not to stress? *Circulation* 2014; **130** (Suppl_2): A11773

14 **Donovan CL**, Marcovitz PA, Punch JD, Bach DS, Brown KA, Lucey MR, Armstrong WF. Two-dimensional and dobutamine stress echocardiography in the preoperative assessment of patients with end-stage liver disease prior to orthotopic liver transplantation. *Transplantation* 1996; **61**: 1180-1188 [PMID: 8610415 DOI: 10.1097/00007890-199604270-00011]

15 **Findlay JY**, Keegan MT, Pellikka PP, Rosen CB, Plevak DJ. Preoperative dobutamine stress echocardiography, intraoperative events, and intraoperative myocardial injury in liver transplantation. *Transplant Proc* 2005; **37**: 2209-2213 [PMID: 15964381 DOI: 10.1016/j.transproceed.2005.03.023]

16 **Harinstein ME**, Flaherty JD, Ansari AH, Robin J, Davidson CJ, Rossi JS, Flamm SL, Blei AT, Bonow RO, Abecassis M, Gheorghiade M. Predictive value of dobutamine stress echocardiography for coronary artery disease detection in liver transplant candidates. *Am J Transplant* 2008; **8**: 1523-1528 [PMID: 18510630 DOI: 10.1111/j.1600-6143.2008.02276.x]

17 **Plotkin JS**, Benitez RM, Kuo PC, Njoku MJ, Ridge LA, Lim JW, Howell CD, Laurin JM, Johnson LB. Dobutamine stress echocardiography for preoperative cardiac risk stratification in patients undergoing orthotopic liver transplantation. *Liver Transpl Surg* 1998; **4**: 253-257 [PMID: 9649636 DOI: 10.1002/lt.500040415]

18 **Ramrakhiani C**, Bacon BR, St Vrain J, Befeler AS, Ramrakhiani S, Labovitz AJ. 2-D and Dobutamine Stress echocardiography in the pre- operative evaluation of patients undergoing orthotopic liver transplantation. *Gastroenterology* 2001; **120**: A371-A371 [DOI: 10.1016/S0016-5085(08)81844-6]

19 **Tsutsui JM**, Mukherjee S, Elhendy A, Xie F, Lyden ER, O'Leary E, McGrain AC, Porter TR. Value of dobutamine stress myocardial contrast perfusion echocardiography in patients with advanced liver disease. *Liver Transpl* 2006; **12**: 592-599 [PMID: 16555336 DOI: 10.1002/lt.20651]

20 **Umphrey LG**, Hurst RT, Eleid MF, Lee KS, Reuss CS, Hentz JG, Vargas HE, Appleton CP. Preoperative dobutamine stress echocardiographic findings and subsequent short-term adverse cardiac events after orthotopic liver transplantation. *Liver Transpl* 2008; **14**: 886-892 [PMID: 18508373 DOI: 10.1002/lt.21495]

21 **Snipelisky D**, Levy M, Shapiro B. Utility of dobutamine stress echocardiography as part of the pre-liver transplant evaluation: an evaluation of its efficacy. *Clin Cardiol* 2014; **37**: 468-472 [PMID: 24719365 DOI: 10.1002/clc.22283]

22 **Patel S**, Kiefer TL, Ahmed A, Ali ZA, Tremmel JA, Lee DP, Yeung AC, Fearon WF. Comparison of the frequency of coronary artery disease in alcohol-related versus non-alcohol-related endstage liver disease. *Am J Cardiol* 2011; **108**: 1552-1555 [PMID: 21890080 DOI: 10.1016/j.amjcard.2011.07.013]

23 **Baker S**, Chambers C, McQuillan P, Janicki P, Kadry Z, Bowen D, Bezinover D. Myocardial perfusion imaging is an effective screening test for coronary artery disease in liver transplant candidates. *Clin Transplant* 2015; **29**: 319-326 [PMID: 25604507 DOI: 10.1111/ctr.12517]

24 **Kryzhanovski VA**, Beller GA. Usefulness of preoperative noninvasive radionuclide testing for detecting coronary artery disease in candidates for liver transplantation. *Am J Cardiol* 1997; **79**: 986-988 [PMID: 9104922 DOI: 10.1016/S0002-9149(97)00030-1]

25 **Senzolo M**, Bassanello M, Graziotto A, Zucchetta P, Cillo U, Maraglino G, Loreno M, Bellotto F, Davià G, Burra P. Microvascular autonomic dysfunction may justify false-positive stress myocardial perfusion imaging in patients with liver cirrhosis undergoing liver transplantation. *Transplant Proc* 2008; **40**: 1916-1917 [PMID: 18675088 DOI: 10.1016/j.transproceed.2008.05.069]

26 **Kandiah K**, Steeds R, Thorburn D. The role of Myocardial Perfusion Imaging (MPI) in the assessment of cardiovascular risk in patients referred with end-stage liver failure for liver transplantation. *J Hepatol* 2009; **50**: S178 [DOI: 10.1016/S0168-8278(09)60476-1]

27 **Oprea-Lager DE**, Sorgdrager BJ, Jukema JW, Scherptong RW, Ringers J, Coenraad MJ, van Hoek B, Stokkel MP. Clinical value of myocardial perfusion scintigraphy as a screening tool in liver transplant candidates. *Liver Transpl* 2011; **17**: 261-269 [PMID: 21384508 DOI: 10.1002/lt.22234]

28 **Davidson CJ**, Gheorghiade M, Flaherty JD, Elliot MD, Reddy SP, Wang NC, Sundaram SA, Flamm SL, Blei AT, Abecassis MI, Bonow RO. Predictive value of stress myocardial perfusion imaging in liver transplant candidates. *Am J Cardiol* 2002; **89**: 359-360 [PMID: 11809445 DOI: 10.1016/S0002-9149(01)02244-5]

29 **Aydinalp A**, Bal U, Atar I, Ertan C, Aktaş A, Yildirir A, Ozin B, Mudderisoglu H, Haberal M. Value of stress myocardial perfusion scanning in diagnosis of severe coronary artery disease in liver transplantation candidates. *Transplant Proc* 2009; **41**: 3757-3760 [PMID: 19917381 DOI: 10.1016/j.transproceed.2009.06.219]

30 **Zoghbi GJ**, Patel AD, Ershadi RE, Heo J, Bynon JS, Iskandrian AE. Usefulness of preoperative stress perfusion imaging in predicting prognosis after liver transplantation. *Am J Cardiol* 2003; **92**: 1066-1071 [PMID: 14583357 DOI: 10.1016/j.amjcard.2003.06.003]

31 **Bezinover D**, Bowman J, Baker S, Kadry Z, Uemura T, McQuillan P, Mets B, Chambers CE. Use of myocardial perfusion imaging for the evaluation of liver transplant candidates. *Liver Transpl* 2013; **19**: S108 [DOI: 10.1002/lt.23661]

32 **Bhutani S**, Tobis J, Gevorgyan R, Sinha A, Suh W, Honda HM, Vorobiof G, Packard RR, Steadman R, Wray C, Busuttil R, Tseng CH. Accuracy of stress myocardial perfusion imaging to diagnose coronary artery disease in end stage liver disease patients. *Am J Cardiol* 2013; **111**: 1057-1061 [PMID: 23337839 DOI: 10.1016/j.amjcard.2012.12.023]

33 **Nguyen P**, Plotkin J, Fishbein TM, Laurin JM, Satoskar R, Shetty K, Taylor AJ. Dobutamine stress echocardiography in patients undergoing orthotopic liver transplantation: a pooled analysis of accuracy, perioperative and long term cardiovascular prognosis. *Int J Cardiovasc Imaging* 2013; **29**: 1741-1748 [PMID: 23974907 DOI: 10.1007/s10554-013-0275-x]

34 **Picano E**, Molinaro S, Pasanisi E. The diagnostic accuracy of pharmacological stress echocardiography for the assessment of coronary artery disease: a meta-analysis. *Cardiovasc Ultrasound* 2008; **6**: 30 [PMID: 18565214 DOI: 10.1186/1476-7120-6-30]

35 **de Jong MC**, Genders TS, van Geuns RJ, Moelker A, Hunink MG. Diagnostic performance of stress myocardial perfusion imaging for coronary artery disease: a systematic review and meta-analysis. *Eur Radiol* 2012; **22**: 1881-1895 [PMID: 22527375 DOI: 10.1007/s00330-012-2434-1]

36 **Krag A**, Bendtsen F, Burroughs AK, Møller S. The cardiorenal link in advanced cirrhosis. *Med Hypotheses* 2012; **79**: 53-55 [PMID: 22537409 DOI: 10.1016/j.mehy.2012.03.032]

37 **Singhal A**, Mukerji AN, Thomaides A, Karachristos A, Maloo M, Sanchez B, Keresztury M, Santora TA, Jain A. Chronotropic incompetence on dobutamine stress echocardiography in candidates for a liver transplant. *Exp Clin Transplant* 2013; **11**: 546-553 [PMID: 24344945 DOI: 10.6002/ect.2012.0295]

38 **Krahwinkel W**, Ketteler T, Gödke J, Wolfertz J, Ulbricht LJ, Krakau I, Gülker H. Dobutamine stress echocardiography. *Eur Heart J* 1997; **18** Suppl D: D9-15 [PMID: 9183605 DOI: 10.1093/eurheartj/18.suppl_D.9]

39 **Pellikka PA**, Nagueh SF, Elhendy AA, Kuehl CA, Sawada SG; American Society of Echocardiography. American Society of Echocardiography recommendations for performance, interpretation, and application of stress echocardiography. *J Am Soc Echocardiogr* 2007; **20**: 1021-1041 [PMID: 17765820 DOI: 10.1016/j.echo.2007.07.003]

40 **Meneghelo RS**, Araújo CGS, Stein R, Mastrocolla LE, Albuquerque PF, Serra SM. III Diretrizes da Sociedade Brasileira de Cardiologia sobre teste ergométrico. *Arq Bras Cardiol* 2010; **95**: 1-29 [DOI: 10.1590/S0066-782X2010002400001]

41 **Geleijnse ML**, Krenning BJ, van Dalen BM, Nemes A, Soliman OI, Bosch JG, Galema TW, ten Cate FJ, Boersma E. Factors affecting sensitivity and specificity of diagnostic testing: dobutamine stress echocardiography. *J Am Soc Echocardiogr* 2009; **22**: 1199-1208 [PMID: 19766453 DOI: 10.1016/j.echo.2009.07.006]

42 **Russo MW**, Pierson J, Narang T, Montegudo A, Eskind L, Gulati S. Coronary artery stents and antiplatelet therapy in patients with cirrhosis. *J Clin Gastroenterol* 2012; **46**: 339-344 [PMID: 22105182 DOI: 10.1097/MCG.0b013e3182371258]

43 **Yong CM**, Sharma M, Ochoa V, Abnousi F, Roberts J, Bass NM, Niemann CU, Shiboski S, Prasad M, Tavakol M, Ports TA, Gregoratos G, Yeghiazarians Y, Boyle AJ. Multivessel coronary artery disease predicts mortality, length of stay, and pressor requirements after liver transplantation. *Liver Transpl* 2010; **16**: 1242-1248 [PMID: 21031539 DOI: 10.1002/lt.22152]

44 **Azarbal B**, Poommipanit P, Arbit B, Hage A, Patel J, Kittleson M, Kar S, Kaldas FM, Busuttil RW. Feasibility and safety of percutaneous coronary intervention in patients with end-stage liver disease referred for liver transplantation. *Liver Transpl* 2011; **17**: 809-813 [PMID: 21425429 DOI: 10.1002/lt.22301]

45 **Diedrich DA**, Findlay JY, Harrison BA, Rosen CB. Influence of coronary artery disease on outcomes after liver transplantation. *Transplant Proc* 2008; **40**: 3554-3557 [PMID: 19100436 DOI: 10.1016/j.transproceed.2008.08.129]

46 **Filì D**, Vizzini G, Biondo D, Pietrosi G, Volpes R, Palazzo U, D'Antoni A, Petridis I, Luca A, Gridelli B. Clinical burden of screening asymptomatic patients for coronary artery disease prior to liver transplantation. *Am J Transplant* 2009; **9**: 1151-1157 [PMID: 19422340 DOI: 10.1111/j.1600-6143.2009.02589.x]

47 **Kertai MD**, Boersma E, Bax JJ, Heijenbrok-Kal MH, Hunink MG, L'talien GJ, Roelandt JR, van Urk H, Poldermans D. A meta-analysis comparing the prognostic accuracy of six diagnostic tests for predicting perioperative cardiac risk in patients undergoing major vascular surgery. *Heart* 2003; **89**: 1327-1334 [PMID: 14594892 DOI: 10.1136/heart.89.11.1327]

48 **Wang LW**, Fahim MA, Hayen A, Mitchell RL, Baines L, Lord S, Craig JC, Webster AC. Cardiac testing for coronary artery disease in potential kidney transplant recipients. *Cochrane Database Syst Rev* 2011; **12**: CD008691 [PMID: 22161434 DOI: 10.1002/14651858.CD008691.pub2]

49 **Wang LW**, Masson P, Turner RM, Lord SW, Baines LA, Craig JC, Webster AC. Prognostic value of cardiac tests in potential kidney transplant recipients: a systematic review. *Transplantation* 2015; **99**: 731-745 [PMID: 25769066 DOI: 10.1097/TP.0000000000000611]

50 **Soldera J**, Camazzola F, Rodríguez S, Brandão A. Dobutamine stress echocardiography, myocardial perfusion scintigraphy, invasive coronary angiography, and post-liver transplantation events: Systematic review and meta-analysis. *Clin Transplant* 2018; **32**: e13222 [PMID: 29436036 DOI: 10.1111/ctr.13222]

P- Reviewer: Gencdal G, Milovanovic T **S- Editor**: Ji FF
L- Editor: A **E- Editor**: Tan WW

World Journal of Hepatology

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

World J Hepatol 2018 November 27; 10(11): 887-891

DOI: 10.4254/wjh.v10.i11.887

ISSN 1948-5182 (online)

CASE REPORT

Trapped vessel of abdominal pain with hepatomegaly: A case report

Sirisha Grandhe, Joy A Lee, Ankur Chandra, Christopher Marsh, Catherine T Frenette

Sirisha Grandhe, Department of Gastroenterology and Hepatology, University of California Davis Medical Center, Sacramento, CA 95817, United States

Joy A Lee, Department of Internal Medicine, Scripps Green Hospital, La Jolla, CA 92037, United States

Ankur Chandra, Department of Vascular Surgery, Scripps Green Hospital, La Jolla, CA 92037, United States

Christopher Marsh, Catherine T Frenette, Scripps Center for Organ Transplant, Scripps Green Hospital, La Jolla, CA 92037, United States

ORCID number: Sirisha Grandhe (0000-0001-6310-733X); Joy A Lee (0000-0001-6760-5176); Ankur Chandra (0000-0001-9481-4870); Christopher Marsh (0000-0002-6517-076X); Catherine T Frenette (0000-0002-2245-8173).

Author contributions: Grandhe S and Lee JA designed and wrote the report; Chandra A collected the patient's data and contributed the images; Marsh C and Frenette CT collected the patient's clinical data and edited the paper.

Informed consent statement: The patient agreed to allow her case to be published including any relevant laboratory data and images.

Conflict-of-interest statement: The authors declare that they have no conflicts of interest.

CARE Checklist (2013): The authors have read the CARE checklist and the manuscript was prepared and reviewed according to the CARE checklist.

Manuscript source: Unsolicited manuscript

Correspondence to: Catherine T Frenette, MD, Academic Research, Attending Doctor, Doctor, Scripps Center for Organ Transplant, Scripps Green Hospital, 10666 North Torrey Pines Road, La Jolla, CA 92037, United States. frenette.catherine@scrippshealth.org
Telephone: +1-858-5544310

Received: June 4, 2018
Peer-review started: June 4, 2018
First decision: July 10, 2018
Revised: August 15, 2018
Accepted: October 10, 2018
Article in press: October 10, 2018
Published online: November 27, 2018

Abstract

Abdominal pain with elevated transaminases from inferior vena cava (IVC) obstruction is a relatively common reason for referral and further workup by a hepatologist. The differential for the cause of IVC obstruction is extensive, and the most common etiologies include clotting disorders or recent trauma. In some situations the common etiologies have been ruled out, and the underlying process for the patient's symptoms is still not explained. We present one unique case of abdominal pain and hepatomegaly secondary to IVC constriction from extrinsic compression of the diaphragm. Based on this patient's presentation, we urge that physicians be cognizant of the IVC diameter and consider extrinsic compression as a contributor to the patient's symptoms. If IVC compression from the diaphragm is confirmed, early referral to vascular surgery is strongly advised for further surgical intervention.

Key words: Liver imaging; Abdominal pain; Hepatic circulation; Ischemia/reperfusion

Core tip: Common etiologies of abdominal pain with elevated transaminases are clotting disorders and trauma. In this article, we present a rare case of external compression of the diaphragm as the cause of these symptoms that requires surgical intervention to relieve the obstruction.

Grandhe S, Lee JA, Chandra A, Marsh C, Frenette CT. Trapped vessel of abdominal pain with hepatomegaly: A case report. *World J Hepatol* 2018; 10(11): 887-891 Available from: URL: http://www.wjgnet.com/1948-5182/full/v10/i11/887.htm DOI: http://dx.doi.org/10.4254/wjh.v10.i11.887

INTRODUCTION

Inferior vena cava (IVC) obstruction presenting with abdominal pain and hepatomegaly is generally seen in patients with venous thromboses, including Budd Chiari syndrome, infective phlebitis, or in iatrogenic cases such as post-liver transplant or vascular catheter placement[1-4]. In the realm of obstetrics and gynecology, IVC obstruction is more commonly seen as vessel compression in the late second trimester of pregnancy[5]. Occasionally, tumors, such as renal cell carcinomas, may be initially detected due to compression of the IVC. Herein, we present a unique case of abdominal pain due to IVC constriction from extrinsic compression of the diaphragm. Previously this has been documented in patients with congenital chest wall abnormalities, such as pectus excavatum; however, this patient described below is one of the first to have this pathology without this birth defect[6].

CASE REPORT

A 49-year-old female was referred for further evaluation of hepatomegaly, abdominal pain, and thrombocytopenia. On interview, she endorsed a several year history of right upper quadrant abdominal pain and very mild dyspnea with exertion. She reported that the abdominal discomfort was worse in a sitting position. At the time of initial evaluation she was feeling well with no symptoms of jaundice, pruritus, abdominal pain, nausea, vomiting, edema, or ascites. She also reported no constitutional symptoms.

Past medical history was notable for a 10-year history of mild thrombocytopenia (platelet count 90000-130000) of unclear etiology with negative laboratory workup. Past surgical history was remarkable for an enlarged and nodular appearing liver observed during laparoscopic cholecystectomy performed one year prior due to the same symptoms. The patient has been followed by a hematologist as an outpatient, and a recent liver spleen single photon emission computed tomography scan had confirmed hepatomegaly without splenomegaly. Abdominal ultrasound characterized the liver as 18.1 cm in size with no evidence of cirrhosis or portal hypertension. Patent vasculature was reported throughout with normal hepatopedal flow. The patient had also previously undergone a computer tomography-guided liver biopsy, which showed mild perivascular and pericellular fibrosis but no evidence of advanced fibrosis or cirrhosis.

Physical examination revealed hepatomegaly 4 cm below the costal margin but was otherwise unremarkable. Initial labs included a complete blood count (white count: 4.6, hemoglobin: 13.5, platelets: 112000), comprehensive metabolic panel (sodium: 141, potassium: 3.8, urea nitrogen: 11, creatinine: 0.8, alkaline phosphatase: 51, total protein: 7.3, aspartate aminotransferase: 30, and alanine aminotransferase: 29), and coagulation panel (international normalized ratio: 1.0). Additional workup revealed a ferritin of 28. Antimitochondrial antibody and actin IgG were negative. Ceruloplasmin level was 32. Patient also tested positive for antinuclear antibody titer (1:80, diffuse pattern) and epstein-barr virus IgG. An elevated transient elastography score of 9.6 was also noted.

Due to concern for early cirrhosis in the setting of thrombocytopenia and an elevated transient elastography score, the patient was advised to pursue a healthy lifestyle and abstain from alcohol. A magnetic resonance venography of the abdomen showed no evidence of thrombosis or obstruction.

At this point in time the patient reported worsening, intermittent, epigastric abdominal discomfort that radiated to the right upper quadrant of her abdomen, often waking her up at night and only improved with standing upright or walking. She occasionally felt nauseous but otherwise reported no jaundice, pruritus, edema, ascites, chest pain, or dyspnea. Physical examination showed a positive hepatojugular reflux, consistent with hepatic congestion. The patient was evaluated by a cardiologist, and a transthoracic echocardiogram showed pericardial thickening but no evidence of constrictive pericarditis or systolic or diastolic dysfunction.

The patient then underwent a transjugular liver biopsy with intravenous ultrasound and pressure measurements, which showed an elevated central venous pressure at 13-15 mmHg, wedged right hepatic vein pressure with occlusion balloon measuring 16-17 mmHg, and a dilated IVC of 3 cm cephalad to the patent veins prior to reentry into the right atrium. Significant respiratory variation involving near-collapse of the retrohepatic IVC at end-expiration was noted. There was question of intraluminal narrowing of the retrohepatic IVC down to approximately 10-15 mm, which had significantly improved upon Valsalva maneuver. Right heart catheterization showed hepatic congestion with normal intracardiac and pulmonary artery pressures.

Table 1 Laboratory data pre and post-venolysis

	Pre-venolysis	Post-venolysis
Sodium	137	137
Potassium	4.1	3.7
Chloride	109	104
Bicarbonate	24	31
Blood urea nitrogen	14	10
Creatinine	0.8	0.8
Glucose	111	98
Calcium	7.4	8.3
Alkaline phosphatase[1]	39	---
Albumin[1]	2.9	---
Total protein[1]	5.6	---
Aspartate aminotransferase[1]	49	---
Alanine aminotransferase[1]	43	---
Bilirubin, direct[1]	0.1	---
Bilirubin, total[1]	0.6	---

[1]Post-venolysis alkaline phosphatase, albumin, total protein, aspartate aminotransferase, alanine aminotransferase, bilirubin direct and total were unable to be obtained due to loss of insurance.

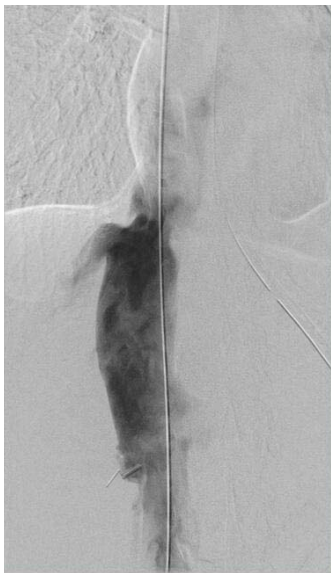

Figure 1 Venogram of the inferior vena cava (pre-lysis). Significant stenosis noted at level of diaphragm prior to exploratory laparotomy, inferior vena cava venolysis, and division of the diaphragmatic constriction.

A multidisciplinary conference among the hepatology, vascular surgery, and cardiology services was held. It was suspected that the diaphragm, *via* the diaphragmatic hiatus through which the IVC was passing, was causing extrinsic compression of the vessel, thereby eliciting symptoms of epigastric and right upper quadrant pain. Repeat transient elastography was still elevated and the patient underwent an exploratory laparotomy with IVC venolysis and division of the diaphragmatic constriction. After the above intervention, resolution of previously identified constriction was noted *via* repeat venogram (Figures 1 and 2) and intravascular ultrasound.

Intraoperative liver biopsy revealed sinusoidal congestion with dilatation in the perivenular areas, features consistent with extrahepatic venous outflow obstruction. The patient recovered remarkably well from the laparotomy. The available pre- and post-venolysis labs are presented in Table 1. At her four week postoperative follow up visit, she reported resolution of her abdominal discomfort and complete ability to perform her activities of daily living without the use of any pain medications. Unfortunately, follow-up liver enzymes were unable to be obtained due to losing her health insurance.

DISCUSSION

The most common etiologies of IVC obstruction are from hypercoagulable states, inflammation, trauma, or recent surgery. Budd Chiari syndrome and hepatic vena cava syndrome, more common conditions associated with hepatic vein outflow obstruction, and hepatic vena cava syndrome may present subacutely or even arise from congenital strictures of the hepatic segment of the IVC[1,2]. While the cause of IVC obstruction is usually due to the abovementioned causes, it is important to consider extrinsic compression from neighboring tumors or even native structures, such as the diaphragm, as in the case outlined above. To date, very few cases have been published attributing abdominal pain or hepatomegaly to compression of the IVC by the diaphragm[7].

Chronic IVC obstruction may be silent in presentation or manifest late with acute symptoms of abdominal pain, hepatomegaly, renal dysfunction, or even unilateral limb symptoms such as leg heaviness, pain, swelling, or even cramping[8,9]. These unusual features may be anatomically related to the extensive network of collateralization of the natural and tributary vessels near the IVC.

The symptoms that arise from extrinsic compression and intrinsic occlusion of the IVC can be explained by understanding the embryological development of the large vessel. As the IVC develops near the liver and diaphragm, new outgrowths from hepatic veins and

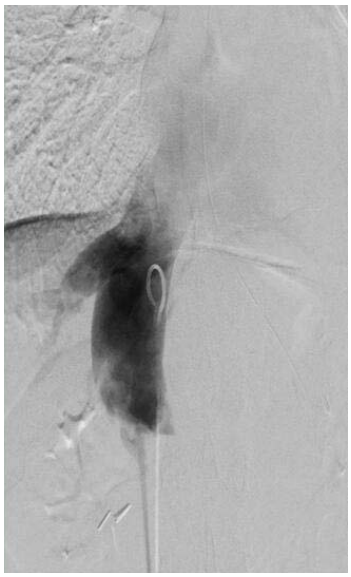

Figure 2 Venogram of the inferior vena cava (post-lysis). Resolution of the stenosis noted at level of diaphragm after exploratory laparotomy, inferior vena cava venolysis, and division of the diaphragmatic constriction.

the infrarenal IVC may make this site more prone to developmental anomalies such as strictures and webs[8]. The patency of the iliac vein is important to collateral function, and occlusion of this vessel usually precipitates acute symptoms of abdominal pain[9]. However, the extent of collateralization may actually prevent patients from developing significant hepatic or renal dysfunction. In addition to the rich vasculature, studies analyzing the interaction between the diaphragm and the IVC during inspiration are limited, but they all support the idea that the size and shape of the lumen of the IVC can be altered by the contraction or anatomy of the diaphragm[7].

The diagnostic workup of IVC obstruction includes a color Doppler sonography and contrast-enhanced computed tomography, magnetic resonance imaging, or venography[9]. Intravascular ultrasound with pressure measurements and cavography provide an additional assessment of hepatic and collateral vein obstructions and thromboses and may indicate if these obstructions are subacute or chronic in nature[4]. Our patient showed evidence of IVC obstruction based on venography and intravascular ultrasound. In terms of therapeutic intervention, endovascular management of hepatic vein outflow obstruction usually includes portocaval shunts or balloon angioplasties with stent implantation[9,10]. Stent implantation *via* balloon angioplasty has proven to be safer with fewer complications of restenosis compared to open surgery[9].

IVC obstruction continues to remain an infrequent cause of abdominal pain and chronic liver disease. While this condition may be rare, it may lead to chronic abdominal pain, cirrhosis, and portal hypertension if not recognized and treated appropriately. Nonetheless, whether from intravascular obstruction, thrombosis, or extrinsic compression from neighboring structures, it is important to keep a broad differential and consider atypical causes of this phenomenon once common etiologies have been ruled out. Referral to vascular surgery may be necessary for surgical intervention, which will ultimately provide symptomatic relief for these patients.

ARTICLE HIGHLIGHTS

Case characteristics

Patients who present with abdominal pain and hepatomegaly are commonly diagnosed as having Budd Chiari or another type of obstruction of the inferior vena cava (IVC) whether it is intrinsic due to thrombosis or an obstruction. However, extrinsic compression, although rare, can also be the culprit of the patient's symptoms.

Clinical diagnosis

Right upper quadrant and epigastric pain and hepatomegaly.

Differential diagnosis

Budd Chiari, infective phlebitis, intravascular obstruction, thrombosis, or external compression from neighboring structures including the diaphragm, kidney, or uterus.

Laboratory diagnosis

Complete blood count, comprehensive metabolic panel, coagulation panel, in addition to labs evaluating causes of cirrhosis including ferritin, anti-mitochondrial antibody, anti-smooth muscle antibody, antinuclear antibody, and ceruloplasmin.

Imaging diagnosis

Color doppler sonography and contrast-enhanced computed tomography, magnetic resonance imaging, or venography.

Pathological diagnosis

Sinusoidal congestion with dilatation in the perivenular areas, features consistent with extrahepatic venous outflow obstruction.

Treatment

Portocaval shunts or balloon angioplasties with stent implantation.

Related reports

A case of IVC compression from the diaphragm has been reported only once in the literature from Louisiana State University Health Science Center in a patient with Pectus Excavatum. Interestingly an article from 1992 demonstrated how radiography can help identify how the IVC can be obstructed, but never specifically discussed a case in which the IVC was externally compressed by the diaphragm.

Experiences and lessons

This case will guide clinicians to think of other etiologies that can cause abdominal pain and hepatomegaly in patients with unremarkable laboratory data. Biopsies are not necessary for this diagnosis. With consideration of this diagnosis, patient care will be expedited with quicker referrals, thereby minimizing the delay in treatment and resolution of symptoms.

REFERENCES

1 **Schaffner F**, Gadboys HL, Safran AP, Baron MG, Aufses AH Jr. Budd-Chiari syndrome caused by a web in the inferior vena cava. *Am J Med* 1967; **42**: 838-843 [PMID: 6024240 DOI: 10.1016/0002-9343(67)90100-3]

2 **Shrestha SM**, Kage M, Lee BB. Hepatic vena cava syndrome: New concept of pathogenesis. *Hepatol Res* 2017; **47**: 603-615 [PMID:

28169486 DOI: 10.1111/hepr.12869]
3 **Shin N**, Kim YH, Xu H, Shi HB, Zhang QQ, Colon Pons JP, Kim D, Xu Y, Wu FY, Han S, Lee BB, Li LS. Redefining Budd-Chiari syndrome: A systematic review. *World J Hepatol* 2016; **8**: 691-702 [PMID: 27326316 DOI: 10.4254/wjh.v8.i16.691]
4 **Shrestha SM**, Okuda K, Uchida T, Maharjan KG, Shrestha S, Joshi BL, Larsson S, Vaidya Y. Endemicity and clinical picture of liver disease due to obstruction of the hepatic portion of the inferior vena cava in Nepal. *J Gastroenterol Hepatol* 1996; **11**: 170-179 [PMID: 8672764 DOI: 10.1111/j.1440-1746.1996.tb00056.x]
5 **Ryo E**, Okai T, Kozuma S, Kobayashi K, Kikuchi A, Taketani Y. Influence of compression of the inferior vena cava in the late second trimester on uterine and umbilical artery blood flow. *Int J Gynaecol Obstet* 1996; **55**: 213-218 [PMID: 9003945 DOI: 10.1016/S0020-7292(96)02760-9]
6 **Yalamanchili K**, Summer W, Valentine V. Pectus excavatum with inspiratory inferior vena cava compression: a new presentation of pulsus paradoxus. *Am J Med Sci* 2005; **329**: 45-47 [PMID: 15654179 DOI: 10.1097/00000441-200501000-00008]
7 **Pearson AA**, Sauter RW, Oler RC. Relationship of the diaphragm to the inferior vena cava in human embryos and fetuses. *Thorax* 1971; **26**: 348-353 [PMID: 5089504 DOI: 10.1136/thx.26.3.348]
8 **Raju S**, Hollis K, Neglen P. Obstructive lesions of the inferior vena cava: clinical features and endovenous treatment. *J Vasc Surg* 2006; **44**: 820-827 [PMID: 16926084 DOI: 10.1016/j.jvs.2006.05.054]
9 **Srinivas BC**, Dattatreya PV, Srinivasa KH, Prabhavathi, Manjunath CN. Inferior vena cava obstruction: long-term results of endovascular management. *Indian Heart J* 2012; **64**: 162-169 [PMID: 22572493 DOI: 10.1016/S0019-4832(12)60054-6]
10 **Kohli V**, Pande GK, Dev V, Reddy KS, Kaul U, Nundy S. Management of hepatic venous outflow obstruction. *Lancet* 1993; **342**: 718-722 [PMID: 8103826 DOI: 10.1016/0140-6736(93)91712-U]

P- Reviewer: Coelho JCU, Gencdal G, Kohla MAS, Roohvand F, Zhu Y
S- Editor: Cui LJ **L- Editor**: Filipodia **E- Editor**: Tan WW

Published by **Baishideng Publishing Group Inc**
7901 Stoneridge Drive, Suite 501, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.f6publishing.com/helpdesk
http://www.wjgnet.com