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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.

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Hepatitis C virus and human immunodeficiency virus transmission routes: Differences and similarities

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Abstract

Bouare *et al* found that hepatitis C virus (HCV) infection in Malian women is mainly transmitted through medical procedures with contaminated supplies, and that human immunodeficiency virus (HIV) transmission is predominantly sexual. The results of this study confirm those of a recent case-control study in New York and Oregon which demonstrated that healthcare exposures represent an important source of new HCV infections in United States. HCV seroprevalence was only 0.2% in pregnant, young Malian women, indicating that hygiene improved in healthcare facilities over time. Heterosexual transmission of HCV is exceptional, and can occur, from males to females, in extremely rare occasions in case of vaginal mucosal damage or less rarely through anal intercourse. The Malian study did not show an association between HIV infection and hospitalization, transfusion, tattoo, dental care. Transmission by needle-stick injury occurs in 0.9%-2.2% of exposures from HCV-infected subjects and in 0.1%-0.3% of exposures from HIV-infected individuals. HCV is therefore more transmissible through percutaneous exposure.

Key words: Hepatitis C virus; Human immunodeficiency virus; Transmission; Sub-Saharan Africa; Pregnant women

Core tip: The results of a number of studies have shown that hepatitis C virus (HCV) infection is mainly transmitted through medical procedures with contaminated supplies, whereas human immunodeficiency virus (HIV) transmission is predominantly sexual. Heterosexual transmission of HCV is exceptional and can occur, from males to females, in extremely rare occasions in case of vaginal mucosal damage or less rarely through anal intercourse. Transmission by needle-stick injury occurs in 0.9%-2.2% of exposures from HCV-infected subjects and in 0.1%-0.3% of exposures from HIV-infected individuals; therefore HCV is more transmissible through percutaneous exposure.

Cainelli F. Hepatitis C virus and human immunodeficiency virus transmission routes: Differences and similarities. *World J Hepatol* 2013; 5(5): 234-236 Available from: URL: http://www.wjgnet.com/1948-5182/full/v5/i5/234.htm DOI: http://dx.doi.org/10.4254/wjh.v5.i5.234

COMMENTARY ON HOT TOPICS

Bouare *et al*[1], studying 1000 pregnant women in six reference health centers, and 231 older women who attended general practice in two hospitals in Mali, found that hepatitis C virus (HCV) infection is mainly transmitted through medical procedures with contaminated supplies rather than through blood transfusion, whereas human immunodeficiency virus (HIV) transmission is predominantly sexual.

The results of this study confirm those of a very recent case-control study done in three health departments that performed enhanced viral hepatitis surveillance in New York and Oregon and included reported cases of symptomatic acute hepatitis B and hepatitis C

occurring in persons ⩾ 55 years of age from 2006 to 2008; healthcare exposures were found to represent an important source of new HBV and HCV infections in United States[2]. Many other studies also found the same in different countries[3-6]. It is reassuring that HCV seroprevalence was only 0.2% in pregnant (young) Malian women[1], possibly indicating that hygiene improved in healthcare facilities over time. The results also confirm that heterosexual transmission of HCV is exceptional[7-10]. Indeed heterosexual transmission of HCV from males to females can occur in extremely rare occasions in case of vaginal mucosal damage[11] or less rarely through anal intercourse[12,13].

What about HIV? The Malian study did not show an association between HIV infection and hospitalization, transfusion, tattoo, dental care. A significant decrease of HIV seroprevalence was detected in young women who used condoms for contraception more than for other purposes, whereas surprisingly HIV seroprevalence was significantly increased in young women using condoms mainly to prevent sexual infections[1]. The authors interpreted these findings as suggestive of awareness of transmission and prevention of HIV infection only after contagion. However knowledge of vaginal sex as an HIV transmission risk and condom use as an HIV prevention strategy were associated with a higher likelihood of HIV infection in Mozambique and elsewhere in sub-Saharan Africa[14], inconsistent condom use was not related to the probability of HIV transmission per coital act in a study of Ugandan HIV discordant couples[15], and condom use was not negatively associated with incident HIV infection in a large study conducted in Benin, Ghana, India, Nigeria, and South Africa[16]. At least the latter of these surprising findings are likely to derive from the inaccuracy of self-reported data[17].

Transmission by needle-stick injury occurs in 0.9%-2.2% of exposures from HCV-infected subjects[18,19] and in 0.1%-0.3% of exposures from HIV-infected individuals[20]; therefore HCV is more transmissible through percutaneous exposure. It has not been definitively established why HCV is much less transmissible than HIV by heterosexual contact, and more infectious through parenteral exposure. Although low infectivity of HCV by vaginal intercourse has been related to low titres in genital secretions, titres of free HIV are also low. It may be that as infection of tissue dendritic DC-SIGN(+)-DC cells and localised replication in cervico-vaginal tissues are of fundamental importance for HIV infection of exposed individuals[21], the lack of target cells in the genital tract may prevent infection by HCV through vaginal intercourse.

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TOPIC HIGHLIGHT

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Strategies to reduce hepatitis C virus recurrence after liver transplantation

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Abstract

Hepatitis C virus (HCV) is a major health problem that leads to chronic hepatitis, cirrhosis and hepatocellular carcinoma, being the most frequent indication for liver transplantation in several countries. Unfortunately, HCV re-infects the liver graft almost invariably following reperfusion, with an accelerated history of recurrence, leading to 10%-30% of patients progressing to cirrhosis within 5 years of transplantation. In this sense, some groups have even advocated for not re-transplanting this patients, as lower patient and graft outcomes have been reported. However, the management of HCV recurrence is being optimized and several strategies to reduce post-transplant recurrence could improve outcomes, decrease the rate of re-transplantation and optimize the use of available grafts. Three moments may be the focus of potential actions in order to decrease the impact of viral recurrence: the pre-transplant moment, the transplant environment and the post-transplant management. In the pre-transplant setting, it is not well established if reducing the pre transplant viral load affects the risk for HCV progression after transplant. Obviously, antiviral treatment can render the patient HCV RNA negative post transplant but the long-term benefit has not yet been fully established to justify the cost and clinical risk. In the transplant moment, factors as donor age, cold ischemia time, graft steatosis and ischemia/reperfusion injury may lead to a higher and more aggressive viral recurrence. After the transplant, discussion about immunosuppression and the moment to start the treatment (prophylactic, pre-emptive or once-confirmed) together with new antiviral drugs are of interest. This review aims to help clinicians have a global overview of post-transplant HCV recurrence and strategies to reduce its impact on our patients.

Key words: Hepatitis C virus; Recurrence; Liver; Transplantation; Outcomes

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INTRODUCTION

Hepatitis C virus (HCV) is a major health problem that leads to chronic hepatitis, cirrhosis and hepatocellular carcinoma (HCC)[1]. HCV-cirrhosis is the most frequent indication for liver transplantation (LTx) in Europe and America[2]. Although accepted as the standard of care for end-stage liver disease[3], the progression of liver disease is variable, leading to re-transplantation and lower survival rates. Because of these results, concerns have been expressed regarding the appropriateness of re-transplantation for HCV and the optimal timing of surgery in an era of organ shortage[4].

The diagnosis of post-transplant HCV recurrence ideally should be histological, by protocol-driven biopsies as biochemical and serological markers for HCV are inaccurate in the post LTx population[5,6]. Although non-invasive procedures such as fibroscan are able to provide similar results in detecting early and rapidly progressive fibrosis, its use is not widely extended[7]. HCV re-infects the liver graft almost invariably following reperfusion[8]. Histological patterns of acute HCV appear between 4 and 12 wk post-transplant, followed by a concomitant rise in the HCV viral load[8,9]. Serum transaminases and HCV RNA levels usually settle but spontaneous viral clearance has not been observed post LTx. A healthy HCV carrier state does not ensue, and histological features of chronic HCV can be demonstrated in 70%-90% of recipients after 1 year and in 90%-95% after 5 years[10].

The natural history of recurrent HCV is accelerated in LTx recipients, with 10%-30% progressing to cirrhosis within 5 years of transplantation. When cirrhosis is present after liver transplantation, the rate of decompensation is > 40% at 1 year and > 70% at 3 years in liver transplant recipients with established cirrhosis versus < 5% and < 10%, respectively, in immunocompetent patients[10-12]. Some patients (2%-5%) develop a severe form of cholestatic fibrosing hepatitis, with extremely high levels of serum and intrahepatic HCV RNA, and histological cholestasis, the majority of which rapidly progress to graft failure and death[10].

Strategies to reduce post-transplant HCV recurrence aim to improve outcome, to decrease the rate of re-transplantation and optimize the use of available grafts. Potential areas where recurrence can be influenced are pre-transplant antiviral treatment (AVT), modifiable perioperative and donor variables, and post-transplant immunosuppression AVT regimens

PRE-TRANSPLANT STRATEGIES

The rationale

The aim of pre transplant AVT is to achieve a sustained virological response (SVR) or to clear HCV RNA at time of transplant. Low HCV RNA before transplant has been shown to be associated with a reduced risk of severe recurrence. The HCV RNA load has also been shown to be an independent factor for fibrosis progression and survival[13,14]. By reducing pre transplant viral load the severity of HCV recurrence has the potential to be reduced and patient survival improved. However, it is not established if reducing the pre transplant viral load affects the risk for HCV progression after transplant[15-17].

Historically, cirrhotic patients were not considered for AVT as it was felt they would not be able to tolerate therapy and be at risk of decompensation. Currently available AVT is also less effective when started in the presence of advanced fibrosis and cirrhosis. Pegylated interferon (PEG IFN) and Ribavirin are emerging as the standard therapy for HCV and appear to be tolerated by cirrhotic patients[18,19].

Predictors of antiviral therapy response and tolerance

Drawbacks to undertaking antiviral treatment in the pre transplant population are the prevalence of genotype 1, the side effects of IFN and Ribavirin preventing full dose from being achieved, and complications related to the underlying liver disease[18,20,21]. To achieve a SVR at transplant the patient generally will need to be observed for 24 wk after completion of the antiviral course. This is difficult to achieve and may represent too long a period for a patient on the waiting list. Predictors of viral clearance include non-genotype 1 and an early virological response[22-25]. The absence of a ⩾ 2 log10 reduction in HCV RNA between baseline and wk 4 has a strong negative predictive value[22-24,26,27]. This absence of an early virological response can be used as a guide to stopping treatment[26]. Pre transplant higher viral load has been reported as another predictor of SVR[22,25].

To make AVT more tolerable, various dosing strategies have been used including tailoring dose to liver function, shortening the duration of therapy or using haematopoietic growth factors[22,24,25]. In decompensated cirrhotics, reported rates of neutropaenia, thrombocytopaenia, anaemia, infection or liver decompensation range from 50%-60%, 30%-50%, 30%-60%, 4%-13% and 11%-20% respectively[22-25]. Child-Turcotte-Pugh (CTP) C patients are unable to tolerate a course of treatment[23,28]. The CTP score appears to be a more reliable predictor than Model for End-stage Liver Disease (MELD) for serious side effects that lead to discontinuation of therapy, hepatic decompensation or death[28].

Evidence regarding pre-transplant antiviral therapy

Over the past decade, there have been a small number of non-controlled studies that have assessed the efficacy of IFN and Ribavirin in HCV patients on the waiting list (Table 1). Many of the reported studies were designed to focus on the safety and tolerability of AVT, rather than HCV recurrence patterns and clinical course post LTx[22-24,26-31]. A number of different antiviral regimens and treatment periods have been studied. Non-pegylated IFN and Ribavirin using a low accelerating dose regime (LADR), appears to be well tolerated with 39% clearing HCV RNA on treatment and 21% with a SVR[23]. The best results have been reported with PEG IFN and Riba-

Table 1 The pre transplant hepatitis C virus antiviral therapy studies

Study	No.	Study design	Regime	Period	GFs	CTP/MELD mean	Genotype	Tx Naive	On Tx virolocal response %		SVR %		CessationTx	Dose reduction	% HCV neg post LTx[2]	Period FU postLTx
									G1/4	G2/3	G1/4	G2/3				
Massoumi *et al*[28]	90	Cohort	90-180 mcg IFNα2a + 400-1200 mg RBV *od*	8 wk	Y	6.7/11.2	77% G1/4	62%	48	67	10	29	33%	18%	40% (2/5)	9.6 mo
A2ALL LADR 2009[1]	79	Prospective semirandomised	0.75 mcg/kg per wk PEG IFNα2b + 600 mg RBV *od*	11.4-14.6 wk	NA	NA	59% G1/4	NA	NA	NA	NA	NA	NA	75% adverse event	40% (2/5)	3 mo
Everson *et al*[23]	124	Cohort	IFNα2b 1.5-3 mu x 3/wk + RBV 600-1200 mg *od*	6-12 mo	Y	7.4/11	70% G1	NA	30	83	13	50	13%	71%	80% (12/15)	6 mo
Iacobellis *et al*[24]	66	Case match	PEG IFNα2b 1 mg/kg per wk + RBV 800-1000 mg *od*	24 wk	Y	8/14.2	67% G1/4	NA	30	83	7	44	20%	40%	0% (0/0)	30 mo
Crippin *et al*[30]	15	Randomised	IFNα2b 1-3 mu *od*/wk ± RBV 400 mg *bd*	12 wk	N	11.9/NA	73% G1	NA	18	10	NA		NA	87% adverse event (study closed)	0% (0/0)	1 mo
Thomas *et al*[27]	20	Cohort	IFNα2b 5 mu *od*	14 mo	Y	12/13/2010	67% G1	100%	56	100	NA		0%	0%	33% (4/12)	33.6 mo
Forns *et al*[22]	30	Cohort	IFNα2b 3 mu *od* + RBV 800 mg *od*	12 wk	Y	50% CTP A, 50% B/C	83% G1	80%	30	82	NA		25%	63%	67% (6/9)	46 wk
Carrion 2009	51	Case match	PEG IFNα2a 180 mcg/wk + RBV 800-1200 mg *od*	15 wk	Y	48% CTP A, 43% B	88% G1/4, 10% G2/3	NA	47		20		43%	49%	67% (10/15)	6 mo
Tekin *et al*[31]	20	Cohort	PEG IFNα2a 135 mcg/wk + RBV 1-1.2 g *od*	48 wk	N	30% CTP A, 70% B	All G1b	NA	45	-	30	-	40%	65%	33% (1/3)	14 mo

[1]Not published presented at American Association for the Study of Liver Diseases 2009. Substudy of Adult to Adult Living Donor Liver Transplant Study (A2ALL) low accelerated dose regime (LADR); [2]% hepatitis C virus (HCV) neg post LTx = Patient No. HCV neg post LTx/HCV neg at LTx (actual patient nos). GFs: Growth factors; CTP: Child Turcotte Pugh; MELD: Model for End Stage Liver Disease; Tx: Treatment; SVR: Sustained virological response; neg: negative; LTx: Liver transplant; FU: Follow up, mcg: Micrograms; IFN: Interferon; RBV: Ribavirin, *od*: Once daily; *bd*: Twice daily; Y: Yes; N: No NA: Not available.

virin, with a SVR of up to 50% depending on genotype. The trials reporting more favourable SVR often had restrictive entry criteria such as patients with clinical, biochemical or haematological evidence of decompensation being excluded. A further factor adding to the variation in SVR reported was the selection of patients who were treatment naïve or previous responders[16,32].

Overall nearly one third of patients stopped treatment due to side effects, and the remaining patients often required dose reduction to improve tolerability. The actual number of patients who were HCV RNA negative coming to transplant was small. Of the responders, up to 80% remained HCV RNA negative after transplant[22,23,26] but long-term follow up was limited. From the available data, in the selected few, AVT can render the patient HCV RNA negative post transplant but the long-term benefit has not yet been fully established to justify the cost and clinical risk.

Who to treat?

It is estimated that 93% of listed HCV patients have a MELD ⩽ 18, equivalent to CTP ⩽ 7 suggesting that the majority of listed patients have stable liver function and should tolerate AVT[16]. Recommendations of the International Liver Transplant Society consensus conference (ILTS) for the selection of HCV cirrhotic patients for IFN based treatment, is to consider therapy when MELD ⩽ 18/CTP ⩽ 7, to use selectively when MELD 18-25/CTP 8-11 and avoid when MELD > 25/CTP > 11[16], as High MELD/CTP have an unacceptable risk of complication[23,28,30].

HCV genotype may influence the severity of HCV recurrence. European centres have reported worse outcome in genotype 1b, which may be related to host immune re-

sponse[33] but this has not been verified in American studies. However, genotype is the major determinant of response to treatment. Genotypes 2 and 3 should be considered for treatment, whereas the benefit of treating genotype 1 has not been fully established.

Patients who are previous non responders to IFN and Ribavirin are not suitable as the likelihood of achieving undetectable HCV RNA is very low < 10%. Prior relapsers would be suitable for treatment as their response would be predicted to be high[34]. The ideal group for waiting list AVT are patients who are previous responders, are treatment naïve or low MELD/CTP. Typically these are patients with living donors, or have compensated cirrhosis and HCC. Living Donor Liver Transplant (LDLT) recipients typically have a lower MELD than patients with decompensated cirrhosis awaiting deceased donor liver transplantation (DDLT).

Treatment regimens

Cytopenia is more common and severe with PEG IFN compared to non PEG IFN and thus, has to be balanced against higher virological response rates observed with PEG IFN. A LADR has been recommended by ILTS, as it is thought to be better tolerated in the cirrhotic patient[16,23]. Starting doses of IFN α2b 1.5 million units three times a week, PEG IFN α2b 0.5 μg/kg per week or PEG IFN 2α 90 μg per week, all with ribavirin 600 mg/d have been recommended. The dose of ribavarin should be reduced in renal impairment. Close monitoring of haematological and biochemical parameters is required, with dose adjustments being made every two weeks to allow full dose treatment to be achieved as tolerated by the patient. Patients with no virological response after 12 wk should have therapy discontinued[26]. Estimated duration of treatment is 6 mo for genotype 2 and 3, and 12 mo for genotype 1[16]. Treating for one year may not be practicable and the best group to treat is those with low MELD scores (< 18) who would be more likely to complete the treatment. LDLT recipients are a perfect group to be treated as the transplant can be timed according to HCV RNA clearance. A minimum of 12 wk, but up to 24 wk prior to LDLT is recommended[16].

Haematological growth factors

IFN is associated with cytopenia though bone marrow suppression, whereas Ribavirin can cause anaemia by a combination of haemolysis and bone marrow suppression. Patients developing anemia during HCV therapy often have inappropriately poor serum erythropoietin responses probably related to their underlying liver disease[35]. Patients with cirrhosis receiving AVT have a high incidence of haematological side effects[30]. To try and counter this Granulocyte-Colony Stimulating Factor (G-CSF) and erythropoietin (EPO) analogues have been used to avoid antiviral dose reduction with the aim of maintaining a good virological response. Severe anemia develops in about 10% of treated patients, and requires close monitoring of hemoglobin and RBV dose reduction, which may compromise sustained virologic response[36]. The impact of haematological growth factors on avoiding complications or improving virological response has not been clearly demonstrated. Aggressive use of bone marrow analogues to allow continued AVT or to avoid a dose reduction has not translated into higher treatment success rates[28,37-39].

DONOR AND PERI-TRANSPLANT FACTORS

Donor age

Several donor factors have been identified that influence HCV recurrence, impacting on both graft and patient survival. Age has been the most widely studied. Berenguer *et al*[40]. identified donor age higher than 60 years as a risk factor for developing cirrhosis [HR = 1.02 (1.008-1.05)] and worse graft survival [HR = 1.05 (1.03-1.07)]. Other studies have demonstrated a relationship between accelerated fibrosis and poorer outcome in grafts from older donors[41,42]. Machicao *et al*[41] and Wali *et al*[42] reported that donors aged 50 years or more, had a median fibrosis progression rate of 2.7 units/year and time to cirrhosis of 2.2 years post transplant. In contrast, Samonakis *et al*[43] found that absence of maintenance steroids and azathioprine but not donor age influenced severity of HCV recurrence. Lake *et al*[44] analyzed data from the American Scientific Registry of Transplant Recipients, looking at the effect of donor age on the outcome of 778 hepatitis B, 3463 hepatitis C and 7429 non-viral recipients. Donor age was not a risk factor for HBV recipients, but was the strongest predictor for graft loss in HCV recipients. The risk was identifiable with donors > 40 years [HR = 1.67 (1.34-2.09); $P < 0.001$] and > 60 years [HR = 2.21 (1.73-2.81); $P < 0.001$]. Donor age was also a strong predictor for graft loss in non-viral recipients, although the age range was higher (> 60 years) and the statistical strength was lower than for HCV recipients [HR = 1.89 (1.61-2.23); $P < 0.001$]. Donor age (> 50 years) was also found to be a strong factor in determining the likelihood of AVT success as measured by SVR[45]. Although there are no clear data defining the donor age at risk of severe HCV recurrence, donors over 60-70 years are generally regarded as higher risk.

Donor graft steatosis

The influence of donor microvesicular steatosis on HCV recurrence is not addressed in the literature, possibly because it is regarded as a mild and reversible condition[46]. Nevertheless, a recent study has reported that microvesicular steatosis increased the risk of initial poor graft function (IPF) (OR = 1.38 per 1 SD = 9.3%; $P < 0.021$)[47] and work is required to establish if it influences HCV recurrence.

The adverse influence of donor macrovesicular steatosis on graft and patient outcome has been widely studied[48-50]. Two recent publications reported that macrosteatotic grafts were safe to use in HCV recipients. Botha *et*

al[51] found that recipients receiving mildly macrosteatotic grafts (< 15% in their classification) had a good outcome, although only 3 out of 113 donors had macrosteatosis greater 30%. Burra *et al*[52] reached the same conclusion, although they classified mild macrosteatosis as < 30% and only 5 patients in their series had macrosteatosis > 30%. The small number of macrosteatotic grafts assigned to HCV recipients in these series makes it difficult to draw firm conclusions. In contrast, Briceño *et al*[53] reported that donor graft macrosteatosis (> 30%) was a risk factor for more frequent, earlier and severe HCV recurrence post LTx. Their study had 29 recipients receiving a moderately (30%-60%) and 19 recipients receiving a severely (> 60%) steatotic graft, although lack of protocolized biopsies was a limitation to this study[54], they reported a clear relationship between donor graft steatosis > 30%, earlier viral recurrence and the development of a more severe graft fibrosis.

The significance of acquired post-LTx macrosteatosis and HCV recurrence is unclear. Baiocchi *et al*[55] in 1998 suggested that macrosteatosis was highly specific for HCV recurrence and sensitive in detecting HCV disease recurrence, at 3 and 12 mo, but with low specificity and not genotype-related. However, Machicao *et al*[56] found that macrosteatosis developing after LTx did not predict severity of HCV recurrence in the first 12 mo. The development of macrosteatosis is influenced by several factors, including body mass index (BMI), immunosuppression, alcohol and diabetes. When the factors behind macrosteatosis development in the LTx population are fully elucidated its relationship to HCV recurrence may become clearer.

Type of graft

Garcia-Retortillo *et al*[57] reported an analysis of 117 LTx in 116 HCV recipients, of which 22 were LDLT. Type of transplant was the only independent predictor of severe recurrence (OR = 2.5; 95%CI: 1.13-5.68; $P = 0.025$) and the 2-year probability of severe recurrence was higher in LDLT compared to DDLT (45% *vs* 22%, $P = 0.019$). Suggested mechanisms, to explain the more aggressive HCV recurrence observed after LDLT, included shared HLA matching, the type of immunosuppression, a higher incidence of biliary complications, and the effect of liver regeneration. However, a prospective controlled trial by Shiffman *et al*[58] using protocol liver biopsies in 23 LDLT and 53 DDLT found no association between graft type and HCV recurrence in terms of recipient and graft survival or fibrosis progression over 3 years. Guo *et al*[59] reported similar results from a retrospective study of 15 LDLT and 52 DDLT, with no difference in histological HCV recurrence rates or graft survival over 2 years. Similar short-term results have also been reported by Schmeding *et al*[60]. with first-year fibrosis rates and graft survival being similar between DDLT and LDLT.

Split liver transplantation shares with LDLT the issue of liver regeneration as a possible risk factor for HCV recurrence, but reported studies show no difference in histological recurrence of HCV or in survival between recipients of deceased donor whole and split livers[61,62]. These studies share the limitation that donors suitable for split grafts are usually younger than 40 years and this may be an important confounding factor.

A recent study from Selzner *et al*[63] on 46 LDLTs and 155 DDLTs followed up with protocol biopsies showed that the mean fibrosis stage (Metavir) was significantly higher at 12 to 48 mo post LTx, and the rate of fibrosis progression tended to be faster after DDLT than LDLT (0.19 *vs* 0.11 stage/year, $P < 0.05$). In multivariate analysis, donor age was the only variable independently associated with both surrogate outcomes. Thus, donor age > 45 years carried a relative risk of 8.17 (95%CI: 2.6-25.5, $P = 0.001$) for reaching fibrosis stage 3 or 4 at 2 years post LTx, suggesting that donor age rather than graft type determines progression of recurrent HCV.

HCV positive donors and co-infections

The increasing organ shortage has necessitated the use of both older and HCV+ organs. There are a small number of studies examining the use of HCV+ grafts. Donor Hepatitis C status does not seem to affect graft or recipient survival and using HCV+ livers for transplantation in HCV+ recipients seems to safely expand the organ donor pool[64]. Interestingly, a recent study has analyzed the effect of HCV+ donors stratified by age. Demonstrating a negative impact of older donor age (> 50 years) on survival and fibrosis progression in patients transplanted with HCV+ organs. According to the current evidence, using HCV+ grafts from young donors (< 50 years) for HCV recipients can produce good results, but further experience is required to establish the validity of this approach[65].

The influence of co-infections on HCV recurrence has been investigated by a number of groups. Humar *et al*[66] studied cytomegalovirus (CMV) and human herpesvirus-6 (HHV-6). No correlation was found between CMV or HHV-6 serum peak and HCV viral load. But on subgroup analysis HHV-6 infection was associated with the development of more severe recurrence (hepatitis and/or fibrosis score > 2). Also, fibrosis scores at last follow up were higher in patients with CMV disease or HHV-6 infection. Burak *et al*[67] have also identified CMV co-infection as a risk factor for graft failure and severe fibrosis on biopsy. Considering that CMV infection occurs in approximately one quarter of HCV-infected LTx recipients, CMV donor and recipient status may be an important modifiable factor to consider.

Duclos-Vallée *et al*[68] reported poorer survival of co-infected HIV/HCV patients (35 patients) than that of HCV mono-infected patients (44 patients). The 2 and 5 years survival rates were 73% and 51% in co-infected, and 91% and 81% in mono-infected patients, respectively. Additionally, fibrosis-free survival rates were markedly low in co-infected patients after the second year post-transplant, whereas the majority of mono-infected patients only experienced mild recurrent hepatitis. These

results are similar to our series, in which 5 of 7 HCV-infected patients died after LT at 95-784 d (median 161 d), of whom 4 patients died of recurrent HCV infection and sepsis, despite antiviral therapy in 3[69]. Longer follow-up in larger series is required before a conclusive directive can be provided for HCV/HIV co-infected patients requiring LT and the advent of more effective anti-viral therapy may transform the outlook for this group.

Ischemia-reperfusion injury

Ischemia-reperfusion injury (IRI) is a result of several peri-operative factors that can define the extended criteria donor. Factors influencing the severity of IRI include donor status (cardiac or brain death), cold and warm ischemia time, donor age, preservation solution and technical factors during retrieval. Other factors influencing IRI are type of reperfusion used and graft-related quality factors such as macrosteatosis. Because of the complexity of IRI, its inclusion in multivariate analysis usually becomes a confounding factor that is difficult to study. A study from Watt *et al*[70] showed worse survival outcome in HCV recipients receiving grafts with preservation injury (PI). The 1- and 3-year survival rates for these 2 groups were 78% and 59% in HCV-PI(+) versus 100% and 88% in HCV-PI(-). In addition, more patients in the PI(+) group had progressed to stage 3 or 4 fibrosis, compared to the group with no PI (-) (43% *vs* 9%, $P = 0.02$). In 2008, Killackey *et al*[71] reported a significant correlation between peak alanine transaminase (ALT) and the severity of IRI on reperfusion biopsy among 477 HCV recipients (of which 44 were LDLT). However, there was no correlation between the severity of IRI and the incidence or timing of histologic HCV recurrence or incidence of acute cellular rejection (ACR). Briceño *et al*[72] also looked at the effect of marginal donor variables on outcome in HCV recipients and IRI was a prognostic factor on univariate analysis. The same group additionally reported that, when moderate to severe IRI was associated with macrovesicular steatosis > 30%, graft survival was decreased[53]. This association has not been previously reported and deserves further study.

Immunological factors

The role of human leukocyte antigen (HLA) matching between donor and recipient in post-transplant rejection and survival has been widely studied. It has been proven to increase graft survival after kidney, heart, and other organ transplants In contrast, HLA matching is not routinely performed in LTx because its importance remains unclear. A recent meta-analysis of over 16 studies found that good HLA compatibility reduces the incidence of ACR but had no influence on graft outcomes[73].

Early acute rejection and steroid bolus treatment are considered as risk factors for HCV recurrence[74]. Langrehr *et al*[75] published a retrospective analysis on 165 LTx in HCV recipients with complete donor/recipient HLA typing, analyzing HCV recurrence and outcome. In this study it was shown that HLA matching reduced rejection episodes, but the severity of fibrosis progression within the first year after LTx was enhanced. Interestingly, this did not result in impaired survival in the better-matched grafts. Belli *et al*[76] reported on the association of MHC alleles and donor/recipient mismatch with the occurrence and the severity of recurrent HCV, drawing two conclusions. Firstly, a fully mismatched donor/recipient pair at the DRB1 locus was associated with HCV recurrence and severity. And secondly, donor age, full HLA-DRB1 donor-recipient mismatch, and HLA B14, were independent risk factors for the development of severe fibrosis.

Balan *et al*[77] studied HLA mismatch in a cohort of 883 LTx recipients. Overall graft survival decreased according to the total mismatch score and there was a negative effect of mismatching at the A locus on patient survival. Interestingly, there was a subgroup with DR-locus mismatch with increased recurrence of autoimmune hepatitis and primary biliary cirrhosis, while mismatch in the A locus was associated with recurrence of HCV ($P = 0.01$, HR = 1.6) and primary sclerosing cholangitis ($P = 0.03$, HR = 2.9). Yoshizawa *et al*[78] reported two cases of LDLT between identical twins. Despite the avoidance of immunosuppression, a rapid increase in serum HCV RNA and histological recurrence of HCV by 1 mo was observed. The contribution of HLA mismatch to HCV recurrence remains unclear and other immunological factors may be involved. To date, cytokine gene polymorphisms in allograft tumor-necrosis-factor β (TNF-β), Interleukin 16 (IL-16)[79], TGF-β, IL-10, and INF-γ[80] have been proposed as novel markers to predict the severity of HCV recurrence. The innate lymphocyte population of CD56+ lymphocytes, NK and NT cells provide an important first line of defense against viral infection. Rosen *et al*[81] showed that the number of CD56+ lymphocytes and NK cells in peripheral blood prior to LTx was significantly lower in recipients who developed severe HCV recurrence compared to those with mild histological recurrence. There was no association between NK and viral levels, suggesting that the severity of HCV recurrence is independent of viral level and high levels of CD56+ lymphocytes are protective against recurrence More work is required to confirm the genetic components that contribute to both NK cell-mediated control of HCV recurrence and to liver injury in the transplant setting[82,83].

Recent research emphasizes the important part that host genetics play in the ability to clear acute HCV infection and to achieve SVR. Polymorphism of the IL-28B gene, which encodes the endogenous antiviral cytokine IFN-λ, are associated with SVR and natural viral clearance[84]. The prevalence of the rs8099917 G allele in HCV/HIV-1 co-infected patients is strongly associated with treatment failure in HCV genotype 1-infected patients[85]. These polymorphisms may well serve, at least in the start, as a predictor of achieving SVR.

Diabetes mellitus

Diabetes and insulin resistance have been associated with progression of fibrosis[86]. Hyperinsulinemia may cause

direct stimulation of hepatic stellate cell mitogenesis and synthesis of collagen[87]. The synergy between donor age and recipient diabetes as a factor for aggressive HCV recurrence was observed by Foxton *et al*[88] who reported that patients with diabetes receiving a liver from a donor older than 55 years was associated with an 8.38-fold risk of progression to severe fibrosis.

In 2008, Hanouneh *et al*[89] performed an analysis on liver biopsies to assess the impact of metabolic syndrome (MS). Overall median rate of fibrosis progression was 0.08 units per month. On univariate analysis, high HCV RNA at 4 mo post LTx, diabetes, CMV, and MS were associated with progression of fibrosis, whereas on multivariate analysis, MS was independently associated with fibrosis progression 1 year after LTx [OR = 6.3 (1.4-28.7); P = 0.017].

More recently, Veldt *et al*[90] evaluated the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) to identify insulin-resistant recipients at risk for rapid fibrosis progression after LTx. They found that when this index was elevated (> 2.5) there was a risk for rapid fibrosis progression and treatment with insulin had no effect on the fibrosis progression rate, suggesting that there might be a role for treatment with insulin sensitizing agents.

The association between immunosuppression with tacrolimus, HCV and post-transplant diabetes or impaired fasting glucose has been reported[91]. The choice of immunosuppressive treatment might be decided on the basis of the patient's pretransplantation status.

POST-TRANSPLANT IMMUNOSUPPRESSION AND ANTIVIRAL TREATMENT

Anhepatic initiated therapy

Thymoglobulin induction: Thymoglobulin is an antithymocyte polyclonal antibody that depletes the T cell pool and can be used as an induction immunosuppressant agent during the anhepatic phase. By pre-treating with thymoglobulin and minimizing immunosuppression, cellular host immunity against HCV re-infection may be improved[92]. A retrospective study on the use of thymoglobulin induction found that lower levels of Tacrolimus were achieved but HCV recurrence rates were similar between patients who had received thymoglobulin induction therapy and tacrolimus versus tacrolimus and steroids. However, HCV RNA loads were significantly lower in the thymoglobulin group[93]. More work is required to see whether this observation is clinically significant and alters the pattern of HCV post transplant recurrence.

Adoptive immunotherapy: HCV viral levels post often exceed pre LTx, as immunosuppression suppresses the host response to HCV replication. Adoptive immunotherapy has been studied in a phase 1 trial where lymphocytes extracted from liver allograft perfusate were able to mount an anti HCV response. Activated liver allograft-derived NK cells were isolated from the perfusate (IL-2 stimulated and anti-CD3 monoclonal antibody treated to deplete T cells), and injected intravenously into the transplant recipient. Early data from the pilot study reported lower HCV RNA titers at one month post-transplant, but the effect was transient[94]. Augmentation of the NK cell response, which plays a pivotal role in innate immunity, may be an alternative approach to preventing HCV recurrence and is an area of active research[95].

Post-transplant immunosuppression

Immunosuppression is considered to be a major factor in accelerated HCV recurrence and has been an area of extensive research. The immunosuppression strategy in HCV LTx recipients was evaluated in 81 LTx programs in an international survey. The most common regimen used (41%) was based on triple therapy [Tacrolimus, Mycophenolate Mofetil (MMF) and steroids]. Steroid-free protocols were used by 7.4% of transplant groups, while 11% discontinued steroids within a week, 56% within three months and 98% within the first year[96].

Steroids: High dose steroid boluses to treat rejection increase viremia and may lead to premature HCV recurrence. It has been shown that HCV recurrence is associated with the number of rejection episodes[97]. Although the data on increased HCV viral load with steroid bolus is convincing, the effect of steroid maintenance remains controversial. Moreover, a rapid reduction in steroid dosage may be harmful for HCV recurrence[98]. Interestingly, Vivarelli *et al*[99] reported that it is the way that steroids are administered what impacts the recurrence; in fact while rapid steroids tapering and withdrawal exert a negative effect, low-dose steroid maintenance in the first 24 post-operative months seems to reduce the severity of HCV recurrence, in particular the degree of fibrosis associated with recurrent hepatitis.

The link between steroids and viral replication has prompted many centres to practice steroid withdrawal. However there is a lack of robust data showing the efficacy of this approach. A meta-analysis of 13 trials[100] showed that steroid avoidance was associated with lower HCV recurrence (RR = 0.90, P = 0.03), although no individual trial reached statistical significance. The heterogeneity, short-term follow-up and relatively small size of many of the trials, as well as the lack of information on steroid dosage, make conclusions less robust. Larger multicenter trials are required to clarify the influence of steroid-free regimens on HCV recurrence.

Calcineurin inhibitors: The association between oversuppression and HCV progressive disease is well recognized. However, the effect of different calcineurin inhibitors (CNIs) on HCV replication and/or progression of recurrent HCV remain controversial. Cyclosporine A (CsA) has been found to inhibit HCV replication *in vitro* while tacrolimus does not[101]. But the effect of CsA on

HCV replication *in vivo*, in the setting of LTx is not clear. A meta-analysis comparing tacrolimus to CsA-based immunosuppression in HCV recipients[102] assessed the clinical, virological, and histologic post-transplant outcomes. A total of 5 randomized control trials (1995-2006) which included 366 patients were analyzed. No significant differences in mortality, graft survival, biopsy proven ACR, steroid resistant ACR or fibrosing cholestatic hepatitis between the regimens was found. But the use of different immunosuppression regimens, an era-effect, and the lack of protocol liver biopsies limit this meta-analysis.

In the LIS2T trial, immunosuppression regimens based on CsA or tacrolimus were compared according to HCV status[103]. All patients received a combination of either CsA (n = 250) or tacrolimus (n = 245) with steroids and some additionally received azathioprine (AZA). The incidence of ACR was similar in patients receiving combination therapy with AZA, whether with CsA or tacrolimus, independent of the HCV status. In HCV patients, death or graft loss was higher with tacrolimus (16%) compared to CsA (6%) ($P < 0.03$). The HCV recurrence rate was similar for tacrolimus and CsA but time to histological diagnosis of recurrence was longer with CsA than with tacrolimus (100 ± 50 d *vs* 70 ± 40 d; $P < 0.05$).

On the basis of current data, it is not possible to conclude that CsA by itself has a significant effect on viral replication or on the course of HCV recurrence. However, choice of CNI may influence the efficacy of AVT. As co-treatment with IFN and CsA has been shown to achieve greater inhibition of HCV replication and higher SVR rates compared to IFN alone[104]. Furthermore, SVR after IFN therapy has been found to be higher with CsA compared to tacrolimus (46% *vs* 27%; $P = 0.03$)[105]. In a pilot study[106], 38 patients with HCV recurrence receiving PEG IFN α2a and Ribavirin were randomized to continue tacrolimus or to be switched to CsA. CsA led to a modest decrease in HCV RNA levels and appeared to enhance the antiviral response to IFN and Ribavirin, but there was no difference in SVR. However this study was limited by small sample size and randomization at transplant rather than at time of significant HCV recurrence. Further randomized trials are needed to establish whether the presently available CNIs affect HCV recurrence.

Post-transplant antiviral treatment

Prophylactic therapy: Treatment with neutralizing antibodies is effective in patients transplanted for HBV, but currently there is no evidence that this strategy is effective in preventing HCV recurrence. Both polyclonal and monoclonal anti-envelope antibodies can capture and neutralize HCV in vitro[107-109]. The main target for neutralizing antibodies appears to be the various epitopes in the E2 envelope glycoprotein. HCV antibody therapy starts in the anhepatic phase and then is continued for 12 to 14 wk after transplant. Three trials[110-112] comparing high dose HCV antibody vs. low dose HCV antibody were included in a Cochrane meta-analysis[113]. There was no difference in patient and graft survival, virological response or fibrosis on histology. Discontinuation of therapy occurred in up to 35% of patients with high dose antibody and 17% with low dose antibody. Considering both the lack of clinical benefit and occurrence of side effects, there is currently no evidence to recommend prophylactic HCV antibody.

Pre-emptive therapy: Antiviral therapy after transplantation but before clinical evidence of reinfection. HCV recurrence manifests in the first 6 mo post LTx. Initiation of AVT in the early post transplant period has been proposed as a potentially more effective way of preventing it. Pre-emptive therapy refers to the initiation of AVT within 2 to 8 wk after LTx when the viral load is low and histological damage is absent[114]. However, only those patients who are well after transplant and without severe complications can receive AVT. In the general population, AVT with PEG IFN for acute HCV hepatitis can achieve high SVR rates, but in transplant patients the rate is lower at 8%-39%. The high levels of immunosuppression early post LTx make AVT less effective and dose reduction or discontinuation due to adverse events is common (up to 57% of cases)[114].

Several trials have assessed the efficacy of the pre-emptive AVT. IFN, PEG IFN and Ribavirin, alone or in combination. At present, there is no standard timing of commencement of pre-emptive treatment. It has been applied very early post LTx (as soon as patients can tolerate food), to 4-6 wk after transplant. A meta-analysis which included randomized trials assessing the use of pre-emptive AVT showed no benefit in terms of survival, graft rejection, virological response or histological changes. The proportion of patients who discontinued treatment was 31% for PEG IFN, 29% for IFN and 9% for Ribavirin. Currently, there is no evidence to recommend pre-emptive AVT to prevent HCV re-infection.

Antiviral therapy after evidence of reinfection: Directed AVT after evidence of HCV recurrence represents the mainstay of management in HCV post transplant. Most LTx centers commence AVT once liver biopsy demonstrates significant histological damage and therapy with PEG IFN and Ribavirin aims to achieve SVR as this has been associated with improved survival, reduced risk of graft failure and reduced risk of developing complications.

The therapeutic efficacy and side effects of different AVT in patients with HCV re-infected grafts have been compared in a Cochrane review[115]. Eleven trials including 389 LTx were analyzed. Dose reduction or discontinuation due to adverse effects or patient choice, was required in 87.5% and 42.9% respectively. All the trials had high-risk bias and none of them reported decompensation rates or quality of life. The antiviral regimens and dosages, the interval between the LTx and the beginning of the treatment and the duration of the therapy were heterogeneous in all trials. There was no difference in the mortality, graft rejection or re-transplantation between

the intervention and control arms. Nevertheless, a higher SVR (48% *vs* 0%) and improvement in fibrosis occurred in the treatment group of PEG IFN and ribavarin[116]. In the comparison between two doses of PEG IFN (1.5 mg/kg per week *vs* 0.5 mg/kg per week) plus ribavirin, higher rates of SVR were achieved in the high-dose group (63% *vs* 22%). Despite there being no difference in the main outcomes, SVR has been shown to reduce mortality rates in observational studies and it is worthwhile performing further studies to assess this.

Data evaluating the effect of AVT on disease progression are scarce and results are controversial. However, it has been demonstrated that AVT with PEG IFN and ribavirin achieves higher SVR rates in mild HCV recurrence than in severe HCV recurrence[116]. AVT tolerability is a major issue as only 30% of transplant patients reach target dose and duration. Dose reduction of PEG IFN and ribavirin are needed in 39% and 45% respectively, with discontinuation of treatment in 26%. Close monitoring is required and growth factors help to avoid dose reduction/discontinuation due to cytopenia.

A recent study attempted to determine the most cost effective timing for AVT (PEG IFN and ribavirin) in advanced liver disease infected with HCV genotype 1 and concluded that treatment of patients with compensated cirrhosis was the most cost effective[117]. Four different treatment strategies in a hypothetical cohort of 4000, 55 years old, treatment naïve cirrhotics with a 17 year follow-up, were analyzed in a Markov model. The authors concluded that treatment of advanced post LTx recurrence is more cost-effective than no treatment, but it gave less survival benefit at greater cost in comparison with patients treated during compensated cirrhosis.

New drugs for HCV recurrence treatment

In view of the current results of standard antiviral therapy, there is a need to improve treatment strategies. The recent knowledge of the HCV life cycle and of structural features of the HCV proteins has supported the development of many promising directly acting antiviral agents, or "specifically targeted anti-viral therapy for hepatitis C" (STAT-C) compounds. Many of these STATs are currently in phase I -Ⅲ development and will significantly change treatment options for HCV infection in the near future.

Compounds targeting HCV polyprotein procession: ns3/4a protease inhibitors: These compounds provide a high anti-viral efficacy but a low genetic barrier to resistance. However, the frequency of resistance development can be reduced by the additional administration of peg-IFN and Ribavirin. Many of these compounds are under development, however telaprevir (VX-950) and boceprevir (SCH 503034) which are the most advanced HCV NS3 protease inhibitors, have already entered phase-Ⅲ clinical development and are expected to be approved in 2011/2012[118]. Most protease inhibitors and polymerase inhibitors are HCV genotype 1 specific. The PROVE 3 trial[119] has shown that telaprevir is highly effective in the treatment of HCV genotype-1 nonresponders or relapsers. In contrast, addition of boceprevir to standard treatment only revealed a minor impact on SVR rates in non-responders, but further trials are awaited. In addition to telaprevir and boceprevir, many NS3/4A inhibitors with promising anti-viral activities are currently investigated in phase I and II trials.

Compounds targeting HCV replication: (1) HCV NS5B polymerase inhibitors. Nucleoside analogue inhibitors (NIs) [valopicitabine (NM283), R7128, R1626, PSI-7851 or DX184]. Since these compounds can mimic the natural substrates of the polymerase, they are incorporated into the growing RNA chain and tackle the active site of NS5B, then causing direct chain termination. NIs are potentially effective against different genotypes, in contrast to NS3⁄4A inhibitors. There is a relatively high genetic barrier in the development of resistances to NIs. Valopicitabine was the first NI investigated in patients with chronic hepatitis C, but its activity was low. More effective NIs are under development; (2) Non-nucleoside analogues inhibitors (NNIs). These drugs can bind to different allosteric enzyme sites, which results in conformational protein change before the elongation complex is formed. Their application results more frequently in resistance development compared to Nis; and (3) NS5A/NS4B inhibitors. NS4B displays RNA-binding properties that are crucial in HCV-RNA replication. In vitro inhibition of NS4B has been shown to compromise HCV replication significantly. NS5A protein contributes in the regulation of HCV replication[120]. No clinical data on resistance to these compounds have been reported, and thus, results studies using multiple dose and combination therapy have to be awaited.

Conclusion and keypoints

HCV recurrence is a major concern when transplanting HCV+ patients. Several strategies to try and prevent graft infection or, if already infected, to reduce recurrence severity are available.

In the pre-transplant setting, AVT aims to achieve HCV RNA negativity at time of transplant. Presently available antivirals can produce HCV RNA negativity in highly selected patients and undetectable HCV RNA or SVR at time of transplant may influence recurrent HCV.

Probably, the most important strategy in the peri-transplant, especially in an era of organ shortage, is ideal donor-to-recipient matching. Factors like donor age, donor steatosis, recipient co-infections and recipient insulin resistance increase the risk of HCV recurrence and decrease global outcome. Interestingly, the type of graft or using young HCV+ donors does not appear to increase the risk. The role of IRI and HLA mismatch needs to be explored more, although available evidence supports the minimization of both.

In the post-transplant setting, there is no evidence for the use of HCV antibody therapy and adoptive im-

munotherapy is still experimental. Steroid boluses and ACR are factors associated with recurrence, and steroid free immunosuppression maintenance appears to reduce recurrence. Presently available CNIs appear to have equivalent influence on HCV recurrence but CsA in combination with AVT may produce a greater inhibition of HCV replication. Directed AVT after histological evidence of HCV recurrence is the mainstay of management, with no evidence supporting pre-emptive AVT. In the future it is strictly necessary to find out whether SVR can be achieved by combination therapies of different STAT-C compounds without PEG IFN and ribavirin. Future clinical trials need to address whether a long-term suppression of HCV replication or even SVR can be achieved with such direct antiviral combination therapies. The results of LTx for HCV will hopefully continue to improve as a greater understanding of the factors influencing recurrence is achieved.

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REVIEW

Cirrhotic ascites review: Pathophysiology, diagnosis and management

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Abstract

Ascites is a pathologic accumulation of peritoneal fluid-commonly observed in decompensated cirrhotic states. Its causes are multi-factorial, but principally involve significant volume and hormonal dysregulation in the setting of portal hypertension. The diagnosis of ascites is considered in cirrhotic patients given a constellation of clinical and laboratory findings, and ultimately confirmed, with insight into etiology, by imaging and paracentesis procedures. Treatment for ascites is multimodal including dietary sodium restriction, pharmacologic therapies, diagnostic and therapeutic paracentesis, and in certain cases transjugular intra-hepatic portosystemic shunt. Ascites is associated with numerous complications including spontaneous bacterial peritonitis, hepato-hydrothorax and hepatorenal syndrome. Given the complex nature of ascites and associatedcomplications, it is not surprising that it heralds increased morbidity and mortality in cirrhotic patients and increased cost-utilization upon the health-care system. This review will detail the pathophysiology of cirrhotic ascites, common complications derived from it, and pertinent treatment modalities.

Key words: Ascites; Cirrhosis; Hepato-hydrothorax; Hepatorenal syndrome; Spontaneous bacterial peritonitis

Core tip: Ascites is an accumulation of fluid most commonly found in cirrhosis with portal hypertension. Ascites can cause or is associated with a number of complications including spontaneous bacterial peritonitis, hepato-hydrothorax and hepatorenal syndrome. Ascites itself, and these associated complications are a significant cause of morbidity and mortality in cirrhotic patients. The management of ascites is complex, utilizing an array of medications and interventional therapies to maintain appropriate total body volume, prevent multi-organ dysfunction, and manage against increased risk for associated infections.

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INTRODUCTION

Ascites is a very common manifestation of decompensated cirrhosis and represents a pathologic accumulation of fluid within the peritoneal cavity[1-3]. The term "ascites" is derived from the Greek term "*askos*" in reference to its similar appearance to a winebag or sac. This seems rather appropriate, both in description of presentation and as an allusion to a main cause of cirrhosis. The term "ascitic fluid" is also utilized in the literature however it is in a way redundant. The clinical presentation of ascites has been described since antiquity, reasonably inferred from passages in the Egyptian medical text, the *Ebers Papyrus* c. 1550 BCE[4].

Cirrhotic ascitic fluid accumulation results from a

number of factors broadly defined in terms of hormonal and cytokine dysregulation and related volume overload in the setting of portal hypertension[1]. The manifestation of ascites is an important landmark in the progression of cirrhosis: (1) it is the most common cause for hospital admissions and thus contingent costs; (2) it portends increased 1-year mortality; and (3) functions as a risk-stratification marker for orthotopic liver transplantation (OLT)[1,5-7]. This review will characterize the pathophysiology of cirrhotic ascitic fluid formation, the complications surrounding ascites, and basic medical management of these processes.

PATHOPHYSIOLOGY

For the purposes of this discussion, the focus will be on cirrhotic ascites, in the setting of portal hypertension, which comprises approximately 85% of all cases[1,2,5]. Other causes of ascites (non-cirrhotic)can be broadly defined as pre- or post-hepatic in origin. Pre-hepatic causes might include: portal vein thrombosis, lymphoma, abdominal lymphatic injury or obstruction, bowel perforation, renal failure, pancreatitis, peritoneal tuberculosis, or a malignancy with peritoneal implants. Post-hepatic causes include congestive heart failure usually associated with pulmonary hypertension, constrictive pericarditis, the Budd-Chiari syndrome, and stricture/web formation in the inferior vena cava (IVC)[1,5]. This latter category, regarding IVC stricture/web formation, is likely to manifest rather slowly overtime as obstruction to critical flow progresses.

Malignant ascites, which is found in 10% of cases, can occur as a result of any neoplastic disease having peritoneal metastasis, but is more common with breast, bronchus, ovary, gastric, pancreatic or colon cancer. Up to 20% of cases of malignant ascites have a tumor of unknown origin. Most cases of malignant ascites have a high protein content[8-10]. Because there are multiple potential causes of ascites other than liver disease and/or portal hypertensive origin, non-hepatic disease processes should be ruled out through clinical history and by utilizing specific laboratory testing and imaging. As an example, in the setting of chronic pancreatitis with associated pseudocyst and internal fistulae formation, significant fluid can directly enter into the peritoneal cavity and manifest as abdominal distension with pain. In particular an elevated ascitic fluid amylase level, found on diagnostic paracentesis, is strongly diagnostic for this category. The physician might be especially sensitive to this diagnosis in a patient with a significant history of alcohol use, chronic pancreatitis and steatorrhea. Notably, the serum-ascites albumin gradient (SAAG) is a useful tool for segregating ascites-associated disease processes due to portal hypertension, such as cirrhosis, from the many other non-portal hypertensive causes of ascites[11]. A SAAG value $\geqslant$ 1.1 g/dL strongly supports (97% sensitivity) a diagnosis of portal hypertension as causal[11].

Despite its well known presentation, the pathogenesis of ascites remains incompletely understood and continues to evolve. A hybrid theory currently prevails, having arisen out of the "overflow" and "underfill" theories of the past generation[1,2,5]. A brief sketch of these views suggests the following: (1) continuous injury to the liver as a combination of both exogenous factors, *e.g.*, chronic alcohol or viral or non-alcoholic steatohepatitis (NASH) injury; (2) in the setting of an appropriate genetic disposition; and (3) continued micro-processes of inflammation, necrosis and collagen deposition/regeneration, all conspiring to transform the liver from a low-resistance to a high-resistance system, *e.g.*, a spectrum of fibrosis with vascular smooth muscle dysfunction[11]. These continued processes can lead, in aggregate, to increased pressure in the portal vein, *i.e.*, portal hypertension. The portal vein is normally approximately 8 cm in length and usually < 13 mm in diameter. It is formed by the union of the splenic and superior mesenteric vein systems; the inferior mesenteric vein enters one of these vessels, or at their junction, quite variably. Portal hypertension is defined as being 6 mmHg or greater as measured by the wedged hepatic vein gradient, and in particular, ascites formation usually occurs at 8 mmHg or greater. For completeness, it is noted that further clinical decompensation in the form variceal formation (10 mmHg), increased risk of variceal bleeding (12 mmHg) and risk for recurrent variceal bleeding (20 mmHg), correlate nicely with these increasing portal pressures[12-16]. This clinical sequence portends significant morbidity and mortality and can be interlaced with relatedfurther complications of hepatic encephalopathy (HE), spontaneous bacterial peritonitis (SBP), hepato-hydrothorax (HHT) and hepatorenal syndrome (HRS)[12].

Thus in the setting of portal hypertension, backflow and stasis of vasodilatory substances, *e.g.*, nitric oxide, begin to accumulate[17]. This causes, amongst other results splanchnic vasodilation with resultant hypoperfusion (although even when globally euvolemic or hypervolemic) of the renal system. Appropriately in this sense, thereninangiotensin-aldosterone system (RAAS) is activated leading to aggressive fluid retention[18-20]. In brief, renin is secreted from the renal juxtaglomerular apparatus (JGA) around the proximal nephrons in response to changes in vascular pressures, changes in serum sodium, and from activation of the sympathetic nervous system[17]. It in turn will convert angiotensinogen (made in the liver) to angiotensin I which is further converted to angiotensin Ⅱ by angiotensin converting enzyme (ACE) in the lungs[17-19]. Angiotensin Ⅱ has multiple important functions that drive fluid acquisition and retention, including stimulation of the thirst drive, release of aldosterone from the zona glomerulosa of the adrenal cortex, and secretion of vasopressin from the posterior pituitary[17-19]. This excess retained blood volume is thought to leak-out (filtered in a sense) directly from both the liver surface, and the mesenteric vessels. This latter mechanism is due to increased hydrostatics and vascular wall permeability, and concurrently decreased oncotic (osmotic) fluid retention in the form of absolute or relative hypoalbuminemia.

These three parameters, as described in the classical Starling equation, overwhelm the reabsorptive capacity of the peritoneal surface and lymphatic system[17-19].

Normally, the peritoneal cavity is decompressed and has a pressure of 5-10 mmHg, containing approximately 25-50 mL of serous fluid. This fluid normally provides a low resistance film over which bowel can move past each other and further hydrates the serosal surfaces maintaining pliability and integrity. The maximum absorption of fluid out of the peritoneum is approximately 850 mL/d in optimal settings. This property of absorption (selective filtration) provides the theory under which peritoneal dialysis operates[21,22]. It can be observed that alterations in the properties of the lymphatic system or the peritoneal surface area, either by inflammatory, infectious or fibrotic/mechanical processes can alter optimal re-absorption. Thus, continued dysregulation of these parameters can lead to profound ascitic fluid retention.

CLINICAL PRESENTATION

Ascites represents a very common manifestation of decompensated cirrhosis and thus on presentation[1,12] if cirrhosis has not already been defined for the patient, risk factors for its usual precursors, namely alcoholic use, viral hepatitis and NASH should be explored[1,12]. The clinical presentation of ascites is variable: it can occur slowly as observed in common and classical liver diseases, or suddenly as in new mechanical obstruction to the major vessels. For instance, hepatic or portal vein thrombosis, compression of the IVC due to trauma with a hematoma or infection, or acute hepatic failure. In the setting of thrombosis, causes for a hypercoaguable state should be sought: infectious, inflammatory, malignancy or hematologic genetic dispositions.Ascites can be painless, and if it is associated with abdominal pain may simply represent discomfort from mechanical distension, or super-imposed infection as in SBP, or even hepatocellular carcinoma[12,23]. Thus, while ascites represents a natural progression of cirrhosis, its appearance should prompt a careful investigation for other causes and complications as well[12].

An increase in abdominal girth can be due to a few generic processes. An increase in the width of the abdominal wall itself, *i.e.*, an enlarging panniculus; or it can represent the accumulation of solid, gas or liquid within the intestines or peritoneal space. Solid causes can represent retained and accumulating stool in constipation, or a malignant mass such ovarian cancer. Gaseous distension can also be observed in those with constipation or small intestinal bacterial overgrowth. Liquid retention, when focused, can represent a cystic object or loculated ascites. When the liquid is distributed uniformly, one certainly considers non-complicated ascites from liver or other sources (*vida supra*). The most common clinical complaints associated with liver related-ascites are an increase in abdominal girth, abdominal fullness, discomfort or ache, shortness of breath, early satiation and a sense of reduced mobility[12,22,24]. These symptoms are sensibly scaled to the actual amount of volume. Ascites can be of three severities: grade Ⅰ, wherein it is diagnosed by abdominal ultrasound, which requires approximately 100 mL of fluid within the peritoneum (recall that normal volume is approximately 25-50 mL); grade Ⅱ, implying at least 1000 mL of peritoneal fluid, which can be detected with physical examination through the classic exam findings of sagging flanks, shifting dullness, fluid-wave, and the more laborious and rarely utilized Puddle's sign;grade Ⅲ, manifested as a grossly distended abdomen, implying liters of ascitic fluid. This final grade can elicit a severe form of discomfort, and may be described as a tense ascites[1,12,22,24].

PARACENTESIS AND LABORATORY TESTING

Proper evaluation of ascites rests upon direct assessment through paracentesis: to characterize the fluid origin, and whether it is sterile, infectious and/or malignant. Unfortunately, there has been much lore related to the contra-indications and complications of this procedure. As with any procedure, coagulation status is a reasonable concern, and indeed in cirrhotics with ascites their coagulation status is altered but it is not at all obvious in which direction (pro- or anti-coagulant)[25]. Certainly there is a deficiency in the production and/or activity of coagulation compounds as would be indicated by the altered international normalized ratio (INR), but this parameter does not measure all coagulation factors, *e.g.*, protein C - a procoagulant. The idea that these patients are "auto-anticoagulated" is not true, and they can in fact be at real risk for thrombo-embolic disease[26]. Considering this problematic background, one must look at the empiric data, and although limited, suggests that paracentesis has been well-tolerated in patients with platelet counts below 20000 cells/mm^3 and an INR as high as 8.7[27-29]. Complications of wall hematoma requiring transfusion and infection are remote. A reasonable absolute contra-indication would be in disseminated intravascular coagulation[12]. The evidence for requisite transfusions of blood products, by non-hepatology procedural services, to meet the arbitrary limits of an INR < 1.5 or platelets > 50000 cells/mm^3 is unfounded, wasteful in resources and time, and itself incurs risks of transfusion reactions.

A diagnostic paracentesis, as opposed to a therapeutic paracentesis (*vida infra*), requires approximately 30-50 mL, and is mandatory in all cases of new onset ascites or ascites occurring in an individual with a change in clinical status to include fever, abdominal pain, new onset or worsening HE and any sign or symptom of infection generally. Paracentesis may be revealing for SBP even in hospital admissions not thought related to hepatic disease, *e.g.*, a presentation of weakness with painless ascites[24,28]. Ascitic fluid analysis in all cases should include cell counts and differential, albumin and total protein, and ascitic fluid culture aliquoted at the bedside[28]. Other studies depending upon the clinical situation or appearance of the ascitic fluid can include lactate dehydogenase

(LDH), cytology, amylase, glucose, total protein (TP), and triglycerides[12].

In regards gross appearance, ascitic fluid that is non-neutrocytic nor infected should be clear to yellow and transparent. In normal ascitic fluid the neutrophil count should be < 250 cells/mm^3, wherein the neutrophils are usually presented as a percentage of the total white blood cell (WBC) count. A common misinterpretation is to read this percentage as the absolute number of neutrophils, potentially missing a diagnosis of SBP. An elevated WBC count itself is certainly indicative of inflammation, and usually, but not definitively of infection, *e.g.*, SBP[12,28]. Other molecules such as lactoferrin have been evaluated for utility as sensitive ascitic biomarkers of infection but have yet to yield cost-effective results[30,31]. In the setting of peritoneal dialysis patients, lower thresholds for peritoneal infection have been described[32], *e.g.*, > 50 neutrophils/mm^3. In cases of "bloody taps", a correction factor of subtracting 1 neutrophil for every 250 red blood cells (RBCs) should be implemented when defining the type of ascites. If a milky appearance is observed it could suggest a high triglyceride count (chylous ascites from injured lymphatic ducts) of > 100-200 mg/dL[33]. An elevated ascitic fluid amylase level would be very suggestive for pancreatic ascites, *e.g.*, in the setting of a patient with chronic pancreatitis with pseudocysts, and a history of alcohol abuse.

A basic analysis of ascitic fluid albumin can be instructive when compared to serum albumin as the SAAG (where ≥ 1.1 g/dL defines a high albumin gradient) suggests portal-hypertension origin with 97% sensitivity[11]. Accuracy is decreased if the serum and ascitic fluid albumin are not drawn at the same time, or if the serum albumin is < 1.1 g/dL[12]. Note that one cannot infer that portal hypertension is from cirrhosis, although this may be a common cause, but other causes pre- and post-hepatic (*vida supra*) can also present in this fashion as well[12]. For instance cardiac ascites, a post-hepatic cause, with a SAAG ≥ 1.1 g/dL and an ascitic TP > 2.5 mg/dL, is a reasonable conclusion in the appropriate patient who has a history of heart failure, elevated brain natriuretic peptide, and a dilated IVC[34].

INFECTIOUS ASCITIC FLUID TREATMENT

The interface between the bowel, the intestinal microbiota, and the ascitic fluid is a dynamic one[35,36]. There is a constant translocation of bacteria across the bowel wall; the wall integrity is variable in part due to host genetics, nutritional status and local bacterial interactions. There is usually clearance of these invading bacteria by the immune system after surveillance and capture by neutrophils and macrophages with assisted opsonic molecules, *e.g.*, immunoglobulins or complement[37,38]. The generation of SBP thus likely is a manifestation of (1) bacterial type and burden; (2) gut integrity; (3) volume status; and (4) local and global immune function[37-39]. The symptoms of SBP can range from fevers and abdominal pain to a more subtle change in mental status, *e.g.*, HE, to being totally asymptomatic[12].

Infectious ascitic fluid is analyzed conceptually and practically through cell count/differential and fluid culture and is configured into four categories, the most important being SBP, defined as a neutrophil count > 250 cells/mm^3 and a positive mono-microbial ascitic culture[12,40]. If the cell count is < 250 cells/mm^3 and there is a positive ascitic culture this is defined as non-neutrocytic bacterascites (NNBA), whereas a negative ascitic culture with > 250 cells/mm^3 is culture-negative neutrocytic ascites (CNNA). A neutrophil count > 250 cells/mm^3 in the setting of a positive polymicrobial ascitic culture suggests, usually in the setting of bowel perforation, a secondary bacterial peritonitis. This diagnosis is supported by ascitic TP > 1 g/dL, glucose < 50 mg/dL and LDH > 225 U/L, the so-called Runyon's criteria[41]. In practice, with a positive neutrophil count, while culture results are pending, a provisional diagnosis of SBP will be granted and antimicrobial treatment initiated (*vida infra*). Given appropriate clinical indications NNBA and CNNA are treated in similar fashion to SBP. Secondary bowel peritonitis, beyond the utilization of antibiotics to include anaerobic coverage, will necessitate imaging and intervention for presumed bowel leak and/or perforation.

Standard treatment for SBP involves immediate implementation of third-generation cephalosporin such as *iv* ceftriaxone 1-2 g daily for five days, although oral fluoroquinolones have been utilized with success as well[42,43]. Repeat paracentesis is not needed unless there is clinical indication of failing treatment. Given the risks of renal dysfunction, specifically HRS (*vida infra*), in the setting of alterations in effective circulating volume, *iv* albumin has been utilized to maintain oncotic tone and renal perfusion. Initial studies demonstrated a benefit when *iv* albumin was dosed as 1.5 g/kg on day 1 and 1.0 g/kg on day 3, yielding renal protection and improved mortality[44]. Sub-analysis of these patients, further prompted by the large cost of *iv* albumin, suggested that patients with SBP and blood urea nitrogen (BUN) > 30 mg/dL and total bilirubin (TB) > 4 mg/mL would best benefit[45]. Ideally, one would seek for prevention of SBP as opposed to reactive treatment, and in this regard three groups have shown to benefit from antibiotic prophylaxis. In those (1) with prior SBP, oral norfloxacin 400 mg daily or equivalent indefinitely; (2) patients in the setting of gastrointestinal hemorrhage, to receive *iv* ceftriaxone 1 g daily × 7 d or equivalent; and (3) hospitalized patients with ascitic TP < 1.5 g/dL and serum Na < 130 mmol/L or BUN > 25 mg/dL or serum creatinine (Cr) > 1.2 mg/dL; otherwise TP < 1.5 g/dL with Child-Turcotte-Pugh (CTP) score > 9 and TB > 3 mg/dL, to receive oral ciprofloxacin 500 mg daily or oral trimethoprim-sulfamethoxazole double-strength daily[46-52].

NON-INFECTIOUS ASCITIC FLUID TREATMENT

Insofar as ascites represents a component of ongoing

cirrhotic decompensation, reversible behaviors contributing to the primary process, *e.g.*, alcoholic intake in a patient with alcoholic-induced cirrhosis, or diabetes and hyper-lipidemia in NASH patients, should be controlled[53]. Additionally, external therapy support groups and family involvement may prove crucial in helping the patient maintain sobriety and therapeutic compliance. A diet consisting of 2000 mg/d or less of salt (equivalent to 88 mmol/d of Na) is advocated given the physiologic limits of serum Na processing and secretion through the urine[53-56]. Serum Na governs volume status generally, and thus fluid restriction is not required and is likely not practical. Overloaded states with hyponatremia, even to levels between 110-120 mmol/L are common and well tolerated when approached slowly. Adherence to such a restricted Na diet can be evaluated by measuring 24-h urinary Na, wherein at least 78 mmol/d should be excreted (with water following Na) and resultant weight loss. More practically, a spot urine Na to potassium (K) ratio > 1 in the setting of weight gain also suggests dietary non-adherence[24]. Given the prognosis of ascites as common manifestation of decompensated cirrhosis, and the increased risk for mortality, these patients should be evaluated for OLT, the expedience of which is gauged approximately by their model for end-stage liver disease (MELD) score (*vida infra*)[1,6,12]. Although not absolute, a sobriety period, in the case of alcoholic cirrhosis, of approximately 6 mo is required of these patients as a predictor of compliance. Furthermore, certain medications, such as non-steroidal anti-inflammatory drugs (NSAIDs), ACE inhibitors (ACEIs), and antibiotics such as aminoglycosides, should be avoided in patients with cirrhotic ascites. NSAIDs inhibit prostaglandins (which function to dilate afferent arterioles) whereas ACEIs inhibit ACEs (which activate angiotensin Ⅱ, which functions to constrict efferent arterioles). In either case regulation of glomerular perfusion is diminished, increasing potential for renal injury. Antibiotics such as aminoglycosides can be directly nephrotoxic.

Beyond dietary and behavioral measures, or those who cannot tolerate such restrictions, diuretic therapy provides another method for ascitic fluid control[57,58]. The standard combination includes spironolactone, an aldosterone antagonist, which down-regulates Na channels from the apical surface of the principal cells of the renal cortical collecting ducts; and, furosemide a Na-K-2 chloride (Cl) symport inhibitor in the ascending limb of the loop of Henle of the kidney. Spironolactone has a half-life of approximately 24 h, whereas furosemide has a half-life of approximately 1.5 h. They are utilized in a ratio of 100 mg of spironolactone to 40 mg of furosemide, which in theory provides for robust natriuresis with subsequent flow of water, while maintainingnormokalemia[24,56]. Spironolactone is initiated at 100 mg/d and increased every 5-7 d (in 100 mg steps) to a maximum of 400 mg/d, as needed for response. Furosemide is initiated at a dose of 40 mg/d to be increased at 40 mg/d until a maximum of 160 mg/d is achieved[24].

Patients should undergo frequent clinical and biochemical monitoring particularly during the first month of diuretic treatment. The maximum recommended weight loss during diuretic therapy for ascites should be 0.5 kg/d in patients without edema and 1 kg/d in patients with edema. These diuretics have proven to be an excellent method for slow fluid removal and commensurate weight loss. The goal of long-term treatment is to maintain the patient free of ascites with the lowest dose of diuretics. There are no absolute levels in regards to the degree of renal impairment or hyponatremia for which diuretics should not be initiated. However progressive renal injury with a Cr rise to > 1.5 mg/dL and hyponatremia < 120 mmol/L, respectively are sensible parameters which should elicit caution and tapering or cessation of diuretics. In patients with chronic kidney disease (CKD) or transient alterations in renal function, which are common in these patients, likely higher doses of diuretics will be required. The physician should be weary for diuretic-induced pre-renal acute kidney injury (AKI) or the HRS (*vida infra*). In this setting, there are likely to be frequent episodes for hyperkalemia given the usage of the spironolactone[24]. Additionally, intractable muscle cramps may develop, and thus precipitate a reduction of diuretics[58]. Alternative drugs to spironolactone, usually given the side-effects of gynecomastia and/or sexual dysfunction, or those allergic to the sulfa moiety, may be given amiloride. Amiloride is a direct inhibitor of the apical Na channel in the principal cells of the renal cortical collecting duct[59]. Furosemide can be exchanged for bumetanide, a similar acting diuretic, in those not responding to high doses. It is approximately × 40 more potent than furosemide with a similar side-effect profile[60,61].

More recently, a novel class of compounds has been generated to exploit the pathway of vasopressin[62]. Vasopressin is a naturally occurring compound built in the hypothalamus and stored in the posterior pituitary which is then secreted in response to alterations in blood volume and high serum osmolarity. In such settings it will bind the vasopressin-2 (V2) receptor on the basolateral surface of the principal cells of the renal cortical collecting ducts and through intra-cellular signaling promote the insertion of aquaporin 2 channels in the apical surface to allow for free water entry[62]. This process naturally concentrates urine while expanding total body volume.

In particular one compound, tolvaptan, has been approved for use in volume dysregulated states such as cirrhosis, congestive heart failure and syndrome of inappropriate anti-diuretic hormone[63]. By blocking vasopressin from binding the V2 receptor, a massive aquaresis takes place with correction of the volume state and normalization of serum Na concentration. In patients with a serum Na < 135 mmol/L, tolvaptan is dosed at 15 mg/d in an inpatient setting, and can be up-titrated by 15 mg/d to a maximum of 60 mg/d[63]. Significant improvement in serum Na concentration with tolvaptan, compared to placebo, was observed within 8 h of usage. Given the significant aquaresis, (1) patients should not be hypovolemic; (2)

they should have adequate thirst mechanism and access to fluids; and (3) should have their electrolytes monitored closely to prevent overly rapid correction, which in acute settings can lead to osmotic demyelination syndrome[64-66].

AUGMENTED MEDICAL MANAGEMENT OF ASCITES

While diuretics provide excellent maintenance of volume status in decompensated cirrhotics, rapid treatment for ascites, especially tense ascites (grade Ⅲ), is best through a therapeutic large-volume paracentesis (LVP)[12,24,67]. LVP can be performed all at once, wherein a catheter is temporarily placed and removed, or with an indwelling peritoneal drain for up to three days to slowly remove ascitic fluid over that time. Notably, the peritoneal drain method of LVP is not associated with increased frequency of SBP[68]. An initial LVP whether in an outpatient or inpatient setting, should be sent for ascitic fluid cell count/differential and cell culture to assess for SBP. Up to 15% of LVP may be associated with paracentesis induced circulatory dysfunction (PICD), which is characterized by an activation of the RAAS due to true or perceived volume dysregulation: (1) arterial underfilling and unloading of high-pressure baroreceptors; (2) stimulation of non-osmotic hypersecretion of vasopressin; (3) free water retention and dilutional hyponatremia; and (4) associated renal dysfunction[69]. Given these concerns, *iv* albumin replacement (8.5 g/kg for each liter of ascitic fluid removed) is indicated in cases where more than 5 L of ascitic fluid is removed[70-72]. Albumin, the most abundant circulating protein in the plasma, is endowed with an array of non-oncotic effects as well, including functioning as an anti-oxidant, anti-inflammatory and positive inotrope[73].

Despite such success, given the risks inherent in the use of *iv* albumin, as a blood product and its cost, other modalities have been attempted. Terlipressin, with a half-life of 6 h, is a vasopressin analog with selectivity for the V1 receptors on vascular smooth muscle cells, which induces vasoconstriction. In theory the maintenance of vascular tone through terlipressin should reduce, at least in part, some factors that generate PICD[74]. A notable study suggested that in cirrhotic patients with tense ascites who were assigned to receive standard *iv* albumin replacement or terlipressin (total 3 mg *iv*) after therapeutic paracentesis, both were effective in reducing manifestations of PICD. There were no significant differences in arterial blood volume (as measured by plasma renin and aldosterone levels) nor in renal impairment or hyponatremia between either group[75].

Following the LVP, patients should receive the minimum dose of diuretics necessary to prevent re-accumulation of the ascites. A small population of ascites patients may be defined as having refractory ascites: ascites which cannot be adequately controlled through dietary, pharmacologic or LVP modalities[74]. Furthermore, a subgroup of patients maybe intolerant to augmented medical management given symptomatic or biochemical side-effects, and thus classified as diuretic-intractable ascites[74,76,77]. Or, a sub-group of patients may retain significant ascites despite optimized and maximal therapy and thus are classified as diuretic-resistant ascites. These groups of patients may require serial LVP, in some cases up to twice per month, which can be time-consuming, costly and increase the risk for iatrogenic infections. There have been smaller studies examining the role of other pharmacologic modalities in refractory ascites, such as midodrine, an alpha-1 agonist upon arterial and venous vessels, inducing increased vascular tone. Midodrine has been shown to be as effective as *iv* albumin in preventing PICD in such patients with refractory ascites, with minimal side-effects and high cost-efficiency[78]. Compare this to terlipressin, which showed similar outcomes in such patients (*vida supra*)[75]. Note however that in the latter case, terlipressin must be given through intravenous, and it is currently not available in the United States. Interestingly, non-selective beta-blockers, which have shown benefit in cirrhotic patients in preventing variceal hemorrhage, are associated with increased mortality, 4 × higher compared to those not on beta-blockers, when observed specifically in those patients with refractory ascites[79]. It is postulated that these beta-blockers may be inhibiting compensatory cardiac output (*via* a negative inotropic effect) and thus pre-disposing to PICD. Further is the interesting finding that in these patients the CTP score, which includes an ascites parameter, is better at predicting mortality than the MELD score. These results require further validation, but may indicate that in the fraction of patients with refractory ascites, beta-blockers should be contra-indicated.

The prognosis of patients with refractory ascites is very poor, and if eligible, should be referred for OLT and/or transjugular intra-hepatic portosystemic shunt (TIPS) as bridge to OLT[80-85]. TIPS is a procedure that has been evolving since the 1980s[86] and relies on the principle of establishing direct continuity (low-resistance) from a large portal branch to a hepatic vein by way of a shunting stent. This stent bypasses the cirrhotic (high-resistance) parenchymal tissue which had generated the portal hypertension and resultant ascites[83]. Recall the portal hypertension develops in the setting of a HWPG of 6 mmHg or greater, and that at 8 mmHg ascites develops, and at 10-12 mmHg varices develop with increased risk of hemorrhage. TIPS is a quite common procedure and not technically demanding with current radiologic techniques. Procedural complications such as failed TIPS deployment and endotipsitis are rare. Concern for TIPS stent thrombosis post-procedurally is minimal in the era of covered stents[83,87,88]. Its strongest indications are in those with refractory ascites and/or recurrent variceal hemorrhage[83]. Overall TIPS has shown benefit in the decreased requirement for diuretics, improved quality of life, and likely a trend towards improved mortality when compared to repetitive paracentesis in patients with refractory ascites[82-85,87-92]. In the MELD era, a score of 14 or less suggests a good candidate for TIPS procedure, a score of 24 or greater, suggests that OLT is more benefi-

cial, and a score in-between requires individual consideration of a risk/benefit analysis to the patient[83,93].

Whether TIPS is ultimately cost-effective, in which most of the cost is up-front at the time of procedure, compared to LVP, where cost is aggregated over time, is still an open question and likely institution dependant. Total TIPS cost have gone down given the decreased requirement for revision in the era of covered stents. Given the physiologic mechanism by which TIPS operates, certain concerns naturally arise: TIPS is contra-indicated in patients with (1) significant right heart failure or pulmonary hypertension as it will place rapid unduevolume burden upon these organs; (2) patients with recurrent HE, as it will not allow for as much detoxification and regulation of the culprit amines; (3) polycystic liver disease or a liver containing malignancy or abscess; (4) active infection; and (5) severe renal disease, given rapid alterations in vascular volume distribution[83,94,95].

There is a small group of patients with refractory ascites, who for a variety of reasons cannot undergo TIPS or OLT, and for whom serial paracentesis has resulted in too much distress or protein losses. In many cases these represent patients who also have peritoneal malignant implants[96-99]. For these scenarios, a peritoneal-venous shunt (PVS) was envisioned, conserving and directing fluid and protein from the peritoneum into the superior vena cava (SVC). There are two types, the LaVeen and the Denver, both one-way valve stents, which empty into the SVC based upon different opening pressures[96]. Contra-indications include loculated ascites, coagulation disorders, and advanced cardiac or renal failure; hemorrhagic ascites and high ascetic TP can cause drain occlusion. Interestingly, in malignant ascites, limited studies have not demonstrated increased systemic metastasis facilitated by stent transfer into the circulatory system. Overall these shunts have not prolonged survival in these patient populations, nor those with HRS[97]. Shunt patency is poor, with < 20% at 2 years. Furthermore SBP and/or sepsis require PVS removal[97]. In general, PVS should be considered as sub-optimal therapy, after standard therapies of diuretics, LVP and TIPS have failed or are contra-indicated[12,83,97].

PULMONARY COMPLICATIONS

HHT is an accumulation of ascitic fluid within the pleural space that occurs in approximately 10% of cirrhotics. In about 85% of these patients it is right-sided, and in others it can be bilateral or even left-sided alone[100]. The etiology is thought to be from the combination of both hemostatic pressure from the ascites pushing through diaphragmatic defects or rents in combination with the "pull" of the negative intra-thoracic pressure[101]. In some cases this combination can effectively drain the peritoneal cavity such that one may have HHT in the absence of a distended abdomen. Normally the pleural space is a potential one, wherein pleural fluid volume is approximately < 25 mL per lung, providing a low frictional interface between the parietal and visceral pleurae. The normal pleural fluid is generated from the parietal pleura, and to a lesser extent the visceral pleura, and reabsorbed by pleural lymphatics. In the setting chronic disease, lymphatic absorption can increase to > 20 × normal baseline rates[102].

Cirrhotic patients who develop a significant amount HHT (approximately after 1 L) tend to have symptoms of shortness of breath and cough. The accumulation of this fluid can lead to hypoexemia, atelectasis, pneumonia and empyema[99,101]. Initial evaluation can include a lateral and posteroanterior chest X-ray, which will show blunting at approximately 50 and 200 mL, respectively. A CT scan of the chest can also be considered to assess for other causes of these symptoms and signs. Initial management should involve a thoracentesis for both diagnostic and therapeutic purposes[99,101]. Similar to a paracentesis, the most useful testing will be to examine the fluid for cell count/differential, cell culture, albumin and TP, with results that should be similar to classical pleural effusions defined as a transudate rather than exudate by Light's criteria[103]. Infected pleural fluid, *i.e.*, spontaneous bacterial empyema (SBE), should always be of concern, and it has been identified in cases where the ascites did not have SBP and in even cases without any ascites[104]. SBE is diagnosed by a positive culture (usually *Escheria*, *Streptococcus* or *Enterococcus*) or a neutrophil count of > 250 cell/mm^3. Standard treatment includes a third-generation cephalosporin or equivalent antibiotic[104]. Chest tubes should not be attempted given the high risk of procedural complications, *e.g.*, abdominal penetration, bleeding, and infection. There is also justified concern for the chronic loss of pleural fluid protein and serum electrolyte abnormalities[105].

Beyond the initial evaluation with thoracentesis, standard measures of dietary restriction and diuretic therapy should be continued[83]. In cases of persistent HHT that have failed these therapies, TIPS has been attempted under the same principles for treatment of refractory ascites in select patients[106,107]. Another procedure is pleurodesis, a process in which an agent such as tetracycline or talc are introduced into the pleural space after which a robust inflammatory reaction occurs that results in visceral to parietal pleural wall fusion[108]. Unfortunately, in most cirrhotic patients, the flow of ascitic fluid entry across the diaphragm and into the pleural space is so high that there is rarely enough time for the pleurae to maintain good approximation for durable fusion. It should only be considered in those patients who have failed first line therapies, and are ineligible for TIPS or OLT[83].

RENAL COMPLICATIONS

Renal injury, encompassing a spectrum from acute to chronic causes, is very common in decompensated cirrhotic patients given the significant alterations in volume and hormonal regulation, vascular tone, immune function and related infections, and the utilization of numerous medications and contrast-assisted procedures[109-111]. Classically AKI is segregated into pre-renal, renal and post-renal causes, and with severe or sustained insult

this can lead CKD with the possible utilization of renal replacement therapy (RRT) in either case. For instance, pre-renal causes might include hypovolemia or renal artery thrombosis; renal (intrinsic) causes might include toxicity from infection, malignancy or medications and *iv* contrast; post-renal causes might include ureteral stone obstruction or extrinsic ureteral compression by a pelvic malignancy[109,111-114].

The HRS should be considered in all cirrhotic patients who develop pure AKI or AKI within a CKD setting[111,115]. HRS, as with any AKI, should be considered when a rise in serum Cr × 1.5 baseline and decrease in urine output are observed in the setting of cirrhosis, and confounding causes for pre-renal, renal and post-renal mechanisms have been reasonably excluded[109,115]. HRS occurs in approximately 30% of patients with SBP treated with antibiotics and is associated with a poor survival[12]. The exact etiology of HRS is unknown, but does involve (1) RAAS dysregulation with avid fluid retention (*vida supra*); (2) splanchnic vessel dilation and a local vasoconstrictive effect at the level of the nephron driven by renin, angiotensin Ⅱ and other vasoconstrictors; and (3) altered cardiac function[115]. The renal JGA continually perceives an effectively low circulating volume and thus continuously activates these volume retaining and vasoconstrive mechanisms.

The HRS is classified into two distinct subtypes: type 1 HRS is characterized by a rapid and progressive impairment in renal function (increase in serum Cr to ⩾ 2.5 mg/dL or a reduction in the Cr clearance (CrCl) to < 20 mL/min in less than two weeks; type 2 HRS is characterized by a slowly progressive impairment of renal function manifested by an increase in serum Cr to ⩾ 1.5 mg/dL or a CrCl to < 40 mL/min[111,115]. Survival in these patients is rather poor, with 50% mortality at less than one month for type 1 HRS and 50% mortality at 6 mo for type 2 HRS[74,116]. Given the complex intrinsic nature of the HRS, it is not surprising that it is defined by negation, *i.e.*, by that which it is not. The criteria for HRS have been evolving and currently include following criteria: (1) rise in serum Cr to > 1.5 mg/dL; (2) the absence of hypovolemic shock (defined by the withdrawl of diuretics and the failure of serum Cr to fall below 1.5 mg/dL in the setting of at least 1 L of saline or standard albumin fluid bolus); (3) the absence of nephrotoxic medications or recent *iv* contrast; and (4) the absence of intrinsic renal disease as assessed by renal ultrasound and proteinuria < 0.5 g/d and microhematuria < 50 RBCs/high powered field[12,74,111,115,116]. Note that sepsis is not part of the exclusion criteria; HRS is commonly precipitated by SBP in many instances, hence the rationale of antibiotic treatments (*vida supra*).

As the diagnosis of HRS can herald significant morbidity and mortality in cirrhotic patients OLT should be considered as definitive therapy, if they are eligible. Diuretics should be discontinued, and high grade ascites should be reduced with paracentesis as large peritoneal pressures can compress renal arteries (abdominal compartment syndrome), further worsening the renal insult[74]. Meanwhile, medical therapies may be considered as a temporizing measure, and work towards maintaining effective arterial perfusion of the kidneys. Terlipressin, an analog of vasopressin, has been much researched in the HRS, either in comparison to placebo, or in combination with *iv* albumin versus placebo, or in comparison to noradrenaline (norepinephrine), a classical vasoactive alpha adrenergic agonist[116-119]. Studies have supported the benefit of terlipressin in reversing HRS when given for at least 14 d, and which typically yield low relapse rates[116,117]. Further, although more limited, there has been data demonstrating reversal of HRS with noradrenaline similar to terlipressin[119]. Notably, in a few studies when terlipressin was administered with *iv* albumin there was reversal of HRS and improvement in mortality[120], although its value in septic patients is unknown. Terlipressin is usually dosed at 1 mg/6 h, and can be increased to 2 mg/6 h if no improvement in serum Cr is observed.

Similarly, midodrine, a vasoactive alpha adrenergic agonist with a half-life of approximately 4 h, has also demonstrated HRS benefit, and can be dosed at 10 mg three times per day (*tid*) and increased to 15 mg *tid*. Complementarily, octreotide (an inhibitor of splanchnic vasodilators, with a half-life of 1.7 h) is dosed at 100 mcg subcutaneously tid and up to 200 mg *tid* can be utilized. A therapeutic cocktail of these vasoactive agents, *e.g.*, midodrine or terlipressin, and octreotide, with the utilization of *iv* albumin dosed at up to 40 g per day in divided doses have demonstrated benefit in HRS[121-124]. It is preferable to use highly concentrated albumin, *e.g.*, 25% *vs* 5% albumin, given the reduced volume of solution and decreased third-spacing burden upon the patient. Successful treatment will manifest as a decrease in serum Cr, ideally by at least 1 mg/dL and an increase in urine output. If the serum Cr decreases to ⩽ 1.5 mg/dL, diuretics can be restarted at half the prior dosing with subsequent careful monitoring of volume status and serum Cr. Certainly, as with other forms of AKI, these patients should be carefully monitored: vital signs, mental status, urine output, electrolyte abnormalities, and overall for uremic signs, which would require emergent use of RRT such as hemodialysis[125].

Serum Cr is utilized as a practical, albeit imperfect marker, for renal function in the clinical setting. By extension, renal function has itself become a proxy for systemic health, and the importance of this fact is reflected in the integration of the serum Cr into the MELD score[126,127]. The MELD score is comprised of the serum TB, serum Cr and INR, yielding an integer score from 6 to 40 which predicts 90-d mortality in non-transplanted cirrhotic patients[128]. The MELD score has been more successful than prior risk stratification methods in prognosticating mortality and equitably distributing organs for appropriately eligible patients[129-131]. However, in its elegant simplicity, it unsurprisingly does not capture the total biology of cirrhosis. Thus certain modifications have been appended, in the form of exception points, notably to those who are on RRT or with low-staged hepatocellular carcinoma, amongst others[132].

As stated, OLT represents the definitive therapy for HRS types 1 and 2, and that while it may "cure" the HRS, it will still leave behind the residual CKD in many patients. Furthermore, as a result of the surgery itself (with significant volume shifts), and afterwards by the lifetime use of potentially nephrotoxic immunosuppressants and baseline co-morbidities, renal function can be expected to decline further, even necessitating RRT in some instances. Such consequences themselves herald significant morbidity and mortality for these transplanted patients. Given these concerns, simultaneous liver-kidney transplant (SLKT) has become prevalent in the MELD era with the following facts noted: (1) inconsistent eligibility criteria for SLKT that varies by transplant center; (2) there has not been consistent benefit to morbidity and mortality for these patients, as had been hoped; and (3) eligible kidneys are removed out of the pool for solitary kidney transplant recipients[132,133]. These problems represent an area of active research, with more formal guidance in development[133].

CONCLUSION

Ascites is a pathologic accumulation of fluid within the peritoneal cavity that is most commonly found incirrhotic patients, and its presence heralds significant morbidity and mortality[1,6,10]. The generation of cirrhotic ascites is multi-factorial, but is found in the setting of portal hypertension, and in essence is driven by global abnormalities in hormonal/cytokine regulation and effective vascular status, in a feed-forward cycle[17-19]. Ascites is problematic on many levels: directly, by causing symptoms of abdominal discomfort and early satiation[12]; and indirectly, by facilitating significant complications of infections and multi-organ dysfunction such as SBP, HHT and HRS[12,38,41,99,115]. The identification of ascites, once suspected, is easily determined through physical exam and imaging[23]. Diagnostic paracentesis is an integral procedure in determining the etiology of ascites and further delineating any associated infection or malignancy[24,28]. Ascites can be managed successfully by aggressive salt restriction and utilization of a diuretic regimen in most patients, however in some instances LVP or even TIPS may be required[54,56,67,83]. Given the complexity and prognosis associated with ascites, a multi-disciplinary approach is required, with work-up for OLT initiated in eligible patients[133]. The biomedical advances in understanding and treating ascites and its complications have been impressive, but nevertheless much work remains in optimizing patient care and patient outcomes.

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REVIEW

Transient elastography: Kill two birds with one stone?

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Abstract

Assessment of liver fibrosis and steatosis is crucial in chronic liver diseases in order to determine the prognosis, the need of treatment, as well as monitor disease progression and response to treatment. Liver biopsy is limited by its invasiveness and patient acceptability. Transient elastography (TE, Fibroscan®) is a non-invasive tool with satisfactory accuracy and reproducibility to estimate liver fibrosis and steatosis. TE has been well validated in major liver diseases including chronic hepatitis B and C, non-alcoholic fatty liver disease, alcoholic liver disease, primary biliary cirrhosis, and primary sclerosing cholangitis. As alanine aminotransferase (ALT) is one of the major confounding factors of liver stiffness in chronic hepatitis B, an ALT-based algorithm has been developed and higher liver stiffness measurements (LSM) cutoff values for different stages of liver fibrosis should be used in patients with elevated ALT levels up to 5 times of the upper limit of normal. Otherwise falsely-high LSM results up to cirrhotic range may occur during ALT flare. TE is also useful in predicting patient prognosis such as development of hepatocellular carcinoma (HCC), portal hypertension, post-operative complications in HCC patients, and also survival. Unfortunately, failed acquisition of TE is common in obese patients. Furthermore, obese patients may have higher LSM results even in the same stage of liver fibrosis. The new XL probe, a larger probe with lower ultrasound frequency and deeper penetration, increases the success rate of TE in obese patients. The median LSM value with XL probe was found to be lower than that by the conventional M probe, hence cutoff values approximately 1.2 to 1.3 kPa lower than those of M probe should be adopted. Recent studies revealed a novel ultrasonic controlled attenuation parameter (CAP) of the machine is a useful parameter to detect even low-grade steatosis noninvasively. CAP may also be used to quantify liver steatosis by applying different cutoff values. As both LSM and CAP results are instantly available at same measurement, this makes TE a very convenient tool to assess any patients who are suspected or confirmed to suffer from chronic liver diseases.

Key words: Biopsy; Cirrhosis; Fibrosis; Hepatitis; Fatty liver; Steatosis of liver

Core tip: Transient elastography (TE, Fibroscan®) is a non-invasive tool with satisfactory accuracy to estimate liver fibrosis and steatosis. Liver stiffness measurement (LSM) with TE has been well validated to detect advanced fibrosis in most liver diseases. LSM is useful in predicting hepatocellular carcinoma (HCC), portal hypertension, post-operative complications in HCC patients, and survival. The new XL probe increases the success rate of TE in obese patients. A novel ultrasonic controlled attenuation parameter (CAP) of the machine is useful to detect steatosis noninvasively. Simultaneous LSM and CAP results make TE very convenient to assess any patients with suspected or confirmed liver diseases.

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INTRODUCTION

Liver fibrosis is the natural wound-healing response to parenchymal injury in chronic liver diseases. Simple steatosis is a usually reversible and benign condition, but steatosis may be part of the more sinister condition in the setting of steatohepatitis where inflammation and hepatocyte changes co-exist[1-3]. Both liver fibrosis and steatohepatitis may eventually result in liver cirrhosis and its various complications. Sensitive detection and accurate staging of liver fibrosis and steatosis is now essentially indispensable in the decision process of treatment in chronic viral hepatitis as well as predicting disease prognosis[4,5]. It is also vital to monitor disease progression and response to treatment.

LIVER BIOPSY: DRAWBACK OF THIS "GOLD STANDARD"

Liver biopsy has been the "gold standard" for assessing liver fibrosis and steatosis in the last few decades[6,7]. However, it has numerous limitations namely its invasive nature, risk of complications, patient discomfort, and sampling errors[8,9]. Complications associated with liver biopsy are rare but can be severe and even life threatening. Pain and hypotension are the predominant complications for which patients are hospitalized[10]. Clinically significant intraperitoneal hemorrhage is the rarest but most serious bleeding complication of percutaneous liver biopsy, which may happen more often in older age patients with cirrhosis or liver cancer[11]. Inadvertent puncture of gallbladder may lead to choleperitoneum[12]. The mortality rate among patients after percutaneous liver biopsy is approximately 1 in 10000 to 1 in 12000[13]. All these problems make it impractical to perform serial biopsies to assess disease progression in routine clinical practice[5]. The cost of liver biopsy is generally high as an in-patient bed, at least as day admission to hospital, is required. Including the charges of specialist doctor, nursing care and histologic examinations, it usually costs at the range of USD $800 to USD $1200 in Hong Kong.

The diagnostic accuracy of liver biopsy is limited by the sampling variability[14-16]. The average size of biopsy is 15 mm in length, which represents 1/50000 the size of the entire liver. There is significant variability in the histologic assessment of two readings of the same biopsy by the same pathologist, and between two pathologists, even among those who are highly specialized[8]. This variability is low for the diagnosis of cirrhosis (kappa coefficient of concordance $\geqslant$ 0.80), moderate for earlier fibrosis stages (kappa 0.70-0.80), but high for the activity grades (kappa 0.40-0.50)[8]. Fortunately, the variability is usually low for the diagnosis of steatosis (kappa coefficient of concordance $\geqslant$ 0.80)[4]. Given the above limitations to the practice of liver biopsy, a noninvasive transient elastography has been proposed as an alternative tool.

TRANSIENT ELASTOGRAPHY

Working principles of liver stiffness measurement

Transient elastography (TE; Fibroscan®; Echosens, Paris, France) measures liver stiffness[17] in patients suffering from different chronic liver diseases[18]. An ultrasound transducer probe is mounted on the axis of a vibrator. Vibrations of mild amplitude and low frequency (50 Hz) are transmitted by the transducer, inducing a plastic shear wave that propagates through the underlying tissues. Pulse-echo ultrasound acquisition is used to follow the propagation of the shear wave and to measure its velocity, which is directly related to tissue stiffness. The stiffer the tissue, the faster the shear wave propagates. TE measures liver stiffness in a volume that approximates a cylinder 1 cm in diameter and 4 cm in length, between 25 to 65 mm underneath the skin surface. This volume is at least 100 times bigger than a biopsy sample, and therefore should be more representative of the liver parenchyma[17]. Results of liver stiffness measurement (LSM) are expressed in kPa and correspond to the median of 10 validated measurements according to Sandrin *et al*[17]. According to the manufacturer, the examination is considered reliable if $\geqslant$ 10 valid measurements are acquired, the success rate (number of valid acquisitions divided by the number of attempts) is over 60%, and the ratio of the interquartile range to the median of 10 measurements (IQR/M) is $\leqslant$ 0.3[17].

Working principles of controlled attenuation parameter

It is important to assess liver steatosis, not only because non-alcoholic fatty liver disease (NAFLD) is the commonest liver disease[19], but also that steatosis often coexists in other chronic liver diseases likely chronic hepatitis C (CHC)[20]. A new physical parameter based on the properties of ultrasonic signals acquired by the machine has been recently developed to assess liver steatosis by applying the property that liver steatosis affects ultrasound propagation[21]. This controlled attenuation parameter (CAP), is measuring ultrasound attenuation (go and return path) at 3.5 MHz using signals acquired by the M probe of TE machine. Ultrasound attenuation is a physical property of the medium of propagation which corresponds to the loss of energy as ultrasound travels through the medium. Due to attenuation, the intensity of the emitted ultrasound decreases exponentially with depth[21]. At a given frequency, the ultrasound-attenuation coefficient (α) can be expressed in dB/m. The CAP is measured only on validated measurements according to the same criteria used for LSM, and on the same signals. This ensures that the operator obtains a liver ultrasonic attenuation simultaneously and in the same volume of liver parenchyma as the LSM. The final CAP value was the median of individual CAP values using the same valid measurements[22].

Practical issues

TE has the advantages of being painless, rapid (usually

Table 1 Diagnostic performance and suggested cutoff values of liver stiffness measurement for the diagnosis of histologic cirrhosis (F4)

Ref.	Biopsies (*n*)	Prevalence of cirrhosis (F4)	Etiologies	Proposed cutoff values (kPa)	Sensitivity	Specificity	NPV	PPV	Positive LR	Negative LR	AUROC
Castéra *et al*[4], 2005	183	25%	HCV	12.5	87%	91%	95%	77%	9.7	0.1	0.95
Fraquelli *et al*[23], 2007	200	12%	All	11.9	91%	89%	98%	53%	8.3	0.1	0.9
Arena *et al*[24], 2008	150	19.3%	HCV	14.8	94%	92%	98%	73%	11.3	0.07	0.99
Ziol *et al*[25], 2005	251	19%	HCV	14.6	86%	96%	97%	78%	23.1	0.1	0.97
Chan *et al*[26], 2009	161	25%	HBV	13.4	60%	93%	88%	75%	85	0.43	0.93
Marcellin *et al*[27], 2009	173	8%	HBV	11	93%	87%	99%	38%	7	0.08	0.93
Wong *et al*[28], 2010[1]	238	23.5%	HBV	9.0 (normal ALT) 12.0 (elevated ALT)	54%	99%	67%	98%	3.3	0.7	0.88
de Lédinghen *et al*[29], 2006	72	23.6%	HCV-HIV	11.8	100%	92.7%	82%	100%	13.7	0	0.97
Nobili *et al*[30], 2008[1]	52	5.8%	NAFLD	10.2	100%	100%	100%	100%	∞	0	1
Wong *et al*[31], 2010	246	10.1%	NAFLD	10.3	92%	88%	99%	46%	7.5	0.09	0.95
Nahon *et al*[32], 2008	174	53.7%	ALD	22.7	84%	83%	82%	85%	5.24	0.19	0.87
Corpechot *et al*[33], 2006	95 (66 PBC, 29 PSC)	16%	PBC/PSC	17.3	93%	95%	99%	78%	18.6	0.1	0.96
Carrión *et al*[34], 2006	124	11%	HCV-LT	12.5	100%	87%	100%	50%	7.7	0	0.98
Witters *et al*[36], 2009	66	NA	Cystic fibrosis	6.5	100%	81%	NA	NA	NA	NA	0.92
Coco *et al*[75], 2007	228	20.2%	HCV/HBV	14	78%	98%	82%	98%	39	0.2	0.96
Ganne-Carrié *et al*[106], 2006	775	15.5%	All	14.6	79%	95%	96%	74%	15.8	0.1	0.95
Foucher *et al*[107], 2006	354	13.3%	All	17.6	77%	97%	92%	91%	25.7	0.2	0.96
Gómez-Domínguez *et al*[108], 2006	94	17%	All	16	89%	96%	98%	80%	22.3	0.1	0.94
Vergara *et al*[109], 2007	169	38.5%	HCV-HIV	14.6	93%	88%	94%	86%	7.8	0.1	0.95
Rigamonti *et al*[110], 2008	95	17%	HCV-LT	12	93%	93%	99%	74%	14	0.1	0.9
Yoneda *et al*[111], 2007	67	7.5%	NAFLD	17	100%	98%	95%	64%	50	0	0.99

[1]Cut-off values proposed for advanced fibrosis (F3 or above). ALD: Alcoholic liver disease; ALT: Alanine aminotransferase; AUROC: Area under receiver operating characteristics curves; HBV: Hepatitis B virus infection; HCV: Hepatitis C virus infection; HCV-HIV: Hepatitis B virus and human immunodeficiency virus co-infection; HCV-LT: Hepatitis C virus infection recurrence after liver transplantation; LR: Likelihood ratio; NAFLD: Non-alcoholic fatty liver disease; PBC: Primary biliary cirrhosis; PSC: Primary sclerosing cholangitis; NPV: Negative predictive value; PPV: Positive predictive value; NA: Not available..

less than 5 min) and easy to perform at the bedside or in the outpatient clinic. The examination is performed on a non-fasting patient lying supine with the right arm placed behind the head to facilitate access to the right upper quadrant of the abdomen. The tip of the probe transducer is placed on the skin between the rib bones at the level of the right lobe of the liver where liver biopsy would be performed. Once the measurement area has been located, the operator presses the button on the probe to start an acquisition. The software determines whether each measurement is successful or not. The cost of a TE examination ranges from USD $100 to USD $150 in Hong Kong, which is much lower than a liver biopsy examination. It is obvious that TE is a user- and patient-friendly, but it would be even more important to be an accurate tool to assess liver fibrosis and steatosis.

ACCURACY OF TE

Liver stiffness measurement

Reproducibility of TE is an important feature for its widespread clinical application. The reproducibility of LSM was excellent for both inter-observer and intra-observer agreement, with intraclass correlation coefficients (ICC) of 0.98[23]. However, interobserver agreement was significantly reduced in patients with lower degrees of liver fibrosis (ICC for F0-1 and F2 were 0.60 and 0.99 respectively), with liver steatosis (ICC for steatosis < 25% and 25% of hepatocytes 0.98 and 0.90 respectively), and with increased body mass index (ICC for body mass index $\geqslant$ 25 kg/m^2 and < 25 kg/m^2 were 0.98 and 0.94 respectively).

Using TE to assess liver fibrosis has been widely validated in different liver diseases, including CHC[4,24,25], chronic hepatitis B (CHB)[26-28], co-infection with HIV[29], NAFLD[30,31], alcoholic liver disease[32], primary biliary cirrhosis, primary sclerosing cholangitis (PSC)[33], post-liver transplantation setting[34], and in cystic fibrosis[35,36]. In these studies, TE was valid with liver histology being the gold standard. In general, all these studies confirm that TE has good overall accuracy to diagnose advanced fibrosis and cirrhosis (though some uncommon diseases like PSC and cystic fibrosis are under-represented by small numbers of patients), independent of the underlying etiology[37,38]. The remaining controversy is the optimal cutoff values to diagnose advanced fibrosis and cirrhosis, which differ according to particular etiologies. This has significant implication when a clinician interprets TE results. The suggested diagnostic performance and cutoff values for histologic cirrhosis (F4) based on published studies are summarized in Table 1.

Controlled attenuation parameter

In a retrospective study of 115 patients of mixed etiologies of chronic liver diseases, CAP was found efficient to detect low grade steatosis (> 10%), with a sensitivity of

Table 2 Diagnostic performance and suggested cutoff values of controlled attenuation parameter for the diagnosis of liver steatosis

Ref.	Biopsies (*n*)	Study design	Etiologies	AUROC ⩾ S1 (11%)	AUROC ⩾ S2 (34%)	AUROC ⩾ S3 (67%)	Cutoff values for ⩾ S1 (dB/m)	Sen	Spe	NPV	PPV	Cutoff values for ⩾ S2 (dB/m)	Sen	Spe	NPV	PPV	Cutoff values for ⩾ S3 (dB/m)	Sen	Spe	NPV	PPV
Sasso *et al*[21], 2010	115	Retrospective	All	0.91	0.95	0.89	238	91%	81%	87%	87%	259	89%	86%	92%	80%	292	100%	78%	100%	28%
de Lédinghen *et al*[39], 2012	112	Prospective	All	0.84	0.86	0.93	215					252					296				
Myers *et al*[40], 2012	153	Prospective	All	0.81			283	76%	79%	64%	87%										
Beaugrand *et al*[41], 2010	74	Retrospective	ALD	0.81	0.87	0.82															
Beaugrand *et al*[42], 2010	96	Retrospective	ALD/ NAFLD	0.86	0.87	0.77															
Cardoso *et al*[43], 2010	133	Retrospective	CHB	0.82	0.81	-															
Sasso *et al*[44], 2012	615	Retrospective	CHC	0.8	0.86	0.88															

ALD: Alcoholic liver disease; AUROC: Area under receiver operating characteristics curves; NPV: Negative predictive value; PPV: Positive predictive value; Sen: Sensitivity; Spe: Specificity; NAFLD: Non-alcoholic fatty liver disease; CHB: Chronic hepatitis B; CHC: Chronic hepatitis C.

91% and specificity of 81% at a cutoff value of 238 dB/m[21]. The accuracy of CAP was confirmed in two prospective studies of mixed etiologies[39,40], as well as in individual etiology, including CHB, CHC, NAFLD and alcoholic liver disease[41-44]. The suggested diagnostic performance and cutoff values for different degrees of steatosis are summarized in Table 2. As a well-validated tool, TE is also well-investigated in different aspects of clinical applications.

CLINICAL APPLICATIONS OF TE

Pre-treatment assessment of liver fibrosis

The severity of liver fibrosis is the key factor of timing and choice of therapy. This is particularly relevant in chronic viral hepatitis. Current international guidelines recommend antiviral therapy for CHB patients with significant liver fibrosis[45-47]. As TE has been repeatedly shown to have satisfactory accuracy to exclude and diagnose advanced fibrosis and cirrhosis as mentioned above, more than half of the patients might reach treatment decision without the need for confirmatory liver biopsies[26]. TE is also found to be more cost-effective than liver biopsy[48]. TE has been incorporated in the international guidelines of CHB and CHC[45,46]. TE, together with other non-invasive parameters, can also be used as the screening tool for cirrhosis in asymptomatic people[49-51], as well as the diagnostic and/or prognostic tool of NAFLD, such that the need of liver biopsy can be reduced[52,53].

Follow-up assessment of liver fibrosis

A few longitudinal studies have been reported that patients responding to treatment had low or decreased liver stiffness[54]. In fact, both reduction in fibrosis and necroinflammation might contribute to the decrease in liver stiffness[55]. In a prospective study of 71 CHB patients on antiviral therapy, paired liver biopsy and TE were both performed at baseline and at 1 year of treatment[56]. Although TE remained accurate in distinguishing patients with insignificant disease from those with advanced fibrosis or cirrhosis at both time points, the absolute change in liver stiffness correlated poorly with the change in histological fibrosis stage, and resolution of advanced fibrosis could only be assumed with significantly decreased liver stiffness to 5.0 kPa or less after antiviral treatment[56].

Predict portal hypertension and variceal bleeding

TE is found useful to identify cirrhotic patients with higher risk of portal hypertension, and cutoff values of 17.6 kPa and 21.0 kPa having sensitivity ⩾ 90% in order to detect patients with hepatic venous pressure gradient (HVPG) above 10-12 mmHg[57,58]. Presence of varices could be excluded with a liver stiffness below 12.5-19.8 kPa[59,60]. Unfortunately, these suggested cutoff values overlap with those for detecting histologic cirrhosis in most chronic liver diseases. Hence there seems no significant new information pro-

Table 3 Liver stiffness measurement and the risk of hepatocellular carcinoma in chronic hepatitis B or C patients

Chronic hepatitis B patients		Chronic hepatitis C patients	
LSM (kPa)	HR of HCC	LSM (kPa)	HR of HCC
≤ 10.0	Referent	≤ 8.0	Referent
10.1-15.0	17	8.1-13.0	3.1
15.1-20.0	21	13.1-18.0	4.7
20.1-25.0	26	18.1-23.0	5.6
> 25.0	46	> 23.0	6.6

LSM: Liver stiffness measurement; HCC: Hepatocellular carcinoma.

vided by TE regarding screening endoscopy for varices among cirrhotic patients.

Predict hepatocellular carcinoma

TE is also useful to predict the risk other liver-related complications and death. A dose-response relationship between LSM and risk of hepatocellular carcinoma (HCC) was found in both CHB and CHC patients (Table 3). Taking patients with LSM ≤ 10.0 kPa as reference, the hazard ratios of developing HCC were 17, 21, 26, and 46 in patients with LSM at 10.1-15.0, 15.1-20.0, 20.1-25.0 and above 25.0 kPa respectively, in a prospective cohort of 866 CHC patients[61]. Patients with LSM ≤ 8.0 kPa acted as the control group, the hazard ratios of developing HCC were 3.1, 4.7, 5.6 and 6.6 in patients with LSM at 8.1-13.0, 13.1-18.0, 18.1-23.0 and above 23.0 kPa respectively in another cohort of 1130 CHB patients[62]. LSM, as well as FibroTest, can also predict 5-year survival of patients with CHC; the prognostic values of LSM remained even after adjustments for treatment response, patient age, and degree of necroinflammation[63].

Predict post-operative outcomes

LSM is also an important prognostic tool in patients confirmed to have HCC. A prospective study of 105 HCC patients demonstrated that a LSM cutoff of 12.0 kPa had the sensitivity of 86% and specificity of 72% in predication of major post-operative complications[64]. This cutoff might also identify patients with more severe operative blood loss and higher transfusion rate[64]. Another study of 133 HCC patients revealed that patients of LSM ≥ 13.4 kPa had a nearly 2-fold increase in the risk of HCC recurrence compared to those with LSM < 13.4 kPa[65].

Assessment of liver steatosis

Liver steatosis is a common histological feature in the general population and in patients with chronic liver disease. Its prevalence is high: almost 30% in the general population[66,67], 50% in patients with CHC[68], above 80% in severely obese patients[69]. Liver steatosis plays a pivotal role in CHC, as metabolically (instead of virally) induced steatosis is associated with a lower response rate to antiviral treatment[70] and liver fibrosis progression[71]. Steatosis may also increase the risk of HCC[72]. Detection of liver steatosis is also important to the potential donors for liver transplantation, as their extent of steatosis is directly related to primary non-functioning of the graft, which may result in mortality or the need for re-transplantation[73]. Liver steatosis is also a risk factor for post-operative complications and mortality after liver resection[74].

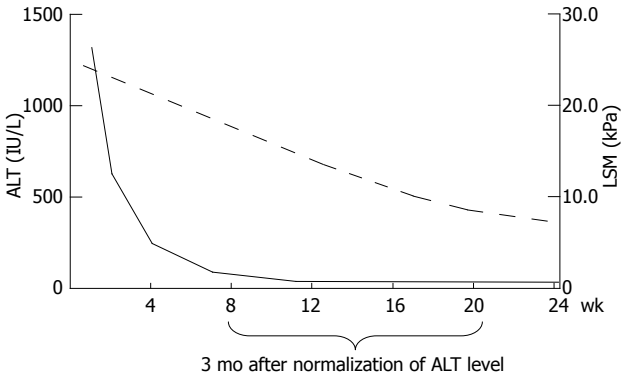

Figure 1 Falsely elevated liver stiffness measurement results in a patient with grossly elevated alanine aminotransferase levels. Liver stiffness measurement (LSM) values decreased considerably after the resolution of acute hepatitis. Modified from Wong *et al*[55]. ALT: Alanine aminotransferase.

There have been enough data to prove TE is accurate and applicable in different clinical settings. How this tool also has a few shortcomings that any user should keep in mind.

LIMITATIONS OF TE

Factors affecting accuracy of measurements

Not only liver fibrosis but also other factors contribute to the liver stiffness. LSM has been consistently found to be falsely elevated in acute hepatitis manifested as alanine aminotransferase (ALT) flares[75,76]. Severe hepatic necroinflammation may lead to LSM values well within the cirrhotic range even in the absence of fibrosis on histology[55,77,78]. In this setting, LSM tends to decrease considerably after the resolution of acute hepatitis. Therefore, applying TE in this scenario can be misleading and not recommended until at least 3 mo after normalization or at least stabilization of ALT levels below 5 times the upper limit of normal[26,76] (Figure 1). An ALT-based algorithm has been developed and higher LSM cutoff values for different stages of liver fibrosis should be used in patients with elevated ALT levels (Figure 2). This also leads to another advantage of liver biopsy over TE that at this stage the necroinflammatory score is only available in histologic assessment.

Extrahepatic cholestasis[79], hepatic congestion[80,81], hepatic amyloidosis[82] and recent food intake (within 60 min)[83] were also found associated with a falsely high LSM values. Fortunately, the degree of liver steatosis does not appear to affect LSM results, therefore TE remains an accurate tool for fibrosis assessment in CHC and NAFLD[24,31]. A recent study found that the correlation between LSM and fibrosis stage was less strong in CHB and NAFLD than in CHC patients[84]. Our recent study showed that NAFLD patients with BMI 30 kg/m^2, the lowest limit of an abnormal BMI in NAFLD, would have

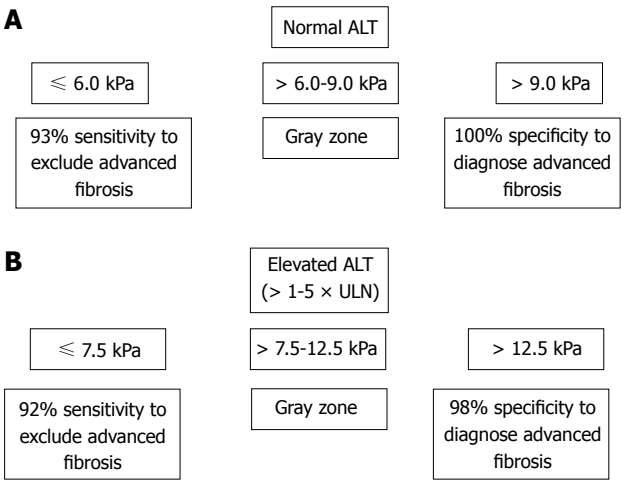

Figure 2 An alanine aminotransferase-based algorithm. A: Normal alanine aminotransferase (ALT); B: Elevated ALT levels up to 5 times of upper limit of normal (ULN) to exclude or establish advanced liver fibrosis for chronic hepatitis B patients. Modified from Chan *et al*[26].

higher LSM values by M probe even in the same fibrosis stage[85]. This provocative finding may lead to concern about the M probe accuracy in obese patients. The emergence of XL probe is a possible solution to this issue.

Factors affecting success rate of measurements

It has been noted that unreliable and failed LSM occur at about 3% and 11.6% to 18.4% in all TE examinations, respectively, and they are independently associated body mass index (BMI) > 30 kg/m² in both Caucasians and Chinese[86,87]. The success rate of LSM with M probe would be as low as 75%[31] in NAFLD patients with BMI > 30 kg/m^2. The low LSM success rate among obese patients is likely related to the thick subcutaneous fat, which hinders the transmission of shear waves and ultrasound waves through the liver parenchyma[87]. Patients with extreme very high and very low BMI were recently found to have higher LSM values in an Indian population[88]. Subjects with narrow intercostal space, high riding liver, hyperinflated lungs, ascites or free peritoneal fluid[17] may also have lower success rate or failed acquisition of LSM.

A recent study challenged the validity of the reliability criteria suggested by the manufacturer of 1165 patients with chronic liver diseases who underwent LSM within 3 mo of liver biopsy. The investigators found the number of successful acquisitions, and its success rate having no influence on the diagnostic accuracy[89]. Furthermore, LSM remained reliable even if the ratio of the interquartile range to the median of 10 measurements (IQR/M) > 0.30, provided that the median LSM < 7.1 kPa. These new findings implied that LSM results were more reliable than what was previously described.

Validity and availability of controlled attenuation parameter

Only a few studies on CAP have been published so far, and a few of them were only in abstract form. Hence CAP needs further validation in larger populations. Furthermore, CAP is not yet available in the measurements with the XL probe, which is designed for overweight and obese patients who are particularly at risk of liver steatosis[69]. Therefore, further development and calibration of CAP in XL probe is warranted.

Table 4 The characteristic of the new S and XL probes comparing to M probe

Probe	Frequency of ultrasound (MHz)	Depth (mm)
S	5	15-40
M	3.5	25-65
XL	2.5	35-75

COMBINING TE WITH SERUM MARKERS

In general, serum markers have modest accuracy to diagnose advanced liver fibrosis[90,91]. TE has certain advantages over serum markers, as TE provides a more direct measurement of fibrosis, is less affected by inter-current health disorders, and is theoretically applicable to all chronic liver diseases. On the other hand, the diagnostic performance was particularly affected in patients with elevated serum ALT levels[55]. Hence a second non-invasive test independent of the serum ALT or AST levels may be a good supplementary test for LSM. Among various serum test formulae, Forns index[92] and Hui index[90] are composed of clinical parameters other than ALT or AST levels. We demonstrated that a combined LSM-Forns algorithm improved the accuracy to predict advanced liver fibrosis in 238 CHB patients[28]. In this combined algorithm, low LSM or low Forns index could be used to exclude advanced fibrosis with a high sensitivity of 95%. To confirm advanced fibrosis, agreement between high LSM and high Forns index could improve the specificity up to 99% to 100%[28].

The combination of TE and FibroTest was found to have the best diagnostic performance compared to either test alone in patients with CHC[4]. When TE and FibroTest matched (present in 70%-80% of cases), results were also concordant, respectively in 84%, 95% and 94% of patients with liver fibrosis ≥ F2, ≥ F3 and F = 4[4]. The combination of LSM and FibroTest allowed exclusion of significant fibrosis (≥ F2) in nearly 80% of 100 CHB patients in inactive carrier stage.

OTHER PROBES OF TE

The development of S and XL probes aim to cater for different population groups of various body-build types (Table 4). S probe contains a higher frequency ultrasonic transducer and shallower measurements below the skin surface, which suit pediatric subjects and those with small body build[93]. XL probe contains a lower frequency and a more sensitive transducer, a deeper focal length, larger vibration amplitude and a higher depth of measurements below the skin surface[94]. This probe serves obese sub-

jects with "XL" body builds. Data concerning the validations of these new probes are emerging.

With the XL probe, LSM could be successfully performed in more obese patients compared to the M probe[95]. In our validation study involving 286 patients, LSM using XL probe documented reliable results in 92% of patients, compared to 80% using M probe (64). In another study of 193 NAFLD patients, a cutoff value had reasonable sensitivity (78%), specificity (78%), positive predictive value (60%), and good negative predictive value (89%) for F3 or greater disease[96]. However, the median LSM by the XL probe was consistently found to be approximately 1.0 to 1.2 kPa lower than that of the M probe at the same stage of liver fibrosis in all of the histologic reports[95,96]. A recent exploratory study of 517 overweight patients having different etiologies, XL cutoff values of 4.8 kPa and 10.7 kPa, 6.0 kPa and 12.0 kPa with the M probe[85], for patients with BMI > 25-30 kg/m^2. Patients with BMI > 30 kg/m^2 might use M probe cut-offs for the XL probe. More studies are warranted to delineate the proper cutoff values of LSM using the XL probe in various etiologies.

SPLEEN STIFFNESS: MEASURES PORTAL HYPERTENSION NON-INVASIVELY

Recent enthusiasm on spleen stiffness measurement (SSM) leads us to the non-invasive evaluation of portal hypertension, which is conventionally assessed by HVPG *via* hepatic angiogram[97]. SSM was recently found accurate to predict portal hypertension and esophageal varices[98,99]. The clinical role of SSM will be explored more in the near future.

NEW IMAGING TECHNOLOGIES

Acoustic radiation force impulse (ARFI) is another new imaging technology based on the shear acoustic waves remotely induced by the radiation force of a focused ultrasonic beam[100]. ARFI may be even more accurate than TE for both significant and severe classes of liver fibrosis in CHC patients[101]. ARFI was also used for SSM in CHB and CHC patients[102]. Another technique called real-time tissue elastography (RTE) is incoporated into B-mode ultrasography machine[103]. RTE is also found accurate to diagnose liver fibrosis and portal hypertension[104,105]. All these new technologies are also promising and should be further validated in the near future.

CONCLUSION

TE is a non-invasive, accurate and reproducible test of advanced liver fibrosis, cirrhosis and steatosis. This tool has been validated in a wide spectrum of liver diseases. TE is also useful to predict patient outcomes. Further studies should explore the appropriate cutoff values of newer XL and S probes, and exploring the prognostic role of CAP.

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BRIEF ARTICLE

Prevalence and risk factors of methotrexate hepatoxicity in Asian patients with psoriasis

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Abstract

AIM: To establish the prevalence of liver fibrosis and to evaluate the possible risk factors for fibrosis and progression in Asian with psoriasis treated with methotrexate (MTX) based on liver histology.

METHODS: Patients with psoriasis treated with MTX referred to the Department of Gastroenterology, Tan Tock Seng Hospital for liver biopsy were identified and retrospectively studied. Patient case notes and electronic records were retrieved from the hospital database and relevant data collated. Histological changes of liver biopsies were staged according to Roengik score. The factors assessed were age, gender, ethnicity, cumulative dose of MTX, presence of comorbid conditions such as diabetes, hypertension, hyperlipidemia, and ethanol use. We also assessed the histological change in those with multiple liver biopsies. Statistical analysis was performed using Stata V.9.2.

RESULTS: There were altogether 59 patients (median age 50 years old, range 22-81 years old, male, 88%) with 98 biopsies liver biopsies; 6 normal [median cumulative dose (MCD), 2285 mg]; 62 grade Ⅰ (MCD 2885 mg), 23 grade Ⅱ (MCD 1800 mg) and 7 grade Ⅲ (MCD 1500 mg). There was no grade Ⅳ or cirrhosis. The prevalence of liver fibrosis (grade Ⅲ) was 12%. Of the factors assessed, diabetes (P = 0.001) and hypertension (P = 0.003) were significant for fibrosis on univariate analysis but not on multivariate analysis. Of the 26 patients who had more than one biopsy (median 2, range 2-6), 57.7% (n = 15) were stable, 34.6% (n = 9) had progression and 7.7% (n = 2) had regression of histological grades. On univariate analysis, non-Chinese ethnicity (P = 0.031), diabetes (P = 0.018), and hyperlipidemia (P = 0.011) were predictive of progression of grades, but these were not significant on multivariate analysis.

CONCLUSION: Liver fibrosis in Asian psoriatic population on MTX is comparable to the West. Cumulative dose was not associated with liver fibrosis. Metabolic syndrome is important factors.

Key words: Hepatotoxicity; Liver fibrosis; Methotrexate; Risk factors; Cirrhosis

Core tip: Old studies have shown that the prevalence of methotrexate (MTX) associated liver fibrosis and cirrhosis to be as high as 50% and 26% respectively. Later studies have shown that the risk is much lower than previously reported. These studies have been based on western patients. To date, there is no study that had assessed MTX hepatotoxicity in Asian patient based on liver histology. This study showed that the risk of methotrexate hepatoxicity in Asian patients with psoriasis is also low and progression is minimal. We showed that ethnicity and presence of diabetes and hyperlipidemia, part of the metabolic syndrome may be important factors.

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INTRODUCTION

Methotrexate (MTX) is used as immune-suppressive therapy for many conditions, and it remain the most commonly used systemic medical therapy for treating psoriasis even after the introduction of biologic therapy[1,2]. Hepatotoxicity associated with MTX in psoriatic patients is well recognised, and in some patients can lead to hepatic fibrosis and even cirrhosis[3].

In the west, the prevalence of MTX associated liver fibrosis and cirrhosis has been reported to be as high as 50% and 26% respectively[3]. Alcohol consumption, diabetes, obesity, chronic viral hepatitis and medications such as arsenic and vitamin A have been reported to be significant risk factors of MTX associated liver fibrosis[4-7]. Previous guidelines recommend that liver biopsy should be considered at intervals of 1500 mg cumulative MTX dose to assess for fibrosis and to decide on whether it is safe to continue MTX therapy[8]. However, recent reports have suggested that MTX may be less hepatotoxic and liver fibrosis may be less prevalent[9-12]. Therefore in 2009, the American Academy of Dermatology has updated their recommendation to consider liver biopsy only after a cumulative dose of 3500 to 4000 mg in patients without any risk factors for hepatotoxicity[13]. Most of these studies have been conducted on the Western population. Data remains scarce on Asian patients treated with MTX. This only study done on Asian patients had shown that MTX was safe based on fibroscan in Korean patients with rheumatoid arthritis[14]. A retrospective study from Malaysia showed that hepatotoxicity based on serum transaminases was uncommon[15].

Fibroscan is now becoming widely used for the assessment liver fibrosis as it is non-invasive. However, fibroscan is user dependent and is not specific for grades Ⅱ and Ⅲ fibrosis. Therefore, liver histology assessment remains the gold standard. To date, there is no study that had used liver biopsy to assess impact of MTX treatment and hepatotoxicity in Asian patients. The aims of our study were to: (1) establish the prevalence of liver fibrosis based on liver biopsy in among multi-ethnic Asian patients with psoriasis treated with MTX; and (2) to evaluate the possible risk factors for fibrosis and progressions.

MATERIALS AND METHODS

Study

We retrospectively reviewed the medical records of all psoriatic patients on MTX referred to our institution for liver biopsy over a seven years period. Pre-biopsy evaluation included history taking, physical examination, basic blood investigations and ultrasound scan of the hepatobiliary system.

Setting

Tan Tock Seng Hospital, Singapore (1300 bedded hospital) is one of the biggest tertiary referral hospital with population catchment close to 1.5 million. It is also the centre that serves the National Skin Centre located close to the hospital where most of patients with psoriasis are treated.

Data

We collected data on the patients' age, gender, ethnicity, cumulative MTX dose, presence of potential risk factors (diabetes, hypertension, alcohol consumption and hyperlipidemia), hepatitis B and C status and the liver biopsy results. There were a total of 101 liver biopsies of which three histological reports could not be traced leaving 98 available liver biopsy reports from 59 patients for the study. Histological slide preparation followed the standardised methods of assessment using hematoxylin and eosin stain and Maisson trichrome strain for fibrosis. Histological changes were graded according to Roengik Classification as shown in Table 1.

Statistical analysis

Statistical analysis was performed using Stata V.9.2 (Stata Corp, College Station, Tx, United States). For comparison, Fisher's Exact Test was used to evaluate the effect of gender, ethnicity (Chinese *vs* non-Chinese), diabetes, hypertension, hyperlipidemia and alcohol consumption on MTX associated liver fibrosis. Mann-Whitney test was applied to compare the effect of age and the distribution of the MCD between patients with and without fibrosis. The binary logistic regression model was subsequently used to assess whether any of the independent risk factors found to be significant on univariate analyses for liver fibrosis and progression of grades on repeat biopsies. For the assessment of the prevalence of fibrosis and the progression of Roenigk histological grades, the latest biopsies results were taken as the end point of progressions.

This study was approved by the Research Ethics Committee of Singapore National Healthcare Group.

RESULTS

Demographics

Over the period, there were 59 patients who were referred and had at least one liver biopsy. The median age was 50 (range 22-81) with majority male (88%) and the ethnic breakdown consisting of Chinese (n = 39, 66%), Malays (n = 13, 22%), Indians (n = 6, 10%) and others (n = 1, 2%).

Thirty-four patients had no risk factor whereas 11 patients had one risk factor, 9 had 2 risk factors and 6 had 3 risk factors. Among the 59 patients, 15 (25.4%) had diabetes, 15 (25.4%) had hypertension, 9 (15.3%) had hyperlipidemia, and 5 (8.5%) were occasional light social drink-

Table 1 Roenigk classification of liver damage

Grade	Fibrosis	Fatty infiltration	Nuclear variability	Portal inflammation
I	None	Mild	Mild	Mild
II	None	Moderate to severe	Moderate to severe	Portal expansion, lobular necrosis
IIIA	Mild (Septa extending into lobules)	Moderate to severe	Moderate to severe	Portal expansion, lobular necrosis
IIIB	Moderate to severe	Moderate to severe	Moderate to severe	Portal expansion, lobular necrosis
IV	Cirrhosis			

Table 2 Distribution of the grades of the liver histology and the median cumulative dose

Histology grade	No.	Median MTX cumulative dose (mg)
Normal	6	2285
I	62	2885
II	23	1800
III	7	1500
IV	0	-
Total	98	2500

MTX: Methotrexate.

ers (less than one drink a week). None of our patients had hepatitis B or C infection. None of the patients had significantly impaired liver profiles especially the serum albumin and bilirubin.

Liver histology

Of the 98 liver biopsy reports, 6 (5.9%) were normal, 62 (61.4%) had grade I histological changes, 23 (22.8%) had grade II changes, 7 (6.9%) had grade III changes and none showed grade IV cirrhotic change. The prevalence of MTX associated liver fibrosis (grade III) was 12%.

Risk factors analysis

MTX cumulative dose: The MCD of MTX was 2500 mg with a range of 875-14433 mg. There was a liver biopsy done at the cumulative dose of 875 mg because of persistent abnormal liver profiles. Table 2 summarises the relationship between MCD and liver histology. There was no difference in the MCD of the patients with MTX associated liver fibrosis compared with those without liver fibrosis (1500 mg *vs* 2500 mg, $P = 0.18$).

Other risk factors: There was no difference in the age of patient with or without fibrosis (50.9 ± 10.2 *vs* 49.8 ± 9.9, $P = 0.901$). On univariate analyses, diabetes and hypertension were risk factor for liver fibrosis whereas age, gender, ethnicity, hyperlipidemia and alcohol consumptions were not (Table 3). Seven of the 20 patients (35%) with either diabetes or hypertension had liver fibrosis with MCD of 1500 mg. None of the 40 patients without risk factor developed fibrosis with MCD of 3000 mg. Among the 7 patients without any history of alcohol consumption, all had either diabetes or hypertension. However, neither diabetes nor hypertensions were significant on multivariate analyses.

Histological changes

Among the 59 patients, 26 (43%) had more than one liver biopsy. There were no change in the grade reported in 57.7% ($n = 15$), progression in 34.6% ($n = 9$) and regression in 7.7% ($n = 2$). On univariate analyses, non-Chinese ethnicity and the presence of diabetes and hyperlipidemia were significant predictors (Table 3) for progression of grades. However, these were no significant on multivariate analysis.

DISCUSSION

Data on MTX in psoriatic patients is well reported in the Western population but data remains scarce on Asian population. The prevalence of liver fibrosis in our psoriatic patients was only 12%, and none of our patients had cirrhosis based on histology evaluation. Generally our findings are consistent with findings of recent studies from the West. Therefore, our findings also suggest that liver fibrosis may be less prevalent (fibrosis 16% and cirrhosis 2%) compared to older studies (fibrosis up to 50% and cirrhosis 26%)[7,8].

Several factors have been reported to accelerate the fibrotic progression in patient treated with MTX, and these include alcohol consumption, presence of diabetes, obesity, chronic viral infections, and use of medications such as arsenic and vitamin A[5-8]. Our study also showed that diabetes and hypertension were significant risk factors, but not alcohol use or hyperlipidemia, age, gender or ethnicity. Correlations with diabetes have been shown but not hypertension. This is perhaps not unexpected considering that hypertension is part of metabolic syndrome, which have also been shown to be an important risk factor. However, further studies are required to assess this correlation.

Among our patients with reported alcohol consumption, none developed liver fibrosis compared to the 12.7% who did not give any history of alcohol consumption. However the latter patients had other risk factors, either diabetes or hypertension. An earlier study showed no association with alcohol consumption[16].

Lipid disorders are common and are also a part of metabolic syndrome. Hence one would expect hyperlipidemia to be an important factor. A recent case series showed that non-alcoholic steatohepatitis (NASH) contribute to MTX hepatotoxicity in patients with psoriasis[17]. In fact NASH is a recognised and an important cause of liver cirrhosis[18,19], in particular those catego-

Table 3 Risk factors analysis for fibrosis and progression of Roenigk grades *n* (%)

Risk factor	Proportion of patients with fibrosis	*P* value[1]	Proportion of patients with progression	*P* value[1]
Gender (male *vs* female)	4/42 (9.5) *vs* 3/17 (17.6)	0.382	5/9 (55.6) *vs* 4/17 (23.5)	0.102
Ethnicity (Chinese *vs* non-Chinese)	4/39 (10.3) *vs* 3/20 (15)	0.594	3/16 (18.8) *vs* 6/10 (60)	0.031
Diabetes mellitus (yes *vs* no)	5/15 (33) *vs* 2/44 (4.4)	0.008	4/5 (80) *vs* 5/21 (23.8)	0.018
Hyperlipidemia (yes *vs* no)	1/9 (11.1) *vs* 6/50 (11.7)	> 0.99	3/3 (100) *vs* 6/23 (26.1)	0.011
Hypertension (yes *vs* no)	5/15 (33) *vs* 2/44 (4.4)	0.008	4/6 (66.7) *vs* 5/20 (25)	0.060
Alcohol consumption (yes *vs* no)	0/5 (0) *vs* 7/54 (12.7)	> 0.99	0/2 (0) *vs* 9/24 (37.5)	0.284

[1]Comparison made with Fisher's Exact Test.

rised as cryptogenic cirrhosis. One reason to account for the lack of significance in our study was probably related to the fact that patients were already on treatment by the time they had their biopsy.

Recent studies have reported that MTX cumulative dose or duration of therapy was not correlated with liver toxicity[7]. Our present study also showed no such correlation. In fact, our patients with normal or grade I changes had higher MCD (2285 mg) compared to those with grade Ⅲ changes (MCD, 1500 mg). This could be explained by the fact that the latter patients had other risk factor, namely diabetes. Among our 35% of patients with either risk factor, they developed liver fibrosis with a MCD of 1500 mg compared to MCD of 3000 mg for those without risk factors. Rosenberg *et al*[5] reported that 96% of their patients with at least one other risk factor (diabetes, being overweight, heavy alcohol consumption, chronic hepatitis B or C) developed liver fibrosis after MCD of 1500 mg. In contrast, 58% without risk factors developed liver fibrosis at a MCD of 2100 mg[6].

Studies based on rheumatoid arthritis and inflammatory bowel diseases have also shown similar results[14,20-22]. A multicentre study looking at the effects of MTX in 46 patients with inflammatory bowel disease showed that advanced fibrosis was only encountered in 6.3% of patients based on elastography scan, with a MCD of 1242 ± 1349 mg. Gender, age, type of inflammatory bowel disorder, or MCD did not have any impact on liver stiffness[20,21]. Another study showed that body mass index (BMI) above 28 kg/m^2 and excess alcohol consumption were important factor but not the cumulative doses[21]. Studies on rheumatoid arthritis also reported low incidence of liver fibrosis and showed similar risk factors.

Majority of our patients who had more than one liver biopsy had stable grade. Progression of histology was seen in only 34.6%. Interestingly, two patients had regression of their histological grade. Non-Chinese ethnicity, diabetes and hyperlipidemia were significant factor for progressions on univariate analysis but not on multivariate analyses. Most progressions were increase of one grade, from grade I to grade Ⅱ which basically indicated increase in the degree of steatosis, nuclear variability and expansion of the portal tract, changes which are also seen in non-alcoholic fatty liver disease. The significance of non-Chinese ethnicity is perhaps related to the higher incidence of overweight among our Malays and Indians population. The small sample size probably also accounted for the non-significance on multivariate analysis. Other studies have also shown that fibrosis progressions among patients continuing on MTX therapy are actually uncommon and some may actually regress[23,24].

Our findings suggest that the interval of repeat biopsy can be prolonged following what have been recommended in the West. The most recent guidelines from the West have recommended liver biopsy only after a cumulative dose of 3500-4000 mg in patients without any risk factors[11,13,25]. Therefore, based on our findings, this recommending can be adapted to the Asian population. In our practice, we also follow the latest recommendations[13,25] and only subject patients to liver biopsies if they have achieved cumulative doses of between 3500 and 4000 mg or those with risk factors such chronic viral hepatitis and disorders (*i.e.*, diabetes, hypertension, obesity) that are part of the metabolic syndrome. However, for patients who are not willing to undergo liver biopsy, we use fibroscan to assess the fibrosis score to decide if patient should go for a liver biopsy. Patients found to have high score or in the grey zone, we will further discuss with patients on the need for liver biopsy.

Currently, it remains uncertain whether use of non-invasive tests can replace liver histology. Based on currently available evidence, use of non-invasive tests such as fibroscan, fibrotest and procollagen Ⅲ N-terminal propeptide (PⅢNP) are not sensitive enough to predict severity of fibrosis[26-28]. Fibrotest have been reported to predict presence of fibrosis whereas fibroscan to predict the absence of fibrosis. Use of serial PⅢNP following the Manchester protocol compared to the American Academy of Dermatology guidelines have been reported to lead to avoidance of liver biopsy with a factor of seven fold, without compromising patient care[26]. Use of PⅢNP has been shown to provide misleading results in patients with psoriatic arthopathy[23]. Whether this can be generalised to patient without arthropathy is unknown. In our setting, we currently do not recommend non-invasive tests as an alternative to liver biopsy as data on Asian patients with psoriasis treated with MTX is lacking.

There are several limitations with our study. First, the sample size was small and may account for the non-significant findings on multivariate analyses. Second, due to its retrospective nature, there were missing or incomplete data, and in our case, incomplete data on patients height made it not possible to assess of the impact of BMI. Despite these limitations, our findings are

consistent with what have been reported in the literatures on MTX and liver fibrosis. The main strength of our study is the use of liver histology which remains the gold standard compared to the available non-invasive tests which all have limitations in terms of sensitivity and specificity.

In conclusion, the prevalence of liver fibrosis in our multi-ethnic Asian patients with psoriasis on MTX is comparable to the rates reported recently which are much lower than the rates reported in older studies. Disorders associated with metabolic syndrome may be important risk factors for fibrosis and progression. Further studies with larger sample sizes will be required to assess the association. Inclusion of non-invasive tests such as fibroscan, fibrotest or PⅢNP to liver histology assessment will also help to assess the reliability of these tests compared to the gold standard.

COMMENTS

Background

Psoriasis is a common chronic skin disorder that is commonly treated with methotrexate (MTX) which is associated with many side effects including hepatotoxicity. Previous studies have reported high prevalence of liver fibrosis and cirrhosis. However, later studies have shown that the risk is much lower than previously reported. These studies have been based on western patients. To date, there have only been few studies on the hepatotoxic effects of MTX in Asian patients.

Research frontiers

This study looked at the prevalence of hepatotoxicity and progression in a multi-ethnic Asian population with psoriasis treated with MTX by using the liver histology, which is the gold standard.

Innovations and breakthroughs

This study showed that significant liver histology is uncommon and the presence of diabetes and hypertension were significant for fibrosis on univariate analysis but not on multivariate analysis. This study also showed that progression is uncommon with most having only change of one grade which indicate increase in steatosis among patients who continued treatment with MTX. A small proportion actually had histology regressions. Non-Chinese ethnicity and the presence of diabetes and hyperlipidemia were predictive of progression of grades, but only on univariate analysis. However, study with larger sample size is required.

Applications

Interval assessment can be delayed in Asian patients treated with MTX until a higher cumulative dose following the recommendations based on Western patients.

Terminology

MTX hepatotoxicity is liver damage secondary to the effect of long term treatment with MTX. Histological assessment can be made using the Roengik Classification which categorise changes in five grades; Ⅰ, Ⅱ, ⅢA, ⅢB and Ⅳ (cirrhosis); Mean cumulative dose is the mean accumulated dose of MTX taken over a period of time and is a factor in deciding when to repeat a liver assessment.

Peer review

This study showed that the risk of MTX hepatoxicity in Asian patients with psoriasis is low and progression is minimal based on assessment of liver histology. Ethnicity, presence of diabetes and hyperlipidemia, part of the metabolic syndrome may be important factors. Based on the findings of this study, repeat liver biopsy assessment can be delayed to higher cumulative dose following updated recommendations.

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BRIEF ARTICLE

Nonalcoholic steatohepatitis in nonalcoholic fatty liver disease patients of Bangladesh

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Abstract

AIM: To explore the prevalence and risk factors for nonalcoholic steatohepatitis (NASH) in nonalcoholic fatty liver disease (NAFLD) patients.

METHODS: We have included 493 patients with sonographic evidence of a fatty change, and 177 of these individuals were evaluated and confirmed after liver biopsy. The exclusion criteria consisted of significant alcohol abuse (< 20 g daily), evidence of hepatitis B and C, evidence of drug-induced fatty liver disease and other specific liver diseases such as hemochromatosis, Wilson's disease or autoimmune liver disease. The patients were assessed for metabolic syndrome, and biochemical, anthropometric and histopathological evaluations were carried out. The degree of disease activity in the NAFLD patients was evaluated using the NAFLD Activity Score. The data were analyzed by SPSS, version 16.0.

RESULTS: Females predominated among the study participants (250, 57.0%), and the mean age was 40.8 ± 10.2 years. The numbers of overweight, obese Ⅰ and obese Ⅱ patients were 58 (13.2%), 237 (53.9%) and 93 (21.2%), respectively. However, there were 422 (96.2%) centrally obese patients. NASH was absent in 10 (5.6%) cases, borderline in 92 (52.6%) cases and present in 75 (42.4%) cases. The presence of diabetes could significantly (P = 0.001) differentiate NASH from simple steatosis. The following parameters did not influence the development of NASH: age, sex, basal metabolic index, waist circumference, serum high-density lipoprotein, triglyceride, insulin resistance index, hypertension and metabolic syndrome. The serum gamma-glutamyl transpeptidase (GGT) level was significantly higher (P = 0.05, 51.7 ± 32.8 and 40.4 ± 22.6 U/L) in the NASH patients, with a sensitivity of 45% and a specificity of only 68%. The serum alanine aminotransferase and aspartate aminotransferase levels were not able to predict NASH.

CONCLUSION: Females were the predominant sufferers of NAFLD in Bangladesh. The prevalence of NASH was high. Diabetes was found to be the main culprit in developing NASH. GGT was the only biochemical marker of NASH. We recommend liver biopsy in NAFLD patients who have diabetes and elevated GGT.

Key words: Fatty liver; Gamma-glutamyl transpeptidase; Nonalcoholic fatty liver disease; Nonalcoholic steatohepatitis; Alanine aminotransferase; Obesity; Basal metabolic index

Core tip: We have designed this study to explore the prevalence of and risk factors for nonalcoholic steatohepatitis (NASH) in nonalcoholic fatty liver disease patients. Other causes of liver disease were excluded. A total of 493 patients with sonographic evidence of fatty change were considered, and 177 of these patients were evaluated and confirmed by liver biopsy after

making exclusions. Females were predominant (250, 57.0%). Central obesity was more prevalent among the patients compared with overall obesity. NASH was observed in 75 (42.4%) of the cases. The presence of diabetes and elevated gamma-glutamyl transpeptidase could differentiate NASH from simple steatosis. Serum alanine aminotransferase and aspartate aminotransferase could not be used to detect NASH.

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is a clinico-histopathological entity with histological features that resemble alcohol-induced liver injury. By definition, this disease occurs in patients with little or no history of alcohol consumption[1]. NAFLD is the most common liver disease in western countries, affecting 20%-30% of the general population[2,3]. The term NAFLD includes a spectrum of histological features, including simple steatosis, steatosis with inflammation, steatosis without inflammation, ballooning degeneration and pericellular fibrosis or Mallory's hyaline (nonalcoholic steatohepatitis, NASH). Primary NASH refers to steatohepatitis that is associated with dysmetabolic syndrome, whereas secondary NASH refers to steatohepatitis that accompanies other syndromes or is caused by certain drugs (for example, amiodarone)[1]. NASH may progress to cirrhosis in up to 20% of patients[4]. Patients with NASH are at risk for progressive liver disease (which can progress to cirrhosis, hepatocellular carcinoma, and death from chronic liver disease), as well as cardiovascular mortality and type-2 diabetes. Reports have suggested that the prevalence of NAFLD among Asian Indians is comparable to that seen in the West, and NASH may be present in approximately 20% of these patients, with a 2- to 3-fold increased prevalence in patients with type-2 diabetes[5]. The average age for NASH patients is 40-50 years, and that for NASH-related cirrhosis is 50-60 years. The progression of fibrosis, as detected by liver biopsy, has been observed in 43% of NASH patients, whereas 54% of patients remained unchanged, and 3% showed a histological improvement during a follow-up from 1-7 years[6]. NASH most likely causes approximately 80% of the cases of cryptogenic (extensive evaluation excluded a recognizable etiology) cirrhosis, which accounts for 10%-20% of all cirrhosis cases and progresses to advanced fibrosis in 32%-37% of patients[7].

In parallel with the epidemic of obesity and metabolic syndrome worldwide, the prevalence of NAFLD in Asian countries has increased rapidly, with a trend toward younger patients, over the last two decades. Childhood NAFLD has also progressively attained clinical importance. The prevalence of NAFLD has been described at 10%-39% in the populations of various locations: North America, Japan, northern and southern Europe, South America, Australia and the Middle East[8]. NAFLD has been associated with insulin resistance and hyperinsulinemia, even in lean subjects with normal glucose tolerance[9]. Diabetes mellitus may be an independent predictor of advanced NAFLD, including cirrhosis and hepatocellular carcinoma[10]. NAFLD is now recognized as the hepatic component of metabolic syndrome, which includes hyperlipidemia, glucose intolerance, obesity, and systemic hypertension. The risk and severity of NAFLD increase with the number of components of metabolic syndrome that are present[11]. The contrasting clinical course of NASH *vs* non-NASH fatty liver (NNFL) indicates that these two conditions diverge early in the course of NAFLD, although a small number of patients most likely transition from NNFL to NASH. A progression to cirrhosis is usually preceded by longstanding histological NASH and is infrequent during NNFL. Longitudinal studies with serial biopsies have shown that approximately one-third of NASH patients develop advanced fibrosis (stage 3 or 4 fibrosis) over the course of 5-10 years from the time of the initial diagnosis[4,12]. Although it is usually relatively slow, the progression to cirrhosis can occur in as little as 2-3 years. NASH is a common cause of "cryptogenic" cirrhosis, which accounts for 10%-20% of all cirrhosis cases[13]. Among patients diagnosed with NASH-related cirrhosis, the risk of developing portal hypertension (a major complication) is 17%, 23% and 52% at 1, 3 and 10 years, respectively. Among patients with early-stage NASH, the overall mortality over 10-15 years is approximately 10%-12%, being significantly higher in the NASH *vs* the NNFL patients, compared to the general population. The risk of developing decompensated cirrhosis is 5%-10%, and that of hepatocellular cancer is 1%-2%. There is a ten-fold increased risk of cirrhosis relative to the general population[14].

A complete diagnosis of fatty liver disease should ideally define the histology, the stage and grade of the disease and its etiology. In Bangladesh, NAFLD has never been sufficiently addressed by the medical community. NASH is a potentially dangerous condition that requires medical intervention. The prevalence of NASH and the potential risk factors for it have not been previously explored. We have designed this study protocol to estimate the prevalence of NASH in NAFLD patients and the risk factors for developing NASH in the context of Bangladesh; the results will contribute to future scientific knowledge and interventions.

MATERIALS AND METHODS

Study population

We initially included 439 patients from the Outpatient Department of Hepatology in the University Hospital during the period of March 2010 to December 2012 who

were referred due to fatty filtration in the liver according to ultrasonography. The exclusion criteria consisted of significant alcohol abuse (> 20 g daily), evidence of hepatitis B and C, evidence of drug-induced fatty liver or other specific liver diseases such as hemochromatosis, Wilson's disease or autoimmune liver disease. These patients underwent clinical evaluations, anthropometric measurements, and blood tests. Liver biopsies were performed in 190 patients, but 4 of these biopsy samples were inadequate to assess for histopathology, and another 4 patients withdrew from the study. The histopathological reports of 182 patients were available, but 5 of them did not present fatty change upon microscopy. We therefore included 177 patients for further analysis. The study was approved by the Institutional Review Board, and all of the individuals provided written informed consent prior to enrollment in the study. Metabolic syndrome was defined according to Asian criteria[15], and three of the five listed criteria were considered: waist circumference ≥ 80 cm for women and ≥ 90 cm for men, serum triglyceride ≥ 150 mg/dL (1.7 mmol/L), serum high-density lipoproteins (HDL) cholesterol < 50 mg/dL (1.3 mmol/L) for women and < 40 mg/dL (1 mmol/L) for men, elevated blood pressure (systolic blood pressure ≥ 130 and or diastolic blood pressure ≥ 85 mmHg or drug treatment for hypertension) and plasma glucose concentration ≥ 100 mg/dL (5.6 mmol/L) or drug treatment for diabetes.

Clinical and biochemical evaluation

All of the patients were clinically evaluated, and blood pressure, body mass index (BMI) and waist circumference were recorded for every patient. Liver function tests were performed prior to the liver biopsy. Blood samples were obtained under fasting conditions, and the following tests were performed using standard laboratory methods: alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, gamma glutamyl transpeptidase (GGT), international normalized ratio, blood glucose (fasting and 2 h after breakfast), lipid profile, and insulin level (which was assessed using the method of indirect chemiluminescence). Insulin resistance was calculated according to the Homeostatic Model Assessment index.

Histological assessment

Liver biopsy specimens of the 177 patients were analyzed by a pathologist who was blinded to the clinical and biochemical results of the patients. The diagnosis of NASH was based on the criteria of Brunt *et al*[16], as modified by Kleiner *et al*[17]. In this scoring system, the degree of disease activity in NAFLD was evaluated using the NAFLD Activity Score (NAS), which was calculated as the unweighted sum of the scores for steatosis (0-3), lobular inflammation (0-3), and hepatocyte ballooning (0-2); therefore, the score ranged from 0 to 8. A NAS of 5 or more was diagnosed as "definitive NASH", a NAS of 2 or less as "non-NASH", and a NAS of 3 or 4 as "borderline NASH". Diagnoses other than NASH were considered to be NNFL. The hepatic fibrosis staging was as follows: 0 = no fibrosis; 1 = zone 3 fibrosis only; 2 = zone 3 and portal/periportal fibrosis; 3 = bridging fibrosis; and 4 = cirrhosis.

Statistical analysis

The results are presented as the mean ± SD for the quantitative data and as numbers or percentages for the categorical or qualitative data. The statistical differences in the quantitative data were assessed using a *t* test or one-way analysis of variance. The qualitative data were compared using the χ^2 test. For all of the tests, significance was achieved at $P < 0.05$.

RESULTS

Patient characteristics

A total of 177 patients were included in this study. There were 104 females (58.8%) and 73 males (41.2%). The mean age of the sample was 40.1 ± 9.5 years. Most of the affected individuals were aged 31 to 40 years (66, 37.3%), and the remainder were aged 41 to 50 years (59, 33.3%). The majority of the population comprised house wives (94, 53.1%), but there were also service holders (26, 14.6%), businessmen (23, 13.0%) and students (34, 19.3%). Hypertension and diabetes were present in 41 (23.1%) and 39 (22.1%) of the patients, respectively, but metabolic syndrome was present in 93 (52.3%) of the patients. Triglycerides were high in 130 (73.9%) of the patients. The BMI was normal in 24 (13.5%) of the patients, overweight in 14 (8.1%) of the patients, obese I in 88 (49.3%) of the patients and obese Ⅱ in 51 (29.1%) of the patients, according to the criteria for Asians[18]. Most of the patients presented central obesity (171, 96.5%), with a waist circumference above normal. The ALT, AST and GGT levels were 56.7 ± 35.9, 46.6 ± 50.5 and 46.2 ± 28.6 U/L, respectively. The insulin resistance index was higher than normal in 79 (44.6%) of the patients.

Histological changes

The histopathological reports of 177 patients were available for further analysis. There was no significant difference between the biopsied and non-biopsied patients regarding the clinical, anthropometric and biochemical variables. Steatosis of < 33% was observed in 73 (41.2%) of the patients, steatosis of 33%-66% was observed in 82 (46.3%) of the patients, and steatosis of > 66% was observed in 22 (12.4%) of the patients. Lobular inflammation was absent in 10 (5.6%) of the patients, mild in 93 (52.5%) of the patients, moderate in 70 (39.5%) of the patients and severe in 4 (2.3%) of the patients. Ballooning was absent in 5 (2.8%) of the patients, there was a small amount of ballooning in 138 (78.0%) of the patients, and there was prominent ballooning in 34 (19.2%) of the patients. No fibrosis was observed in 28 (15.8%) patients, stage I was observed in 94 (53.3%) patients, stage Ⅱ was observed in 40 (22.5%) patients, and stage Ⅲ was observed in 15 (8.3%) patients. None of the pa-

Table 1 Clinical, anthropometric and biochemical characteristics of the non-nonalcoholic steatohepatitis fatty liver and nonalcoholic steatohepatitis patients

Variable	NNFL *n* = 102	NASH *n* = 75	*P* value
Age, yr, (mean ± SD)	39.3 ± 9.4	41.0 ± 9.7	0.24
Sex: male/female	42/60	31/44	1.00
Body mass index (kg/m^2)	27.8 ± 3.9	27.8 ± 4.6	0.998
Waist (cm)			
Male	93.0 ± 5.5	93.0 ± 9.8	0.081
Female	95.8 ± 9.9	95.6 ± 11.0	0.927
HDL (mg/dL)			
Male	36.3 ± 8.9	34.2 ± 6.5	0.337
Female	39.8 ± 10.3	39.2 ± 10.3	0.801
Serum triglyceride (mg/dL)	225.2 ± 165.8	239.8 ± 111.6	0.509
Insulin resistance index	1.8 ± 1.3	1.5 ± 0.7	0.337
Diabetes (present/absent)	13/86	25/48	0.001
Hypertension (present/absent)	17/65	17/48	0.555
Metabolic syndrome (present/absent)	41/41	39/32	0.328
ALT (U/L)	56.9 ± 38.8	56.3 ± 31.8	0.603
AST (U/L)	46.9 ± 63.7	46.1 ± 22.2	0.916
GGT (U/L)	40.4 ± 22.6	51.7 ± 32.8	0.05

NNFL: Non-nonalcoholic steatohepatitis fatty liver; NASH: Nonalcoholic steatohepatitis; HDL: High-density lipoproteins; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: Gamma-glutamyl transpeptidase.

Table 2 Multivariate regression analysis for the factors influencing the development of nonalcoholic steatohepatitis

Model	Unstandardized coefficients		Standardized coefficients	*t*	Sig.
	B	SE	Beta		
Constant	1.247	0.517		2.411	0.018
BMI	0.014	0.018	0.124	0.780	0.438
Diabetes	0.260	0.125	0.227	2.084	0.040
Serum triglyceride	0.000	0.000	-0.105	-0.919	0.361
GGT	0.005	0.002	0.289	2.473	0.015
Waist circumference	-0.004	0.008	-0.077	-0.491	0.624

BMI: Body mass index; GGT: Gamma-glutamyl transpeptidase; Sig.: Significance.

tients presented stage Ⅳ fibrosis.

According to the NAS scoring system, NASH was absent in 10 (5.6%) cases, borderline in 92 (52.6%) cases and positive in 75 (42.4%) cases. Consequently, NNFL was present in 102 (57.6%) of the cases, and NASH was present in 75 (42.4%) of the cases.

Factors leading to NASH

The prevalence of NASH in NAFLD was 75 (42.4%). There were no significant differences of age, BMI, waist circumference, serum HDL and triglyceride level, or insulin resistance index. Sex, hypertension, and metabolic syndrome did not exert influences on the development of NASH. The mean age, BMI and waist circumference were similar in NNFL and NASH patients. The mean triglycerides were higher in the NASH cases, and the mean HDL was lower in the NASH cases, but this difference was not significant. The presence of diabetes could significantly (P = 0.001) differentiate NASH from NNFL. The serum ALT and AST levels could not be used to distinguish NASH from NAFLD. However, the serum GGT level in the NASH cases was significantly (P = 0.05) higher than that in the NNFL cases (Table 1). The GGT level for the NASH patients was 51.7 ± 32.8 U/L, and it was 40.4 ± 22.6 U/L for the NNFL patients. Multivariate regression analysis was also used to explore whether the presence of diabetes could influence the development of NASH (P = 0.04) and whether GGT could differentiate NASH from NNFL (P = 0.01) (Table 2). However, the area under the curve was 59.3% for GGT to differentiate NASH, with a sensitivity of 45% and a specificity of only 68% at 44.5 U/L (Figure 1).

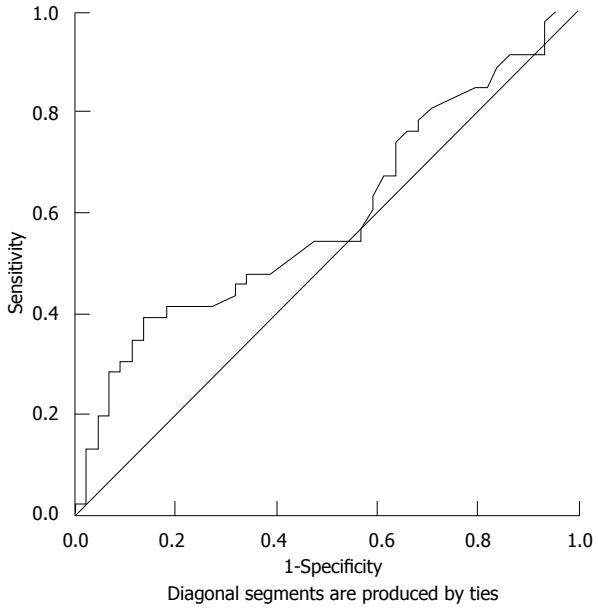

Figure 1 Receiver operating characteristic curve for gamma-glutamyl transpeptidase to differentiate nonalcoholic steatohepatitis from non-nonalcoholic steatohepatitis fatty liver.

DISCUSSION

This study is the largest series from Bangladesh in NAFLD. Reports of biopsy-confirmed NASH and NNFL are also rare. The university hospital is a tertiary care "center of excellence" hospital, and the patients are referred from all across the country. Consequently, this study may be representative of the prevalence of NASH in NAFLD throughout Bangladesh. The population-based prevalence of NAFLD had not previously been examined in Bangladesh. Most of our NAFLD patients were between 30 and 50 years of age; this result is similar to that of several reports from Asia[6,19,20]. However, age did not influence the development of NASH. The female preponderance in NAFLD conflicts with reports from developed counties. Many recent studies have reported that males are at a higher risk for fatty liver disease[21]. For example, in a study of 26527 subjects undergoing medical checkups, the prevalence of NAFLD was 31% in men and 16%

in women[22]. The female preponderance (250, 57.0%) observed in our study may be the result of the socially conservative attitude that led many of the women in our study to remain at home to attend to household activities without a job, leading to a sedentary life style. A similar female preponderance was observed in a population study from India[23]. However, in accordance with previous studies, sex did not influence the development of NASH from NAFLD.

Central obesity was observed in 171 (96.5%) of the patients, which was more common than overall obesity. The prevalence of NAFLD was higher following increases in BMI or abdominal circumference, according to a report from Japan[24]. However, other reports concluded that waist circumference served as an independent predictor of the advanced histological changes in NAFLD, rather than BMI[25,26]. However, waist circumference was similar between the NASH and NNFL cases in our series. This result could be explained by the fact that waist circumference indicates visceral obesity but has no influence on the pathogenesis of NASH at the stage of the 2nd hit. Hypertriglyceridemia was very common (130, 73.9%) in this study, but there was no difference between the NASH and NNFL patients. TG has long been considered to be a major factor in the development of NAFLD[5-8], but there is mounting evidence that these non-TG lipid molecules are implicated in the pathogenesis of NASH via the process of lipotoxicity. Conversely, the formation of TG may actually be a cytoprotective mechanism in the liver[27,28]. Our study observed that the prevalence of NASH was 42.4% (75 cases) among NAFLD patients, which is high. This rate is alarming for a country such as Bangladesh. This issue has been neither addressed previously nor considered by other studies. In a previous review, NAFLD was highly prevalent (15%-45%) in modern societies, but only 10%-25% of cases developed NASH, hepatic fibrosis leading to cirrhosis, end-stage liver disease or hepatocellular carcinoma[29]. In other studies, the prevalence of NASH was 10%-30% in NAFLD[30] and was less in Asian populations than in European populations[31,32]. We were unbiased in selecting patients to undergo liver biopsy, and the choice was made irrespective of the clinical, biochemical and anthropometric status of the study population. Consequently, these results can be considered to be representative of the prevailing conditions in Bangladesh society. This finding warrants further extensive study on the prevalence of NASH in Bangladesh and indicates that the awareness of the clinician is essential to diagnose NASH and to offer advice regarding possible interventions as early as possible.

The presence of diabetes indicated the presence of NASH in our study population ($P = 0.001$). Metabolic syndrome was observed in 188 (42.9%) of the patients. NAFLD is strongly associated with insulin resistance (IR) and other components of the metabolic syndrome, such as type-2 diabetes mellitus, central obesity, hyperlipidemia, and hypertension[33]. The pathogenesis of NASH appears to be a multi-factor process. The initial insult is the development of macrovesicular steatosis with the accumulation of hepatic fat from decreased hepatic free fatty acid oxidation and/or increased hepatic de novo lipogenesis and/or decreased lipid export from the liver. Although IR can contribute to this dysregulation of lipid metabolism, once fatty liver develops, it can worsen hepatic IR and diabetes, contributing to a vicious cycle[34].

Serum ALT and AST levels were similar in the NASH and NNFL patients in this study. However, GGT was significantly higher ($P = 0.05$) in the NASH cases than in the NNFL cases. NASH has been associated with a slight elevation of liver enzymes, mostly ALT[35]. In other reports, NAFLD patients typically present with asymptomatic serum aminotransferase levels that are 2-3 times higher than normal[36]. This difference was due to variations in the selection criteria. GGT is a sensitive indicator of liver damage[37]. An excess deposition of fat in the liver is associated with an elevated serum GGT[38]. Recent reports suggest that an increased GGT level is a risk factor for advanced fibrosis in NAFLD, and in combination with weight loss, a decrease in GGT activity is predictive of improved lobular inflammation and fibrosis of the liver[39].

The limitation of this study was that it was not performed at the community level but rather at the tertiary level hospital in the country.

In conclusion, females were the predominant sufferers of NAFLD in Bangladesh. The prevalence of NASH was high in NAFLD patients. Diabetes was the main contributor to the development of NASH in NAFLD cases. GGT was the only biochemical predictor of NASH, but it suffered from a low sensitivity and specificity. We recommend liver biopsy in NAFLD patients with diabetes and increased GGT.

COMMENTS

Background

Nonalcoholic fatty liver disease (NAFLD) is the most common liver disease, and it encompasses a histological spectrum that ranges from simple steatosis to hepatic steatosis with a necroinflammatory component, as well as nonalcoholic steatohepatitis (NASH) that may progress to cirrhosis.

Research frontiers

The area of research was the prevalence of NASH and the clinical and biochemical predictors to detect NASH in fatty liver.

Innovations and breakthroughs

Transaminases are minimally elevated in NASH, but the reports are conflicting. It was previously thought that fatty liver was a consequence of obesity. However, many normal weight people are suffering from the disease, especially in Asia.

Applications

Females were the predominant sufferers of NAFLD in Bangladesh. The prevalence of NASH was high. Diabetes was mainly associated with NASH. Gamma-glutamyl transpeptidase (GGT) was the only biochemical predictor of NASH, but it showed a low sensitivity and specificity.

Peer review

The authors offer the first report on a hospital-based cohort of NAFLD patients from Bangladesh. The cohort consists of 493 patients with an echogram-based diagnosis of fatty liver. The authors provide further analysis on 177 patients who underwent liver biopsy. The authors find that more females were affected than males, which NASH was associated with diabetes, and that GGT was significantly higher in the NASH patients.

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CASE REPORT

Primary biliary cirrhosis and hereditary hemorrhagic telangiectasia: When two rare diseases coexist

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Core tip: This case report shows the coexistence of two rare diseases, primary biliary cirrhosis and hemorrhagic hereditary telangiectasia, in a single patient. We think that this case would be worthwhile to publish because this is the first manuscript, to our knowledge, in which the coexistence of these two rare diseases has been reported. In this setting, the relevance of an accurate medical history, the role of liver histology and the characterization of liver involvement through dynamic imaging techniques can be emphasized.

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Abstract

Primary biliary cirrhosis is a slowly progressive cholestatic autoimmune liver disease that mainly affects middle-aged women with an estimated prevalence ranging from 6.7 to 402 cases per million. Hereditary hemorrhagic telangiectasia, or Rendu-Osler-Weber disease, is an autosomal dominant disorder characterized by angiodysplastic lesions (telangiectases and arteriovenous malformations) that can affect many organs, including liver, with a prevalence of 1-2 cases per 10000. We describe the coexistence, for the first time to our knowledge, of these two rare diseases in a 50-year old Caucasian woman. In this setting, the relevance of an accurate medical history, the role of liver histology and the characterization of liver involvement through dynamic imaging techniques can be emphasized.

Key words: Primary biliary cirrhosis; Hereditary hemorragic telangiectasia; Focal nodular hyperplasia; Ursodeoxycholic acid; Immunostaining

INTRODUCTION

Primary biliary cirrhosis (PBC) is a slowly progressive cholestatic autoimmune liver disease that mainly affects middle-aged women with an estimated prevalence ranging from 6.7 to 402 cases per million[1]. Hereditary hemorrhagic telangiectasia (HHT), or Rendu-Osler-Weber disease, is an autosomal dominant disorder characterized by angiodysplastic lesions (telangiectases and arteriovenous malformations) that can affect many organs, including liver, with a prevalence of 1-2 cases per 10000[2]. This condition may lead to portal hypertension due to the presence of intrahepatic shunts between hepatic artery and portal vein[3], and to ischemic lesions of the biliary ducts causing the development of strictures and/or dilatation[4,5]. Liver function tests (LFTs) abnormalities commonly observed in HHT are elevation of alkaline phosphatase (ALP) and gamma glutamyl transpeptidase (GGT)[6], thus resembling those observed in cholestatic liver diseases such as PBC. We describe the coexistence of these two rare diseases in a 50-year old Caucasian woman.

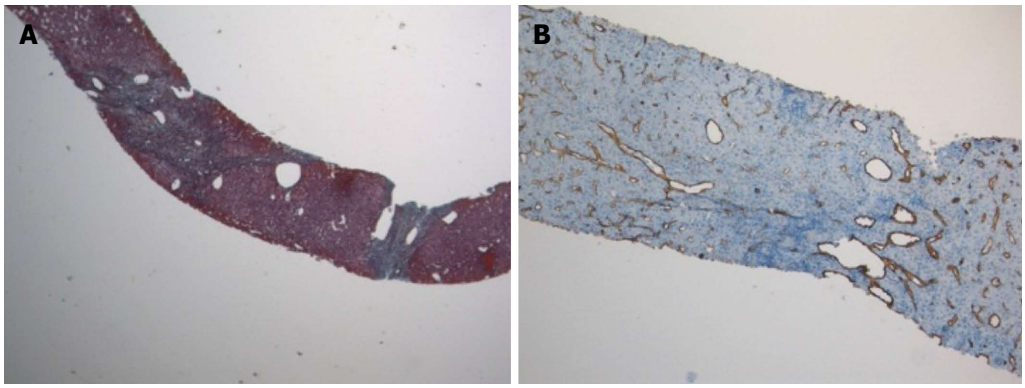

Figure 1 Focal nodular hyperplasia. A: Fibrous septa (green) giving a nodular appearance to liver parenchyma. Masson's trichrome staining; original magnification × 40; B: Diffuse CD34 positive immunostaining in the sinusoids; original magnification × 100.

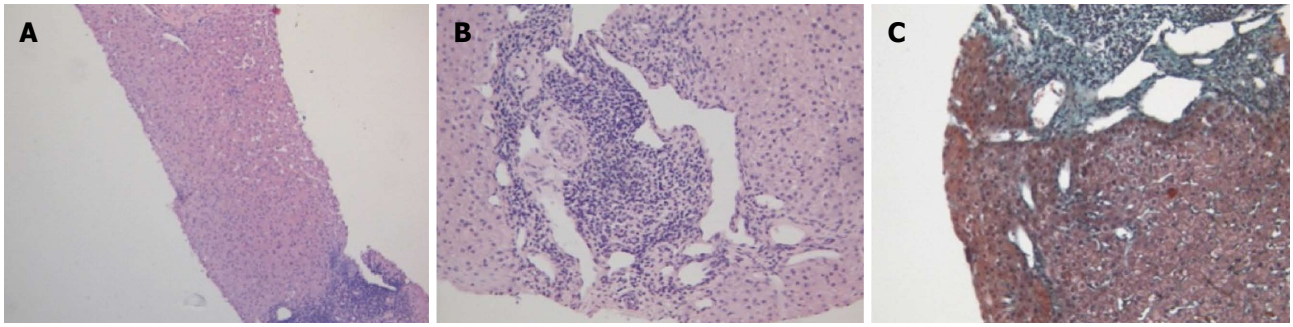

Figure 2 Hereditary hemorrhagic telangiectasia. Liver parenchyma, devoid of fibrous intra-parenchimal septa, with widespread angiectases in portal and peri-portal areas. A: Hematoxylin-eosin staining; original magnification × 100; B: Hematoxylin-eosin staining; original magnification × 200; C: Masson's trichrome staining; original magnification × 400.

CASE REPORT

A 50-year-old Caucasian woman with PBC stage I according to Scheuer's classification [antimitochondrial antibodies (AMA) type M2 positivity, titer 1:320, and histological diagnosis five years earlier] was admitted to our Unit in September 2011 for the evaluation of a hypoechoic focal liver lesion (segment Ⅶ, longest diameter 20 mm) detected with routine abdominal ultrasound.

At the time of PBC diagnosis, GGT and ALP levels were approximately five times the upper limit of normal, while other LFTs were normal. Subsequent therapy with ursodeoxycholic acid (UDCA, 15 mg/kg per day) had obtained a rapid reduction, up to normalization, of cholestatic liver enzymes. Furthermore, a thorough medical history revealed recurrent epistaxis and two episodes of gastrointestinal bleeding with negativity of both esofagogastroduodenoscopy and colonoscopy in 2003 and 2004. The causes of death of her parents were unknown.

Physical examination upon admission was unremarkable, except for two clearly visible telangiectasias, one on the lower lip and the other one on the tongue. All routine laboratory tests, including LFTs, were normal. Non-organ specific autoantibodies evaluation (tested by indirect immunofluorescence for antinuclear antibodies, AMA, smooth muscle antigens and by immunoblotting for AMA type M2, type I liver-kidney microsomes, lebercytosol 1, soluble liver antigen/liver-pancreas antigen) confirmed AMA (title 1:1280) and AMA type M2 positivity. Esofagogastroduodenoscopy and colonoscopy did not show any lesion. Multiphasic contrast-enhanced helical computed tomography (CT) scans of the liver revealed multiple focal hypervascular areas (the greater in segment Ⅶ) without a fully exhaustive radiological characterization. Ultrasonically guided fine needle biopsy of segment Ⅶ focal liver lesion showed fibrous septa intersecting the parenchyma, giving an appearance of nodular liver. Staining the fibrous septa in green through Masson's trichrome, the nodular architecture was highlighted (Figure 1A). In addition, CD34 immunohistochemical assay showed diffuse CD34 positive staining in the sinusoids (Figure 1B). These features were limited to the hypoechoic focal liver lesion, in keeping with a diagnosis of focal nodular hyperplasia (FNH). Biopsy of the adjacent liver parenchyma was devoid of fibrous intra-parenchimal septa but showed widespread vascular ectasias in portal and peri-portal areas (Figure 2). Furthermore, an intra-portal granuloma, portal immunoglobulin M (IgM) lymphoplasmacytic infiltrate (polyclonal anti-IgM; Novocastra, Newcastle, United Kingdom), bile duct damage and absence of cytokeratin 7 positive bile ducts in some portal tracts were evidenced both in the focal lesion and in the adjacent parenchyma (Figure 3). These features were all consistent with the previous diagnosis of PBC. No

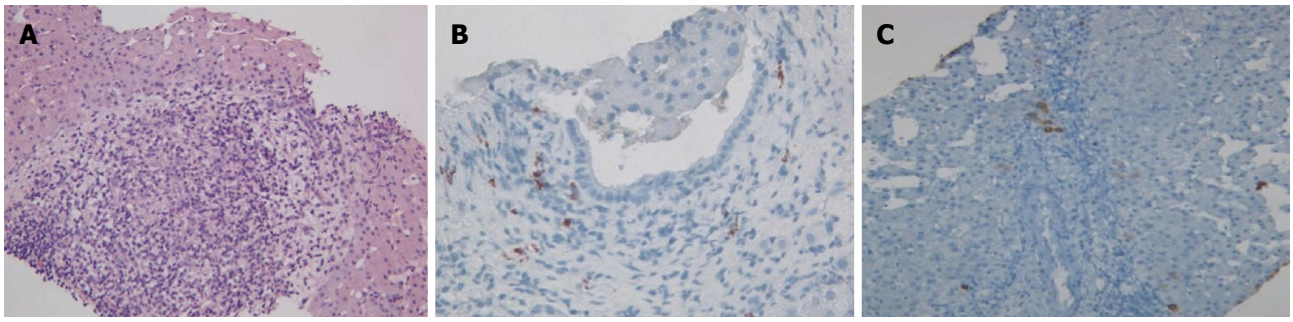

Figure 3 Primary biliary cirrhosis. A: Intra-portal granuloma. Hematoxylin-eosin staining; original magnification × 200; B: Portal immunoglobulin M (IgM) lymphoplasmacytic infiltrate and bile duct damage. IgM immunostaining; original magnification × 400; C: Absence of cytokeratin 7 (CK7) positive bile ducts in some portal tracts. CK7 immunostaining; original magnification × 200.

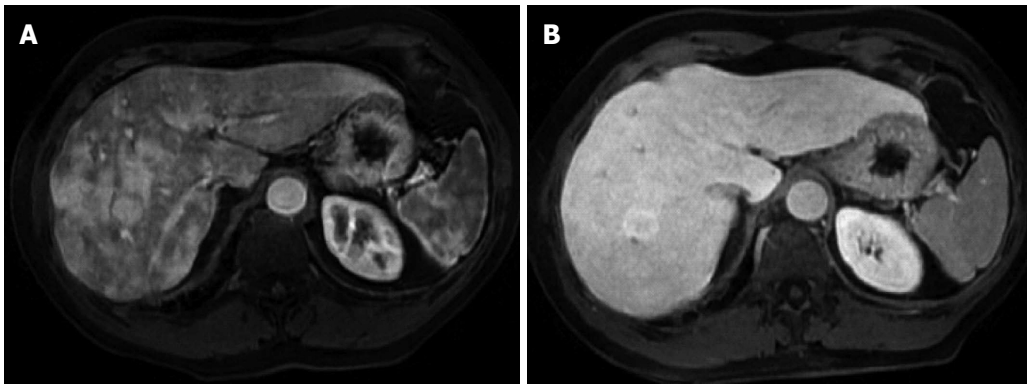

Figure 4 Magnetic resonance. A: Fat saturation gradient echo T1-weighted hepatic arterial phase magnetic resonance (MR) shows multiple ill defined hypervascular areas in the the right liver lobe, due to arterovenous shunts, and a round, clearly visible, 3 cm lesion surrounded by a hypointense halo; B: On hepatobiliary phase MR, arteriovenous shunt are no longer visible, while lesion, proven to be focal nodular hyperplasia at core biopsy, is hyperintense in comparison to surrounding liver parenchyma.

complications occurred after the procedure. Afterwards, multiphasic abdominal magnetic resonance (MR) showed hypervascular areas mainly in the right liver lobe, due to arteriovenous shunts, a clearly visible 3 cm lesion consistent with FNH and enlarged hepatic artery and hepatic veins (Figure 4). MR cholangiography did not show any alteration of the biliary tract. Finally, a CT angiography of the chest was negative for pulmonary arteriovenous malformations.

UDCA therapy was continued at the same dosage. At the last follow-up visit (January 2012) the patient was still asymptomatic, and all LFTs were persistently normal.

DISCUSSION

PBC is a rare chronic autoimmune disease whose diagnosis is mainly based on AMA type M2 positivity and liver histology; it primarily affects middle-aged women with a prevalence of less than 1/2000[7]. HHT is an autosomal dominant disease characterized by angiodysplastic lesions that can affect many organs, including liver, with an estimated prevalence of 1/5000[2].

The so-called Curaçao criteria (Table 1) are widely used in clinical practice for the diagnosis of HHT[8]. In our case, HHT diagnosis may be labeled as "definite", because at least three out of four criteria (epistaxis, telangiectases and visceral involvement) were present, while the fourth criterion (family history) was not applicable.

Table 1 Curaçao criteria for clinical diagnosis of hereditary hemorrhagic telangiectasia

Criteria	Description
Epistaxis	Spontaneous and recurrent
Telangiectases	Multiple, at characteristic sites: lips, oral cavity, fingers, nose
Visceral lesions	Gastrointestinal telangiectasias, pulmonary, hepatic, cerebral or spinal arteriovenous malformations
Family history	A first-degree relative with hereditary hemorrhagic telangiectasia according to these criteria

The prevalence of hepatic involvement in HHT has been estimated to range between 32% and 72% by ultrasonography or CT scan[4], even if about 90% of patients are reported to be asymptomatic. High output heart failure is the most common manifestation[3], even if our patient did not show any sign or symptom of cardiovascular disfunction nor abdominal angina, which may rarely occur due to mesenteric arterial steal by vascular liver malformations[6]. Furthermore, ischemic lesions of the biliary tree may cause the development of strictures and/or dilatation[9], but MR-colangiography was negative. LFTs abnormalities commonly observed in HHT

patients are elevation of ALP and GGT[6], which were normal on admission but elevated before the PBC diagnosis and the subsequent introduction of UDCA therapy five years before. Thus, overall clinical picture could be related to the coexistence of HHT and PBC, whose diagnosis was stated again by AMA type M2 positivity and liver histology. In fact, even if bile duct abnormalities and ductopenia may also be secondary to ischemic bile duct injury due to HHT liver involvement[10], the presence of the portal granuloma and the increase of IgM plasma cell number in the portal tract confirmed a definitive diagnosis of PBC[11].

Consequently, even if generally not recommended in a clinical setting of HTT due to a theoretical risk of bleeding[12], liver biopsy was decisive to confirm the diagnosis of PBC.

Finally, the prevalence of FNH in patients with HHT is 100-fold greater than general population[13]. In our case, FNH lesion showed a CD34 immunostaining pattern resembling the one usually observed in hepatocellular carcinoma. Nevertheless, the presence of fibrous septa, highlighted by Masson's trichrome staining, and the absence of cell atypias led to a conclusive diagnosis of FNH, which, as previously reported[14], can show the above mentioned CD34 immunohistochemical pattern. Its histological finding raised the suspicion of HHT liver involvement, which was furtherly confirmed by RM detection of the typical liver vascular abnormalities of HHT and by histologically evident portal and peri-portal angiectases.

To our knowledge, this is the first case in which the coexistence of PBC and HHT has been reported. In this setting, the relevance of an accurate medical history, the role of liver histology and the characterization of liver involvement through dynamic imaging techniques can be emphasized.

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CASE REPORT

A caval homograft for Budd-Chiari syndrome due to inferior vena cava obstruction

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Abstract

Transjugular intrahepatic portosystemic shunt (TIPS) is the standard treatment of Budd-Chiari syndrome (BCS) non responsive to medical therapy. However, patients with inferior vena cava (IVC) obstruction proximal to the atrium do not benefit from TIPS and a surgical approach is mandatory. We report the case of BCS due to intrapericardial IVC obstruction. We describe a novel surgical approach using a fresh caval homograft. An attempt to balloon dilatation of the IVC obstruction was complicated by right atrial disruption with tamponade and ventricular fibrillation. Lately, the patient successfully underwent a reconstruction of the cavo-atrial continuity by the interposition of a fresh caval homograft, a novel surgical approach never described before for BCS. Further follow-up revealed progressive reduction and resolution of ascites, and overall clinical improvement. IVC obstruction near to the atrium can be surgically approached with a new technique consisting in inferior vena cava resection and replacement with a caval homograft.

Key words: Budd-Chiari syndrome; Inferior vena cava; Occlusion; Surgery; Liver transplantation

Core tip: We describe a novel surgical approach using a fresh caval homograft for inferior vena cava (IVC) obstruction proximal to the atrium. An attempt to balloon dilatation of the IVC obstruction was complicated by right atrial disruption with tamponade and ventricular fibrillation. Lately, the patient successfully underwent a reconstruction of the cavo-atrial continuity by the interposition of a fresh caval homograft, a novel surgical approach never described before for Budd-Chiari syndrome. Further follow-up revealed progressive reduction and resolution of ascites, and overall clinical improvement.

Mancuso A, Martinelli L, De Carlis L, Rampolli AG, Magenta G, Cannata A, Belli LS. A caval homograft for Budd-Chiari syndrome due to inferior vena cava obstruction. *World J Hepatol* 2013; 5(5): 292-295 Available from: URL: http://www.wjgnet.com/1948-5182/full/v5/i5/292.htm DOI: http://dx.doi.org/10.4254/wjh.v5.i5.292

INTRODUCTION

The management of Budd-Chiari syndrome (BCS) non

responsive to medical therapy relies on the possibility of further interventional [angioplasty/stenting or transjugular intrahepatic portosystemic shunt (TIPS)] or surgical (shunts or liver transplantation) approaches[1,2]. However, apart from liver transplantation, surgical approach is performed in a minority of patients[3]. This is probably the consequence of the absence of a well established surgical strategy for BCS, the huge difference in the outcome reported after surgery and the good response homogeneously reported after interventional approaches[4-13]. In fact, TIPS has recently become the standard treatment of BCS non responsive to medical therapy[3]. However, a subgroup of patients with BCS, namely those with inferior vena cava (IVC) obstruction proximal to the atrium, do not benefit from TIPS and a surgical approach is mandatory. The combination of side-to-side portacaval shunt (SSPCS) and cavoatrial shunt seems to be the most reliable surgical technique according to a single centre experience[6].

Herein, we report the case of a patient with intrapericardial inferior vena cava obstruction successfully treated with the reconstruction of the cavo-atrial continuity by the interposition of a fresh caval homograft.

CASE REPORT

A 47 year old man was referred to our centre for ascites and radiological evidence of IVC obstruction.

Due to a Ⅲ degree congenital atrio-ventricular block, a bicameral pacemaker (PM) with epicardial leads had been positioned at the age of 2 years old. Later on, many endocardial leads were implanted and, when he was referred to our centre for ascites, he had 5 catheters in place: two inactive right ventricle (RV) leads trough the left subclavian vein (one since 1980 and one since 1997) and three active leads trough the right subclavian vein (a bipolar atrial catheter since 2010, a RV bipolar catheter since 2010 and a monopolar catheter in the coronary sinus for left ventricle pacing; the 3 active catheters were linked to a ABIV PM).

At admission, examination revealed ascites and leg swelling. Biochemistry revealed hemoglobin 11.1 g/dL, platelets 173000/mm^3, albumin 4.2 g/dL, normal aspartate aminotransferase/alanine aminotransferase, only slightly increased alkaline phosphatase and gamma-glutamyltransferase, bilirubin 1.8 mg/dL, international normalised ratio 1.3. Splenomegaly was evident clinically and at ultrasound (14 cm). A contrast-enhanced computed tomography confirmed a complete IVC occlusion at the level of the connection with the right atrium. At that level, one of the inactive leads formed a loop with presence of evident ingrowth of fibrotic tissue. The angiography confirmed the occlusion of the IVC with an extension of 2 cm.

In November 2011, in the cardiac hybrid suite, an attempt to balloon dilatation of the IVC obstruction was performed but was complicated by right atrial disruption with tamponade and ventricular fibrillation. An emergency sternotomy was performed and the atrial tear was repaired with a running suture without the institution of the extracorporeal circulation. The patient was then admitted to the intensive care unit and slowly recovered despite progressive worsening of ascites and leg swelling.

In December 2011, an elective surgical treatment was planned. A jugulo-pubic incision with median sternotomy was performed. Hepatic hilus was prepared and through a phrenic incision the junction between the inferior vena cava and the right atrium was thoroughly isolated. The extracorporeal circulation was instituted between aorta, superior vena cava and right femoral vein. On beating heart, the right atrial wall was opened in the site of the previous tear and all the leads were removed. The obstruction of the vena cava was due to the excess of fibrous tissue around one of the old inactive electrodes which migrated deeply into the vein. All the scarred tissue was removed and the hepatic vena cava was opened free with the supra-hepatic veins widely pervious. Then the liver and the right atrium were connected with a segment of vena cava harvested from a cadaveric multi-organ donor and preserved in electrolytic solution. The extracorporeal circulation was interrupted and the inferior caval flow restored. Three new epicardial electro-catheters were implanted, respectively, one on the left ventricle, one on the RV and one in the right atrium. Few days after surgery ascites and swelling started to resolve and the patient was discharged on day 12 with low dose of diuretics that were subsequently stopped.

Histological examination of the liver biopsy performed during surgery confirmed a BCS picture and revealed severe centro-central bridge and sinusoidal fibrosis.

At the programmed examinations, respectively three, six and thirteen months after surgery, the patient was well, with no signs of inferior venous congestion and normal liver biochemistry. Moreover doppler echography revealed a normal flow from IVC and right atrium.

DISCUSSION

BCS is a rare and serious disease with a generally worsening outcome without intervention. Although definition of response to treatment is a debated topic, when medical treatment alone is not sufficient to prevent progression, further treatments are needed[1,13].

It is widely accepted that the management of BCS should follow a step by step strategy. In fact, medical therapy is the first-line treatment, angioplasty/stenting or TIPS the further step and liver transplantation (LTx) the last chance[1,2].

Apart from LTx, surgical treatment is generally not contemplated in the BCS management by recent guidelines[1,2]. In fact, the most used treatment for BCS non responsive to medical therapy is TIPS, LTx is used as a rescue therapy, while surgical treatments are limited to a strict minority of patients[3].

TIPS is surely the mostly used treatment for BCS when medical therapy fails[3]. In early experiences, TIPS has proved effective as BCS treatment, also in the technically difficult case of extension of thrombosis to the por-

tal vein tree[10,11]. Recently, a multi-centre study provides long-term data on TIPS treatment for 147 BCS with a 10 year survival of 69%[12]. A less used radiological treatment is angioplasty/stenting[8,9].

Surgical shunts for BCS can be very successful, but have been associated with rapid decompensation and in-hospital mortality can be high (about 25%), primarily due to the patients' poor general condition[4-8]. In the past years, a surgical approach has been traditionally considered the first choice. In some series, an excellent outcome with long-term follow-up has been reported (95% survival, 3 to 28-year follow-up)[6]. However, in this series, the SSPCS was used, an approach that cannot be used when there is IVC thrombosis or significant compression. In the same series, the mortality rates of patients with IVC involvement were very high after traditional surgery (mesoatrial shunts) and better results were described with another technique (SSPCS + cavoatrial shunt) in 18 patients, all survived after a follow-up of 5-25 years[6]. However, outcome after surgical portosystemic shunt is variable and worse results were reported by others[4,5,7,8]. In most of the above series, patients with liver failure were not considered for surgery but for liver transplantation[4-8].

LTx is the last chance for BCS syndrome non responsive to either medical therapy and re-canalization/decompression. A multi-centre study reported a 10 year survival of 68%[14].

Patients with BCS due to a IVC obstruction near to the atrium constitute a difficult to treat subgroup of BCS patients, whose management can benefit from endovascular dilatation/stenting or surgical treatment.

Because of obvious reasons, TIPS does not by-pass IVC obstruction, resulting ineffective. Although some study describe the possibility of endovascular management as safe and with good long-term patency, data are scanty and need confirmation on a larger scale[15].

The patient we describe underwent angiographic IVC obstruction balloon dilatation that was complicated with atrial laceration and pericardial tamponade , requiring immediate sternotomy, pericardiotomy, internal cardiac massage, pericardial blood drainage and atrial suture. Surgical approach to BCS due to a IVC obstruction near to the atrium relies on the possibility of surgical shunts or LTx . Although the long-term results of LTx for BCS are good, we believe that LTx should be the last change, since surgical correction avoid successive immunosuppression and the possibility of LTx complications[14].

The widest experience with a surgical shunt approach for BCS due to a IVC obstruction is that recently published, and the best experience was reported after a combination of SSPCS and cavo-atrial shunt: 18 patients, all survived after a follow-up of 5-25 years[6]. Other surgical techniques reported in that and other series either had unacceptable or non reported outcomes[4,5,7,8].

The case here reported had an excellent outcome after the replacement of the obstructed segment of the IVC with a caval homograft. This technique requires a strict collaboration with the hepatic and the cardiac team, with experience both in cardiac and hepatic transplantation, and the aid of the extracorporeal circulation. The key of success is to obtain a clear surgical field, with all the blood drained from the right atrium and from the liver, allowing a perfect control of the anastomotic site. Moreover the use of a fresh homograft facilitates immensely the surgical performance.

Acquired inferior vena cava obstruction near to the atrium can be surgically approached with a new technique consisting in IVC resection and replacement with a caval homograft.

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CASE REPORT

Gram-negative bacteria causing infective endocarditis: Rare cardiac complication after liver transplantation

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Abstract

Bacterial endocarditis is a rare complication amongst solid organ transplant recipients and is often linked to bacteremia. Majority of these recipients do not have underlying valvular heart disease or congenital valvular abnormalities. *Staphylococoocusaureus* and *Enterococcus* species are the most commonly isolated organisms. There are very few reports of gram-negative bacteria causing endocarditis in liver transplant recipients. We report a 51-year-old male, a liver transplant recipient, who developed bacterial endocarditis of the mitral valve due to extended spectrum of betalactamase producing strain of *Escherichia coli* and was managed successfully with antibiotics.

Key words: Bacteria; Infective endocarditis; Liver transplantation

Core tip: A pre-transplant cardiac assessment should include a careful evaluation for underlying valvular pathology. Bacterial endocarditis can however still occur in liver transplant recipients with normal cardiac valves. Gram negative bacteria though rare can be a causative agent for infective endocarditis. High index of suspicion for bacterial endocarditis is essential when investigating transplant recipients for fever of uncertain origin.

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INTRODUCTION

Infective endocarditis is a rare complication affecting solid-organ transplant recipients. Common isolates include *Staphylococoocusaureus* (*S. aureus*), *Enterococci* and *Aspergillus*. Gram negative bacilli causing bacterial endocarditis of mitral valve has hitherto not been reported. We report bacterial endocarditis of the mitral valve due to extended spectrum of betalactamase (ESBL) producing strain of *Escherichia coli* (*E. coli*) following a deceased donor liver transplantation.

CASE REPORT

A 51-year-old male, a hypertensive, underwent a deceased donor liver transplant (LT) for ethanol induced end-stage

liver disease. Pre-transplant cardiac work up included an echocardiogram (ECHO), adenosine stress SPECT study and coronary angiogram. ECHO showed a mild dilation of the left atrium, normal left ventricular systolic function with an ejection fraction of 65%. There was annular calcification of the mitral valve with extension into the posterior leaflet and a mild regurgitation. The aortic valve was sclerotic. Pulmonary artery systolic pressure was 40 mmHg with mild tricuspid regurgitation. Adenosine stress SPECT study revealed a small area of inducible ischemia in the left anterior descending artery but the coronary angiogram showed no significant disease in the epicardiac arteries. Right heart study showed normal pulmonary artery pressures.

Three weeks prior to LT, the patient had fever. Urine and blood cultures grew Escherichia coli and the patient was treated with appropriate antibiotics for 2 wk. Subsequent cultures of both blood and urine prior to LT were sterile. Donor and recipient cultures on the day of transplant were also sterile. Post-operative period was uneventful and he was discharged with good graft function after 3 wk. One month later, the patient attended the liver clinic with high-grade fever and chills. On examination, he was haemodynamically stable, conscious, oriented but febrile (temperature 39 degree Celsius). Cardiovascular system examination revealed a grade 3/6 pansystolic murmur at the mitral area. Other organ system examination was normal.

Investigations revealed hemoglobin of 7.2 gm/dL, total white cell count of 14400 cell/mm^3 with predominant neutrophils (86%), elevated ESR (92 mm/h) and C reactive protein of 289 mg/L. Serum creatinine was 1.8 mg/dL. Urine examination showed proteinuria and plenty of red blood cells. Blood and urine cultures were positive for ESBL producing strain of *E. coli*, which was sensitive to Meropenem, Tigecycline and Amikacin. ECHO showed multiple echogenic mobile masses on the mitral valve leaflet especially the posterior leaflet with moderate to severe grade of mitral regurgitation.

A diagnosis of infective endocarditis of the mitral valve due to ESBL *E. coli* was made. Patient was treated with meropenem and tigecycline for 6 wk though cultures were sterile a week after initiation of antibiotics. Follow-up ECHO at 8 wk showed mild mitral regurgitation with vegetations on the valve. Repeat ECHO after 48 wk showed mild mitral regurgitation without vegetations on mitral valve.

DISCUSSION

Bacterial infections are common in the post LT period and occur in 33%-68% of LT recipient[1]. In a recent study from our centre, *Klebsiella* and *E. coli* were the two common organisms responsible for post LT infection[2]. Common sources were the respiratory tract, urinary tract and blood stream infection[2].

Infective endocarditis after LT is rare. The prevalence of bacterial endocarditis among liver transplant recipients has been reported to be around 1.7%. Unlike in general population, bacterial endocarditis in transplant recipients can occur even in normal cardiac valves. The common causative agents for valvular endocarditis and mural endocarditis are *S.aureus* and *Aspergillus* respectively[3]. Other uncommon bacterial organisms causing infective endocarditits are *Enteroccocusfecalis*, ESBL producing *Klebsiellaterrigena* and *Propionibacterium acnes*[4,5]. *E.coli* causing infective endocarditis has been reported in pulmonary valve[6]. To our knowledge, this is the first report of ESBL producing *E. coli* induced mitral valve endocarditis.

Bacteria producing ESBL enzymes are resistant to most betalactam antibiotics such as penicillin and cephalosporins. ESBL is found exclusively in Gram negative organisms such as *Klebsiellapneumoniae*, *Klebsiellaoxytoca*, *E. coli* species and *Acinetobacterburkholderia*. Risk factors for infection with ESBL producing organisms include prolonged hospital or intensive care unit stay, prolonged mechanical ventilation, central venous or arterial catheters, bladder catheter, emergency abdominal surgery and prolonged exposure to antibiotics.

In conclusion, a high index of suspicion for endocarditis is essential, especially in the setting of new auscultation findings at cardiac valve areas. Multiple blood cultures are necessary to make the correct diagnosis. Initiating empirical broad-spectrum antibiotics at the earliest prior to getting culture sensitivity is important in LT recipients.

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Acknowledgments

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5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

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