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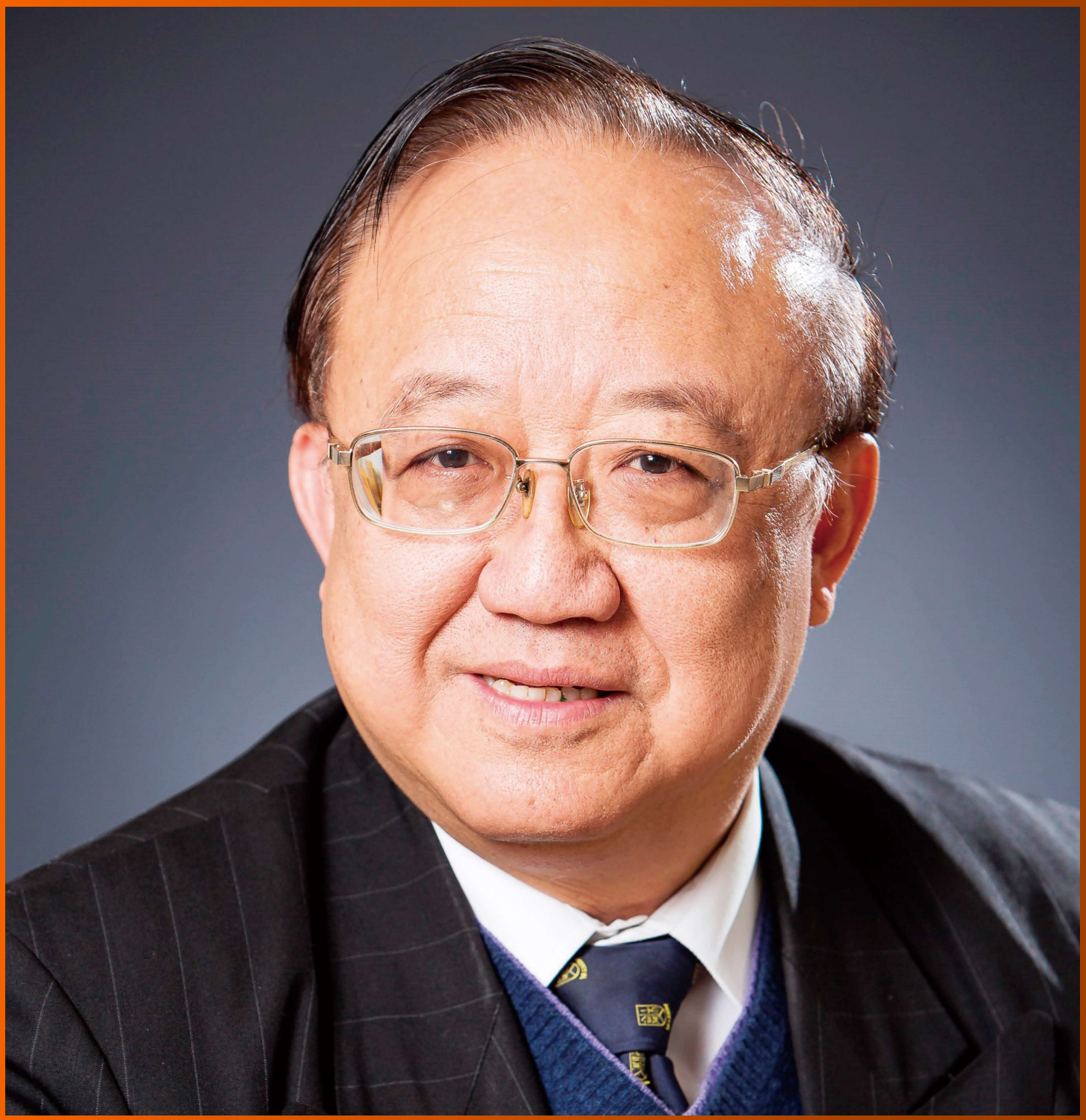

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Contents

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EDITORIAL

Wnt-/-β-catenin pathway signaling in human hepatocellular carcinoma

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Abstract

The molecular basis of the carcinogenesis of hepatocellular carcinoma (HCC) has not been adequately clarified, which negatively impacts the development of targeted therapy protocols for this overwhelming neoplasia. The aberrant activation of signaling in the HCC is primarily due to the deregulated expression of the components of the Wnt-/-β-catenin. This leads to the activation of β-catenin/T-cell factor-dependent target genes that control cell proliferation, cell cycle, apoptosis, and cell motility. The deregulation of the Wnt pathway is an early event in hepatocarcinogenesis. An aggressive phenotype was associated with HCC, since this pathway is implicated in the proliferation, migration, and invasiveness of cancer cells, regarding the cell's own survival. The disruption of the signaling cascade Wnt-/-β-catenin has shown anticancer properties in HCC's clinical evaluations of therapeutic molecules targeted for blocking the Wnt signaling pathway for the treatment of HCC, and it represents a promising perspective. The key to bringing this strategy in to clinical practice is to identify new molecules that would be effective only in tumor cells with aberrant signaling β-catenin.

Key words: Carcinoma; Hepatocellular; Wnt signaling pathway; Beta catenin; Wnt proteins; Receptors

Core tip: The Wnt signaling pathway is decisive in the rule of mechanisms of proliferation and survival, as well as the differentiation of liver cells during hepatic embryogenesis and morphogenesis. The atypical initiation of signaling in the hepatocellular carcinoma (HCC) is primarily because of deregulated expressions of the components of the Wnt-/-β-catenin. The mechanisms that are considered more functional and that sustain aberrant activation of signaling pathways act *via* alterations in the β-catenin gene or the *AXIN1/2*-gene's encoding axin, a protein necessary for the degradation of β-catenin. The development of targeted therapeutic molecules for the blockade of the Wnt-signaling pathway for the treatment of HCC depends on the identification

of molecules that would be effective only in tumor cells that carry an aberrant signaling β-catenin.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is distributed globally. It is the second most important cause of cancer deaths and caused approximately 750000 deaths in 2012[1].

Management options for HCC are restricted, and this neoplasm can just be cured by radical treatments, such as hepatectomy or liver transplantation, when the diagnosis is made while the tumor still has small proportions. However, the diagnosis is often made at a late stage in the development of HCC, when the cancer has already grown too much and/or is widespread. Moreover, malignant tumors also have significant resistance to multidisciplinary treatment protocols[2].

The increasing incidence of HCC triggered an intense phase of research to clarify the main molecular, genetic, and cellular mechanisms involved in its pathogenesis, which could encourage the development of more effective treatments for this neoplasia[3,4]. However, the molecular basis of the carcinogenesis of HCC still has not been adequately clarified[5,6], which impairs the development of targeted therapy protocols for this overwhelming neoplasia[2].

MOLECULAR PATHOGENESIS IMPORTANCE

Complex processes are involved in several steps within the molecular pathogenesis of HCC. The normal hepatocytes can have their phenotype transformed by an accumulation of aberrant genetic mutations or epigenetic nature, as well as by signaling the pathway's activation of growth factors[7-13].

In chronic hepatic disease, it is decisive to recognize the mitogenic signaling pathways that do not participate in liver regeneration. Also, the mitogenic signaling pathways are essential to trigger the emergence of a clonal expansion that promotes tumor growth. This identification can prevent the occurrence of adverse side effects on the eventual use of a target therapy for the steps involved in the carcinogenesis of HCC. However, non-transformed hepatocytes can show higher molecular redundancy and continue to proliferate, though some signaling pathways are inhibited. Moreover, due to the fact that the altered hepatocytes might provide incomplete terminated molecular mechanisms, they may block specific pathways that control cell growth and survival[5].

The signaling of those specific anomalies and inherited and epigenetic mechanisms related to risk features are specific cellular changes and are considered the center of initiation and development of HCC[5]. Due to the complex genetic heterogeneity of HCC, the investigation targets the molecular signaling pathways and their shared molecular mechanisms[4].

PATHOGENIC MECHANISMS

The main determinants' mechanisms of liver carcinogenesis are related to cirrhosis renewal subsequent to liver injury caused by hepatitis, contaminants, or metabolites and mutations in oncogenes (sole or multiple) or in neoplasm suppressor genes. These mechanisms are associated with the main changes in cell signaling pathways with interest from the therapeutic point of view because its lock can reverse, delay, or prevent hepatocarcinogenesis[14].

The atypical initiation of signaling in HCC is primarily related to a deregulated expression of the components of the Wnt-/-β-catenin. These activate β-catenin/TCF-dependent target genes that monitor cell proliferation, cell phase, apoptosis, and cell motility. The Wnt pathway is the input constituent of the physiological processes implicated in the embryonic progress and homeostasis of human tissues[3].

WNT SIGNALING PATHWAY

Although it is inactive in adult livers, the Wnt pathway participates in liver pathobiology[15]. This route is markedly decisive in the active surroundings of hepatic development and controls the progressions of proliferation, survival and the differentiation of hepatocytes. Abnormal initiation of this pathway has also been recognized in hepatoblastoma as well as in HCC[5].

Under normal conditions, the β-catenin level of a hepatocyte is lowered, because the complex activity destruction of the β-catenin protein, involving the APC, axin, and GSK-3 proteins, and the fact that it connects itself to β-catenin molecules, phosphorylating it, with consecutive deprivation in the proteasome[4]. Stimulation of the non-canonical Wnt-/-β-catenin pathway is started by the connecting of extracellular ligands of the transmembrane receptor's Wnt-/-FZD-related protein and low-density lipoprotein receptor, which afterward liquefies the complex configuration destruction of β-catenin proteins, which results in the increase of β-catenin in the cytoplasm[4]. The β-catenin proteins are able to displace to the nucleus and forming a binding complex with the transcription factor LEF-/-TCF proteins. This binding complex promotes an activation of target genes that regulate cell proliferation, migration, invasion, cell cycle progress, and metastasis propagation[4] (Figure 1). For that reason, the constitutive start of this pathway could possibly be significant for establishing and maintaining the malignant liver phenotype[4].

In liver carcinogenesis, early deregulation of the

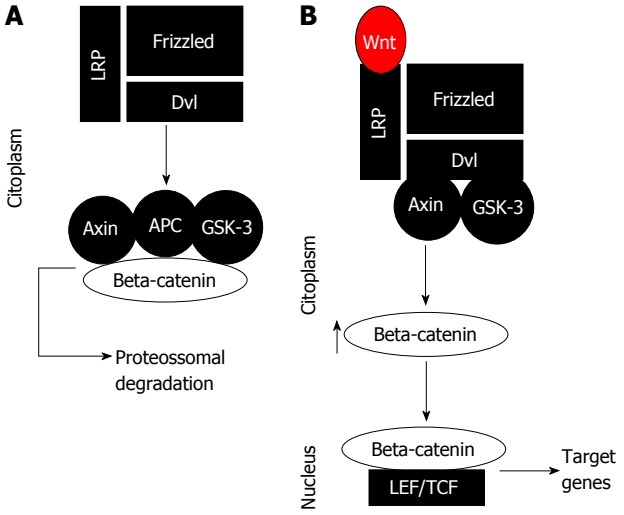

Figure 1 Wnt-/-β-catenin signaling pathway. A: APC protein, axin, and GSK-3 that forms the complex destruction of the β-catenin protein in the proteasome; B: Wnt binds to Frizzled and LRP receptors, dissolving the destruction-complex, which results in an increase of β-catenin in the cytoplasm and nucleus. β-catenin forms a binding complex with the transcription factor LEF-/-TCF proteins to promote the activation of target genes. LRP: Lipoprotein receptor; Dvl: Dishevelled; APC: Adenomatous polyposis coli; GSK3: Glycogen synthase kinase 3; LET/TCF: Lymphoid enhancer factor/T-cell factor.

Wnt pathway occurs. An aggressive phenotype was associated with HCC, since this pathway is implicated in the proliferation, migration, and invasiveness of cancer cells, within the course of the cell's survival[2].

The start of this pathway happens when a ligand connects to a Wnt receptor Frizzled (FZD) on the cell membrane. The routes identified in the Wnt signaling pathway are the non-canonical pathway and the canonical pathway, where β-catenin protein is involved[3].

The Wnt signaling pathway mediated by the β-catenin protein involves the binding of 1 or more of the 19 Wnt ligands to 1 or more of the FZD transmembrane cell surface receptors of the tumor cells. This stimulates the activation of the associated β-catenin canonical pathway and the non-canonical pathway in which the participation of c-Jun NH2-terminal kinase (Jnk) plus protein kinase C occurs, both of which are primarily active during embryogenesis and adult tissue homeostasis[3].

The mechanisms considered more functionally aberrant and the sustained initiation of the signaling pathway happen *via* the β-catenin mutations in the genes or AXIN1-/-2 axin genes encoding a protein essential for the degradation of β-catenin[5].

Kan *et al*[6] described the complete genome sequencing of 88 HCC, 81 of which are positive for the hepatitis B virus (HBV). The author identified genes with genetic modifications and signaling pathways involved in HCC related to HBV. They found the β-catenin gene (15.9%) and TP53 (35.2%), the oncogene and tumor suppressor gene, respectively, to be the most often mutated. The signaling pathway Wnt-/-β-catenin and Janus kinase protein (JAK)-/-STAT were changed from 62.5% to 45.5% of the patients, respectively, and were considered probable to perform as the two main oncogenic conductors in HCC. The mutation/activation of JAK 1 was found in 9.1% of patients[6].

The deregulated signaling cascade Wnt-/-β-catenin was observed in 95% of HCC[16]. Moreover, this route can likewise be initiated by deletions or mutations in the β-catenin gene, thus making non-degradable proteins by destruction complex. This event facilitates the increase of β-catenin protein in the cytoplasm. In turn, such a molecule translocates to the nucleus and activates genes related to cell growing[9]. Most mutations/deletions are located in the N-terminus of beta-catenin, thus leading to a change in beta-catenin protein turnover after failure of phosphorylation (GSK3, CK1). Thus, the overexpression of FZD Wnt ligands and receptors leads the activation of these ligands and receptors as the primary mechanism causing the increase of β-catenin protein in the cytoplasm and the displacement of β-catenin to the cell nucleus[4].

An important ligand that is involved is the Wnt3, which is normally overexpressed in CHC. After attaching to the FZD7, it triggers the canonical signaling triggered by hepatitis B and hepatitis C virus[17,18]. In this context, the interruption of the interaction involving the ligands and Wnt-/-FZD receptors was suggested as a mechanism of inhibition of Wnt signaling/β-catenin to reduce the migration and invasiveness of tumor cells of HCC[18].

Wnt ligands focus on the cell surface for linking with heparan sulfate proteoglycans; then, the Wnt ligand is unrestricted and able to interact with the FZD receptors to start the signaling pathway of β-catenin[4].

The mechanism further clarified that the mutations in the β-catenin gene or CTNNB1, which were observed in about 20% to 40% of all cases of HCC[19,20] concerned the activation of β-catenin in HCC. The mutations have also been reported in the constituents of the complex of β-catenin degradation, including AXIN1 gene mutation, which was observed in 3% to 16% of all cases of HCC[19,20] and the *AXIN2* gene and in approximately 3% of all cases of HCC[21]. Interestingly, HCC occurs in HCV patients, up to 40% of whom show an incidence of *CTNNB1* gene mutations[22]. Further, the HCV patients led to an increased expression of the gene Wnt1 in HCC cells due to mechanisms not yet completely understood[23]. Studies of HCC occurring in patients with HBV have implicated protein X of the HBV to stimulate the activation of β-catenin, representing an independent *CTNNB1* gene mutation[24]. Interestingly, the majority of the functionally important mutations that cause the activation of Wnt signaling are those that affect the *CTNNB1* gene and correlate significantly with the concentration of the nuclear β-catenin protein. The ultimate consequence of continued signaling pathway Wnt-/-β-catenin brings about an amplified expression of genes' β-catenin dependents, which influence the whole tumor[25,26].

Mutations of the β-catenin gene mostly have been described as late events in HCC[27], while other authors reported that these mutations are early events[28,29]. The tumors with the mutated β-catenin gene have been

reported as having less vascular invasion[30] and higher grades of cell differentiation[30,31]. These mutations have been associated with better prognoses for patients with HCC. However, other authors observed a higher nuclear and cytoplasmic concentration of the β-catenin protein in HCC with the most micro- and macro-vascular invasion[32,33], increased neoplastic cell proliferation, and poorly differentiated tumors[29].

Moreover, it is interesting that a diminutive but important number of patients with HCV developed HCC with no confirmation of fibrosis[34]. The HCV has a preference in the use of the Wnt pathway as an HCC development mechanism[35]. Similarly, it is worth noting that the diminutive number of hepatic adenomas that develop into HCC often have mutations in the gene for β-catenin[36]. The neoplastic conversion of hepatic adenomas in HCC usually takes place in the healthy liver without confirmation of fibrosis. Thus, this finding indicates the involvement of the mutation of the β-catenin gene, regardless of the presence of liver fibrosis[5].

CONCLUSION

The findings of the Wnt signaling pathway activity in HCC suggest that the activation of β-catenin is sometimes found in up to 90% of HCC. However, in 40% to 60% of HCC patients, mutation of the *CTNNB1* or *AXIN1*/*AXIN2* genes was not observed. Actually, this finding may reflect the significant participation of Wnt-/-β-catenin signaling pathway in the maintenance of the normal function of hepatocytes in liver parenchyma, even without the presence of neoplastic cells in its interior[5].

The interruption of the signaling cascade Wnt-/-β-catenin has shown antineoplastic activity in HCC, although therapeutic molecules are not currently blocking the Wnt signaling pathway for the treatment of HCC[2]. Still, proteins that are part of the Wnt signaling pathway are considered potential targets for pharmacological therapy[3,37]. However, the complexity of transcription - dependent mechanisms of β-catenin becomes the challenge of ambitious drug therapy. Moreover, such medicaments may have important side-effects in organs, such as the intestine, where the Wnt-/-β-catenin is significant for the regeneration of tissues. The key toward this strategy coming into clinical practice is to identify new molecules that would be effective only in tumor cells that carry an aberration that signals β-catenin.

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TOPIC HIGHLIGHT

2015 Advances in Liver Transplantation

Strategies to optimize the use of marginal donors in liver transplantation

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Abstract

Liver transplantation is the treatment of choice for end stage liver disease, but availability of liver grafts is still the main limitation to its wider use. Extended criteria donors (ECD) are considered not ideal for several reasons but their use has dramatically grown in the last decades in order to augment the donor liver pool. Due to improvement in surgical and medical strategies, results using grafts from these donors have become acceptable in terms of survival and complications; nevertheless a big debate still exists regarding their selection, discharge criteria and allocation policies. Many studies analyzed the use of these grafts from many points of view producing different or contradictory results so that accepted guidelines do not exist and the use of these grafts is still related to non-standardized policies changing from center to center. The aim of this review is to analyze every step of the donation-transplantation process emphasizing all those strategies, both clinical and experimental, that can optimize results using ECD.

Key words: Liver transplantation; Extended criteria donors; Marginal donors; Results; Survival

Core tip: This review analyzes the donation-transplantation process when using extended criteria donors. Every step, from donor selection to transplantation, is discussed emphasizing experimental and clinical strategies that can lead to optimize results.

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INTRODUCTION

Liver transplantation (LT) is the treatment of choice for patients with end stage liver disease. Due to improvement in surgical techniques, immunosuppressive strategies, and patient management, the number of candidates has dramatically grown in the last decades while the number of donors has remained stable. This gap has stimulated the development of innovative strategies to increase the donor pool. Currently, the ideal liver donor - younger than 40 years; trauma as the cause of death; donation after brain death; hemodynamic stability; without macrovescicular steatosis, infection(s) or chronic liver disease[1] - is less frequent due to demographic changes in the general population[2]. The concept of extended criteria donors (ECD) was introduced to indicate donors associated with a higher risk of primary non function (PNF) of the liver graft, delayed graft function (DGF), and a poorer prognosis after transplantation. Elderly donors (> 60 years), donors with malignancies, infections, macrovescicular steatosis > 30%, donors after cardiac death (DCD), hypernatremia, hemodynamic instability, prolonged cold ischemia time (CIT), split liver grafts, and living donor liver transplants (LDLT) are all included in this category[3-5].

Despite numerous studies, the impact of each donor variable on recipient outcome is still debated due to controversial results. Some authors reported that careful liver graft selection provides comparable results *vs* optimal donor grafts, and some recent studies confirm these findings[4-5]. Nevertheless, the reported results may be related to specific donor demographic characteristics (*i.e.*, healthier life styles) or to the experience of transplant teams with management of these donors[6]. The aim of the present review is to appraise all strategies that can be implemented in view of optimization of use of ECD in LT.

DONOR EVALUATION

Age

Old donors should be carefully evaluated as age is related to allograft failure and post-transplant death[7]. Nevertheless, the progressive aging of the population and the decreasing incidence of trauma-related deaths have made elderly donors a considerable resource in many countries. In our recent study the mean donor age was 70 years[5], and similar results were reported by the Spanish liver donor registry[8]. Old organs develop brown atrophy, show a decrease in weight and number of cells, thickening of endothelial cell lining, endothelial cell fenestrations, reduction of blood flow, reduced synthetic capacity resulting in a diminished response to external stressors and a limited regeneration rate[9-13]. Short term complications using these grafts include PNF - defined as an irreversible graft dysfunction requiring liver re-transplantation within 10 d - initial poor function (IPF) and vascular complications[14]. Long-term complications include reduced patient and graft survival, especially in HCV positive recipients, and ischemic type biliary lesions (ITBL)[14]. These grafts are extremely sensitive to hemodynamic instability, and an appropriate donor management is pivotal with adequate systemic blood (> 100 mmHg) and central venous pressures (> 10 cm H_2O), a hematocrit > 25%, normal body temperature, and diuresis greater than 1 mL/kg per hour in order to avoid hypoperfusion and low oxygen support to the liver graft[15]. A rapid procurement technique with minimal organ manipulation and double perfusion (aortic and portal) should be preferred[15]. In order to minimize the ischemia/reperfusion injury (I/R), CIT should be as short as possible[14,15]. Many series using graft older than 70 years showed optimal results when CIT is shorter than 8 h, whilst a CIT > 12 h is associated with a twofold risk of graft failure[16]. Thus, procurement in distant hospitals should be carefully evaluated and allocation to more stable patients who can better tolerate some degree of organ dysfunction should be warranted[16,17]. Older liver grafts are preferentially allocated to low biochemical model for end-stage (MELD) score patients and HCV-negative recipients with hepatocarcinoma[5,15].

Hemodynamic instability

Previous United Network for Organ Sharing (UNOS) data have shown that organs subjected to prolonged hypotension do not show any significant increase in post-transplant graft loss[17]. However, graft loss increased in transplants from donors receiving norepinephrine[17]. Some studies showed that dopamine dose > 10 μg/kg per minute[18], or 6 μg/kg per minute[19] had a significant impact on early graft function. Systemic blood pressure should be kept above 90-100 mmHg as low pressure is related to increased preservation injury[20]. The use of dopamine is indicated to increase the mesenteric and renal flows at doses of 2-5 μg/kg per minute. Higher doses can lead to renal impairment and a dopamine dose > 15 μg/kg per minute is considered a marginality criterion[21,22].

Hypernatremia

Hypernatremia is considered a risk factor for graft dysfunction, but the mechanism of hypernatremia-related injury to liver cells is not clear[23,24]. One hypothesis is that a sudden change in extracellular osmolality in a liver graft obtained from a hypernatremic donor might cause intracellular water accumulation and cell swelling[25]. However, high serum sodium concentrations may promote accumulation of osmoles within the liver allograft cells. Subsequent transplantation of these livers into recipients with normal serum sodium levels may promote intracellular water accumulation, hepatocyte lyses, and death[23]. Avolio *et al*[23] suggested that donor hypernatremia may adversely affect the outcome of LT and showed a direct correlation between the donor serum sodium concentrations and the recipient liver enzyme levels [aspartate aminotransferase (AST) and alanina aminotransferase (ALT)] after surgery[23]. González

et al[24] showed that donor hypernatremia correlates with hepatic allograft dysfunction, whilst Figueras *et al*[25] reported that donor hypernatremia is associated with high bilirubin levels post-operatively and graft loss within the first month post-transplantation. Totsuka *et al*[26] showed that both graft function and survival were improved by correction of donor hypernatremia and suggested that latent changes in hepatocytes induced by hypernatremia are reversible and might be attenuated by appropriate donor management. Recent studies have found that donor hypernatremia does not affect graft survival in liver and kidney transplantation[27].

Infections

Hepatitis B virus: In the presence of antibody to hepatitis B core antigen (anti-HBc) IgM-positivity or circulating hepatitis B virus (HBV)-DNA levels, some centers decline using these organs for donation. Anti-HBc IgG-positive donor grafts can be safely used, provided use of anti-HBV prophylaxis with oral antiviral agents in HBV naïve recipients[28-30]. The addition of anti-hepatitis B surface antigen immunoglobulin does not seem to provide superior protection rates *vs* oral antivirals alone[29].

In pediatric transplantation, organs from anti-HBc-positive donors are still used with caution after an individualized risk-to-benefit evaluation[28-30].

Hepatitis C virus: The use of hepatitis C virus (HCV)-positive donors for LT was originally debated and not widely practiced due to concerns about an increased risk of HCV-related graft failure after transplantation[31-34]. In the last decade, long-term follow-up data confirmed that use of HCV-positive donor grafts in HCV-positive recipients was safe and did not affect graft survival[31]. In this setting, post-transplant HCV recurrence rates were 55.54% *vs* 41.74% for recipients of HCV-negative grafts[32]. Patient and graft survival at 4 years post-transplantation are similar in recipients of either HCV-positive or HCV-negative liver grafts[32].

A recent UNOS-based study on 1695 HCV patients transplanted with HCV-positive grafts has confirmed no difference in patient and graft survival *vs* HCV-positive recipients transplanted with HCV-negative liver grafts[33]. An European, multicenter study has also shown similar overall patient and graft survival rates in this category of patients[34]. HCV recurrence was reported to be more rapid in the group of patients who received anti-HCV-positive grafts, although it did not reach statistical significance ($P = 0.07$)[34]. The authors suggested appropriate use of anti-HCV-positive donor grafts, especially if HCV-RNA is positive, as their use might be associated with more rapid fibrosis progression[34]. The recent introduction of direct antiviral agents for treatment of HCV infection will likely reshape this practice.

Malignancies

According to the UNOS database, 2.7% of deceased donors have a history of cancer[35]. Between 2000 and 2005, more than 800 LT procedures were performed using grafts from donors with a history of malignancy, and only two donors transmitted a fatal disease[35]. The most common cancers were non melanoma skin neoplasms followed by central nervous system malignancies[35].

Melanoma is one of the most commonly reported donor-derived malignancies and might have one of the highest transmission rates and associated mortality if inadvertently transmitted to the recipient. As its biological behavior is complex and characterized by late recurrences (tumor dormancy) donors with an history of malignant melanoma should always be discarded also in case of cured disease[36]. Donors with central nervous system malignancies should be carefully evaluated as certain risk factors are associated with malignancy transmission; organs from donors having high grade (Ⅲ or Ⅳ) tumors, ventriculo-systemic shunts or history of extensive cranial surgery that disrupts the blood-brain barrier are associated with a transmission rate of 45% and should not be considered for transplantation; in cases where the underlying etiology of brain death is unclear, a rapid limited brain autopsy should be conducted[37].

Data derived from the United Kingdom Transplant Registry showed that 18 solid organ recipients developed cancer from 16 donors (0.06%): 3 were donor-derived cancer (0.01%) and 15 were donor-transmitted cancer (0.05%)[38]. Of the 15 donor-transmitted cancers, 6 were renal; 5 were lung; 2 were lymphoma; 1 was neuroendocrine, and 1 colon cancer[38].

Some recent Italian series have shown no disease transmission with use of grafts from donors with low-grade malignancies or neoplasms of low metastatic potential[39,40]. An accurate donor evaluation coupled with histological information of tumor grade allows to reduce to acceptable rates the risk of donor-to-recipient transmission[39,40]. Donors with a documented history of malignancy should not discarded *per se*, especially for low-grade central nervous system tumors and malignancies treated successfully with long-term disease-free survival rates. However, there is still variability in guidelines and practices across countries[39,40].

Steatosis

Steatosis is a very common chronic liver disease and it is estimated to occur in more than 65% of obese patients[41]. Microvescicular steatosis is accumulation of small fatty droplets not displacing the cell nucleus, and even if diffuse it does not entail a higher risk for graft loss after LT[42]. Macrovescicular steatosis is characterized by large droplets displacing the nucleus to the cell periphery and is associated with a significant risk factor for PNF[42,43]. It can be classified based on the proportion of hepatocytes affected, being mild < 30%, moderate from 30% to 60%, and severe > 60%[43]. Most transplant centers do not use grafts with more than 30% of macrovescicular steatosis. However, use of these latter grafts is suggested reducing cold storage within 6 h[44]. Steatotic livers show heightened sensitivity to I/R

injury and several mechanisms have been proposed to explain this. The liver might be more subjected to lipid peroxidation[45], and a more accentuated pro-inflammatory response with release of mediators, such as tumor necrosis factor (TNF)-α, and an increased neutrophil infiltration[46]. Animal models showed narrowed and tortuous microvessels with reduced hepatic and sinusoidal blood flow, mitochondrial dysfunction and decreased energy levels[47].

INTERVENTIONS

Several approaches have been suggested in order to reduce the sensitivity of livers to I/R injury. Physical exercise and dietary interventions are reserved to living donors, but it may take long before providing histologic changes in liver cells[48]. Drug schedules have been used to decrease liver cell lipid intake. Urso-deoxycholic acid was used in a clinical trial, but its results are controversial[49]. Pentoxifylline was used based on its effect on reducing TNF-α levels and increasing glutathione activity[50]. To date, only bezafibrate was reported in steatotic living liver donors before transplantation[51].

Ischemic preconditioning is based on intermittent clamping before cold flushing and has been shown to reduce lipid peroxidation, hepatic microcirculation failure and neutrophil accumulation when applied to steatotic livers[52]. Volatile anesthesia has been shown to be superior to the intravenous one in preventing liver injury after reperfusion in previous studies on liver resection[53], but a recent multicenter trial comparing propofol with sevoflurane in LT has shown no difference in terms of acute organ injury and clinical outcomes between the two regimens[54].

Several experimental strategies can be applied to either the donor or the graft. Pharmacological preconditioning was successfully used in rats with resulting reduced inflammatory responses, parenchymal dysfunction, and injury[55]. Heat-shock preconditioning is a method to induce endogenous protective heat-shock proteins by exposure to heat, and is applied 3-48 h before organ procurement[56]. This leads to a decrease in TNF-α, an increase in nitric monoxide and improvement of microcirculation and inhibited platelet aggregation[56]. Some pharmacological additives can be used during cold preservation to ameliorate metabolism and suppress inflammation, such as interleukin-6, pentoxifylline, L-carnitine, carvedilol, epidermal growth factor, and insulin like growth factor 1[57]. Venous systemic oxygen persufflation during static cold storage (SCS) preservation was described in the Nineties to supply gaseous oxygen to livers, and it utes was demonstrated that application for 90 min may rescue steatotic livers after extended SCS preservation[58]. The use of machine perfusion has recently been introduced in some centers and may preserve steatotic livers by continuous supply of nutrients, removal of waste products, and maintenance of ideal microcirculation conditions[59].

ALLOCATION STRATEGIES

In LT setting, several allocation policies have been proposed over the recent years, but none is complete in evaluating all clinical aspects of a liver disease patient. Patient based policies includes: Urgency principle and utility based principle. The urgency principle is based on MELD[60], and although widely practiced it has raised criticism over the years. The components of the formula are not always objective, due to inter-laboratory variability[61]; symptom-based exceptions may be under- or mis-scored, and extra-points are assigned almost arbitrarily[62]. The first-come-first-served principle did not take into consideration the individual patient gravity with the resulting risk of increased death on the waitlist of sickest patients. The utility based principle is based on survival benefit concept and was introduced as a way to balancing the risk of death after LT with the risk of mortality while on the list, thus avoiding futile transplantation[63]; survival benefit computes the difference between the mean lifetime with and without LT so that a graft goes to the patient with the greatest difference between the predicted post transplant lifetime and the predicted waiting list lifetime for this specific donor. Donor-based policies were introduced with the increasing use of ECD, as graft and patient survival was greatly reduced for some unfavorable donor-to-recipient matching categories[64,65]. Feng *et al*[1] introduced the concept of a donor risk index (DRI) assessing donor variables that can affect transplant outcomes, thus providing formal assessment to clinical donor-related variables. Main limitations of DRI are: First DRI was reported before introduction of MELD, second DRI is mainly related to donor age, third DRI takes into consideration only data at the time of procurement. Combined donor-recipient based systems have been proposed widely; balance of risks (BAR) score includes: MELD, recipient age, retransplant, life support dependence prior to LT, donor age and CIT thus establishing a threshold at 18 points. BAR score is mainly determined by MELD balanced by other factors both of recipient and donor[66]. Actually the ideal matching is still a theory based more on myth than reality. To date, every system that has been proposed appears to not be statistically robust enough[65].

ORGAN RETRIEVAL

Preservation solutions

A recent study was conducted on 42869 first liver transplants performed in Europe with the use of either University of Wisconsin solution (UW; n = 24562), histidine-tryptophan-ketoglutarate (HTK; n = 8696), Celsior solution (CE; n = 7756) or the Institute Georges Lopez preservation solution (IGL-1; n = 1855)[67]. The overall 3-year graft survival was higher with UW, IGL-1 and CE (75%, 75% and 73%, respectively), compared to HTK (69%) ($P < 0.0001$)[67]. The same trend was observed with a total ischemia time > 12 h or for grafts

used for patients with cancer ($P < 0.0001$)[67].

Retrieval techniques

During liver procurement for deceased donation, rapid *en bloc* procurement with minimal manipulation after clamping the donor aorta achieved better early graft function post-transplantation[68].

In DCD, most surgeons use some modification of the super rapid recovery technique[69]. The donor is prepared as well as the surgical instruments. After the declaration of death the surgeons expeditiously perform aortic cannulation. Thereafter, the thoracic or supraceliac aorta is cross-clamped, and the vena cava is vented into the right chest. The portal system can be flushed by *in situ* cannulation of the inferior mesenteric vein or on the back table. Organs can be removed separately or *en bloc.* Cannulating the donor pre-mortem may decreases warm ischemia time[69]. It is necessary to cannulate both femoral artery and vein before support withdrawl in order to perfuse with cold preservative solution immediately after declaration of death. Thereafter, a median sternotomy and midline abdominal incisions are made and the intra-abdominal organs are topically ice cooled and then removed *en bloc* or separately[69].

In donors from brain death, a randomized prospective study was performed to test the impact of the donor harvesting technique on post-transplantation outcomes in ECD. A modified double perfusion (MDP) technique was compared with the single aortic perfusion (SAP) technique. Thirty-five suboptimal grafts were randomly assigned to either technique (18 MDP livers *vs* 17 SAP livers). Variables were comparable in the 2 study groups. The SAP group presented higher blood transaminases and bilirubin levels after LT. Graft primary dysfunction was also significantly higher ($P = 0.01$) in the SAP group (35%) *vs* the MDP group (5%). In the SAP group, 5 cases required re-LT (< 30 d). Patient and graft survival rates were higher in the MDP (100% in both cases) than in the SAP group (68% and 58%, respectively) so that the study was stopped[70].

Perfusion with fibrinolytic drugs

Plasminogen activators have been tested in LT to prevent microthrombosis, improve microcirculation and oxygen supply[71]. Liver grafts from non-heart-beating donors (NHBD) are additionally affected by microvascular alterations, including erythrocyte aggregation and thrombi formation, which might hamper appropriate equilibration of the preservation solution to the graft microvasculature[71]. Streptokinase was used in experimental models to observe post-preservation viability in NHBD. Streptokinase preflush resulted in a relevant and significant improvement of structural integrity as well as functional and metabolic recovery[71,72].

ITBL have a multifactorial origin but I/R injury and microthrombosis are considered to be the most relevant[73]. In order to decrease its incidence, urokinase perfusion has been tested[74]. In a prospective study by Lang R *et al*[74], the arterial system of the donor liver was perfused twice with urokinase during cold perfusion and after trimming of the donor liver. The incidence of ITBLs resulted lower than in the control group[74].

CIT

Prolonged CIT is an independent risk factor for DGF and PNF[75]. The European Liver Transplant Registry survey showed a lower 5-year survival rate with CIT over 15 h if compared with CIT less than 12 h[76]. Similar results were reported in a United States survey[77].

Liver grafts from elderly donors and/or donors with steatosis are even more affected by prolonged CIT, which should be kept below 8 h[78]. In our previous series, we showed that, albeit not statistically significant, graft survival was lower for grafts > 80 years with a CIT > 8 h (3-year survival 82.6% *vs* 61.9%, $P = 0.078$)[5].

Biopsy

Biopsy can be a valuable tool to determine the utility in pursuing donation in ECDs, particularly with liver-only donors[79]. Nevertheless, there are still no guidelines on its routine use in this kind of donors. In our previous experience, we performed on demand biopsies based on surgical evaluation at procurement and discarded livers in the presence of macrovescicular steatosis > 30%, necrosis > 5%, fibrosis > 2% as per Ishak's score, severe micro and macroangiopathy, and severe inflammation[5]. In a recent review some authors stated that pre-transplant histopathological evaluation is a time-effective, accurate, and reliable tool to assess liver quality from candidate deceased donors[80]. Pre-transplant biopsies are of value in the selection of donor livers for transplantation, especially in case of ECD, and should be performed more frequently in order to avoid unnecessary loss of organs suitable for transplantation and transplantation of inappropriate organs[80]. Correlation of histopathological findings with clinical conditions is essential and requires excellent communication between pathologists, surgeons, and the other members of the transplant team.

Machine perfusion and machine preservation

Machine perfusion and/or preservation (PM) consists of a pump creating a flow of blood or preservative solution through the organ[81]. This continuous perfusion allows better preservation, oxygenation and removal of metabolites[81]. Another advantage is the possibility to monitor the performance of the graft and to provide adjuvant substances[81,82]. PM can be divided into 3 groups based on the temperature of preservation: Hypothermic (HMP) at 4 ℃; normothermic (NMP) at 37 ℃, and subnormothermic (SNMP) at 20 ℃-25 ℃. Different flow regimes and pressures (pulsatile *vs* unpulsatile), single (artery) *vs* dual perfusion (artery and portal vein), oxygenated *vs* nonoxygenated[82].

The HMP, by lowering the metabolism but providing metabolic substrates, is reported to protect grafts from ischemic insults related to reperfusion[83]. Guarrera *et al*[83] were the first to analyze the impact of this method in

humans observing an attenuation of biochemical markers of liver injury, less biliary complications and hospital stay. They concluded that HMP of donor livers provided safe and reliable preservation[83]. The addiction of oxygen to perfusion solution (hypothermic oxygenated perfusion) in animal models showed further improvements[84,85].

The SNMP lowers the liver metabolic demand in sub-physiological temperature conditions, however maintaining sufficient metabolism for viability testing and improvement of graft function[86,87]. In an animal model, a beneficial effect with lower transaminases was found, while rising total bilirubin levels suggested inadequate prevention of I/R or hypothermia-induced biliary damage[86].

This technique was tested on livers discarded from transplant and showed a preservation of liver function with minimal injury and an improvement in various post-ischemia hepatobiliary parameters[87].

The NMP system seems the most promising technique as it allows to maintain livers in an environment similar to human body with normal temperature and metabolite and oxygen supply[88]. Moreover, it allows to monitor liver function parameters such as pH, transaminases, and the bile output[88]. It has been recently tested on a human setting with optimal results showing favorable safety and feasibility profiles, whilst costs seems to limit its widespread applicability.

Back-table

The major back table concerns using ECD are related to arterial structure and anatomy. When using grafts from old donors, arterial evaluation plays a pivotal role as aneurysms or severe atherosclerosis may led to graft discharge[5]. Graft arterial reconstruction of a right replaced hepatic artery using a safe and rigorous technique does not enhance the risk of arterial complications or graft loss, and the technique using the GDA stump is to be recommended for routine use[89].

In order to reduce the incidence of ITBL, some authors reported on the use of back-table arterial pressure perfusion to achieve reliable perfusion of the capillary system of the biliary tract, which may be impaired by the high viscosity of UW solution[90]. A highly significant difference in the incidence of ITBL was found when this technique was used when compared to standard perfusion with lower peak AST and ALT levels[91]. The authors' conclusion was that arterial back-table pressure perfusion is an easy and reliable method for preventing ischemic biliary lesions in LT and suggested it should be standard in liver procurement[90,91].

Split liver grafts

Split liver transplant (SLT) is a technique used to increase the donor pool that creates two allografts from a single liver graft. Technical and logistical issues in both donors and recipients prevent its worldwide usage and it accounts for only 4% of LT in the United States. Splitting was originally performed as an *ex-vivo* bench procedure but it was after performed as an *in-situ* procedure as well in order to reduce CIT and prevent blood loss after reperfusion[92]. SLT in adults is associated with significant increase (10%) of graft failure and recipient morbidity. Results are notably better in children[93].

Even if procured from ideal donors these grafts should be considered as extended criteria as the volume is lower and may lead to hepatic failure in the post-operative course. Moreover non-optimal positioning in the recipients may lead to compromised venous outflow and complications as biliary leakage, hepatic artery thrombosis (HAT), IPF are more frequent than in whole organ LT[94].

SLT for two adults has been performed reporting worst results with the left segment and is actually considered a high risk procedure due to insufficient parenchymal volume and complex vascular anastomosis[94-96].

The use of left allografts should be primary considered for pediatric patients while the use of right allografts in adults marginally increases risks of graft failure so that SLT should be considered as a safe technique to expand the donor pool.

TRANSPLANTATION

At transplantation, the main strategies encompass the modality of graft reperfusion and use of temporary porto-caval shunts[97-100]. Graft reperfusion can be sequential or simultaneous. In the sequential mode, the liver graft is perfused first *via* portal vein or hepatic artery, while in the simultaneous technique the arterial anastomosis is fashioned during the anhepatic phase and both the porta and the hepatic artery are perfused simultaneously[97,98].

Sequential reperfusion is associated with a shorter CIT. However, if the porta is perfused first the delay of arterial revascularization is associated with more pronounced microvascular disturbances, while if the hepatic artery is perfused first this might cause an increased blood flow called reactive hyperemia[98]. Simultaneous graft reperfusion results in improved oxygenation but may entail a longer CIT[97-99].

The use of temporary porto-caval shunt (TPCS) is controversial. The hemodynamic and immunological consequences of portal vein clamping are poorly characterized. In animal models an interruption of portal flow for up to 90 min induces edema of the gut with mucosal damages. The use of TPCS was initially advocated for patient with acute liver failure without collaterals. It was thought to be useless in cirrhotic patients as the presence of collaterals resulted in little hemodynamic changes during portal clamping.

In a prospective randomized trials Figueras *et al*[101] demonstrated a beneficial effect of TPCS in terms of decreased blood transfusions especially in patients with severe portal hypertension and high portal flow.

Renal impairment is a common sequel to LT. Impaired renal perfusion, vascular instability and the release of cytokines at reperfusion contribute to a reduction in renal

function[102].

In a study by Ghinolfi *et al*[100] it has been shown to improve hemodynamic stability and renal function in patients undergoing orthotopic LT. Lower graft survival rates were reported in patients of high DRI liver grafts when a TPCS was not used[100]. TPCS improves the perioperative outcome, this being more evident when high-risk grafts are allocated to high-risk patients[100].

Another series by Pratschke *et al*[103] showed reduced hepatic injury and increased portal flow after reperfusion. Retransplantation rate was decreased and long term survival increased. This effect was more pronounced when using ECD[103].

POST-TRANSPLANT COMPLICATIONS

ITBL

Biliary complications continue to be a major issue in LT ranging between 10% and 30%[104,105]. Anastomotic strictures (AS) are mainly related to the surgical technique and to ischemia to the distal bile stump[104,105]. Non-AS (NAS) are thought to be caused by three different types of injuries: I/R, immune-mediated mechanisms, and cytotoxic injury from bile salts[106]. The highest incidence of NAS has been reported for DCD livers as they suffer from an additional warm ischemia time during organ retrieval[107]. NAS with a patent hepatic artery are generally referred to as ITBL. The incidence of ITBL in ECD is higher, due to a major vulnerability to I/R injury and to warm ischemia time and CIT, and are reported in up to 14% *vs* 3% for younger donors.

Several strategies have been suggested to reduce the incidence and severity of ITBL[108-111]. The relative importance of portal venous blood flow in developing ITBL was outlined by Farid *et al*[108] because these lesions were diagnosed in patients with a normal arterial flow but with portal thrombosis. In order to reduce the incidence of graft microangiopathy and thrombosis, back-table pressure arterial perfusion and the use of plasminogen activators have been proposed with favorable results. Simultaneous graft revascularization seems to be associated with a lower incidence of ITBL than sequential revascularization[109,110]. Viscous preservation solutions may negatively impact on efficacy of flushing of the bile ducts capillaries, resulting in residual bile crystallization and obstruction[111-113]. Use of less viscous solutions, like HTK, seems to provide better results in reducing the incidence of biliary tract injuries, despite the recent results of the European liver transplant registry data[67,112].

In a large study, a CIT > 10 h was found to be associated with a higher incidence of ITBL and every effort should be made not to exceed this limit[113].

Vascular complications

HAT represents more than 50% of all arterial complications following LT and it is divided into early (< 4 wk from LT) and late HAT (> 4 wk from LT)[114-116]. Early HAT is generally related to technical problems and can have serious consequences[115]. Emergent interventions are usually needed with early HAT because of its related ischemia/necrosis of the bile duct system[114]. Although urgent re-transplantation is considered the main treatment for early HAT, endovascular interventions including percutaneous transluminal angioplasty (PTA), intra-arterial thrombolysis (IAT) in selective cases, and stent placement may be alternative treatments[117,118]. Currently many centers consider interventional radiology as first choice for the management of early HAT[118,119]. IAT can be considered but is related to high risk of hemorrhage in patients with recent (< 2 wk) surgery[120]. Late HAT can be silent in up to 50% of patients with only mildly elevated liver function tests[116]. Symptomatic patients often present with biliary complications with recurrent cholangitis, abscess and biliary leakage or stricture, and the presentation may be insidious[116]. Late HAT is usually due to ischemic or immunologic injuries and can be treated with biliary stenting and/or endovascular interventions[116,117].

Hepatic artery stenosis (HAS) has been treated both with PTA and stent placement with comparable results[117-120]. The use of PTA for HAS can reduce the rate of HAT[120]. Solitary stenosis are usually treated with PTA while angioplasty is used for tandem lesions[117-120]. These procedures are related to complications and risks that have to be taken into consideration and moreover are, in some cases, ineffective so that surgical intervention such as anastomotic reconstruction or re-transplantation must be applied[120].

Aneurysms and pseudoaneurysms of the hepatic artery are very rare complications after LT, but they are associated with high mortality rates (> 50%)[121]. Both can be treated by either surgical or endovascular procedures[121].

A series from the UNOS database reported that the risk of HAT with loss of the graft increases progressively with each decade of donor age > 50 years, such that a 61% risk was associated with use of donors older than 70 years[122]. A recent experience with donors older than 70 years showed a lower incidence of HAT (4.7%) and improved results were attributed to better management[123]. Ghinolfi *et al*[5] in their series showed a 3.6% of severe vascular complications: 10 (1.2%) HAT and 7 HAS (0.8%) with no differences across all donor age groups. There were no differences in terms of donor age for the 11 (1.3%) cases of portal thrombosis as well[5].

Venous complications are more frequent in LDLT[124]. Compared with the arterial complications, venous adverse events usually have a better response rate to endovascular interventions, such as angioplasty or stent placement[124,125]. Endovascular procedures are considered as the first choice for post-transplant portal vein complications with high success rates[125].

CONCLUSION

The imbalance between the number of potential

recipients and available donors still represents a major concern in LT so that the expansion of donor pool continues to be a priority.

Improvements have been made in order to better define ECD but many lacks still exist regarding their use. Some centers routinely use ECD but their results seem to be related more to their practical experience and can be reproduced with difficulties.

Some ethical considerations should also be carried out; the use of ECD can constitute a risk for recipients in terms of PNF, DGF and surgical complications so that some authors advocate the use of an informed consent about allograft specific risks. Moreover some combinations such as ECD with HCV recipients have been proved to be dangerous in terms of recurrence and survival but they have never been clearly censored by the scientific community.

It has finally to be taken into consideration that this is an expanding field in LT so that applying too strict rules on ECD use may preclude further advancement. Many efforts should be carried out in order to establish an international consensus on ECD use and to create guidelines that could be largely adopted.

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REVIEW

Hepatocellular carcinoma: A comprehensive review

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Abstract

Hepatocellular carcinoma (HCC) is rapidly becoming one of the most prevalent cancers worldwide. With a rising rate, it is a prominent source of mortality. Patients with advanced fibrosis, predominantly cirrhosis and hepatitis B are predisposed to developing HCC. Individuals with chronic hepatitis B and C infections are most commonly afflicted. Different therapeutic options, including liver resection, transplantation, systemic and local therapy, must be tailored to each patient. Liver transplantation offers leading results to achieve a cure. The Milan criteria is acknowledged as the model to classify the individuals that meet requirements to undergo transplantation. Mean survival remains suboptimal because of long waiting times and limited donor organ resources. Recent debates involve expansion of these criteria to create options for patients with HCC to increase overall survival.

Key words: Liver transplantation; Hepatectomy; Milan Criteria; Sorafenib; Living donor liver transplantation; Transarterial chemoembolization; Expansion Milan Criteria; Hepatocellular carcinoma; Mammalian target of rapamycin inhibitors; University of California San Francisco Criteria; Salvage liver transplantation

Core tip: Hepatocellular carcinoma (HCC) is the prominent Primary Hepatic tumor. Survival rates average between 6 and 20 mo, making Liver transplantation is the most efficient treatment. The established Milan Criteria is now widely accepted around the world for choosing patients suffering with HCC as liver transplant candidates. Due to high mortality rates, additional variables and tumor characteristics have been researched (example, University of California, San Francisco Criteria) in order to include more patients as candidates, so as to increase overall survival. In this comprehensive review, the pathophysiology, diagnostic modalities, and treatment options are thoroughly discussed.

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INTRODUCTION

Hepatocellular carcinoma (HCC) has become the most common primary hepatic malignancy, with average survival rates between 6 and 20 mo[1]. It now ranks sixth in the world among all malignancies, contributing to the third leading cause of mortality attributed to cancer[2]. Incidence worldwide has increased, likely due to the rising incidence of chronic hepatitis B and C infections. Since 1963 when first performed by Starzl *et al*[3], liver transplantation has seen dramatic changes, though initial outcomes were suboptimal. Attempts to treat HCC with liver transplantation showed poor results. At this point, it was determined that a narrow spectrum of selection criteria was needed to increase survival during the time after transplant. In 1996, Mazzaferro *et al*[4], in his revolutionary paper, proposed stricter criteria for liver transplantation. The four-year rate of survival was 75% with an 83% survival rate without recurrence[4]. From this landmark study, the Milan Criteria (MC) was established. The MC includes three major points: an isolated malignancy $\leqslant$ 5 cm, or 2-3 tumors each < 3 cm, that does not have any evidence of invasion into the vascular system or dissemination outside the liver. The MC became accepted for assessing individuals that have HCC as candidates for transplantation[5]. Given the high mortality associated with HCC, there has been a recent discussion on expanding the current criteria to include more patients as potential transplant candidates, and, therefore, increase overall survival.

In the hopes of improving disease-free survival, there may be certain ways to help incorporate more candidates with HCC. These may include expanding the current Milan and University of California San Francisco (UCSF) criteria to include tumor markers and histology, increasing the number of living donor transplants for HCC, using sorafenib post transplant, and utilizing alternative immunosuppressive regimens.

ETIOLOGY

Worldwide, chronic hepatitis B contributes to the greatest number of HCC. Chronic hepatitis C is primarily the cause in Southern Europe and North America. Individuals that have chronic hepatitis B may develop HCC without evidence of cirrhosis[5]. However, 70%-90% of patients suffer from concurrent cirrhosis[6]. Some factors, such as elevated viral loads, and having hepatitis B envelope and surface antigens are believed to contribute to HCC incidence[7,8]. Advanced age, being male, obesity, alcohol abuse, diabetic, and family history, are variables associated with increased risks for developing HCC[6,9]. Hepatitis B and C co-infection have a cumulative effect in contributing to the formation of HCC[9,10]. Additional variables of risk for HCC are in Table 1[11,12]. The United States, as well as other developed countries, have increasingly seen non-alcoholic steatohepatitis (NASH) as a primary contributor. It is assumed that the obesity epidemic and prevalence of diabetes has played a significant role. Associated factors include: Age, male gender, hepatitis C virus (HCV)/hepatitis B virus, alcohol abuse, severity of non-alcoholic fatty liver disease/NASH, diabetes/obesity, iron overload, and genetic variants (PNPLA3, APOB, TERT)[13].

PATHOPHYSIOLOGY

The pathophysiology of HCC is an evolving topic and appears to be multifactorial. In 1981, after Beasley linked hepatitis B infection to HCC development, its cause was thought to have been identified[14]. Subsequently further research linked other etiologies of underlying cirrhosis to HCC[15]. Ongoing studies have linked metabolic syndrome as a significant cause[16]. Research has shown that repeated inflammation facilitates carcinogenesis[17]. HCC predominantly arises in a cirrhotic liver where repeated inflammation occurs along with fibrogenesis. Inflammation and fibrogenesis predispose the liver to dysplasia and subsequently malignant transformation[17]. An inflammatory microenvironment plays a prominent part in starting the advancement towards HCC[17,18].

The pathogenesis of HCC is made up of different genetic/epigenetic aberrations and alterations with many signaling pathways that lead to a known heterogeneity of the diseases biologic and clinical behavior[19]. The majority of specimens are from hepatectomies and, thus reflect a minority of patients. Cancer genetic heterogeneity of HCC is quite magnificent. Difference exist between patients including variations within stages of tumor development in a similar patient, such as in the nodules, as well as diversity within a tumor[16,20].

Recent analysis has been sought to investigate the genetic pathways that are affected during hepatocarcinogenesis[21]. p53, PIK3CA, and β-catenin appear to be frequently mutated in patients. Additional research is needed to identify the signal pathways that are disrupted, leading to uncontrolled division. Two pathways in cellular differentiation (*i.e.*, Wnt-β-catenin, Hedgehog) appear frequently altered. Up-regulated WNT signaling is believed to link preneoplastic adenomas with greater chances for malignant transformation[22,23].

Ongoing studies are looking at inactivated mutations of ARID2, a chromatin-remodeling gene, in the major subtypes of HCC[17]. Eighteen point two percent of individuals with HCV-associated HCC, primarily in Europe and the United States, had inactivation mutations of ARID2, suggesting this as a common mutation subtype in a tumor suppressor gene.

DIAGNOSIS

Patients who are high risk require surveillance. High risk groups include: Cirrhotic hepatitis B carriers, patients with hepatitis C cirrhosis, stage 4 primary biliary cirrhosis, other causes of cirrhosis, Asian males older than 50 years of age that are hepatitis B carriers, a known family member having HCC in hepatitis B carriers, and African/Northern American blacks having hepatitis B[24]. Surveillance

		Arterial phase hypo- or iso-enhancement		Arterial phase hyper-enhancement		
Diameter (mm)		< 20	⩾ 20	< 10	10-19	⩾ 20
"Washout"	None:	LR-3	LR-3	LR-3	LR-3	LR-4
"Capsule"	One:	LR-3	LR-4	LR-4	LR-4 / LR-5	LR-5
Threshold growth	⩾ two:	LR-4	LR-4	LR-4	LR-5	LR-5

Figure 1 Liver Imaging Reporting and Data System. Adapted from American College of Radiology (www.acr.org).

Table 1 Etiology of hepatocellular carcinoma[12]

Risk factors for hepatocellular carcinoma
Chronic hepatitis C infection with advanced fibrosis or cirrhosis
Chronic hepatitis B infection with/without cirrhosis
Alcoholic liver disease with cirrhosis
Hereditary hemochromatosis with cirrhosis
Alpha1-antitrypsin deficiency with cirrhosis
Autoimmune hepatitis with cirrhosis
Porphyrias
Wilson's disease
Non-alcoholic fatty liver disease
Nonalcoholic steatohepatitis with cirrhosis
Primary biliary cirrhosis
Type 1 hereditary tyrosinemia
Type 1 and 2 glycogen storage disease
Hereditary ataxia-telangiectasia
Hypercitrullinemia
Aflatoxin exposure
Other carcinogens
Thorotrast
Polyvinyl choloride
Carbon chloride

includes ultrasound at 6-mo intervals[24,25]. Nodules found on ultrasound that are < 1 cm must routinely be followed by ultrasound every three to six months. If nodules are stable then routine surveillance every six months can be resumed. Nodules > 1 cm require further investigation by quadruple phase computed tomography (CT) scan or dynamic enhancement magnetic resonance imaging (MRI) with contrast[26]. Because a tumor gets its vascular source through the hepatic artery, it demonstrates a classic vascular pattern on multiphase CT scans. This pattern of enhancement during the early phase of arterial enhancement has quick washout in the delayed or portal venous phase. Diagnosis can be made purely by radiology. Saborido *et al*[27] reported a higher recurrence rate among patients who underwent tumor biopsy before liver transplantation. Currently, a pre-transplant tissue diagnosis is not required in cirrhotic patients that have the classic imaging findings for HCC[12,28]. If an imaging study does not reveal this typical vascular pattern, then another imaging study with enhancement using a different modality should be performed, or tissue diagnosis must be pursued[5]. However, the differential diagnosis between dysplastic nodules and early HCC might be cumbersome even for an experienced liver pathologist, because stromal invasion, a typical malignant feature, could be absent[23].

Liver Imaging Reporting and Data System (LI-RADS) first came about around March 2011, with widespread acceptance by many in practice. LI-RADS is a method to help standardize the assessment and ability for CT and MRI in recognizing HCC in individuals that demonstrate risk factors[29,30]. LI-RADS categorizes a liver lesion on imaging by its likelihood of being benign, HCC, or alternative diagnosis. The criteria to categorize a lesion into LI-RADS depends on the diameter as well as identifying the four primary variables useful for diagnosing HCC. These include enhancement during the arterial phase, washout following hyperenhancement, the development of a capsule, and growth compared with previous studies[29] (Figure 1). LI-RADS is in constant expansion and critique, garnering input from multiple specialists.

Another imaging study, contrast-enhanced ultrasound, is useful for identifying hepatic lesions. It can help characterize cirrhotic nodules from HCC using microbubble contrast agents[31,32]. In general, HCC does not have Kupffer cells (reticuloendothelial cells). These cells came of importance when Sonazoid, an agent used to enhance imaging about ten minutes after its administration, was introduced. Since the tumor lacks Kupffer cells, there is no enhancement in the post vascular phase, while benign lesions show continued enhancement[33].

TUMOR MARKERS AS CRITERIA FOR HCC

Historically, alpha-fetoprotein (AFP) has been used to aid in diagnosing HCC[24]. Typically, levels greater than 400 ng/mL are considered diagnostic. However, recent data has shown its sensitivity and specificity to be unreliable. AFP can be elevated in other disease manifestations such as metastatic colon cancer or intrahepatic cholangiocarcinoma[34,35]. Therefore, its use may be limited as the only tool for surveillance or diagnosis. Diagnosis should be made purely on radiological appearances and histology[26]. Interestingly, recent studies have shown that AFP may be significant in anticipating the reappearance of HCC after liver transplantation.

Other markers that aid in determining recurrence have included the size and quantity of lesions, bi-lobar disease, an involvement of macrovascular invasion

Table 2 Criteria for listing for liver transplantation and hepatocellular carcinoma: Various expansion beyond the Milan Criteria

Criteria	Ref.	No. of patients	Selection criteria	Survival rate at 5 yr	Survival rate at 5 yr using MC
MC	Mazzaferro *et al*[4]	48	Solitary HCC < 5 cm or 3 nodules < 3 cm	75% (4 yr)	-
Up to seven criteria	Mazzaferro *et al*[86]	283	Sum of the number of tumors and diameter of the largest tumor ≤ 7 cm	71.2%	73.3%
Toronto Criteria	DuBay *et al*[109]	294	Dominant lesion not poorly differentiated on biopsy, no restriction on tumor size and number	68%	72%
UCSF Criteria	Yao *et al*[81]	70	Solitary tumor ≤ 6.5 cm or 3 nodules ≤ 4.5 cm in diameter with a total tumor diameter ≤ 8 cm	75.2%	72%
Clinica universitaria de Navarra Criteria	Herrero *et al*[110]	154	Solitary tumor ≤ 6 cm or ≤ 3 nodules ≤ 5 cm in diameter	68%	66%
Kyoto Criteria	Ito *et al*[41]	125	≤ 10 nodules all ≤ 5 cm in diameter protein induced by vitamin K absence or antagonist-Ⅱ ≤ 400 mAU/mL	Overall survival 68.3%	No difference
Asan Criteria	Lee *et al*[111]	186	≤ 6 nodules with a maximum tumor diameter of ≤ 5 cm	76%	76.3%
Bologna Criteria	Del Gaudio *et al*[112]	177	Solitary HCC ≤ 6 cm or 2 nodules ≤ 5 cm or < 6 nodules ≤ 4 cm and sum diameter ≤ 12 cm	71% (3 yr)	71% (3 yr)
Metroticket Calculator	Mazzaferro *et al*[86]	> 1000	International Liver Transplant Society meeting in 2005 as a Web-based survey. Predict 5 yr survival based on tumor size	50%-70%	75%-80%
Toso Criteria	Toso *et al*[113]	288	Total tumor volume ≤ 115 cm^3	80%	82%
Silva Criteria	Boin *et al*[114]	257	≤ 3 nodules with a maximum tumor diameter of ≤ 5 cm and total tumor diameter < 10 cm	69%	62%
Hangzhou Criteria	Zheng *et al*[87]	195	Total tumor diameter < 8 cm with grate Ⅰ or Ⅱ tumor on biopsy and AFP < 400 ng/mL	72%	78%

HCC: Hepatocellular carcinoma; MC: Milan Criteria; AFP: Alpha feto protein.

and tumor satellites, and tumor-specific biomarkers[36]. Tumor differentiation and microvascular invasion are also substantial risks, but these features are not determined until after the evaluation of the explant. Biomarkers that consist of AFP and des-gamma-carboxy prothrombin are reported to correlate with a post-transplant recurrence of HCC[37]. In a recent study, an AFP over 400 ng/mL supplemented with the total tumor volume was recommended as a predictor following transplant[38]. In another investigation by Hameed *et al*[39], an AFP level > 1000 ng/mL was highly favorable in predicting recurrence of HCC, with a comparison to vascular invasion. Individuals that have elevated preoperative AFP levels > 1000 ng/mL, were found to have 1- and 5-year rates of survival, without reappearance of HCC, of 90% and 52.7% respectively, with levels ≤ 1000 ng/mL showing 95% and 80.3% 1-5 year survival rates. Levels of > 1000 ng/mL led to excluding 4.7% of the individuals with a reduction in the recurrence rate for HCC of 20%[39].

Another recent marker for tumor growth, antagonist-Ⅱ (PIVKA-Ⅱ), might have benefit for listing criteria in HCC patients. This tumor marker is a protein brought about by the deficiency of vitamin K[40]. The Kyoto Criteria (Table 2), was created at Kyoto University by Ito *et al*[41], where they looked at 125 patients that had HCC, 70 of which were inside MC, and the rest 55 who were outside. All patients had no extrahepatic or macrovascular disease. They identified individuals who had no more than 10 tumors, of at most 5 cm with PIVKA-Ⅱ < 400 mAU/mL, demonstrating five-year rates of survival of 86.7%, similar to individuals who fell within MC[41].

Systemic inflammation has been found to have an association with worsening outcomes and recurrence of tumor in patients with HCC. The detection of inflammation has led to identifying various indicators, including the neutrophil-to-lymphocyte ratio (NLR). A Japanese study demonstrated individuals having levels of at least 5 were found to have diminished rates of survival; multivariate analysis identified NLR elevation as being the main predictor of recurrence-free survival[42].

C-reactive protein (CRP) has been another marker of inflammation frequently studied. A meta-analysis done with 1885 patients confirmed an elevation of serum CRP > 10 mg/L showed poor overall [hazard ratio (HR) = 2.15] rates of survival and diminished recurrence-free rates of survival (HR = 2.66). Levels of at least 10 mg/L were comparative to invasion of the vascular system [odds ratio (OR) = 3.05], tumor growth (OR = 2.36), increasing size (OR = 3.41) and advanced stage (OR = 3.23)[43]. Based on these various findings a score has been proposed that is a combination of elevated CRP and low albumin levels, known as the Inflammation-based index[44].

STAGING

According to the American Association for the Study of Liver Disease (AASLD), the system to categorize HCC must incorporate the stage, the individual's functional status, and the underlying function of the liver. Different systems to stage HCC have been created and validated, in various degrees. The American Joint Committee on Cancer revised the tumor, lymph nodes, and metastasis (TNM) classification of malignant tumors staging system

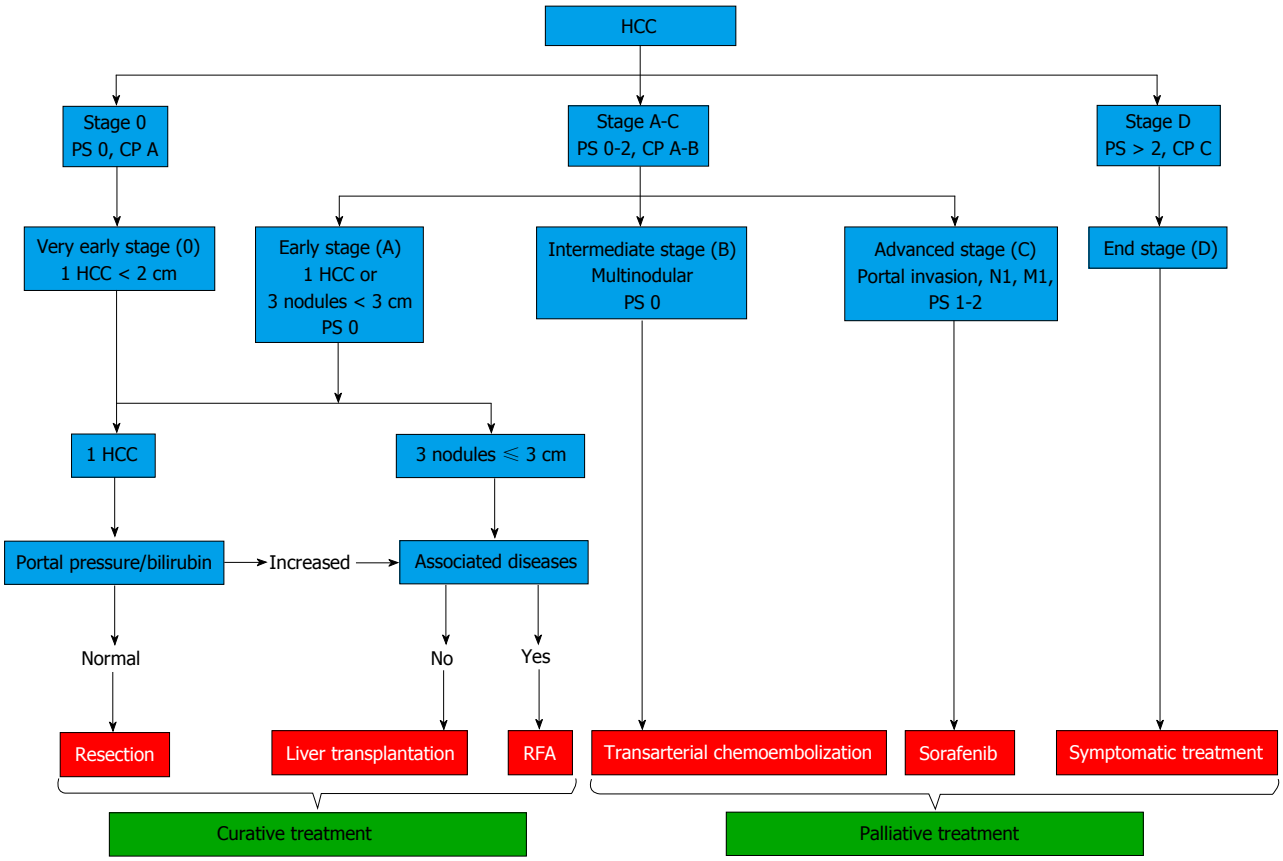

Figure 2 Barcelona-Clinic Liver Cancer Staging System[24]. HCC: Hepatocellular carcinoma; RFA: Radiofrequency ablation; CP: Child-Pugh.

in 2010[45]. Like the 2002 classification, this incorporates the number of lesions, and existence and extent of any invasion into the vasculature. However, compared to the 2002 staging system, changes surrounding the improved prognosis of multiple HCC lesions *vs* major vascular invasion was incorporated[46]. The TNM staging system has been the basis for allocating exception points for the Model for End-stage Liver Disease (MELD). The MELD score validated discriminating different stages of individuals undergoing hepatic resection.

The Okuda staging system, developed in 1985, by Okuda *et al*[47], includes the length of the tumor and three markers identifying the degree of cirrhosis. This includes the total bilirubin, albumin, and quantity of ascites. In one study, the noted survival was 8.3, 2.0, and 0.7 mo for patients that were untreated with stages Ⅰ, Ⅱ, and Ⅲ, in the Okuda System respectively[48]. The Okuda system appears to be purely clinical, and patients staged in this system are not candidates for resection. This staging system does not stratify patients by extrahepatic or macrovascular involvement. The Cancer of the Liver Italian Program score (CLIP), proposed in 1998, combines features of the tumor (macroscopic tumor morphology, serum AFP levels, and any evidence or lack of portal vein thrombosis) with a cirrhosis index of severity to reach a prognostic score between 0 and 6[46,49]. The CLIP staging system was found to have some limitations, especially in determining rates of survival in patients planning for surgical resection with HCC[47].

The Barcelona-Clınic Liver Cancer (BCLC) staging system (Figure 2) came about from data obtained in multiple studies done by the Barcelona-Clinic Liver Cancer Group[50]. The BCLC became a standardized measure of identifying prognosis for patients with HCC[11]. The primary benefit of the BCLC system has been its ability to identify patients having early HCC that may be helped by curative therapies. It differentiates itself from other individuals having a progressive disease that may demonstrate assistance with other life-sustaining therapies. This compares to Child-Pugh (CP), which evaluates only how severe the underlying hepatic dysfunction is in cirrhotic patients. BCLC takes into account the individuals performance capability, tumor burden, the involvement of the vasculature, metastatic disease, CP stage, and evidence of portal hypertension[1].

TUMOR HISTOLOGY

Well-differentiated, clear cell and fibrolamellar tumors, and the presence of tumor encapsulation are associated with a better prognosis[51]. Some suggest the utility of using tumor grade to select patients for treatment (*e.g.*, liver transplantation), although this has not yet been accepted into practice[51]. Also, this creates another invasive procedure in the pre-transplant workup, and biopsy has the potential risk of seeding the tumor through the needle tract. There have been reports of tracking and seeding within the soft tissue, peritoneum,

and intermittent involvement of the proximal ribs many months and years after the biopsy[52].

CURRENT MANAGEMENT OF HCC

With the establishment of the MELD system, five-year survival without HCC therapy, with local tumor ablation, surgical resection and liver transplantation was 15.2%, 37.6%, 55.5% and 77.2% respectively[53]. Current management of HCC includes surgical resection/hepatectomy, liver transplantation (deceased and living), thermal or chemical ablation, chemoembolization, and medical treatment.

LOCAL REGIONAL THERAPY FOR HCC

Because of the scarcity of donor grafts, some patients with HCC may experience long waiting times, which varies based on geographical location, during which their disease may progress or recur. Local treatment has been a mainstay to slow or arrest the advancement of the disease while patients are waiting for transplantation[54]. Transarterial chemoembolization (TACE) and radiofrequency ablation (RFA) have become prominent clinical tools of therapy[55]. TACE can be used to manage unresectable and multifocal HCC and to downstage lesions prior to liver transplantation, but not as a primary curative procedure[24]. During the progression of HCC, it exhibits extreme neo-angiogenic activity[55]. TACE uses an infusion of a cytotoxic agent deployed inside the artery followed by the embolization of blood vessels that supply the tumor. This results in a cytotoxic and ischemic effect[26]. TACE combines the delivery of chemotherapy, *via* a catheter, mixed with various agents followed by stagnation of the vasculature achieved with embolic agents. It is relatively safe. However, complications like post-embolization syndrome can affect up to 50% of patients that may induce acute liver failure, with an associated risk of post-procedure mortality[56]. Absolute contraindications to TACE include no hepatopetal flow (thrombus in the portal vein), hepatic encephalopathy, and evidence of obstruction in the biliary system. Some relative contraindications include bilirubin > 2 mg/dL, lactate dehydrogenase > 425 unit/L, aspartate aminotransferase > 100 unit/L, tumor load involving > 50% of the liver, cardiac or renal insufficiency, ascites, recent variceal bleed, or significantly low platelets[57]. RFA is the most common local ablation therapy[58]. It has been one of the best alternative therapies for patients having early HCC that cannot undergo surgical removal or transplantation. Percutaneous ethanol injection (PEI), like RFA, can be utilized as alternative therapy in small HCC for patients deemed poor surgical candidates for resection, given limited hepatic reserve. Injecting 95% ethanol into the tumor *via* a needle produces local coagulation necrosis and fibrosis, with thrombosis of tumor microvasculature and tissue ischemia[58]. Ideal applicants to undergo PEI should have a tumor with a size encompassing less than 30% of the encompassing liver. PEI shouldn't be used for individuals that demonstrate spread outside the liver, with evidence of a thrombus in the portal vein, CP class C with a prothrombin time > 40% of standardized level, thrombocytopenia of > 40000/micro/L[59]. The introduction of ethanol and RFA were found to be as efficient in lesions < 2 cm in size[60]. However, RFA has more predictable necrotic effects for all tumor sizes, with superior efficacy as compared to alcohol injection for bigger tumors[24]. Most programs use the MC as the endpoint of down-staging, and this must be maintained for at least 3-6 mo[16,61,62].

RESECTION

Hepatic resection is a possible curative therapy, considered ideal for individuals with maintained hepatic reserve[25]. Patients with single lesions and without any evidence of invasion of the vasculature can be offered resection. Individuals without any proof of cirrhosis or having preserved synthetic function with cirrhosis, standardized levels of bilirubin and the pressure gradient of < 10 mmHg in the hepatic vein (Grade Ⅱ recommendation) are potential candidates[24,63]. In addition, EASL guidelines (Table 3) also recommend platelet counts being over 100000[26,64]. Rates of continued survival without recurrence averaged 40% or better, with a five-year survival of 60%, but results up to 90% are reported for certain individuals. Perioperative mortality is low, reported as 2%-3% with less than 10% requirements for blood transfusions[26]. Current guidelines, notably AASLD and EASL, recommend RFA if patients are not suitable for surgical resection. Recent debates have argued that RFA may be a decent alternative to surgical resection with similar outcomes and side effect profiles. A total of 19 studies comparing resection to RFA were reviewed, of which three were randomized controlled trials with the rest being retrospective observational studies. The conclusion was that for small HCC (< 2 cm) RFA was a reasonable option, until further studies become available. Small HCC presents an easy access, without any significant technical limitations, with complete necrosis, including the desired safety margin, being most likely achieved. This is compared to nodules greater than 2 cm, especially if greater than 3 cm, and/or in locations where tumor ablation may not be effective or safe, surgical removal is preferred. This often correlates to subcapsular locations, making atypical resections possible[65].

Despite curative resection, recurrence remains common[66]. Recurrence develops either from the microscopic residual disease that remains after resection or from *de novo* cancer that comes about in hepatitis or cirrhosis[67]. Most often, recurrence occurs in the liver. Controversy does exist over whether resection or transplantation offer better options for individuals with a low MELD and fall within MC. This also depends on the wait time of a particular country or United Network for Organ Sharing region. In a recent study by Squires *et*

Table 3 Clinical practice guidelines for liver transplantation in hepatocellular carcinoma - European Association for the Study of the Liver and European Organization for Research and Treatment of Cancer

Guideline	Level of evidence	Strength of recommendation
Liver transplantation is considered to be the first-line treatment option for patients with single tumors less than 5 cm or ⩽ 3 nodules ⩽ 3 cm (Milan criteria) not suitable for resection Perioperative mortality and one-year mortality are expected to be approximately 3% and ⩽ 10%, respectively	2A	1A
Extension of tumor limit criteria for liver transplantation for HCC has not been established. Modest expansion of Milan Criteria applying the "up-to-seven" in patients without microvascular invasion achieves competitive outcomes, and thus this indication requires prospective validation	2B	2B
Neoadjuvant treatment can be considered for loco-regional therapies if the waiting list exceeds six months due to good cost-effectiveness data and tumor response rates, even though impact on long-term outcome is uncertain	2D	2B
Down-staging policies for HCCs exceeding conventional criteria cannot be recommended and should be explored in the context of prospective studies aimed at survival and disease progression end-points Assessment of downstaging should follow modified RECIST criteria	2D	2C
Living donor liver transplantation is an alternative option in patients with a waiting list exceeding six to seven months, and offers a suitable setting to explore extended indications within research programs	2A	2B

Adapted from the EASL-EORTC clinical practice guidelines: Management of hepatocellular carcinoma. *J Hepatol* 2012; 56: 908. The level of evidence and strength of recommendation are based on the National Cancer Institute classification and GRADE system, respectively. HCC: Hepatocellular carcinoma; RECIST: Response Evaluation Criteria In Solid Tumors.

al[68], they looked at 257 patients, of which 131 individuals had transplant compared to 126 that underwent resection. MC was met in all transplant patients, and only in 45 (36%) patients who had a resection. Follow up median was 30 mo, and the average time waiting for transplantation was 55 d, without having any individuals being dropped from the list while waiting.

Individuals within MC demonstrated greater five-year comprehensive survival (65.7% *vs* 43.8%; $P = 0.005$) and RFA (85.3% *vs* 22.7%; $P < 0.001$) compared to resection. Individuals having hepatitis C, with transplant, ($n = 87$) showed significant improvement in 5-year results as correlated with individuals within Milan have undergone resection ($n = 21$; OS: 63.5% *vs* 23.3%; $P = 0.001$; RFS: 83.5% *vs* 23.7%; $P < 0.001$)[68]. In this study, they showed that transplant not only increased longevity from recurrence but improved five-year survival, illustrated as well for subjects having preserved synthetic function or low MELD.

Salvage liver transplantation is postulated as a possible option in the reappearance of HCC after surgical resection. It is promising in that it could relieve the burden of increasing waiting times for listed patients as well as limited organ resources, but it still has not been thoroughly evaluated. Recently, in a study by Hu *et al*[69], they retrospectively monitored outcomes and factors that influenced the survival of 53 individuals that underwent salvage liver transplantation from 2004-2012 in a single center Zhejiang University in China. Patients that had salvage liver transplantation were found inside MC, Hangzhou criteria (Table 2) or outside both Milan and Hangzhou criteria. Results showed that individuals not within Milan but inside Hangzhou criteria showed one and three-year rates of survival of 70.1% and 70.1%, comparable to patients inside MC. Tumor-free survival was also similar[69].

LIVER TRANSPLANTATION FOR HCC

Current reports that came out of the Organ Procurement and Transplantation Networks (OPTN) and European Liver Transplant Registry revealed HCC being the cause of 17.2% of liver transplantation for the United States[70]. HCC was initially a primary reason for transplantation. It was believed that this would get rid of the tumor and provide a cure for the primary liver disease[71]. However, it came to fruition that the amount of tumor load correlated with the success of transplantation; patients who had diffuse disease did not have favorable outcomes, whereas individuals that had minimal tumor quantity may have the opportunity for a cure. Selection of patients was a source of constant debate, given a worldwide organ shortage, controlling the amount of tumor present during the time till transplant, exploring live donors, and different immunosuppressive or supplementary therapy[71]. During this period, it was found that patients who had incidental lesions found in their explants postoperatively had similar outcomes to patients who had a nonmalignant disease. Individuals identified with minimal tumor load from HCC during surgery that was not seen through imaging because of the small size had excellent results similar to patients without malignant disease[72]. The size of less than 5 cm was the cutoff. As stated earlier, Mazzaferro's study established the MC, creating guidelines for selecting patients to undergo transplantation for HCC[4]. An agreement among guidelines is that transplantation has become the best option to treat cirrhotic's in Child's class B that may or may not having portal hypertension, being within Milan. Surgical resection still is an accepted initial therapy in early HCC with maintained hepatic reserve[26]. Individuals require a detailed review to evaluate the size and amount of tumors and to exclude any involvement outside

Table 4 Organ Procurement and Transplantation Networks classification system for nodules seen on images of cirrhotic livers

OPTN class 0	
Incomplete or technically inadequate study	Repeat study required for adequate assessment; automatic priority MELD points cannot be assigned on basis of an imaging study categorized as OPTN class 0
OPTN class 5	
Meets radiologic criteria for HCC	May qualify for automatic exception, depending on stage
Class 5A: ⩾ 1 cm and < 2 cm measured on late arterial or portal venous phase images	Increased contrast enhancement in late hepatic arterial phase AND washout during later phases of contrast enhancement AND peripheral rim enhancement (capsule or pseudocapsule)
Class 5A-g: Same size as OPTN class 5A HCC	Increased contrast enhancement in late hepatic arterial phase AND growth by 50% or more documented on serial CT or MR images obtained ⩽ 6 mo apart
Class 5B: Maximum diameter ⩾ 2 cm and ⩽ 5 cm	Increased contrast enhancement in late hepatic arterial phase AND either washout during later contrast phases OR peripheral rim enhancement (capsule or pseudocapsule) OR growth by 50% or more documented on serial CT or MR images obtained ⩽ 6 mo apart (OPTN class 5B-g)
Class 5T: Prior regional treatment for HCC	Describes any residual lesion or perfusion defect at site of prior UNOS class 5 lesion
Class 5X: Maximum diameter ⩾ 5 cm	Increased contrast enhancement in late hepatic arterial phase AND either washout during later contrast phases OR peripheral rim enhancement (capsule or pseudocapsule)

Adpated from Wald *et al*[30]. HCC: Hepatocellular carcinoma; OPTN: Organ Procurement and Transplantation Networks; MELD: Model for End stage Liver Disease; CT: Computed tomography; MR: Magnetic resonance; UNOS: United Network for Organ Sharing.

the liver with or without vascular spread (*i.e.*, Tumor thrombus in the hepatic or portal system). If a nodule is found with CT or MRI in a patient with cirrhosis, based on OPTN, it should have an organization of LI-RADS nodules[70].

Individuals with HCC have minimal use from the MELD criteria, as minimal liver dysfunction was often concurrently present and did not progress until later in their disease course. This was in comparison to individuals with hepatic dysfunction from other etiologies, presenting with worsening hepatic dysfunction at the time of diagnosis. HCC individuals are often on the waiting list for some time, having a range of 104 to 387 d, with a wide overall fluctuating timeline[73]. These individuals may also be dropped from the list for numerous reasons including: Tumor progression beyond MC, metastatic disease, vascular invasion, progression of their liver disease or complications (infection, renal failure), or non-tumor related contraindications (*i.e.*, alcohol relapse). Therefore in 2002, the idea of the MELD exception points was created. Now, individuals that have ALTSG stage T2 HCC (which is a primary HCC lesion within 2 and 5 cm, with at most three lesions all no greater than 3 cm) will be assigned a higher priority MELD score[74]. They are awarded 22 MELD points because their 3-mo mortality approximates patients with liver failure and a score of 22. Patients receive an increase by 10% every three months, only if their disease remains within MC. Patients having T1 HCC (an isolated lesion < 2 cm) had been formerly allocated additional MELD points, but this practice was abandoned after a further study showed excellent 3-mo survival with such small lesions[75]. With the allocation points for HCC, the individuals receiving MELD exception was escalated from 10.5% in 2002 to 15.5% in 2008. The guidelines to help classify HCC in the UNOS/OPTN system (Table 4)[30] was developed to help continue MELD exception point allocation for individuals having HCC that was capable of being diagnosed without doubt, through imaging. The OPTN class 5 nodules correlate with definite imaging interpretation for HCC. Class 5B and 5T nodules can also account for continuous allocation for a greater MELD score of 22.

Class 5B and 5T nodules can also account for continuous allocation for a greater MELD score of 22.

A Mayo Clinic trial compared a new approach for allocating organs and looked at pre and post-MELD time span. There was statistical significance favoring improved principles including: The time span until liver transplant (LT) - 2.28 years *vs* 0.69 years ($P < 0.001$), individuals transplanted 0.439 transplant/person-years *vs* 1.454 transplant/person-years ($P < 0.001$), waiting list survival after five months of 90.3% *vs* 95.7% ($P < 0.001$) with the rate of falling off the list in five months, of 16.5% *vs* 8.5% ($P < 0.001$)[75]. These findings illustrated that this novel incorporation criterion improved in increasing rates of the incidence of Deceased Donor Liver Transplant (DDLT) for HCC individuals. Also, five-month rates to fall off the list greatly diminished, with increased survival rates in this time span, while noted to be waiting during the post-MELD period[76]. These findings indicated these novel MELD allocation criterion showed definite benefit to candidates with HCC for transplantation.

RECURRENCE POST TRANSPLANTATION

The recurrence of HCC, post-transplant, remains a clinically relevant problem. Based on the literature in the post-transplant period, HCC recurrence uniformly occurs with an incidence of 10%-20%[18]. Recurrence post-transplant typically occurs within the first two years. Repeated transplantation has not been encouraged due to diminished rates of survival and lack of organs, with the average survival rate lower than one year[77]. In a study where 60 LT recipients were evaluated, the overall median survival measured post reappearance was roughly ten and a half months (ranging from one-136), with primarily delayed recurrence as well as being eligible to undergo resection were felt to correlate in a positive

manner with overall survival[19]. Another meta-analysis, by Chen *et al*[9] studied 1198 patients and showed that the presence of involvement into the vasculature, tumor diameter > 5 cm, tumor status beyond Milan, and poor differentiation were felt as prominent variables for the risk for recurrence of HCC[31]. The gross features of HCC, including the size and total amount of the lesion, both variables part of Milan, are identified as the greatest predictors of results. The entire tumor size, defined as the total of all tumor diameters, was found to correlate with a fourfold increase in tumor recurrence if greater than 10 cm[30]. Despite this, there is currently no precise formula to predict recurrence accurately. In the post-transplant period, roughly 10% to 15% of individuals with HCC inside Milan, undergo recurrence[78]. Further evidence suggests that independent variables beyond the size of the tumor and total number of tumors may be linked to a more aggressive tumor biology, resulting in an increased chance of HCC recurrence post-transplant[39,79].

The diagnostic accuracy of MRI and CT has shown to be in the range of 45%-60% and for cases with lesions under stage, noted for 21%-43%[36]. This is likely because the relationship comparing imaging criterion and histopathology for cirrhotic hepatic explants needs further investigation. Also, the sensitivity of different multidetector-row CT for HCC less than 1 cm is not as sensitive[22].

Recent studies have tried to find characteristics to predict better tumor recurrence including tumor markers, inflammatory markers, tumor histology, explant pathology. Because of the risk of recurrence, some have been intimidated to expand the current criteria used in guidelines to list patients with HCC. Nevertheless, it is an avenue that needs to be investigated.

EXPANSION OF CRITERIA FOR LIVER TRANSPLANTATION FOR HCC

Although undergoing transplantation provides positive outcomes, when it comes to HCC, the limited number of viable organs restricts the number of patients getting transplanted. Allocation guidelines will have to incorporate that individuals with HCC may come off the waitlist as their tumor progresses while also taking into account the patients that have inherent liver disease waiting for transplantation[80]. Irrespective of the reason for transplantation, the purpose is to provide individuals with the utmost benefit despite the limitations of resources from deceased and living donors, in an impartial, ethical, and fiscal manner. The initial studies of the MC determined that the earlier stages of HCC yielded significant benefit from transplantation. The latter stages, in whom transplantation could potentially offer some benefit, are not included, and this has created open forums about the potential necessity to expand the criteria, to incorporate more patients[26].

In 2001, Yao *et al*[81] studied 70 subjects that had expanded guidelines for HCC and liver transplantation. Their results showed that when having these certain observed criteria [isolated tumor size, 1 lesion < 6.5 cm, or < 3 nodules with the biggest lesion diameter < 4.5 cm and the entire tumor burden diameter < 8 cm] survival rate were 90% and 75.2%, at 1 and 5 years, respectively, after orthotopic liver transplantation (OLT) *vs* 50% 1-year survival when individuals were outside these guidelines ($P = 0.0005$)[81]. This widely used UCSF criteria showed that modest expansion showed similar results to MC and, therefore, allowed a greater number of patients with HCC the opportunity for transplantation.

In 2006 Decaens *et al*[82] suggested expanding the criteria to incorporate the characteristics of the lesion within the explanted liver. This study was a large independent series testing the utility of the suggested criteria for pre-transplant evaluation. Four hundred and seventy nine patients were listed, between 1985 and 1998, and 467 underwent LT for HCC. Individuals were categorized into both the Milan and UCSF categories, according to pre or post-transplantation tumor characteristics, with imaging at the time listed and the time of liver transplant, respectively. The survival rates for five years were measured utilizing Kaplan-Meiers method in comparison to the log-rank test. Pre-transplant UCSF guidelines were measured by the principle for the intention-to-treat. With these criteria, 279 subjects were categorized within Milan, 44 outside Milan while within UCSF (this being the subgroup that could benefit from expanding criteria), and 145 subjects were outside both Milan and UCSF.

Given the minimal time frame of four months, the 5-year survival was 60.1%, 45.6%, and 34.7%, respectively ($P = 0.001$). Survival rates were mathematically decreased for the group within UCSF but outside Milan, in comparison with patients within Milan. However, it was noted that the results were not significant ($P = 0.10$). Five-year survival was 70.4%, 63.6%, and 34.1%, for subjects within Milan ($n = 184$), within UCSF but outside Milan ($n = 39$), as well as individuals both outside UCSF and Milan ($n = 238$), correspondingly ($P = 0.001$). Results for five-year survival showed no difference when comparing individuals inside Milan and those inside UCSF but not within Milan ($P = 0.33$). This data was extrapolated for pre-transplant assessment and demonstrated that the UCSF guidelines correlate to a 5-year survival below 50%[72,82].

CHALLENGING THE MC

To some, the MC seem too constrained, and various clinical studies have challenged their limits with suggestions of new parameters to select patients[76].

Even with these findings, the AASLD guidelines do not suggest expanding the current transplant criteria past the MC[24]. The EASL guidelines state that the HCC limit extension guidelines are currently not identified. The broadening of MC, utilizing "up-to-seven" in individuals with no evidence of microvascular invasion, has noted favorable outcomes and requires further prospective

confirmation[26]. Individuals that will be transplanted while in Milan have 5-year survival rates of 70% or greater, as compared to other congregations that demonstrated 5-year survival rates being approximately 50% when undergoing transplantation in expanded criteria[41,72]. The survival rate is arguably the lowest that is accepted, but given the minimal amount of available organs, extending criteria is still an ongoing debate[83]. Therefore, many patients who have a possibility of doing well post-transplant are not viable candidates at most transplant centers. Because of this, different expansion criteria have been proposed with varying degree of success (Table 3).

The Metroticket project was introduced at the International Liver Transplant Society meeting in 2005 as a Web-based survey in an attempt to gather an appropriate amount of subjects to aid with robust statistical analysis[84,85]. The project collected data from more than 1000 individuals outside of MC that underwent transplantation. The result of the project is the Metroticket calculator, which can be used to predict 5-year survival based on a patient's tumor characteristics (size of the total nodules, length of the largest nodules, and involvement into the vasculature if available)[76,86]. The Metroticket predicts survival beyond the MC, the upper limit of liver transplantation being the "rule of 7" where the length of the biggest nodule and the total amount of nodules cannot exceed 7[72]. In a study by Lei *et al*[84], they found that he was able to calculate the rates of survival in 230 situations, by utilizing the Metroticket model. The three- and five-year survivals are 64.7% and 56.2% respectively, and what had been seen was 71.3% and 57.8%, respectively. However, the predicted five-year rate of survival was 43.5%, with observations being only 8.7%, implying that validity for HCC with macro-invasion may need to be revised[84].

LIVING DONOR LIVER TRANSPLANT FOR HCC

With current listing guidelines of HCC, only a limited number of patients can qualify to be placed on LT lists. The demand for donor livers has continued to grow over the last two decades, and this has placed greater weight on the need for efficient and effective means of increasing the supply. Over the last few years, while having the ability to perform living donor liver transplantation (LDLT), various institutions thought to broaden their guidelines[87]. At this time, the use of LDLT makes up roughly fewer than 5% of adult LTs, disproportionately lower when compared to renal transplantation, which has similar donors comprising 40% of the population[26]. In Asian countries, the majority of liver transplantations for HCC patients are LDLT, and these account for 96% of liver transplantation for HCC[88-90].

The current benefits of LDLT are an intensive donor evaluation, time available for optimization before transplantation, as well as a nominal time for cold ischemia[91-93]. There is also a reduction in the mortality for recipients in comparison with deceased donors[91]. Some ethical issues have arisen with LDLT with respect to overall well-being of the donor as well as possible monetary exchange for organs[93]. People who oppose LDLT state it is not acceptable to bring healthy donors into such long-term risk for disability or even mortality. Currently it is estimated that right hepatic lobe transplantation mortality is approximately 0.5%[91]. Another important issue regarding a variable that influences the recurrence of HCC following LDLT is the procedure itself. This may represent more risk when measured with DDLT. A large multicenter cohort trial from Japan and Korea demonstrated that when applying MC and UCSF guidelines for LDLT there were equivalent long-term outcomes when compared to DDLT, but some authors recently illustrated a greater incidence of recurrence of HCC in LDLT in comparison to DDLT[89]. Six studies compared DDLT and LDLT for HCC, and there was no any conclusive data demonstrating a difference in outcome between the grafts. There was a greater risk of HCC recurring in patients that were fast-tracked, as LDLT may lengthen the time from the diagnosis until the transplant. This would not allow the necessary time for the tumor to behave biologically and materialize[71]. In 2010, the consensus conference recommended that individuals that had HCC, who opted to have LDLT, may benefit from the consideration of a 3 month observation period prior to transplant because of this finding[71]. LDLT remains another promising, yet controversial option for patients with HCC, who face increased mortality waiting for transplantation.

MEDICAL TREATMENT

The pathophysiologic complexity of HCC has made medical treatment of HCC challenging. It has been difficult to provide adequate tumor therapy but at the same time maintaining liver function.

Sorafenib, which is an oral tyrosine kinase inhibitor, was the original therapy that demonstrated any improvement in mortality for progressive HCC[94-96]. It is recommended as initial treatment for individuals with the maintained hepatic reserve but cannot attain advantages from surgical removal, transplantation, ablation or TACE (grade- Ⅰ recommendation)[24]. Sorafenib is still shown to be the exclusive treatment that has shown any mortality benefit in this category. Tamoxifen, anti-androgens, octreotide or herbal drugs are not recommended.

The sorafenib HCC Assessment Randomized Protocol trial illustrated safety and mortality benefit in individuals that have progressive HCC. This randomized study of 602 patients, with maintained hepatic reserve (> 95% CP A) were on a continuous regimen of Sorafenib 400mg twice-daily, or a placebo[96]. If patients did not respond or had deleterious effects to Sorafenib, there was no second line agent available[26].

More recently, there have been further studies using sorafenib as neoadjuvant therapy or as bridging therapy prior to LT[96]. Some preclude that sorafenib may also have a role in preventing tumor relapse[97]. The studies in

the last three years have been small ranging from 1-39 patients but do provide some optimism that sorafenib may have a role in decreasing tumor recurrence post transplantation[98].

In the recent sorafenib as Adjuvant Treatment in the Prevention of Recurrence of Hepatocellular Carcinoma trial, effectiveness and safety with supplementary sorafenib was tested. Eligibility criteria included individuals that underwent local ablative therapy or resection surgically, with the intention of a cure but developed a significant risk of recurrence[99]. The main criteria for inclusion included: CP score between five and seven, ECOG PS 0, with the lack of any redevelopment measured by CT or MRI. The study's criteria of exclusion was made up of ascites, the redevelopment of HCC, any spread outside the liver or involvement of major vasculature, and any prior major systemic HCC therapy. Individuals were separated by curative treatment, geographical location, risk of recurring, and their CP score. They were arbitrarily assigned in an equal distribution, consisting of two therapeutic arms: Sorafenib 400 mg twice daily or placebo therapy, for a length of at most four years. The main endpoint included survival without recurrence of HCC documented by an independent reviewer. Secondary goals were the timeframe until HCC recurred and the overall survival[100].

In this randomized study, 1114 patients were included (556 received Sorafenib and 558 were randomized to placebo). There was no noted variation in survival free recurrence, time to recurrence and overall survival benefit. There was a shorter median therapy time for sorafenib (12.5 mo *vs* 22.2 mo) and smaller average daily doses (578 mg *vs* 778 mg). The rates for cutting short sorafenib treatment were much higher due to adverse effects (24% *vs* 7%) and withdrawal of consent (17% *vs* 6%). Unfortunately, their primary endpoint was not met. Initial smaller studies do suggest that further extensive studies need to investigate the possibility of supplementary treatment with sorafenib for HCC.

IMMUNOSUPPRESSION POST TRANSPLANT: MTOR INHIBITORS

Mammalian target of rapamycin (mTOR) inhibitors have significant roles with monitoring cell growth, propagation, and continuation through cytoplasmic serine/threonine kinase. These inhibitors might play a part in targeting cancer cells. Overexpression using mTOR has been identified within 15% to 41% of HCC, and their inhibitors have demonstrated properties on cancer cells of HCC as well as animal models[101].

Sirolimus has also been proposed as a better option in individuals with HCC because of its antiproliferative activity[102]. In many transplant centers, sirolimus has been used as monotherapy or in adjunct, for patients who have had adverse effects of calcineurin inhibitors[103]. Also, for patients who have developed non-hepatic malignancies post transplant, some LT centers have switched patients to sirolimus, again, because of its anti-angiogenic properties[102,104].

The ongoing phase 3 SILVER study (Immunosuppression in Patients Undergoing Liver Transplantation for Hepatocellular Carcinoma) could demonstrate the effect immunosuppression with sirolimus will have with HCC reappearance after undergoing transplantation for this primary hepatic malignancy (Clinical Trials government identifier NCT00355862). The data obtained should provide greater certainty when inquiring about situations with mTOR inhibitor/SFN combinations[83]. There is an obvious risk of cancer recurrence with immunosuppression post transplantation, including the recurrence of HCC. It is postulated that HCC patients would benefit from personalized regimens after transplantation[105]. Even after identifying the potential benefits of mTOR inhibitors on HCC, the studies done are not prospective and are uncontrolled. A large prospective, case-controlled data analysis demonstrated a significant improvement in survival with sirolimus than tacrolimus. In a retrospective, systematic review study by Cholongitas *et al*[101], they looked at 3666 HCC liver transplanted patients in around 42 clinical studies from January 2007 to October 2013. Their results showed patients on calcineurin inhibitors (CNIs) had higher rates of redeveloping HCC, as compared to individuals undergoing therapy with mTORi (448/3227 or 13.8% *vs* 35/439 or 8%, $P < 0.001$). CNI therapy had greater recurrence for HCC in Milan (74% *vs* 69%) with fewer episodes through involvement micro vascularly, in comparison with mTORi therapy treatment (22% *vs* 44%) ($P < 0.05$). It was noted that everolimus treatment demonstrated quite an improvement for HCC recurrence, when compared to sirolimus and CNIs (4.1% *vs* 10.5% *vs* 13.8%, correspondingly, $P < 0.05$)[101].

Few initial studies have shown induction of partial remission or stability with sirolimus in advanced HCC[101]. Further studies are needed to evaluate the promising role of mTOR inhibitors and HCC, with a recent ongoing trial looking at this very data post transplant[106].

Post-transplant, side effects of sirolimus include thrombosis of the hepatic artery, delayed wound healing, incisional hernias, hyperlipidemia, bone marrow suppression, mouth ulcers, skin rashes, albuminuria, and pneumonitis, among others[102,107]. These risks are difficult to quantify because the incidence (and even the presence) of side effects varies widely by reporting. Because of the side effect profile of sirolimus and everolimus, specifically hepatic artery thrombosis, it is recommended that mTOR inhibitors not be used in the initial three months after transplantation[103,108].

CONCLUSION

HCC has become a significant burden globally, contributing to major morbidity and mortality. Since the 1980's, the number of cases domestically has been increasing. Undergoing transplantation offers excellent

results, and the MC provides guidelines for patients who should undergo transplant evaluation[24]. Many patients who may have potentially positive outcomes after transplant are often excluded, leaving them with dismal treatment options. Recent discussions have involved expansion to the guidelines to include greater tumor size, tumor quantity, and incorporating tumor markers and histology in the listing criteria. Also, with the addition of chemotherapy, changes in immunosuppression regimens, increasing the use of living donors and salvage transplantation post resection, the data looks promising, with comparable survival rates. These findings may support future studies investigating these possibilities, with goals of improving mortality for individuals with HCC.

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REVIEW

Challenges of liver cancer: Future emerging tools in imaging and urinary biomarkers

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Abstract

Chronic liver disease has become a global health problem as a result of the increasing incidence of viral hepatitis, obesity and alcohol misuse. Over the past three decades, in the United Kingdom alone, deaths from chronic liver disease have increased both in men and in women. Currently, 2.5% of deaths worldwide are attributed to liver disease and projected figures suggest a doubling in hospitalisation and associated mortality by 2020. Chronic liver diseases vary for clinical manifestations and natural history, with some individuals having relatively indolent disease and others with a rapidly progressive course. About 30% of patients affected by hepatitis C has a progressive disease and develop cirrhosis over a 20 years period from the infection, usually 5-10 years after initial medical presentation. The aim of the current therapeutic strategies is preventing the progression from hepatitis to fibrosis and subsequently, cirrhosis. Hepatic steatosis is a risk factor for chronic liver disease and is affecting about the half of patients who abuse alcohol. Moreover non-alcoholic fatty liver disease is part of the metabolic syndrome, associated with obesity, hypertension, type Ⅱ diabetes mellitus and dyslipidaemia, and a subgroup of patients develops non-alcoholic steatohepatitis and fibrosis with subsequent cirrhosis. The strengths and pitfalls of liver biopsy are discussed and a variety of new techniques to assess liver damage from transient elastography to experimental techniques, such as *in vitro* urinary nuclear magnetic resonance spectroscopy. Some of the techniques and tests described are already suitable for more widespread clinical application, as is the case with ultrasound-based liver diagnostics, but others, such as urinary metabonomics, requires a period of critical evaluation or development to take them from the research arena to clinical practice.

Key words: Virus hepatitis; Liver cancer; Ultrasound; Fibrosis; Urinary biomarkers

Core tip: There is an increasing need for non-invasive

assessment of liver disease. New techniques to assess liver damage from transient elastography to experimental techniques, such as *in vitro* urinary nuclear magnetic resonance spectroscopy are currently investigated. The guidelines of sustainability in countries with limited resources, facilities and low financial income can be seen as an opportunity for addressing research toward low-cost diagnostics and for driving clinical practice toward more streamlined technology, with ultimate benefits for the populations of poorer countries around the world. In this perspective, urinary biomarkers of liver cancer and ultrasound imaging are two complementary models.

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INTRODUCTION

"Nothing strengthens authority so much as silence": Leonardo da Vinci

Many relevant reviews are available on chronic liver disease and on one of its major complications, hepatocellular carcinoma (HCC), and the most significant advances in diagnosis, management, and long-term outcome are appropriately considered and well-focused[1]. By contrast, this brief overview has the aim of summarizing some epidemiological and clinical concepts, highlighting some of the current diagnostic criteria used for addressing personalized therapeutic choices, and discussing briefly some practical and ethical challenges. The latter problems are also the consequence of a global economy and of the current research approach, which we have to consider from the perspective of sustainability[2].

The most frequent liver cancer, accounting for 80%-90% of all primary liver cancers, is HCC, but there are critical differences in the diagnostic algorithms. Differences are due not only to the skills and knowledge of pathologists, but also to the actual availability of such diagnostic facilities in most countries, particularly where liver cancer is more frequent. Sadly, there are countries in which only one surgical pathology laboratory is available: This is the case of Zambia, a country with 12 million inhabitants, while until recently, Liberia had no diagnostic laboratory services, owing to the ravages of a prolonged civil war. With this limitation and shortage of expertise, frequent even in developed countries, surrogate tools for reliable diagnosis are warranted, beside the fact that reducing the number of invasive procedures also has great appeal. The Italian Association of Pathologists, "Patologi Oltre Frontiera", has been working in Africa since 2004 to create a virtual laboratory with telemedicine[3]. This is a very important approach, equally useful in countries with large populations, such as China[4]. The use of this approach as a continuous tool for training, by plain e-learning technology, provides valuable results in both the developed and the developing world[5,6], while new developments using emerging e-learning technologies and smartphone applications are steadily becoming a reality. In general, international research partnerships and potential clinical applications are receiving ever greater attention, given that technology has diminished the restriction of geographical barriers with the effects of globalisation becoming more evident, and populations increasingly more mobile[7].

The implications are manifold, and among them, the opportunity of assessing new diagnostic tools where there are emerging or more prevalent diseases, such as viral hepatitis, non-alcoholic fatty liver disease (NAFLD) and liver cancer. In this setting, international collaboration should move research goals away from pure market forces and towards humanitarian aims. Indeed, encompassing this second aim, the profession and the mission of medical intervention will contribute to peaceful cohesion and ultimately to shared economic profits.

The pathophysiology of HCC has been attributed to chronic inflammation associated with a variety of disease processes, but on a worldwide scale, mainly due to viral hepatitis B virus (HBV) and hepatitis C virus (HCV). Nonetheless, cirrhosis is not the absolute pre-condition for the further development of cancer. HCC onset is associated with active HBV or HCV infection, but also its incidence increases with age, alcohol consumption, smoking, human immunodeficiency virus (HIV) infection, obesity and diabetes. In this regard, the concurrent effect of NAFLD is becoming all the more important, owing to an increasingly obese population, not only in Western Europe and North America, but also in the developing world[8]. Since it is possible to reverse much of the pathology seen in fatty liver disease through lifestyle change, such as exercise and dietary modification[9,10], it is quite surprising that the two paths of research and therapeutic intervention are not more closely allied in a translational approach, as in many places they are still separated, much like the tracks of a railroad[11].

The increase seen in chronic liver disease and HCC, confirmed and increasing in Europe for 20 years or more, is due to a multiplicity of factors, and not just to HCV-induced hepatitis[12]. The same increasing trend of incidence, possibly due to improved diagnostic tools, is reported worldwide for cholangiocarcinoma[13], the causes of which[14] are even less directly attributable to the same factors as HCC[15]. Cholangiocarcinoma is more insidious in its onset, with elusive clinical features devastating and scarcely responsive clinical progress.

It is clear that a strategy for eradication of HBV and prevention of its consequences must include an effective campaign of successful widespread vaccination, with birth dose vaccination in the developing world, owing to the high prevalence of mother to baby transmission. As this is not possible yet for HCV infection, antiviral therapy regimens for HCV have been burdensome for

the patient and expensive for healthcare systems, and until recently, with poor sustained viral response rates to antiviral medications. Nonetheless, overall there is only weak evidence, if any, of a beneficial effect of viral eradication on the subsequent occurrence of liver cancer in such treated patients with cirrhosis[16].

The mainstay of diagnosis has been liver biopsy for many years, but now a variety of alternatives are beginning to emerge.

Liver biopsy techniques

A liver aspirate was performed for the first time in 1883 by a German physician, Paul Ehrlich, while the percutaneous liver biopsy technique dates back to the 1920s. Fifty years later the radiologist Charles Dotter invented the transjugular approach[17]. Several pre-procedural precautions need to be taken, including prior knowledge of the patient's anatomy with a screening liver ultrasound, an up-to-date platelet count and a clotting screen. Many conditions, such as high bleeding risk, are contraindications to standard percutaneous approaches, partially or completely[18]. However, liver biopsy remains the gold-standard for assessing the severity of chronic liver diseases.

Requirement for biomarkers of liver fibrosis

Effective antiviral therapies and the advent of antifibrotic drugs have led to an increasing demand for non-invasive, accurate and reliable biomarkers of hepatic disease severity. It is recognised that the current "gold standard" for monitoring the severity of fibrosis, histological analysis of liver biopsy, has limitations and engenders risk to the patient with a defined morbidity, including pain, bleeding, time off work and a mortality rate of between one in 1000 and one in 10000 cases[18]. The specimen retrieved by standard liver biopsy is just 1/50000 of the total volume of the liver, and in about 16% of cases the sample exceeds the optimal length for adequate histological assessment of 25 mm[19]. This causes sampling variability and errors since inflammation, hepatic fibrosis and steatosis may all have an irregular distribution within the liver. In addition, as histological scoring systems are semi-quantitative categorical assessments of a continuous process (fibrogenesis), there is appreciable intra- and inter- observer variability.

A safe, reliable, non-invasive imaging approach for detecting hepatic fibrosis would obviate the hazards associated with liver biopsy and allow patients to be monitored serially with a view to prevent the decline towards cirrhosis and its complications. Accordingly, there are potential health economic benefits from prevention of end-stage disease and the reversal of less severe fibrosis.

Non-invasive assessment of chronic liver diseases

The non-invasive assessment of the severity of chronic liver disease includes the development of serum (or blood) markers, which may be divided into direct or indirect tests, either singly, or combined as serum panel markers, and the application of imaging-based technologies, such as ultrasound and magnetic resonance (MR) techniques.

Serum markers

Serum may be obtained at routine venepuncture, making it quick and acceptable to most patients. Sampling variability is negated, although site-specificity to hepatic processes may be questioned. Serum markers may broadly be divided into indirect and direct markers of hepatic fibrosis. Indirect markers are those where the indices measured correlate with fibrosis stage, but are not integral to the pathogenesis of disease. Such markers include "so-called" liver function tests, such as aspartate aminotransferase (AST) and alanine aminotransferases, and composite or panel markers, such as the AST-to-platelet ratio index (APRI) and the fibrotest/actitest markers.

On the other hand, direct markers are those measuring intermediates or metabolites of fibrogenesis, such as hyaluronic acid and panel markers such as the Enhanced Liver Fibrosis test, consisting of metalloproteinase-1, procollagenase 3 and hyaluronic acid. The performance of a number of these tests for the detection of cirrhosis is displayed in Table 1[20].

Imaging-based markers

Imaging techniques, particularly those based on ultrasound and MR, often provide hepatic structural information. Although a number of structural changes are associated with cirrhosis and portal hypertension, these signs alone are neither sensitive nor specific enough to stage chronic liver disease. A number of specialized applications have, however, shown promise and imaging techniques have the added benefit of providing real-time information to the operator and patient.

Transient elastography

Transient elastography is an ultrasound based technique that evaluates the velocity of propagation of a low-frequency shear wave through the liver (Figure 1). This is dependent on the "stiffness" of the liver and reflects the degree of fibrosis. FibroScan® (Echosens, Paris, France) is the equipment dedicated to apply this technology and its performance has been scrutinised in a large number of studies over the last decade[21].

However, while transient elastography performs well for the assessment of cirrhosis, with sensitivity and specificity quoted between 77% and 100%, there has been less clear separation of stages of pre-cirrhotic disease[21]. Cut-off values reported by different studies to assess histological stages are variable according to the kind of patients selected and aetiology of disease.

More recently, liver stiffness has been shown to increase in flares of viral hepatitis and in acute hepatitis, even up to the levels seen in cirrhosis, but in the absence of clinically significant fibrosis. Further studies have demonstrated a compelling correlation between liver stiffness and portal pressure, while cardiac failure,

Table 1 Examples of serum markers for the assessment of fibrosis

Name	Constituents	Accuracy (%)			
		Se	Sp	PPV	NPV
Indirect markers					
APRI	AST, platelets	41	95	88	64
FibroTest	α2 macroglobulin, α2 and γ globulin, total bilirubin, apolipoprotein A1, γGT	87	59	63	85
Direct markers					
ELF	PⅢNP, HA, TIMP-1, (age)	90.5	41	99	92
FibroSpect	HA, TIMP-1, γ2 macroglobulin	77	73	74	76

APRI: Aspartate aminotransferase (AST) to platelet ratio index; γGT: γ-glutamyl transpeptidase; HA: Hyaluronic acid; PⅢNP: Amino terminal of procollagenase Ⅲ; TIMP-1: Tissue inhibitor of matrix metalloproteinase 1; ELF: Enhanced Liver Fibrosis test; Se: Sensitivity; Sp: Specificity; PPV: Positive predictive value; NPV: Negative predictive value.

Figure 1 Transient elastography. A specialist nurse places the probe perpendicular to the surface of the liver. A low frequency shear wave is generated along the same axis as the ultrasound transducer. The velocity of the shear wave through the liver is measured by a high frequency ultrasound signal and the output displayed as stiffness, in kPa, alongside a two-dimensional "elastogram". The output is the median of 10 measurements, with a success rate of > 66% and an interquartile range of measurements < 1/3 of the median considered satisfactory.

infiltrative conditions and even hepatic steatosis may affect stiffness values.

On the contrary of what was initially assumed, the sole liver stiffness cannot be considered a measure of hepatic fibrosis, but rather the result of different processes including fibrosis. Thus the results should be interpreted according to the clinical context and corroborated by other non-invasive techniques.

Use of ultrasound contrast agents

Microbubble contrast agents are small, stabilised gas-filled phospholipid bubbles (about 3 μm) that resonate when subjected to ultrasound, amplifying the reflected signal, thus enhancing intravascular signal for several minutes after intravenous injection and increasing signal from vessels and tissues. Safety and tolerance with current agents is excellent. When quantified, the resultant signal intensity change is proportional to microbubble concentration[22].

A simple microbubble-enhanced ultrasound test to measure hepatic vascular transit time (HVTT) by timing the arrival of contrast agent in the hepatic artery and subsequently the hepatic vein has been developed. A curve of signal intensity against time can be plotted, with shorter arrival times correlating with increased severity of liver disease due to circulatory changes, such as arterialisation of liver sinusoids, hyperdynamic circulation and extra- and intra-hepatic shunting (Figure 2). A study on 85 chronic hepatitis C patients assessed by HVTT showed 100% sensitivity and 80% specificity for cirrhosis[22]. Moreover microbubbles allowed to stratify mild and moderate disease (95% sensitivity and 86% specificity) suggesting that other processes, besides portal hypertension, may contribute to effects observed.

MR techniques

Both MR imaging (MRI) and MR spectroscopy (MRS) techniques have been applied to assess the severity of chronic liver diseases[23]. MRI techniques include dynamic superparamagnetic iron oxide and gadolinium enhanced studies, which have been shown to demonstrate reticular-nodular patterns, thought to represent septal hepatic fibrosis, allowing the qualitative discrimination of moderate to severe, from mild fibrosis. Objective stratification of fibrosis severity in patients with chronic hepatitis C has been reported using diffusion-weighted MRI[24]. Furthermore, MR elastography, which like transient elastography measures liver stiffness, allows visualisation of a map of hepatic liver stiffness[25]. MRS examines the chemico-physical environment of nuclei in a region of interest, providing metabolic information in the form of a spectrum, of relevance in chronic liver disease.

MR spectroscopy

In vivo ^{31}P MRS is a safe, reproducible technique which provides biochemical information on hepatic metabolic processes. Typical *in vivo* ^{31}P MR liver spectra contain phosphomonoester (PME), phosphodiester (PDE), inorganic phosphate (Pi) and ATP resonances, reflecting cellular energy state, intermediates of carbohydrate metabolism, precursors of cell membrane synthesis and breakdown. These resonances are multicomponent, but more detailed biochemical information may be obtained with *in vitro* MRS at higher magnetic field strengths (11.7 T-14.0 T) than in clinical studies (1.5 T-3.0 T).

In vivo phosphorus-31 (^{31}P) MRS provides metabolic information useful to evaluate fibrogenesis. The PME/PDE ratio has been used as an index of cell membrane

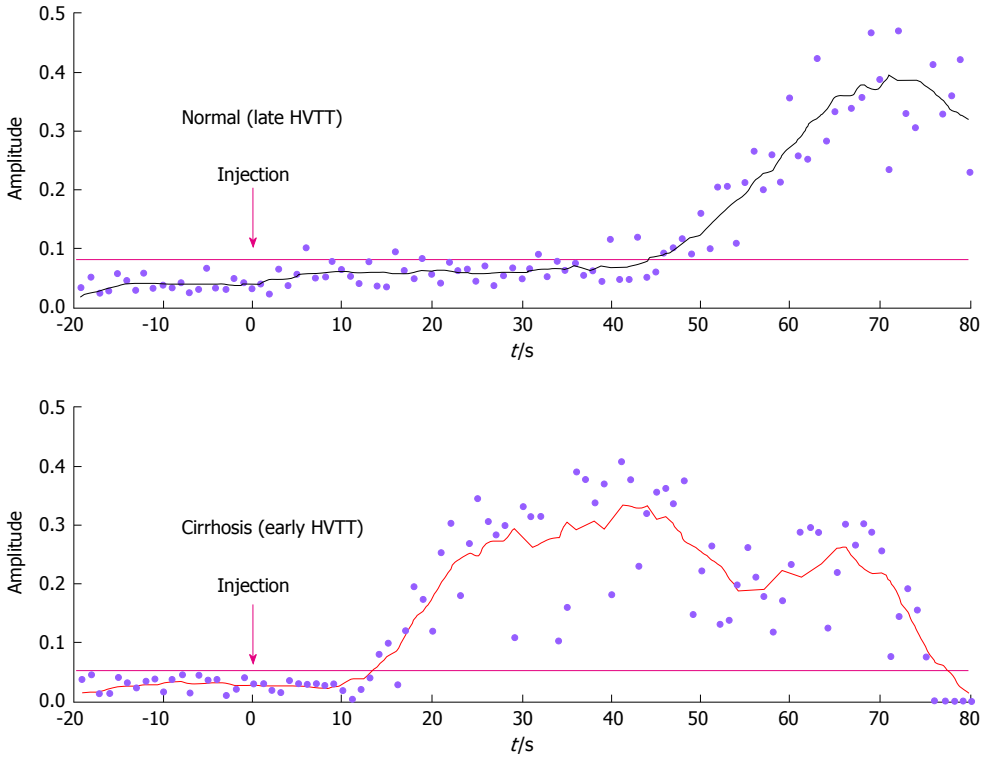

Figure 2 Hepatic vascular transit times. Time intensity curves from the hepatic vein plotted in a normal patient and a patient with cirrhosis, showing earlier arrival of contrast in the cirrhotic liver. Adapted from Lim *et al*[22] 2005. HVTT: Hepatic vascular transit times.

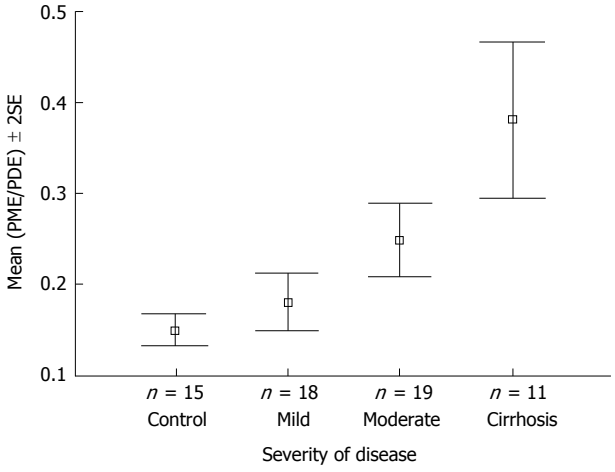

Figure 3 ^{31}P magnetic resonance spectroscopy. PME/PDE ratios obtained from *in vivo* hepatic ^{31}P MRS varying with severity of hepatitis C-associated liver disease. Adapted from Lim *et al*[26] 2003. MRS: Magnetic resonance spectroscopy; PME: Phosphomonoester; PDE: Phosphodiester.

turnover and correlates with histological stages. MRS has good sensitivity (82%) and specificity (81%) to detect cirrhosis and could differentiate it from mild hepatitis and moderate hepatitis[26] (Figure 3).

Recent longitudinal work in chronic hepatitis C has demonstrated a change in PME/PDE ratios in response to antiviral treatment, separating virological responders from non-responders.

MRS in hepatic steatosis

In hepatic steatosis, proton (^{1}H) MRS can provide information on the amount of liver fat[27]. More recent studies have demonstrated the potential to measure lipid composition non-invasively, which may change with disease state and with dietary intervention. Typical hepatic spectra contain water, fat and choline resonances, which can be quantified using external reference standards or expressed as a percentage relative to the total MR signal (Figure 4). ^{1}H MRS is readily accessible to all centres that have an MR scanner and most machines have the capability to perform such sequences as an addition to a standard MRI examination.

The future of biomarkers of chronic liver disease

Inflammation, steatosis and fibrosis are complex multistep processes. It would be surprising if a single biomarker were able to describe liver disease completely. Accordingly, combinations of markers and modalities may describe disease more accurately and reproducibly than one marker alone. Studies of marker combinations should be performed to establish optimal combinations, in terms of numbers of tests, accuracy of combinations and the provision of complementary information from the test components. Candidate markers differ widely in the equipment and expertise required, so cost-benefit analyses compared to routine liver biopsy are warranted. Serum markers and imaging techniques need to be investigated longitudinally in response to intervention in a number of disease states. As histological assessment of liver biopsy is itself a surrogate marker of liver disease, the challenge is to develop and validate protocols correlated to clinically meaningful outcome measures.

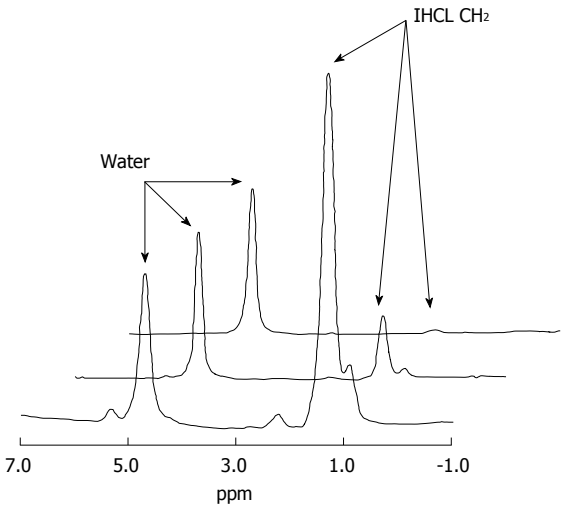

Figure 4 ^{1}H magnetic resonance spectroscopy. Proton (^{1}H) MR Spectra (left to right) from: (1) a patient with significant hepatic steatosis; (2) a patient with mild hepatic steatosis; and (3) a healthy volunteer. The intrahepatocellular (IHCL) lipid resonance is many times larger in (1) than (3), with the hepatic water resonance scaled to the same height for comparative purposes. Candidate markers for hepatocellular carcinoma which have been proposed in the literature. Most reflect high cellular turnover exhibited by tumours, but the majority lack sensitivity and specificity (see text for further explanations). Reproduced from Thomas *et al*[27] 2005. ppm: Parts per million; IHCL CH_2: Intrahepatocellular lipid.

Further research into non-invasive technologies for the assessment of chronic liver disease is required to correlate these techniques with clinical outcomes and to optimise them, in order to create validated management algorithms.

The challenge of a reliable diagnosis by non-invasive imaging

"Don't spend time beating on a wall, hoping to transform it into a door" Coco Chanel: According to the United States Center for Disease Control, May 2015, (http://www.cdc.gov/hepatitis/HCV/HCVfaq.htm#section1) every 100 persons infected with HCV, 75-85 will go on to develop chronic infection, 60-70 will develop chronic liver disease and over a period of 20-30 years 5%-20% will become cirrhotic. The death for liver disease, for cirrhosis or liver cancer involves the 1%-5% of patients infected, since the yearly incidence of HCC in people with cirrhosis is 3%-5%. This is very similar to the recommendation of WHO, which specifies that development of HCC is rare in patients with chronic hepatitis C not complicated by cirrhosis (http://www.who.int/csr/disease/hepatitis/whocdscsrlyo2003/en/index3.html).

The current treatment indications for HCV are under a state of flux, owing to the advent of a raft of new directly-acting antiviral agents (DAAs). The current modus vivendi is to allow the use of new drugs according to the presence of severe grades of fibrosis in some countries, but decompensating cirrhosis in others[17,28]. The grade of fibrosis can be assessed by liver biopsy, but modern algorithms now allow the use of non-invasive ultrasound procedures, such as transient elastography (Fibroscan®) and Acoustic Radiation Force Impulse (ARFI), two methods suitable for measuring the "stiffness" of the liver (LSM).

In the United States, as in Italy, there are reference criteria, *i.e.*, cut-off values, for defining the presence of severe fibrosis. For instance, according to the criteria of the Harvard Pilgrim Health Care, LSM of ⩾ 7.5 kPa on Fibroscan® should allow the use of DAAs; while according to the Idaho criteria Fibroscan® measurement > 12.5 kPa or ARFI value > 1.75 m/s or APRI score > 1.5 should be used. These striking differences are mirrored by the different results reported in the past and are well discussed in the EASL-AASLD clinical practice guidelines[29]. These variances in cut-off values for treatment are all the more complicated, given how prone the "gold standard", liver biopsy, is to sampling error and underscoring of fibrosis[18,19].

With this in mind, we set out to define the non-invasive measures most suitable to be used as cut-off values to define cirrhosis in a prospective case-control study, taking into account co-temporaneous liver biopsies, ARFI and TE measurements in chronic liver disease with different severity of fibrosis, measured by the Ishak grading system. Patients with liver cancer were excluded. According to the results in our patients, there is optimal correlation of the non-invasive measures of fibrosis, either ARFI or Fibroscan®, with each other and with the Ishak score. The cut-off values for cirrhosis that we identified are: ⩾ 10.25 kPa for Fibroscan®, and ARFI of the left liver lobe m/s 1.77, ARFI of the right liver lobe m/s. 1.92 for the overall group of patients. These cut-off values for the presence of cirrhosis were lower in subjects with previous HBV, compared to HCV, and even lower in subjects without any evidence of viral hepatitis, such as NAFLD. This observation strengthens the need of using the chosen cut-off for specific disease groups.

Differently, detection of liver nodules by any imaging method, particularly by ultrasound, is a defendable screening procedure, even if it encounters several limitations. It is suggested that combining alpha-fetoprotein (AFP) with ultrasound is the method of choice for screening patients at high risk for developing HCC, but this is not a widely accepted criterion due to the lack of sensitivity and specificity[30]. The use of plain ultrasound, even without contrast, is still the most suitable approach. Small nodules (< 1 cm) should be followed up in 3 subsequent months repeating ultrasound. If the lesion is no longer detectable, or if it is stable, it should be watched every 3 mo according to a monitoring strategy. In the case that the nodule enlarges, further imaging is needed. Equally, nodules that are > 1 cm require an immediate work up with computed tomography or MRI[31]. Despite the great interest and intervention aimed at screening HCC in high-risk populations[32-34], the actual results are controversial and, more importantly, the cost-benefit ratio in terms of outcome is still disputed[35,36]. This is the current situation, despite the benefits of the use of effective drugs[37-39] and, to a lesser degree, of

United States-guided treatments. Of course, surgical procedures are still the first line therapy in appropriate settings. Unfortunately, this is not the most frequent situation, less than 20% are suitable candidates for resection due to either multifocal unresectable tumors or their underlying chronic liver disease[1]. In this subset pharmacological therapy, with the current available drugs and other emerging molecules or associations, which will be available in the future, remains the more sustainable and rewarding option. The use of biomarkers, also derived from proteomic profiles, was found to have some utility in the prediction of clinical response to therapy[40], but other investigations in this field are needed.

SUSTAINABLE AND RELIABLE DIAGNOSIS: THE PLACE OF URINARY BIOMARKERS FOR LIVER CANCER DIAGNOSIS

Better three hours too soon than a minute too late. William Shakespeare

Urine has long been known to possess diagnostic features; Indian scriptures reporting its sweet taste are probably the earliest proof of this. In the XVII century, Thomas Willis identified the sweet taste of urine in diabetic polyuria. These characteristics have been used in daily practice since the diagnostic potential of urine has been applied in dipstick reagents. Some examples are the diagnosis of proteinuria, through detection of protein amine groups; haematuria, thanks to peroxidise activity of lysis-released haemoglobin; infection, through the detection of leukocyte esterase and nitrites; glycosuria, thanks to the conversion to hydrogen peroxide by glucose oxidase and pregnancy, with a strip-based immunoassay for rapid determination of beta human chorionic gonadotropin[41,42].

Urinary biomarkers

To be a urinary biomarker, a substance must pass through the renal collecting system avoiding tubular re-absorption, after plasma filtration through the renal glomerulus thanks to its size and ionic charge. Indeed the renal glomerulus is a barrier for larger or negatively charged plasma proteins, like albumin, since only molecules of < 20 kDa or 1.8 nm in size may pass through. Only after these passages can a molecule finally be found in enough quantities in urine to allow a diagnosis.

How select a good diagnostic test?

A good test must have high sensitivity (ideally 100%) and specificity, whilst also detecting the disease in its early stages, in order to allow the best treatment. "Gold standard" is the term used for the most accurate test that unfortunately is often expensive or time consuming to use for rapid diagnostics in the daily practice. So a good test must also be cheap, minimally invasive with low risk for the patient, and rapid, providing the needed information quickly, and allowing a management plan. In particular for HCC the early diagnosis is important as lesions detected below 2 cm are treatable with surgical resection or liver transplantation. Moreover HCC affects different populations, and the test should, be applicable across different patients and ethnicities.

The prevalence of HCC is high in the developing world[43], particularly in sub-Saharan Africa, where the attention to expense is of most importance, since often patients spent many days to reach a hospital and have limited resources to move. A urine dipstick test for HCC potentially would fulfil many of these criteria.

How detect cancer through the urine?

A urine biomarker must have three main features: first, correct size (less than 20 kDa) and ionic charge to pass through the renal glomerulus and be not re-absorbed by the tubules. Second, the biomarker should not be a molecule produced as a secondary effect of cancer, but needs to be specific for the type of cancer. Third, the amount of biomarker secreted should be adequate for early detection. The research of markers must be focused on small molecules (50-1000 Da) called metabolites, including bile acids, amino acids, peptides and nucleotides. Different combinations of metabolites could be specific for different conditions, while individually, since they are ubiquitous and involved in most cellular processes, they have minimal diagnostic potential. So a metabolic profile of different altered metabolites in combination may be highly specific for a type of cancer. This field of research has been termed "metabonomics"[44].

Urinary biomarkers of HCC

Nucleosides: The research for a urinary biomarker of HCC dates back to the 1970s when high levels of methylated purines (7-methylguanine, 1-methylhypoxanthine, N-dimethylguanine, 1-methylguanine and adenine) were detected in the urine of patients with HCC compared to both cirrhotic patients and healthy controls[45] (Table 2). This suggested that a rapid ribonucleic acid (RNA) turnover is involved in HCC pathogenesis and the methylation of nucleic acid could be a potentially involved in carcinogenesis. Later, a study using immunoassay technique showed high urinary levels of cyclic guanosine 3′:5′ monophosphate (cGMP) in rats with implanted liver and kidney tumours[46]. These findings were confirmed in 1982 by Dusheiko *et al*[47] in a clinical study on humans. The urinary cGMP excretion, as well as the plasma and ascitic fluid levels of cGMP, were found to be increased in patients with HCC, hepatic disease and other neoplasms[47]. These findings supported the hypothesis of a shift in cyclic nucleotide metabolism toward cGMP in cancer. However, urinary cGMP is not accurate to detect progression of cirrhosis to HCC, nor to differentiate HCC from other cancers.

The case for nucleoside derivatives as tumour markers was supported in 1986 by Tamura *et al*[48] in their study in HCC patients using high performance

Table 2 Urinary markers of hepatocellular carcinoma

Year	Urinary biomarker
Nucleosides and nucleotides	
1974	Methylated purines[45]
1976	Cyclic GMP[46,47]
1986	Pseudouridine[48,49]
Proteins and polyamines	
1990	TGFα and β[50-52]
1998	Neopterin[53-56]
2004	Urinary trypsin inhibitor[59,60]
1998	Spermine, putrescine, spermidine[58]
Metabolite profiles	
2009	Octanedioic acid, glycine and hypoxanthine[61]
2010	Creatinine, carnitine, creatine[62]

GMP: Guanosine 3′:5′ monophosphate; TGF: Transforming growth factor.

liquid chromatography (HPLC). They detected high level of urinary pseudouridine, a C-glycoside isomer of the nucleoside uridine,that showed a high sensitivity (83%) for HCC diagnosis if combined with serum AFP levels[48]. Urinary pseudouridine levels probably reflect the overall cellular proliferation in tumorigenesis and are not specific for HCC, since have also been shown to be elevated in other cancers, such as non-Hodgkin's lymphoma.

Jeng *et al*[49] showed, with an HPLC-based study in Taiwanese subjects, that the nucleosides adenosine, cytidine and inosine were elevated in the urine of patients affected by HCC and if joined with serum levels of AFP, the diagnosis of cancer was reached with a sensivity of 80%[49]. However this study evaluated the potential markers of HCC comparing only with a healthy control group, not considering cirrhotic patients, decreasing the reliability of the study.

Transforming growth factor α and β

In 1990, a study using a modified ELISA assay, showed the presence of transforming growth factor α (TGFα) in the urine of patients affected by HCC[50]. The following year a small study by Chuang *et al*[51] identified low molecular weight epidermal growth factor-related TGFs with functional activity in the urine of a similar group of patients. The same author corroborated their results in a larger study in 1997, showing a correlation of urinary TGFβ1 with the outcome of HCC patients[52]. A functional explanation of TGFs role in carcinogenesis comes from the ability to stimulate non-transformed cells to grow as colonies *in vitro*. However further studies are needed to confirm if these findings are specific for HCC compared to other malignancies.

Neopterin

Neopterin is a protein released from macrophages during the inflammatory response. In 1998 authors showed increased urinary levels of neopterin in Japanese patients with advanced HCC, correlating with lesion size but not with AFP. However this protein was not detected in patients with early HCC[53,54]. Unfortunately, neopterin has been found to be elevated in several cancers and other inflammatory diseases such as HIV infection[55], thus it is not specific for the diagnosis of HCC. Conversely, the combination of HPLC urinary levels of neopterin, pseudouridine and creatinine is a reliable indicator of RNA turnover, indicating neoplastic growth, in adenocarcinoma, non-Hodgkin's lymphoma and HCC[56].

Polyamines

The exact role of the polyamines (putrescine, spermine and spermidine) is unclear, although they are involved in cellular proliferation. Putrescine acts on *S*-adenosylmethionine (SAMe), a methylating molecule, to produce spermine, which in turn acts on further SAMe molecules to produce spermidine[57]. Antoniello *et al*[58], in their study based on reverse phase liquid chromatography, found increased levels of free and acetylated polyamines in the urine of patients affected by HCC compared to both healthy and disease controls (cirrhotic patients)[58]. As other biomarkers polyamines are not specific to HCC and not sensitive enough for early-stage diagnosis.

Urinary trypsin inhibitor

Urinary trypsin inhibitor (UTI) is a 25 kDa trypsin inhibitor used as a marker of hepatocyte function, it is believed to be produced by hepatocytes. A study in 2004 failed to shows a significant difference in levels of UTI in liver cirrhosis and HCC patients[59] reducing its usefulness in the early diagnosis of cancer. Other authors demonstrated that there is a correlation with the severity of liver disease, indeed Kikuchi *et al*[60] found a reduction of UTI plasmatic levels after HCC surgical treatment. Moreover the levels of UTI were correlated also with risk of tumour recurrence. Thus, UTI may be considered a biomarker of hepatic disorders and HCC but, also in this case, it lacks sensitivity for the early detection of cancer.

Metabolic profiling

Wu *et al*[61], in their study based on urinary gas chromatography mass spectrometry of 20 HCC patients, founda marker set of 18 metabolites (including octanedioic acid, glycine and hypoxanthine) distinguishing HCC and healthy Chinese controls, but a disease control group of cirrhotic patients was not considered[61]. This reduces the reliability of those findings in the principal at risk population.

A recent proton magnetic resonance (^{1}H-NMR) spectroscopy study by Shariff *et al*[62] reported a panel of urinary metabolites discriminating patients affected by HCC from both healthy controls and cirrhotic ones, with high sensitivity and specificity, respectively 100% and 93% in the first case and 89.5% and 88.9% in the second one, in a Nigerian groups of patients (Figure 5)[62]. This panel included creatine, creatinine, carnitine and acetone, that mirror an alteration of energy metabolism and cellular growth in such group of patients. Moreover, creatine is a biomarker of cachexia and sarcopenia related to the malignancy condition. These results need to be corroborated by larger studies on different ethnicity,

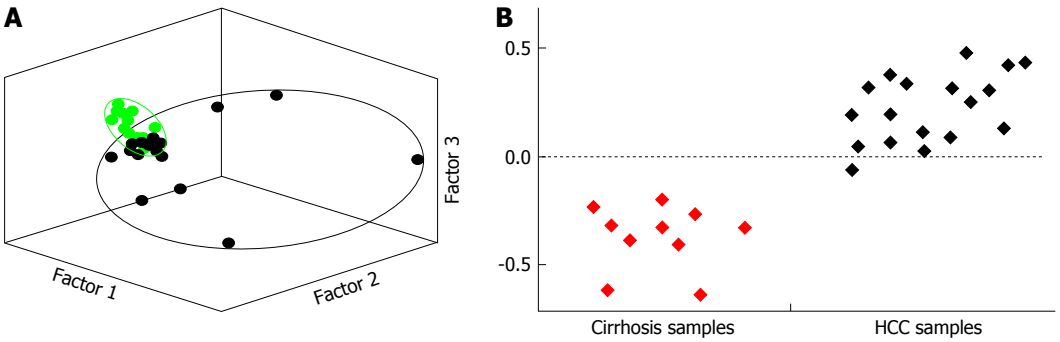

Figure 5 Principal components multivariate statistical analysis (A) and partial least squared discriminant multivariate statistical analysis (B). A: Principal components multivariate statistical analysis scatter plot showing statistical separation of data sets of urinary nuclear magnetic (NMR) spectroscopy information of hepatocellular carcinoma (HCC) subjects (small black spots), compared to urinary data sets from healthy controls (small green spots); B: Partial least squared discriminant multivariate statistical analysis showing statistical differentiation of metabolite information from HCC subjects obtained using NMR spectroscopy, compared to similar urinary data sets from patients with cirrhosis. These are the essential raisons d'être for searching and using urinary biomarkers for the early and reliable diagnosis of HCC, but promising research is still being undertaken to this end (Figure 6).

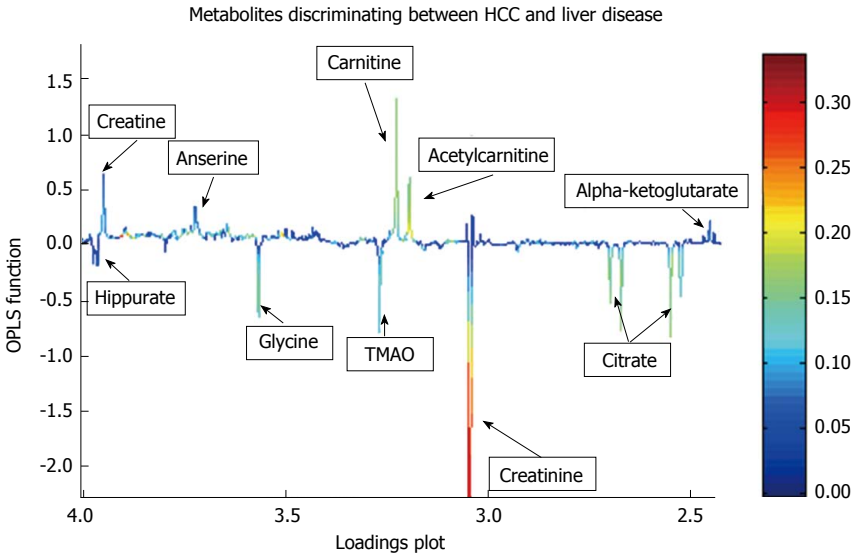

Figure 6 Statistical "loadings plot" of information obtained from an urinary nuclear magnetic resonance spectroscopy data set showing metabolites upregulated in hepatocellular carcinoma (upward peaks: Carnitine, anserine, creatine, acetylcarnitine, alpha-ketoglutarate) and downregulated in hepatocellular carcinoma (downward pointing peaks: Hippurate, glycine, trimethylamineoxide, creatinine, citrate), compared to urinary nuclear magnetic resonance spectroscopy data from patients with cirrhosis. Most metabolites represent alternative energy metabolites as liver tumours are not solely dependent on glycolysis for an energy source. HCC: Hepatocellular carcinoma; TMA: Trimethylamineoxide; OPLS: Orthogonal partial least squares discriminant analysis.

before this panel could be applicable and extended to the varying range of patients affected by HCC.

The information contained in the urine is useful to reach the diagnosis and urinary dipsticks are used in daily practice, allowing the physician to institute rapid management of an underlying condition, ranging from urinary tract infections to pregnancy. A new urinary dipstick test for the diagnosis of HCC would be of great value both in developed countries, where the first screening could be done by general practitioners, and in the resource-poor settings, where patients may not have easy access to serological tests or imaging facilities.

These are the essential raisons d'être for searching and using urinary biomarkers for the early and reliable diagnosis of HCC, but promising research is still being undertaken to this end (Figure 6).

After preliminary studies[62-64] using urinary ^{1}H-NMR spectroscopy in African populations, multiple marker metabolites in the urine do provide clues for the implication of altered energy-related pathways in the pathogenesis and progression of HCC[63]. More importantly from a clinical perspective, metabotypic changes seem to characterize HCC patients with enhanced sensitivity and specificity compared to serum AFP in the published studies to date, although much work needs to be performed on validation of this[64]. These findings suggest panel of urinary metabolites may prove useful for screening HCC in at-risk populations.

Moreover, further investigation in high risk populations for other liver cancers, such as cholangiocarcinoma[64], notably in Northeast Thailand[65], may be a worthwhile direction to pursue, potentially providing an answer to the difficult challenge of early diagnosis of primary cholangiocarcinoma and of monitoring the effects of

treatment, whenever available[66-68]. Furthermore, the rising trend of prevalence of cholangiocarcinoma in Europe, although of uncertain origin[69,70], is a matter of serious concern, but the lesson learnt by the long history of HCC can be useful for future research and applications.

CONCLUSION

Urinary biomarkers have been studied for almost half century, including nucleosides, small proteins, polyamines and recently, metabolites. Some of the techniques and tests described are already suitable for more widespread clinical application, as is the case with ultrasound-based liver diagnostics, but others, such as urinary metabonomics, requires a period of critical evaluation or development to take them from the research arena to clinical practice. The guidelines of sustainability in countries with limited resources, facilities and low financial income can be seen as an opportunity for addressing research toward low-cost diagnostics and for driving clinical practice toward more streamlined technology, with ultimate benefits for the populations of poorer countries around the world[70]. Also medicine, as "science, after all, is essentially international, and it is only through lack of the historical sense that national qualities have been attributed to it" (Marie Curie). Medicine should not exist as a "medical science" with different priorities for low and high-income populations[71]. The most important discoveries and advancements in the field of medicine have required, and probably still require, more focus to the clinical problems along with a sustainable analytical investigation of all the physiological and pathological details.

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MINIREVIEWS

Hepatitis C virus: A global view

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Abstract

Hepatitis C virus (HCV) is a global challenge; 130-175 million are chronically infected. Over 350000 die each year from HCV. Chronic HCV is the primary cause of cirrhosis, hepatocellular carcinoma (HCC), and end-stage liver disease. Management of chronic HCV is aimed at preventing cirrhosis, reducing the risk of HCC, and treating extra hepatic complications. New treatments for chronic HCV has been devoted based on direct-acting antivirals, as pegylated interferon (peginterferon) is responsible for many side effects and limits treatment access. Sofosbuvir is the first compound to enter the market with Peginterferon-free combination regimens.

Key words: Hepatitis C; Peginterferon; Sofosbuvir; Direct-acting antivirals

Core tip: Peginterferon is responsible for many side effects. Direct-acting antiviral drugs represent a breakthrough in hepatitis C virus (HCV) therapy. Sofosbuvir is the first compound to enter the market with Peginterferon-free combination regimens. The next few years are expected to introduce more new drugs in the market of HCV therapy with complete elimination of pegylated interferon and ribavirin combination therapy.

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INTRODUCTION

Hepatitis C is a global health problem as the World Health Organization (WHO), reported 3-4 million people

are newly infected with hepatitis C virus (HCV) per year and 130-170 million people are chronically infected. Over 350000 people die each year from hepatitis C-related liver diseases[1]. The data on the global prevalence are mostly based on HCV seroprevalence studies[2]. HCV-infected people are at high risk for developing chronic liver disease, cirrhosis, and hepatocellular carcinoma (HCC). HCV accounts for about 27% of cirrhotic cases and about 25% of HCC cases worldwide. However, WHO data are based on published studies and data submitted from different countries and regions. Although HCV is a world epidemic, there is great variability in its distribution in different regions of the world[1,2] (Table 1).

The highest prevalence rates are reported from developing poor countries in Africa and Asia, while the developed, industrialized nations in Europe and North America have low prevalence rates. Egypt, Pakistan, and China have the highest rates of chronic infection. Unfortunately, there are no good data from African countries, with the exception of Egypt, Morocco, and South Africa. The major transmission route in these countries is thought to be unsafe injections using contaminated equipment as in the case of Egypt, where the HCV epidemic has been mainly attributed to the prolonged use of parenteral anti-schistosomal treatment (antimony potassium tartrate, tartar emetics) with use of non-disposable glass syringes for more than 30 years. Chronic HCV is the most common cause of cirrhosis and the most common indication for liver transplantation in Egypt[3].

PREVALENCE OF HCV GENOTYPES AND SUBTYPES

HCV classified into seven genotypes (1-7) with multiple subtypes on the basis of phylogenetic and sequence analyses of whole viral genomes[4,5]. HCV strains belonging to different genotypes differ at 30%-35% of nucleotide sites. Strains that belong to the same subtype differ at < 15% of nucleotide sites[6]. The distribution of HCV genotypes depend on modes of transmission and ethnic variability[5].

Genotype 1 is the most common HCV genotype and is estimated to account for 83.4 million (46.2%), with wide geographical distribution, in Northern and Western Europe, Asia, North and South America, and Australia[4,5]. HCV genotype 2 mostly present in West and Central Africa, as its endemic place of origin[7,8]. HCV genotype 3 is the next most common genotype after genotype 1 and account for 54.3 million (30.1%) cases globally, about 75% of this number occur in south Asia[4]. Genotype 4 is characteristic for the Middle East especially Egypt[7]. The predominant HCV genotype among Egyptians was found to be genotype 4, particularly subtype 4a suggesting an epidemic spread of HCV. However, recent studies revealed that other genotypes and subtypes as 1a, 1b, and 2a are also present indicating that HCV genotypes are extremely variable[8,9]. Genotype 5 is present only in South Africa[5,7]. Genotype 6 is endemic in South East Asia especially in Hong Kong and Southern China[5,8]. Genotypes 2, 4, and 6 are responsible for the majority of the remaining cases of HCV worldwide after cases caused by genotype 1and 3, with an estimated 16.5 million (9.1%), 15.0 million (8.3%), and 9.8 million (5.4%) cases, respectively. To date, only one genotype 7 infection has been reported; it was isolated in Canada from a Central African immigrant[10].

MORBIDITY

Twenty-five percent to thirty percent of chronic infected HCV will suffer from cirrhosis after 20-30 years[3]. Twenty-five percent or more of cirrhotic patients will develop end-stage liver disease or hepatocellular carcinoma. However, pre-cirrhotic infection is not benign, and many HCV-infected patients suffer from extra-hepatic manifestations such as fatigue, joint affection, depression, insulin resistance, diabetes mellitus, nephropathy and lympho-proliferative disorders which increase the hospitalization for HCV patients by 15% per year[11-13].

MORTALITY

Chronic HCV infection causing about 2.4 million deaths each year. Recently reported that, the average annual age-adjusted mortality rate of deaths in which HCV was increased by 0.18 deaths per 100000 persons per year[14].

DIAGNOSIS

HCV is often remains undiagnosed for many years and usually diagnosed accidentally. HCV should be suspected in high risk persons and all patients presenting with increased liver enzymes, or cryptogenic chronic liver disease[15]. Infection with HCV is diagnosed by testing for specific antibodies using enzyme immunoassay (EIA), chemiluminescence immunoassays and recombinant immunoblot assays[16]. The introduction of the third generation EIA has brought the specificity of the serological testing to extremely high (greater than 99%)[17]. The presence of HCV antibodies indicate that HCV infection is acute, chronic, or has resolved. HCV-RNA can be detected in the blood using polymerase chain reaction or transcription-mediated amplification[18]. HCV-RNA should be determined before initiating treatment and monitoring of HCV treatment[19]. HCV genotyping is useful in determining treatment duration and predicting the likelihood of treatment response[20-22].

TREATMENT

Treatment indications

The goal of antiviral therapy is to cure HCV with sustained virological response (SVR). Treatment should be recommended in all chronic HCV infection adult patients especially patients who are at risk of developing cirrhosis unless there are therapy contraindications. Treatment of

Table 1 Hepatitis C prevalence rates in developed and developing countries[1]

Country	Prevalence
Egypt	18%-22%
Italy	2.5%-10%
Pakistan	4.9%
China	3.2%
Indonesia	2.1%

Table 2 Pretreatment assessments in patients with chronic hepatitis C virus infection[23]

Medical history, including previous antiviral therapies and response
Psychiatric history, including substance use disorders
Liver function tests, including liver enzymes, serum albumin, serum bilirubin, and prothrombin time
Complete blood count
Thyroid-stimulating hormone
Serum creatinine
Plasma glucose
Pregnancy test (in female of childbearing period)
HIV serology
Hepatitis B surface antigen
Quantitative HCV RNA measurement
Retinal examination
Electrocardiogram

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

chronic HCV with pegylated interferon (PegIFN)-alpha and ribavirin (RBV) containing regimens is absolutely contraindicated in: Uncontrolled depression, psychosis or epilepsy; pregnancy; severe concurrent medical diseases including retinopathy, autoimmune thyroid disorders; liver cell failure[16,23,24].

Now, Pretreatment liver biopsy is not mandatory and instead we can use fibroscan[25]. Other lines of pretreatment assessment are included in (Table 2).

Until 2011, the combination of PegIFN-alpha and ribavirin for 24 or 48 wk was the standard of care for treatment of HCV infection. PegIFNs administered subcutaneously once weekly in combination with oral RBV, resulting overall SVR rates of 40%-50% among treatment-naïve patients[21,26]. SVR rates were lower in specific patient populations, such as African Americans[27]. Adverse events from either PegIFN alpha-2a or alpha-2b, and RBV are similar. The optimal RBV dose appears to be between 800 and 1400 mg per day, based on weight in combination with either PegIFN product[28]. The standard treatment duration of PegIFN and RBV has been 48 wk, except in patients who are slow responders (detectable HCV RNA at 12 wk but undetectable HCV RNA by 24 wk into treatment), in whom extending therapy to 72 wk may be beneficial[29,30].

New drugs for hepatitis C

After 2011, new oral effective drugs have been introduced in the treatment of chronic HCV infection with the cure rate about 90%[31]; suggest that we might soon be able to cure all patients with HCV (treatment-naïve, relapsed patients on previous treatment and resistant patients). These new drugs open a new era in the management of chronic HCV infection after 25 years of HCV discovery. During these 25 years, the classical line of treatment of HCV had many side effects with limited success and low SVR; the new class of drug is called directly acting antiviral agents (DAAs)[32].

DAAs drugs increase the SVR rates with fewer side effects and provide a new hope for chronic HCV either naïve or treated patients with simplified route of administration *via* oral intake and more short period for treatment. First-generation NS3 protease inhibitors introduced in the market of HCV therapy since 2011 are telaprevir and boceprevir, which approved as a new standard line of therapy for genotype 1 HCV patients in addition to standard classical therapy, although low SVR rates were obtained in replasers and previous non-responder to dual therapy[33]. Moreover, many side effects, especially in patients with advanced grade of hepatic fibrosis[34].

Sofosbuvir (SOF), simeprevir (SIM), and daclatasvir (DCV), are new generations of DAAs which increase the SVR rates with fewer side effects and short duration of treatment. These drugs are used with or without PegIFN and/or RBV combination with different duration of treatment according to combination were used. In IFN eligible patients, the optimal regimen is a 12-wk course of PegIFN and RBV plus SOF, SIM, and DCV, but in IFN ineligible patients, the best line of treatment is 24-wk of SOF/RBV, or 12-wk of SOF-SIM or SOF-DCV with or without RBV. Monotherapy with SOF, SIM, and DCV is not recommended[35].

SOF as line of treatment of chronic HCV

SOF is pan-genotypic antiviral HCV-specific nucleotide inhibitor of viral NS5B polymerase that acts as chain terminator when incorporated as a substrate by RNA polymerase in the nascent HCV-RNA genome, leading to inhibition of viral replication which has a high barrier to resistance[36]. SOF is taken at dose of 400 mg once daily oral, without relation to food intake. SOF is taken as prodrug which became active molecule by phosphorylation inside the hepatocytes. SOF is metabolized by dephosphorylation to convert the active molecule to inactive metabolite GS-331007. GS-331007 is excreted through the kidney but the dose modification of SOF is not required if creatinine clearance is $\leqslant$ 30 mL/min. In severe renal impairment and end stage renal disease SOF is not recommended. Dose adjustment is not recommended in patients with mild-to-severe hepatic impairment[37,38].

SOF treatment regimens without PegIFN should not be used for patients with genotype 1, 4, 5 or 6 HCV infection unless the HCV patients had contraindication for PegIFN. Patients with advanced liver fibrosis or cirrhosis, high baseline viral load, previous unresponsiveness to PegIFN and RBV combination therapy may need extended course for 24 wk[39].

GLOBAL PREVENTION AND CONTROL

In many countries, including the developed countries, most patients with HCV infection are unaware about their infection for many years and, so developed cirrhosis and HCC before they known about their HCV infection and also became a big source of HCV infection in their communities[40]. In developing countries, barriers to screening include inadequate awareness of hepatitis C among healthcare providers and their patients. Public health officials in many developing countries do not understand the true burden of HCV infection. Surveillance for HCV infection is very important[41,42]. Linking prevention to testing, and treatment of HCV infection requires a comprehensive approach tailored to meet the needs of individual countries[43].

CONCLUSION

DAAs drugs represent a breakthrough in HCV therapy. The next few years are expected to introduce more new drugs in the market of HCV therapy with complete elimination of PegIFN and RBV combination therapy.

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MINIREVIEWS

Factors associated with the response to interferon-based antiviral therapies for chronic hepatitis C

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Abstract

Hepatitis C virus (HCV) infection is a major health concern worldwide. Interferon-α (IFN-α) therapy has been the main antiviral treatment for more than 20 years. Because of its established antitumor effects, IFN-based treatments for chronic HCV infection still have a clinical impact, particularly for patients with high risk conditions of developing hepatocellular carcinoma, such as older age and advanced liver fibrosis. As a result of exhaustive research, several viral factors, including NS5A amino acid mutations such as the IFN sensitivity-determining region and the IFN/ribavirin resistance-determining region, and mutations of amino acids in the core protein region (core 70 and 91) were shown to be associated with the response to IFN-α treatment. In addition, among the host factors related to the response to IFN-α treatment, polymorphisms of the *interleukin-28B* gene were identified to be the most important factor. In this article, we review the factors associated with the efficacy of IFN-α treatment for chronic HCV infection. In addition, our recent findings regarding the possible involvement of anti-IFN-α neutralizing antibodies in a non-response to pegylated-IFN-α treatment are also described.

Key words: Anti-interferon-α neutralizing antibody; Interferon-α; Direct-acting antiviral; Interferon-free treatment; Chronic hepatitis C

Core tip: Interferon-α (IFN-α) therapy has been playing a central role in anti-hepatitis C virus (HCV) strategies, and several viral and host factors related to the treatment efficacy have been identified. After the development of pegylated-IFN-α (Peg-IFN-α), the clinical impact of anti-IFN-α neutralizing antibodies in the treatment for HCV infection has not been sufficiently addressed. We recently found that anti-IFN-α neutralizing antibodies were associated with a non-response to Peg-IFN-α treatment. Our findings provide important information for the treatment of chronic hepatitis C in the clinical setting.

Enomoto H, Nishiguchi S. Factors associated with the response to interferon-based antiviral therapies for chronic hepatitis C. *World J Hepatol* 2015; 7(26): 2681-2687 Available from: URL: http://www.wjgnet.com/1948-5182/full/v7/i26/2681.htm DOI: http://dx.doi.org/10.4254/wjh.v7.i26.2681

INTRODUCTION

HCV infection is a major health concern worldwide. Approximately 150-160 million individuals are assumed to be infected with hepatitis C virus (HCV), and chronic HCV infection causes fibrotic liver changes and cirrhosis[1,2]. Furthermore, HCV-associated cirrhotic patients are at a high risk of developing hepatocellular carcinoma (HCC). The eradication of HCV is considered to terminate the chronic liver inflammation and decrease the risk of cirrhosis-associated clinical complications. Therefore, the main goal of anti-HCV treatment has been focused on how to eradicated HCV and "cure" the patients. Interferon-α (IFN-α) therapy has been playing a central role in anti-HCV strategies. Exhaustive studies have been carried out to increase the efficacy of IFN-α treatment, and many viral and host factors have been identified that affect the response to treatment[3,4].

Recently, new agents which directly inhibit the replication of HCV have been developed [direct-acting antivirals (DAAs)], and new IFN-free regimens which include only DAAs have been introduced, with promising clinical efficacy. IFN-free treatments are currently approved as standard therapies in the recent guidelines of the American Association for the Study of Liver Disease and the European Association for the Study of the Liver[5,6]. However, even after a HCV infection has been resolved, elderly patients with advanced liver fibrosis still have a high risk at developing HCC, and many Japanese patients have these features[7,8]. Previous studies have shown that IFN therapy significantly reduces the risk for hepatocellular carcinoma in HCV-infected patients[9-11], while the antitumor effect of IFN-free treatment has not yet been sufficiently evaluated. IFN therapy is therefore still considered to have clinical significance, particularly in Japanese HCV-infected patients with a high incidence of HCC. In this article, we review the factors associated with the efficacy of IFN-α therapy for chronic HCV infection. In addition, we also discuss our recent findings regarding the role of anti-IFN-α neutralizing antibodies (NAbs) in IFN-α based therapy.

VIRAL FACTORS ASSOCIATED WITH THE RESPONSE OF HCV INFECTION TO IFN TREATMENT

It was known that there were patients who showed chronic liver damage irrespective of the absence of hepatitis A virus or hepatitis B virus infection (nonA-nonB hepatitis: NANB hepatitis). In 1986, Hoofnagle *et al*[12] reported that recombinant IFN treatment normalized the aminotransferase levels of patients with NANB hepatitis, and suggested the potential clinical utility of IFN treatment for NANB hepatitis. In 1989, HCV was identified by Choo *et al*[13], and many patients with NANB hepatitis were demonstrated to be infected with HCV. In 1992, IFN-α monotherapy was approved as the first antiviral therapy for chronic hepatitis C (CH-C) in Japan. At that time, the factors associated with the efficacy of IFN-α treatment were unclear, and IFN-α treatment was sensationally reported as a "dream" therapy that would cure about 30% of CH-C patients.

However, intensive research demonstrated several factors that were related to the efficacy of treatment[3,4]. Among these factors, serogroup 1 and a high viral load (100 KIU/mL: Currently 5.0 log copies/mL) were particularly associated with poor treatment efficiency, and most Japanese patients who achieved a sustained viral response (SVR) were infected with viruses of serogroup 2 or had a low viral load. Unfortunately, about 60%-70% of Japanese CH-C patients were infected with viruses of serogroup 1 (mostly genotype 1b) and had a high viral load (so-called "1b/high" patients), and fewer than 5% of these patients experienced a successful eradication of HCV by the IFN-α treatment. Therefore, many Japanese "1b/high" patients showed unfavorable outcomes despite their high expectations to be free from HCV infection, and a few years after the approval of IFN-α treatment for CH-C, the "1b/high" patients came to be considered patients who should not be treated using this regimen, because they were predicted to be at risk for experiencing adverse events without achieving a SVR.

Although only a small percentage of patients with 1b/high disease obtained a SVR, this finding indicated that there were patients who achieved a SVR irrespective of infection with "1b/high" viruses. In 1996, Enomoto *et al*[14] compared the amino acid sequences of HCV between patients with a SVR and those without a SVR. They found that the amino acids sequence of the NS (non-structural) 5A region was closely related to the eradication of HCV genotype 1b in response to IFN-α monotherapy. They named the specific region (NS5A 2209-2248) the interferon sensitivity determining region (ISDR). The identification of the ISDR had a high clinical impact; however, the HCV phenotype with many mutations of amino acids in the ISDR was observed in only in a small percentage of "1b/high" patients, and HCV eradication remained a major challenge for Japanese clinicians.

In 2000, ribavirin (RBV) became clinically available, and about 20%-30% of "1b/high" patients succeeded in obtaining a SVR following RBV treatment. This gave physicians the impression that they could also cure patients infected with "1b/high" viruses even without mutations of the ISDR. However, because of the presence of an ISDR-independent response, the identification of additional factors that were associated with the response to IFN-α plus RBV combination therapy was needed. Akuta *et al*[15]

reported that mutations of amino acids in the core protein region (core 70 and 91) were significantly associated with a non-response to combination therapy. Subsequently, a specific region other than the ISDR (NS5A 2334-2379) was found to be related to the treatment response to IFN-α plus RBV therapy, and was reported as the Interferon/Ribavirin Resistance-Determining Region (IRRDR)[16,17]. Overall, the most important viral factors associated with the response to IFN-α treatment for HCV were determined to be the amino acid sequences in the core region and NS5A region, such as mutations of core 70, core 91, ISDR and IRRDR.

HOST FACTORS ASSOCIATED WITH THE RESPONSE TO IFN TREATMENT FOR HCV INFECTION

From 2004, when pegylated-IFN-α (Peg-IFN-α) became available, Peg-IFN-α plus RBV combination therapy came to be a standard treatment, which provided a SVR in about 40%-50% of the patients with "1b/high" infections. Since IFN-α treatment depends on the immune response of patients, the characteristics of HCV-infected patients were considered to affect the treatment efficacy. Some host factors such as aging, sex, and the degree of liver fibrosis, had long been known to be related with the treatment efficacy. However, the major predictive factors for the response to IFN-α treatment were the amino acid sequences of HCV in the NS5A and core regions, and no decisive host factor had been discovered.

In 2009, findings regarding the gene polymorphisms of interleukin 28B (IL28B) were reported[18-20]. A genome-wide analysis showed that patients with a risk allele had about 40-fold higher resistance to Peg-IFN-α plus RBV combination therapy. These three papers were extremely important, because these studies included various races of patients from different counties, thus demonstrating that the involvement of IL28B in the treatment response to the Peg-IFN-α plus RBV combination therapy was not limited to patients with a specific ethnic back ground. In Japan, the *IL28B* gene polymorphism rs8099917 is commonly assessed, and patients with the G allele are predicted to show a poor response to Peg-IFN-α plus RBV combination therapy. Among the host factors associated with the response to the IFN-α treatment for HCV, the IL28B sequence is considered to be the most important factor.

MUTATIONS OF HCV RESULTING IN RESISTANCE TO DAAS

As described above, the Peg-IFN-α plus RBV treatment increased the rate of HCV eradication in patients with "1b/high" infection; however, more than half of the patients with "1b/high" infections still experienced treatment failure. In order to provide a higher SVR rate than Peg-IFN-α plus RBV treatment, DAAs which directly inhibit the replication of HCC were developed, and triple therapy (the Peg-IFN-α plus RBV plus a DAA) became available in Japan. In 2011, telaprevir, which inhibits the activity of the protease in the NS3/4A region, was first approved for clinical use in Japan, and the combination of telaprevir with Peg-IFN-α plus RBV increased the SVR rate of the "1b/high" patients over 60%[21,22]. Recently developed drugs such as simeprevir[23,24] and vaniprevir[25,26] were shown to provide a SVR in over 80% of the "1b/high" patients when one of these agents was administrated in combination with the Peg-IFN-α plus RBV. Although the DAAs showed strong anti-HCV effects, DAA monotherapy induced viruses with drug-resistant mutations, and the main role of a DAA has thus been to increase the treatment efficacy of Peg-IFN-α and RBV. Many viral mutations associated with resistance to DAAs have been reported[27,28]; however, factors associated with the response to the IFN treatment are also considered to be important in the efficacy of DAA-containing triple therapy. Table 1 summarizes the various factors associated with the efficacy of interferon-based treatment.

THE ROLE OF ANTI-IFN-α NEUTRALIZING ANTIBODIES IN IFN-α TREATMENT

Since IFN treatment involves the exogenous administration of the antiviral drug, patients who receive IFN sometimes develop anti-IFN NAbs. Anti-IFN NAbs inhibit the interactions between IFN and its receptor, and diminish the biological activity of IFN. Anti-IFN NAbs were reported to be associated with a poor response of CH-C treated with IFN, particularly in patients treated with non-natural recombinant IFNs[29-32]. With regard to HCV-infected patients receiving rIFN-α, several previous studies have suggested that anti-IFN-α NAb were more frequently detected in the sera of non-responders than in that of responders[29-32]. Because of the difficulty in obtaining a SVR, Japanese HCV-infected patients with "1b/high" sometimes received multiple kinds of IFN therapy, and frequently develop anti-IFN-α NAbs.

Since non-pegylated IFN-α products were unstable in human sera, the administrated non-pegylated IFN-α has a short plasma half-life (3-8 h), and becomes undetectable within one day[33]. Peg-IFN-α maintains serum concentrations that show antiviral effects for a long time (Peg-IFN-α 2a: 168 h and Peg-IFN-α 2b: 80 h) for two reasons. One reason is that the clearance of IFN-α is decelerated because of the biding of the IFN-α with a high weight molecule agent (polyethylene glycol), and the other is that the Peg-IFN-α product, which is enclosed in polyethylene glycol, can escape from recognition and attack by the host immune system[34].

Since Peg-IFN-α products were designed to be protected from the host immune system, the anti-IFN-α NAb were no longer believed to be of clinical significance after Peg-IFN-α was used as the first-line drug. In 2010, Halfon *et al*[35] measured anti-IFN-α NAbs with a

Table 1 Factors associated with the efficacy of interferon treatment

Factors	Main findings	Ref.
Classically identified viral and host factors		
Age, HCV genotype, Viral load, Liver fibrosis	Older age, HCV genotype 1, high viral load, and advanced liver fibrosis were associated with poor treatment results	[3,4]
Viral factors		
ISDR	Mutations of the ISDR (NS5A 2209-2234) were positively related to the HCV eradication with IFN-α monotherapy	[14]
Amino acid mutations of the core region (Nos. 70 and 91)	Mutations of amino acids were associated with a poor response to IFN-α plus RBV treatment	[15]
IRRDR	Mutations of the IRRDR (NS5A 2334-2379) were associated with a favorable response to the IFN-α plus RBV treatment	[16,17]
Drug resistant mutation[1]		[27,28]
Host factors		
IL28B SNPs	The hero/minor allele of IL28B SNPs was related to a poor response to Peg-IFN-α plus RBV treatment	[18-20]

[1]Resistant mutations to DAAs are only associated with the treatment efficacy of DAA-containing triple therapy. HCV: Hepatitis C virus; ISDR: Interferon sensitivity-determining region; IRRDR: Interferon/ribavirin resistance-determining region; RBV: Ribavirin; SNPs: Single nucleotide polymorphisms; Peg-IFN-α: Pegylated-interferon-α; IL28B: Interleukin 28B; DAA: Direct-acting antiviral.

quantitative sandwich enzyme-linked immunosorbent assay and reported that the presence of anti-IFN-α NAb was not associated with an early viral response (≥ 2 $\log_{10}$ copies/mL reduction in HCV-RNA at week 12 relative to baseline values). However, Peg-IFN-α agents are artificially generated drugs as well as conventional IFN agents, and we therefore asked whether anti-IFN-α NAbs were associated with the treatment efficacy of Peg-IFN-α using an antiviral biological assay method[36].

ANTI-IFN NEUTRALIZING ANTIBODIES IN PEG-IFN-α TREATMENT WITH THE ANTIVIRAL BIOLOGICAL ASSAY METHOD

We studied a total of 129 patients who had received Peg-IFN-α plus RBV treatment at our institute, and evaluated the involvement of anti-IFN-α NAb in the response to the Peg-IFN-α plus RBV treatment. An antiviral biological assay revealed that none of the 82 end-of-treatment responders had developed anti-IFN-α NAbs, while anti-IFN-α NAbs were detected in seven of the 47 NR patients (7/47: 14.9%). When we examined the sera of an additional 83 NR patients who had received Peg-IFN-α treatment at other institutions, 12 patients were proven to be anti-IFN-α NAb-positive (12/83: 14.5%). The patients who had IFN-responsive factors, such as HCV serogroup 2 and major allele homozygotes for the *IL28B* gene, were included in the 19 anti-IFN-α NAb-positive patients; however, all of them were non-responders, suggesting that the presence of anti-IFN-α NAb contributed to the non-response to the Peg-IFN-α treatment[36]. Table 2 shows the published reports regarding the possible involvement of anti-IFN-α neutralizing antibodies in the response to IFN-α treatment for chronic hepatitis C.

Since patients with a non-response to Peg-IFN-α plus RBV therapy often show a poor response to robust triple therapy (Peg-IFN-α plus RBV plus a DAA), the factors associated with the response to IFN treatment were also suggested to have an impact on the new DAA-containing therapy. We recently used the HCV-replicon system with genotype 1b to assess the potential role of anti-IFN-α NAb in the response to DAA-containing triple therapy[37]. Although telaprevir (TVR) monotherapy rapidly reduced the HCV-RNA level *in vitro*, the HCV-RNA level was increased again with the emergence of TVR-resistant viruses. Combination treatment with TVR and IFN-α successfully inhibited the replication of HCV for more than 30 d. However, in the presence of anti-IFN-α NAb-positive sera, the levels of HCV-RNA showed a time course similar to that with TVR monotherapy, and TVR-resistant viruses were detected in the conditioned medium. Our findings suggest that the anti-IFN-α NAb decreased the antiviral effects of IFN-α and caused treatment failure even when used in DAA-containing triple therapy. Indeed, we recently experienced a patient who achieved a SVR with an IFN-free regimen, despite that the patient developed the anti-IFN-α NAb and resulted in NR to the triple therapy. The role of anti-IFN-α NAb in triple therapy (Peg-IFN-α plus RBV plus a DAA) should be clarified in further clinical studies.

CONCLUSION

Although IFN-free treatments are currently recommended in the USA and Europe[5,6], the guideline of the Asian Pacific Association for the Study of Liver Disease[38] includes IFN-based antiviral treatments for CH-C. Because of its antitumor effects, IFN treatment is still important in HCV-infected patients, particularly in Japanese patients who are at a high risk of developing HCC. Viral factors (such as serogroup, viral load, mutations of core 70, core 91, ISDR and IRRDR) and host factors (such as aging, sex, the degree of liver fibrosis and IL28B SNPs) have been identified to be

Table 2 Possible involvement of anti-IFN-α neutralizing antibodies in the response to interferon-α treatment for chronic hepatitis C

Treatment	Cohort	Main results	Ref.
IFN-α monotherapy	47	Fifteen of 47 patients (31.9%) developed detectable levels of NAbs within two to eight months after starting treatment. Patients who developed anti-IFN NAbs showed poor responses to IFN (4/15: 26.6%) compared to antibody-negative patients (26/32: 81.3%) (P = 0.0009)	[29]
IFN-α monotherapy	63	Fifteen of 63 patients were positive for neutralizing anti-IFN-α NAbs. The responsive rate of all patients was 60.3% (38/63), while that of patients with anti-IFN NAbs was 13.3% (2/15), showing that NAbs development could significantly affect the therapeutic efficacy of IFN (P < 0.01)	[30]
IFN-α monotherapy	28	Among 28 patients treated with recombinant IFN-α-2a, anti-IFN-α NAbs were detected in 75% (6/8) of the patients who did not respond to IFN therapy. During IFN treatment, the mean ALT level of anti-IFN negative patients was decreased and continuously suppressed during treatment with the 3 MU of IFN-α-2a, while that of anti-IFN positive patients was reelevated without a dose-reduction of IFN	[31]
IFN-α monotherapy	84	In 84 patients with initial responses to IFN-α treatment, anti-IFN-α NAbs developed in 38.5% (5/13) of patients with breakthrough, as compared to 2.8% (2/71) of complete-responder patients (P < 0.0005) The emergence of anti-IFN-α NAbs three months after the initiation of therapy was the only factor to be predictive of breakthrough (RR = 9.5, 95%CI: 1.6-64.7, P = 0.007)	[32]
Peg-IFN-α plus RBV	42	A total of 42 non-response patients to previous conventional IFN treatment were re-treated with Peg-IFN-α-2a plus RBV. A decrease in HCV-RNA greater than 2 log10 copies/mL at week 12 relative to baseline values was not associated with the presence of anti-IFN-α NAbs (7/19, 36.8% in responders *vs* 6/23, 26.1% in non-responders at week 12; P = 0.73)	[35]
Peg-IFN-α plus RBV	129	A total of 129 patients who received Peg-IFN-α plus RBV were studied. Of the 47 patients who did not achieve an end of treatment response, seven patients (14.9%) were positive for anti-IFN-α NAbs, while no anti-IFN-α NAbs were detected in the 82 end of treatment responders (P = 0.0001). Anti-IFN-α NAbs were associated with a non-response to Peg-IFN-α plus RBV treatment, regardless of the patient IL28B-type and other treatment response-related characteristics	[36]

IFN: Interferon; Peg-IFN-α: Pegylated-interferon-α; RBV: Ribavirin; NAbs: Neutralizing antibodies; ALT: Alanine aminotransferase; RR: Relative risk; HCV: Hepatitis C virus; IL28B: Interleukin 28B.

associated with the response to IFN treatment for HCV infection. In addition, viral mutations resistant to DAAs have become problematic in recent triple therapies.

After the development of the Peg-IFN-α, the clinical impact of anti-IFN-α NAb in the treatment of CH-C was no longer considered. Due to the discrepancy in the results of our study[36] and a previous study[35], perhaps because of different detection methods of NAbs, the association between anti-IFN-α NAbs and a non-response to Peg-IFN-α therapy has not been fully confirmed. However, our recent findings suggest that anti-IFN-α NAb abolished the antiviral effects of Peg-IFN-α, and this finding provides important information for the treatment of CH-C in the clinical setting.

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ORIGINAL ARTICLE

Prospective Study

Importance of virological response in the early stage of telaprevir-based triple therapy for hepatitis C

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Abstract

AIM: To investigate the efficacy of virological response (VR) to telaprevir (TVR)-based triple therapy in predicting treatment outcome of hepatitis C.

METHODS: This prospective, multicenter study consisted of 253 Japanese patients infected with hepatitis C virus (HCV) genotype 1b. All received 12 wk of TVR in combination with 24 wk of pegylated-interferon-α (IFN-α) and ribavirin. Serum HCV RNA was tested at weeks 1, 2, 3, 4, 6, 8, 12, 16, 20, and 24. VR was defined as undetectable serum HCV RNA. Sustained virological response (SVR) was VR at 24 wk after the end of treatment and was regarded as a successful outcome.

RESULTS: Of 253 patients, 207 (81.8%) achieved SVR. The positive predictive value of VR for SVR was 100% at week 2, after which it gradually decreased, and was over 85% to week 12. The negative predictive value (NPV) gradually increased, reaching 100% at week 12. The upslope of the NPV showed a large increase from week 4 (40.6%) to week 6 (82.4%). There was a moderate concordance between the SVR and VR at week 6 (kappa coefficient = 0.44), although other VRs had poor concordance to SVR. Multiple logistic regression analysis extracted VR at week 6 ($P < 0.0001$, OR = 63.8) as an independent factor contributing to SVR. In addition, the interleukin-28B single nucleotide polymorphism and response to previous pegylated-IFN-α and ribavirin therapy were identified as independent factors for SVR.

CONCLUSION: VR at week 6, but not at week 4, is an efficient predictor of both SVR and non-SVR to TVR-based triple therapy.

Key words: Chronic hepatitis C; Direct-acting antiviral agent; Rapid virological response; Early virological response; Response-guided treatment

Core tip: Although an undetectable viral level at week 4 or 12 is a good predictor of the outcome of hepatitis C for conventional interferon therapy without direct-acting antiviral agents (DAAs); the transition of the viral level during DAA therapy has not been well documented. In this prospective multicenter study, we frequently tested 253 patients to investigate viral activity during triple therapy containing telaprevir, the first approved DAA, and found that an undetectable viral level at week 6 was the most effective predictor of disease outcome. Our findings suggest that the most predictive time point in DAA therapy is different from conventional therapy markers.

Hiramine S, Furusyo N, Ogawa E, Nakamuta M, Kajiwara E, Nomura H, Dohmen K, Takahashi K, Satoh T, Azuma K, Kawano A, Koyanagi T, Kotoh K, Shimoda S, Hayashi J. Importance of virological response in the early stage of telaprevir-based triple therapy for hepatitis C. *World J Hepatol* 2015; 7(26): 2688-2695 Available from: URL: http://www.wjgnet.com/1948-5182/full/v7/i26/2688.htm DOI: http://dx.doi.org/10.4254/wjh.v7.i26.2688

INTRODUCTION

Since the approval of interferon-α (IFN-α) for the treatment of hepatitis C virus (HCV) infected patients in 1991, treatment regimens have greatly evolved and improved. The rate of sustained virological response (SVR) to dual therapy with ribavirin (RBV) and pegylated IFN (PegIFN) of patients with HCV genotype 1 has remained approximately 50%[1-3], but with telaprevir (TVR), the first direct-acting antiviral agent (DAA) approved in the United States, Canada, the European Union, and Japan, the rate of SVR to triple therapy of PegIFN-α, RBV, and TVR against HCV genotype 1 has reached over 70%[4-6]. New DAAs have since been developed and approved, and it has become common for patients to be treated with IFN therapy that contains a DAA or a DAA based IFN-free oral therapy. Unfortunately, the cost of DAAs can be prohibitive, and some have serious side effects. If patients who will not achieve SVR can be identified before or in the early stage of treatment, they can avoid starting or continuing an expensive treatment that has no possibility of success. Therefore, studies of factors that can be used to predict the outcome of DAA based therapies are needed.

For dual therapy with PegIFN-α/RBV, it has been consistently reported that virological response (VR: undetectable serum HCV RNA) at week 4 or 12 of therapy is strongly associated with outcome[7-10]. Rapid VR (RVR), VR at week 4, and early VR (EVR), VR at week 12, were terms coined before the approval of DAAs, and this criterion is still used for determining the best form of antiviral treatment management, as recommended by international consensus conferences such as the American Association for the Study of Liver Diseases (AASLD)[11] and the European Association for the Study of the Liver (EASL)[12]. However, the viral kinetics during DAA therapy are unclear, and it is possible that the time point most predictive of success might be different than the older regimens.

To clarify the timing of VR most predictive of SVR during DAA based treatment, we measured serum HCV RNA at seven time points during the early stage of TVR-based triple therapy for Japanese patients.

MATERIALS AND METHODS

Patients

Since 2004, the Kyushu University Liver Disease Study Group has conducted prospective, multicenter studies to investigate the efficacy and safety of antiviral treatment for chronic hepatitis C patients[3,6]. For this study, we recruited 253 chronic hepatitis C patients infected

with HCV genotype 1b who started TVR-based triple therapy between December 2011 and December 2012 and completed 24 wk post-therapy follow-up by June 2013. Exclusion criteria were as reported previously[6]. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and was approved by the Ethics Committee of our hospital. Informed consent was obtained from all patients before enrollment. The study was registered as a clinical trial on the University Hospital Medical Information Network (ID 000009711).

Treatment response

VR was defined as undetectable serum HCV RNA. Successful treatment was SVR at 24 wk after the end of treatment. Relapse was defined as VR during the treatment but non-SVR. Patients with HCV RNA detectable throughout treatment were classified as non-responders. Patients who had not been previously treated with PegIFN-α/RBV therapy were classified as treatment naïve.

Clinical and laboratory assessment

Clinical parameters included hemoglobin, platelet count, serum albumin, aspartate aminotransferase (AST), alanine aminotransferase, γ-glutamyl-transpeptidase, low-density lipoprotein (LDL) cholesterol, ferritin, and estimated glomerular filtration rate. HCV RNA was tested at baseline, weeks 1, 2, 3, 4, 6, 8, 12, 16, 20, and 24 during the treatment and at weeks 4, 8, 12, and 24 after the end of treatment. We defined the early stage of treatment as the period between day 1 and week 12. The timing of VR in the early stage of treatment was evaluated for candidate predictors of SVR. Liver biopsy was done for 154 (60.9%) patients before the induction of therapy. For each specimen, the stage of fibrosis (F0-4) and grade of activity (A0-3) were established according to the Metavir score[13].

Determination of HCV markers

The baseline and follow-up tests for HCV viremia were done by real-time polymerase chain reaction (PCR) assay (COBAS TaqMan HCV test, Roche Diagnostics, Basel, Switzerland), with a detectability of ⩾ 15 IU/mL and a linear dynamic range of 1.2-7.8 log IU/mL. HCV genotype and the core amino acid substitution at position 70 of the HCV genome were determined before treatment for all patients. HCV genotype was determined by sequence determination in the 5′ non-structural region of the HCV genome, followed by phylogenetic analysis[14].

Interleukin 28B and inosine triphosphate pyrophosphatase polymorphism genotyping

Human genomic DNA was extracted from peripheral blood. Genotyping by the single-nucleotide polymorphism (SNP) of the interleukin 28B (*IL28B*) (rs8099917) gene was done using the TaqMan Allelic Discrimination Demonstration Kit (7500 Real-Time PCR System; Applied Biosystems, Foster City, CA). Patients were genotyped as TT, TG, or GG at the polymorphic site. Similarly, genotyping by the SNP of the inosine triphosphate pyrophosphatase (ITPA) (rs1127354) gene was done using the TaqMan Allelic Discrimination Demonstration Kit. Patients were genotyped as CC, CA, or AA at the polymorphic site. IL28B and ITPA SNPs were not available for only two patients (1.2%). Although rs12979860, another IL28B SNP that is also strongly correlated to the therapeutic outcome, has been reported[15], we determined only rs8099917 because it was previously reported that rs8099917 and rs12979860 represent 98.6% of the Japanese population[16].

Therapeutic protocol

All patients received 12 wk triple therapy that included TVR (2250 mg/day) (Telavic; Mitsubishi Tanabe Pharma, Osaka, Japan), PegIFN-α-2b (60-150 μg/wk) (PEG-Intron; MSD, Tokyo, Japan), and RBV (600-1000 mg/d) (Rebetol; MSD), followed by a 12 wk dual therapy that included PegIFN-α-2b and RBV. TVR (750 mg) was administered orally three times a day at 8 h intervals after each meal. PegIFN-α-2b was injected subcutaneously once weekly at a dose of 1.5 μg/kg. RBV was given orally at a daily dose of 600-1000 mg based on body weight (600 mg for patients weighing < 60 kg, 800 mg for those weighing 60-80 kg, and 1000 mg for those weighing > 80 kg). The above durations and dosages are those approved by the Japanese Ministry of Health, Labor, and Welfare. If marked anorexia, an elevation of serum creatinine, or severe anemia developed, the TVR dose could be reduced to 1500 mg/d (750 mg at a 12 h interval, after meals). The method of RBV/TVR dose reduction in the case of anemia was as reported[17]. The completed assigned total cumulative dosages of each drug were calculated by reviewing the patients' medical records and by counting the pills not consumed by each patient. The actual dosage of TVR given was calculated as the percentage of target TVR (2250 mg/d). The dosages of PegIFN-α-2b and RBV were calculated individually as averages on the basis of body weight at baseline.

Definition of positive predictive value and negative predictive value

To evaluate the precision rate of on-treatment VR for predicting outcome, we calculated the positive predictive value (PPV) and the negative predictive value (NPV). PPV is defined as the probability that a patient with a given on-treatment VR will achieve SVR. In contrast, NPV is defined as the probability that a patient without a given on-treatment VR will not achieve SVR.

Statistical analysis

Statistical analyses were performed using the SAS system, version 9.1.3 (SAS Institute, Cary, NC, United States). Continuous data are expressed as median with interquartile range. Univariate analyses were performed using the χ^2 test, Fisher's exact test, paired *t*-test, or

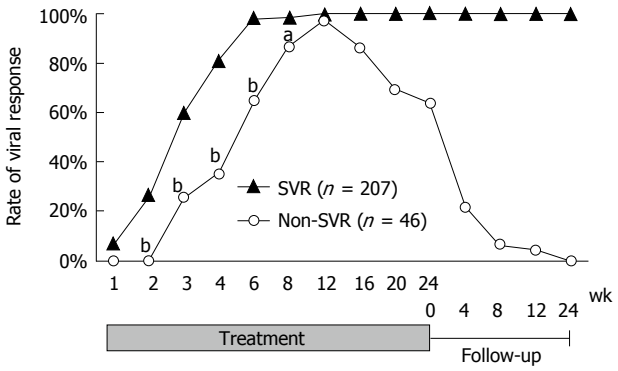

Figure 1 Transition of the virological response rate by sustained virological response status. The virological response (VR) rates were significantly higher in the sustained VR (SVR) than the non-SVR group between weeks 2 and 8 (26.9% *vs* 0.0%, 59.8% *vs* 25.6%, 81.6% *vs* 35.0%, 98.5% *vs* 65.0% and 98.5% *vs* 86.5% at weeks 2, 3, 4, 6 and 8, respectively. $P < 0.0001$ at weeks 2, 3, 4 and 6. $P = 0.0027$ at week 8), although there was no statistical difference at week 1 or 12 (7.3% *vs* 0.0% and 100% *vs* 97.3%, respectively). $^{a}P < 0.01$, $^{b}P < 0.0001$, *vs* SVR group.

Mann-Whitney *U* test, as appropriate, with SVR as the outcome. Kappa coefficient was used for the analysis of the concordance between SVR and VR at the seven time points. To identify independent factors predictive of SVR, variables that reached the $P < 0.1$ level in univariate tests were used as candidates in the multiple logistic regression analysis. Continuous parameters that were significant in univariate analysis were converted into categorical variables by dichotomizing at the round number closest to their median for analysis in the multiple logistic regression model. Because liver histology data was missing for 99 (39.1%) patients, it was excluded from the multiple logistic regression model. A *P* value less than 0.05 was regarded as statistically significant in all analyses.

RESULTS

Transition of VR rate during telaprevir-based triple therapy and follow-up

Of the 253 patients, 207 (81.8%) achieved SVR, 37 (14.6%) relapsed, and nine (3.6%) were non-responders. The VR rates increased dramatically over the first 6 wk (5.9%, 22.0%, 53.4%, 74.0%, and 93.1% at weeks 1, 2, 3, 4, and 6, respectively). Two hundred and forty-four patients (96.4%) had achieved VR by week 12. The rate gradually decreased to 81.8% after the end of treatment. A graph of the VR rates classified by SVR status is shown in Figure 1. Comparison of the VR rates of the SVR and non-SVR groups in the early stage of treatment showed that although there was no statistical difference at weeks 1 or 12 (7.3% *vs* 0.0% and 100% *vs* 97.3%, respectively), the rates were significantly higher for the SVR than for the non-SVR group for weeks 2 to 8 (26.9% *vs* 0.0%, 59.8% *vs* 25.6%, 81.6% *vs* 35.0%, 98.5% *vs* 65.0%, and 98.5% *vs* 86.5% at weeks 2, 3, 4, 6, and 8, respectively. $P < 0.0001$ at weeks 2, 3, 4, and 6. $P = 0.0027$ at week 8).

Demographic and clinical features of patients, by SVR status

The patient characteristics are summarized by the clinical outcome in Table 1. Sex, age, genotype of IL28B SNP (rs8099917), hemoglobin level, platelet count, serum albumin, AST, and LDL-cholesterol at baseline were significantly correlated with SVR in the univariate analysis (all $P < 0.05$). The rate of non-responders to previous PegIFN-α/RBV therapy was significantly higher in the non-SVR group than in the SVR group (44.8% *vs* 12.5%, $P < 0.0001$). The SVR rate significantly decreased as the stage of fibrosis progressed but was not related to the grade of activity.

Concordance between SVR and VR during the early stage of treatment

The PPV and NPV, calculated on the basis of VR in the early stage of treatment, are shown in Table 2. The PPV of VR for SVR was 100% at week 2, after which it gradually decreased, and it was over 85% to week 12. The NPV gradually increased, reaching 100% at week 12. The upslope of the NPV showed a large increase from week 4 (40.6%) to week 6 (82.4%). Kappa coefficients were calculated to evaluate the concordance between SVR and VRs (Table 2). There was a moderate concordance between the SVR and VR at week 6 (kappa coefficient = 0.44, 95%CI: 0.24-0.76), although the other VRs had poor concordance to SVR.

Multivariate analysis for factors predictive of SVR

Multiple logistic regression analysis was done to determine factors predictive of SVR. VR at week 6, which had the highest kappa coefficient, was included as a candidate in order to compare its predictive power. IL28B SNP (rs8099917) genotype [$P < 0.0001$, odds ratio (OR) = 8.24, 95%CI: 2.81-26.8], response to previous PegIFN-α/RBV therapy ($P = 0.0281$, OR = 3.29, 95%CI: 1.14-9.46), and VR at week 6 ($P < 0.0001$, OR = 63.8, 95%CI: 10.8-563) were extracted as factors contributing to SVR. VR at week 6 had a high statistical correlation with SVR (Table 3).

DISCUSSION

VR in the early stage of treatment has in the past been used to manage the treatment of patients with HCV. Since the advent of DAAs, no studies have been published that describe the detailed transition of serum HCV RNA during DAA therapy. Although the guidelines of AASLD and EASL recommend checking VR at weeks 4 (RVR) and 12 (EVR) for the assessment of initial response to therapy and adherence, other time points, such as weeks 1, 2, 3, 6, and 10, were not mentioned in these guidelines[11,12]. It is likely that RVR and EVR were chosen because they have been traditionally used as markers for PegIFN-α/RBV therapy and because the efficacy of other time points in DAA-containing therapy have not yet been fully investigated. By testing at frequent intervals in this prospective multicenter study

Table 1 Patient characteristics

	All (n = 253)	SVR (n = 207)	Non-SVR (n = 46)	P value
Sex, male (%)	123 (48.6)	108 (52.2)	15 (32.6)	0.0153
Age (yr)	61 (12.5)	60 (12)	63.5 (11.25)	0.0340
Body mass index (kg/m^2)	23.4 (3.9)	23.4 (3.9)	23.9 (3.8)	0.2198
Baseline HCV RNA (log10 IU/mL)	6.5 (0.9)	6.5 (0.9)	6.4 (0.7)	0.4468
IL28B SNP (rs8099917), TT/TG or GG (%)[1]	186/65 (74.1/25.9)	166/40 (80.6/19.4)	20/25 (44.4/55.6)	< 0.0001
ITPA SNP (rs1127354), CC/CA or AA (%)[1]	193/58 (76.9/23.1)	157/49 (76.2/23.8)	36/9 (80.0/20.0)	0.5802
Hemoglobin level (g/L)	138 (22)	140 (21)	134 (20)	0.0031
Platelet count (× 10^9/L)	157 (69)	159 (65)	129 (69)	0.0006
Serum albumin (g/L)	40 (6.0)	40 (6.0)	39 (5.0)	0.0143
Aspartate aminotransferase (U/L)	48 (42)	46 (43)	59 (34.5)	0.0350
Alanine aminotransferase (U/L)	54 (58)	53 (64)	58 (44)	0.4955
γ-glutamyl-transpeptidase (U/L)	40 (51)	39 (47)	46 (59)	0.1270
LDL-cholesterol (mg/dL)	95 (38)	98 (36)	75 (35)	< 0.0001
Ferritin (μg/L)	164.6 (232.3)	160.5 (223.2)	181.7 (253.9)	0.3583
Estimated glomerular filtration rate (mL/min per 1.73 m^2)	79.4 (19.1)	79.7 (18.9)	77.7 (19.4)	0.6210
Response to previous PegIFN-α/RBV therapy				< 0.0001
Treatment naïve, n (%)	92 (36.4)	81 (39.4)	11 (23.9)	
Prior relapse, n (%)	113 (44.7)	100 (48.1)	13 (28.3)	
Prior non-response, n (%)	48 (19.0)	26 (12.5)	22 (44.8)	
Liver histology				
Stage, F0-2/F3-4 (%)	96/58 (62.3/37.7)	87/38 (69.6/30.4)	9/20 (31.0/69.0)	< 0.0001
Grade, A0-1/A2-3 (%)	54/100 (35.1/64.9)	45/80 (36.0/64.0)	9/20 (31.0/69.0)	0.614
Not determined, n	99	82	17	

[1]IL28B and ITPA SNPs were not available for only two patients (1.2%). Continuous variables are expressed as median (interquartile range). P value draws a comparison between SVR and non-SVR patients. SVR: Sustained virological response; HCV: Hepatitis C virus; IL28B: Interleukin 28B; SNP: Single-nucleotide polymorphism; ITPA: Inosine triphosphate pyrophosphatase; LDL: Low-density lipoprotein; PegIFN: Pegylated interferon; RBV: Ribavirin.

Table 2 Precision rate for the prediction of sustained virological response and non-sustained virological response in the early stage of telaprevir-based triple therapy

	Patients who achieved SVR/patients with VR, n	PPV (%)	Patients who did not achieve SVR/patients without VR, n	NPV (%)	Kappa coefficient (95%CI)
Week 1	14/14	100	45/222	20.3	0.03 (0.01-0.05)
Week 2	52/52	100	44/185	23.8	0.12 (0.08-0.16)
Week 3	113/124	91.1	33/109	30.3	0.22 (0.12-0.33)
Week 4	168/182	92.3	26/64	40.6	0.38 (0.24-0.51)
Week 6	202/228	88.6	14/17	82.4	0.44 (0.27-0.61)
Week 8	200/232	86.2	5/8	62.5	0.18 (0.02-0.34)
Week 12	198/234	84.6	1/1	100	-

PPV: Positive predictive value, the probability that a patient with a given on-treatment virological response (VR) will achieve sustained virological response (SVR); NPV: Negative predictive value, the probability that a patient without a given on-treatment VR will not achieve SVR.

Table 3 Factors contributing to sustained virological response

	Univariate analysis		Multivariable analysis	
	OR	P value	OR (95%CI)	P value
Sex (male to female)	2.25	0.0153		
Age (< 60 yr to ≥ 60 yr)	1.79	0.0822		
IL28B SNPs (rs8099917) (TT to TG/GG)	5.19	< 0.0001	8.24 (2.81-26.8)	< 0.0001
Hemoglobin level (≥ 140 g/L to < 140 g/L)	2.13	0.0245		
Platelet count (≥ 150 × 10^9/L to < 150 × 10^9/L)	3.21	0.0005		
Serum albumin (> 35 g/L to ≤ 35 g/L)	2.51	0.0308		
Aspartate aminotransferase (< 50 U/L to ≥ 50 U/L)	2.30	0.0123		
LDL-cholesterol (≥ 95 mg/dL to < 95 mg/dL)	4.39	< 0.0001		
Response to previous PegIFN-α/RBV therapy (naïve/relapse to non-response)	6.38	< 0.0001	3.29 (1.14-9.46)	0.0281
VR at week 6	31.1	< 0.0001	63.8 (10.8-563)	< 0.0001

P value draws a comparison between SVR and non-SVR patients. SVR: Sustained virological response; IL28B: Interleukin 28B; SNP: Single-nucleotide polymorphism; LDL: Low-density lipoprotein; PegIFN: Pegylated interferon; RBV: Ribavirin; VR: Virological response.

of 253 patients infected with HCV genotype 1b, we were able to show that the transition during treatment with a DAA is different than what was seen in the past with PegIFN-α/RBV therapy. VR at week 6 had a high PPV (88.6%), NPV (82.4%), and kappa coefficient (0.44), which indicates its usefulness as a single time point for predicting both SVR and non-SVR during TVR-based triple therapy. In addition, multiple logistic regression analysis that included pretreatment factors, such as the patient's genotype, laboratory parameters at baseline, and response to previous therapy, extracted VR at week 6 as an independent factor contributing to SVR.

For dual therapy with PegIFN-α and RBV, RVR and EVR correlate with outcome and have traditionally been utilized as predictors. It has consistently been reported that RVR has a high PPV, around 90%[7-9], making it a useful marker for the prediction of SVR. In contrast, EVR has a high NPV, over 90%, making it a useful predictor of non-SVR[10]. In our study, the rates of EVR were not significantly different between the SVR and the non-SVR group. Although RVR had a high PPV, the NPV showed a sharp rise, from 45.7% at week 4% to 87.0% at week 6. This suggests that in DAA therapy, which has a direct mechanism and much stronger power to eliminate HCV than dual therapy, the most useful and meaningful time points for predicting the outcome may be different than in PegIFN-α/RBV therapy.

Although the DAAs strongly eliminate HCV, they are costly and some have serious side effects, such as the rash and anemia that often accompany TVR. To avoid unproductive expenditures and side effects, attempts have been made to establish response-guided treatment regimens that include early termination rules for unproductive DAA therapy[5,18]. It has been suggested that patients who have a rapid decline in their viral level can be treated with a shorter treatment duration, while preserving the high rate of SVR, and that treatment can be discontinued earlier for patients who are unlikely to respond the treatment. Our results showed that checking VR at week 6 would contribute to shortening the duration of TVR-based triple therapy. Furthermore, because both SVR and non-SVR can be predicted at a single time point (week 6), unnecessary testing can be eliminated, which will contribute to patient comfort and economic efficiency.

One of the limitations of our study is that TVR is no longer the standard of care in many countries. It is not recommended for the treatment of patients with decompensated cirrhosis or in a post-liver transplantation setting, and it should not be administered as co-medication. In addition, TVR can cause serious rash and anemia. In Japan, IFN-based therapy with RBV and simeprevir, a new nonstructural protein (NS)3/4A inhibitor, has become the standard of care against HCV genotype 1[19]. More recently, a number of novel DAAs, such as NS5A and NS5B inhibitors, have been developed and approved, and the current standard of care in the United States is an IFN-free DAA regimen[11]. Although our results might seem late to the game, TVR-containing treatment will continue to be an option in regions of the world where the newly approved DAAs are not available or in those patients with no other alternative. Another limitation of our study is that the patients were all Japanese and infected with HCV genotype 1b. The rate of SVR significantly differs by the race of the patient and the genotype of HCV[20]. Hence, our results may not be broadly applicable to the up-to-date IFN-free DAA regimens or to every patient with chronic hepatitis C. However, the results are useful because 253 patients were enrolled and frequent HCV RNA testing during DAA-containing therapy was analyzed that included numerous variables, including the genotype and laboratory parameters of each patient in this study. We believe that our study is sufficiently reliable to show that the most efficient time point for checking VR in DAA therapy might be different than the RVR and EVR that was developed for earlier therapies. Our results will need to be validated for the current DAA regimens, and further studies of patients with other HCV genotypes and of other racial cohorts will be necessary.

It is also a limitation of our study that we did not test for mutations of various HCV strains. Many studies have revealed that the variations in the amino acid sequences of HCV affect the antiviral activity of DAAs. Bartels *et al*[21], using a direct-sequencing technique, reported that the mutant strain resistant to NS3/4A protease inhibitors was detected in 2% of treatment naïve patients and that it was the pre-existing dominant strain in some of the patients. Nasu *et al*[22], using an ultra-deep sequencing technique, found some resistant mutations in a surprisingly high percentage of treatment naïve patients. In the coming era of IFN-free regimens, it will be essential to determine the mutations of the patients' HCV strains before treatment.

In conclusion, VR at week 6 is the time point most predictive of both SVR and non-SVR in the early stage of TVR-based triple therapy. This result shows the possibility that the most efficient time point for checking VR in DAA therapy might be different than the conventional RVR and EVR. Our results will need to be validated in light of the newly developed DAA regimens.

COMMENTS

Background

Since direct-acting antiviral agents (DAAs) were approved for the treatment of chronic hepatitis C, the treatment success rate has greatly improved. However, DAAs are costly, and some have serious side effects. To avoid unproductive expenditures and side effects, attempts have been made to predict the treatment response by checking the serum viral load of patients during the early stage of treatment. It has been suggested that patients who have a rapid decline in viral level can be treated with a shorter treatment duration while preserving the high rate of success and that patients who are unlikely to respond treatment should discontinue it early.

Research frontiers

An undetectable viral level at week 4 or 12 has consistently been correlated with outcome of conventional interferon therapy without DAAs, with rapid virological response and early virological response commonly used as predictors of treatment success. The transition of the viral level during DAA

therapy has not been well documented. In this prospective multicenter study, the authors did frequent testing of 253 patients to investigate viral activity during triple therapy containing telaprevir, the first approved DAA.

Innovations and breakthroughs

This is the first study to report the detailed transition of the viral level during the early stage of DAA therapy for chronic hepatitis C. Importantly, it was found that an undetectable serum viral level at week 6, and not at week 4 or 12, is the most efficient predictor of outcome.

Applications

Checking the serum viral level at week 6 would be useful for establishing a response-guided treatment regimen for patients treated with DAAs, which would help reduce the total duration of treatment.

Terminology

Virological response (VR) is defined as undetectable serum hepatitis C virus (HCV) RNA. Sustained virological response (SVR) is VR at 24 wk after the end of treatment and is regarded as successful treatment. The authors evaluated the ability of the VR between weeks 1 and 12 during the treatment to predict SVR or non-SVR.

Peer-review

Hiramine *et al* in this article describe in detail the factors that can be used for the prediction of therapeutic outcome. The limitation of the study is, as mentioned by the authors, that all the results are only for HCV genotype 1 Japanese patients. Interleukin 28B (IL28B) polymorphism is now a known factor influencing treatment response, and the authors have identified this as a predictive factor too. Although the polymorphism at IL28B rs8099917 is studied, another very important polymorphism rs12979860, which is well documented to influence the therapeutic outcome, is not studied. It would be helpful if the authors studied that polymorphism as well in these patients. Overall, the study is well-designed and well written.

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CASE REPORT

Pseudolymphoma (reactive lymphoid hyperplasia) of the liver: A clinical challenge

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Informed consent statement: The patients involved in this study gave their written informed consent authorizing use and disclosure of their protected health information.

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Abstract

Reactive lymphoid hyperplasia (RLH), also known as pseudolymphoma or nodular lymphoid lesion of the liver is an extremely rare condition, and only 51 hepatic RLH cases have been described in the literature since the first case was described in 1981. The majority of these cases were asymptomatic and incidentally found through radiological imaging. The precise etiology of hepatic RLH is still unknown, but relative high prevalence of autoimmune disorder in these cases suggests an immune-based liver disorder. Imaging features of hepatic RLH often suggest malignant lesions such as hepatocellular carcinoma and cholangiocarcinoma. In this report, we discuss two cases of hepatic RLH in patients with autoimmune hepatitis. We also present pathologic and magnetic resonance imaging findings, including one case utilizing a hepatocellular contrast agent, Eovist. Definitive diagnosis of hepatic RLH often requires surgical excision.

Key words: Pseudolymphoma; Nodular lymphoid lesion; Liver; Magnetic resonance imaging; Reactive lymphoid hyperplasia

Core tip: Reactive lymphoid hyperplasia of the liver also known as pseudolymphoma is an extremely rare condition. Because of its rarity, association with underlying inflammatory liver disease and close resemblance to malignant hepatic lesions such as hepatocellular carcinoma and cholangiocarcinoma on imaging studies,

this rare lesion is frequently misdiagnosed. We discuss two cases of hepatic reactive lymphoid hyperplasia (RLH) in patients with autoimmune hepatitis and how we came to the correct diagnosis. Definitive diagnosis of hepatic RLH often requires surgical excision.

Kwon YK, Jha RC, Etesami K, Fishbein TM, Ozdemirli M, Desai CS. Pseudolymphoma (reactive lymphoid hyperplasia) of the liver: A clinical challenge. *World J Hepatol* 2015; 7(26): 2696-2702 Available from: URL: http://www.wjgnet.com/1948-5182/full/v7/i26/2696.htm DOI: http://dx.doi.org/10.4254/wjh.v7.i26.2696

INTRODUCTION

Reactive lymphoid hyperplasia (RLH) also known as pseudolymphoma[1-3] and nodular lymphoid lesion[4,5] is a condition characterized by localized non-neoplastic proliferation of lymphoid tissue at extranodal sites[6]. This rare condition is known to affect various organs including skin, orbit, thyroid, lung, stomach, breast, intestine, spleen and pancreas, however involvement of liver is extremely rare[6] and only 51 such cases have been reported to date[7-10]. Although the pathogenesis of hepatic RLH remains unclear, this condition is found to be associated with a number of chronic inflammatory and immunological conditions including viral hepatitis and various autoimmune diseases including autoimmune hepatitis, primary biliary cirrhosis and autoimmune thyroiditis[6,11,12]. We report two cases of incidentally found hepatic lesion for which surgical excision was performed with a final diagnosis of hepatic RLH. We also describe magnetic resonance imaging (MRI) and pathologic features of RLH, and review of current literature.

CASE REPORT

Case 1

A 41-year-old Hispanic women with autoimmune hepatitis (ANA+, SMA+, IgG greater than 1.1-fold of upper normal limit) had an abdominal MRI at an outside hospital with conventional extracellular contrast agent as a part of elevated transaminase workup. The MRI demonstrated a non-cirrhotic liver with a single 2.5 cm lesion in segment 3 with hypervasular enhancement with washout (Figure 1A-D). Due to recent diagnosis of cervical cancer (stage 1B), positron emission tomography (PET) scan was performed (Figure 1E), which showed PET positivity. Metastatic disease was a concern, and as such, the patient underwent image guided needle biopsy of the liver lesion, which showed indeterminate lesion with unusual florid lymphoplasmacytic infiltrates. To further characterize the lesion, MRI was repeated in our institution with single dose of Eovist (Gadoxetate Disodium, Bayer HealthCare Pharmaceuticals), a liver specific contrast. This showed an arterial enhancement and lack of uptake on hepatocellular phase images most suggestive of a hepatic adenoma (Figure 1F). Due to the diagnostic uncertainties of this liver lesion, the patient underwent a surgical resection of the mass and also core biopsy of the non-tumoral area to assess the condition of the background liver.

Pathologic examination of the resected specimen showed a 2.5 cm relatively well-circumscribed tumoral nodule containing lymphoid proliferation characterized by reactive lymphoid follicles and interfollicular plasma cells within the resected liver parenchyma (Figure 2A and B). Since lymphoma was suspected on initial evaluation, flow cytometric analysis, immunohistochemistry and polymerase chain reaction analysis for immunoglobulin heavy chain gene rearrangement were performed. Briefly, the flow cytometric analysis on the cells obtained from the tumor showed mixed population of CD2, CD5 and CD3 positive T cells with a CD4/CD8 4.5/1 (approximately 40%) and polyclonal CD19, CD20 and CD22 positive polyclonal B lymphocytes (60%) with no abnormal immunophenotype. Also with cytoplasmic kappa and lambda stains, clonality could not be demonstrated. These results were consistent with a reactive process. Immunohistochemical analysis showed numerous CD20 positive B cells mostly confined to follicles (Figure 2C) without abnormal immunophenotype and numerous interfollicular polyclonal CD138 and MUM-1 positive plasma cells (Figure 2D) with kappa/lambda ratio 3/1 within the lesion area. The majority of the plasma cells were IgG positive but negative for IgG4, CD56, CD117 and CD20. CD21 immunostain showed round follicular dendritic networks in follicles. Cyclin-D1 stain was negative. CD3, CD43 and CD5 highlighted the T-cells but they were negative on the B-cells. MIB-1 proliferative index was approximately 30% in interfollicular areas. Although these results were consistent with reactive lymphoid hyperplasia, low grade marginal zone B-cell lymphoma was in the differential diagnosis. Polymerase chain reaction (PCR) analysis did not show monoclonal immunoglobulin heavy chain gene rearrangement and excluded the possibility of a subtle B cell clonal process. In summary, our analysis ruled out a low grade extranodal marginal zone lymphoma and supported the diagnosis of RLH. The pathologic examination of the core biopsy of the liver from non-tumoral area showed steatohepatitis with portal lymphoid aggregates and plasma cells consistent with autoimmune hepatitis (grade 2, stage 2, data not shown).

Case 2

A 60-year-old African-American woman with chronic liver cirrhosis from autoimmune hepatitis (ANA+, SMA+) had a routine surveillance MRI, which showed a 1 cm lesion in segment 2 (Figure 3). The lesion had early contrast enhancement with washout, with features probable for hepatocellular carcinoma (HCC), which is classified as Liver Imaging Reporting and Data System category 4 by American College of Radiology[13,14]. Similar to the above case, at the time of surgery, the initial evaluation of the liver nodule showed atypical lymphoid proliferation and

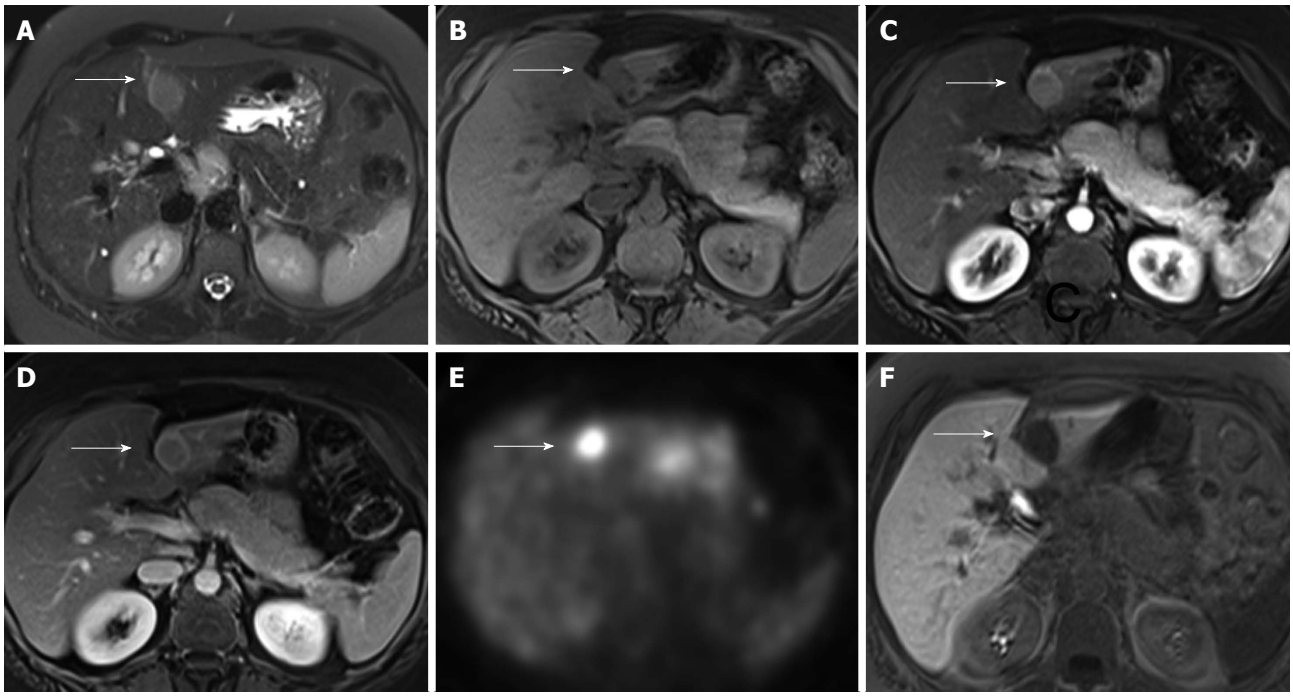

Figure 1 Imaging findings of case 1. A: T2-weighted fat-saturated image showing focal mass (indicated by arrow) in segment 2 with increased signal intensity as compared to background liver; B: T1-weighted fat-saturated image showing focal mass with decreased signal intensity as compared to background liver; C: T1-weighted fat-saturated image after contrast infusion in the late arterial phase. The mass is hypervascular on this phase; D: T1-weighted fat-saturated image after contrast infusion in the portal venous phase. The mass washes out on this phase. A capsule is seen; E: Positron emission tomography scan shows hypermetabolic activity; F: T1-weighted fat-saturated image 20 min after hepatocellular contrast, Gadoxetate Disodium (Eovist) infusion. Lesion does not take up Eovist.

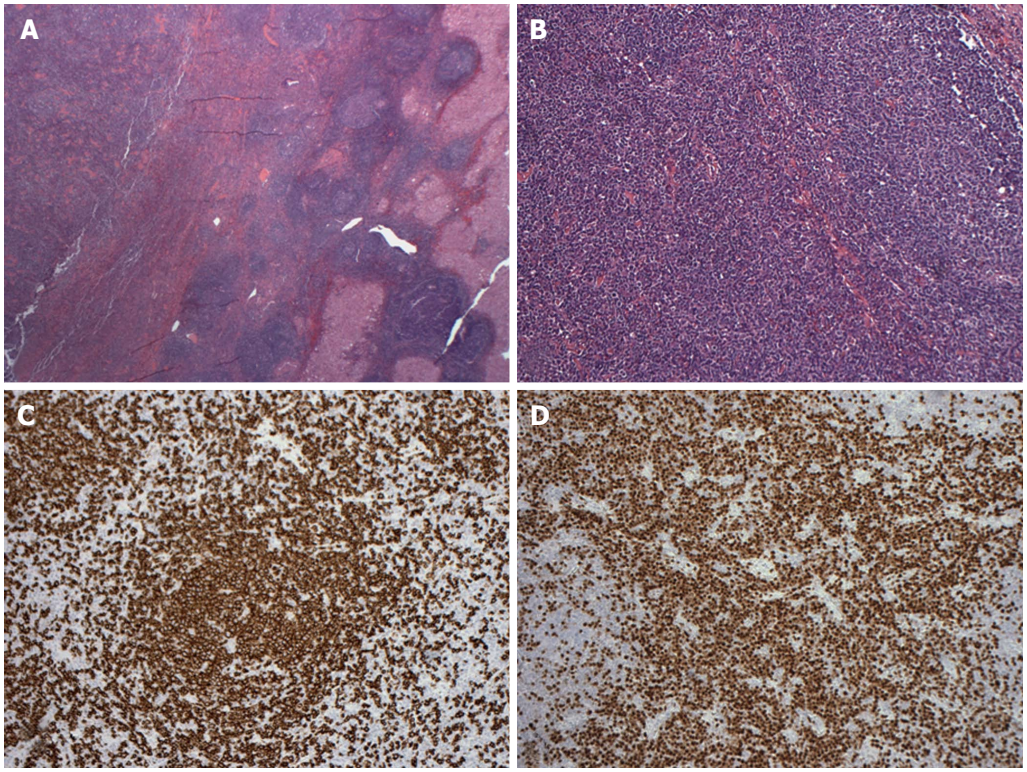

Figure 2 Histopathological findings of case 1. A and B: Tumoral nodule containing lymphoid proliferation characterized by reactive lymphoid follicles and interfollicular plasma cells within liver parenchyma; C: Numerous CD20 positive B cells mostly confined to follicles; D: Numerous interfollicular polyclonal CD138 and MUM-1 positive plasma cells.

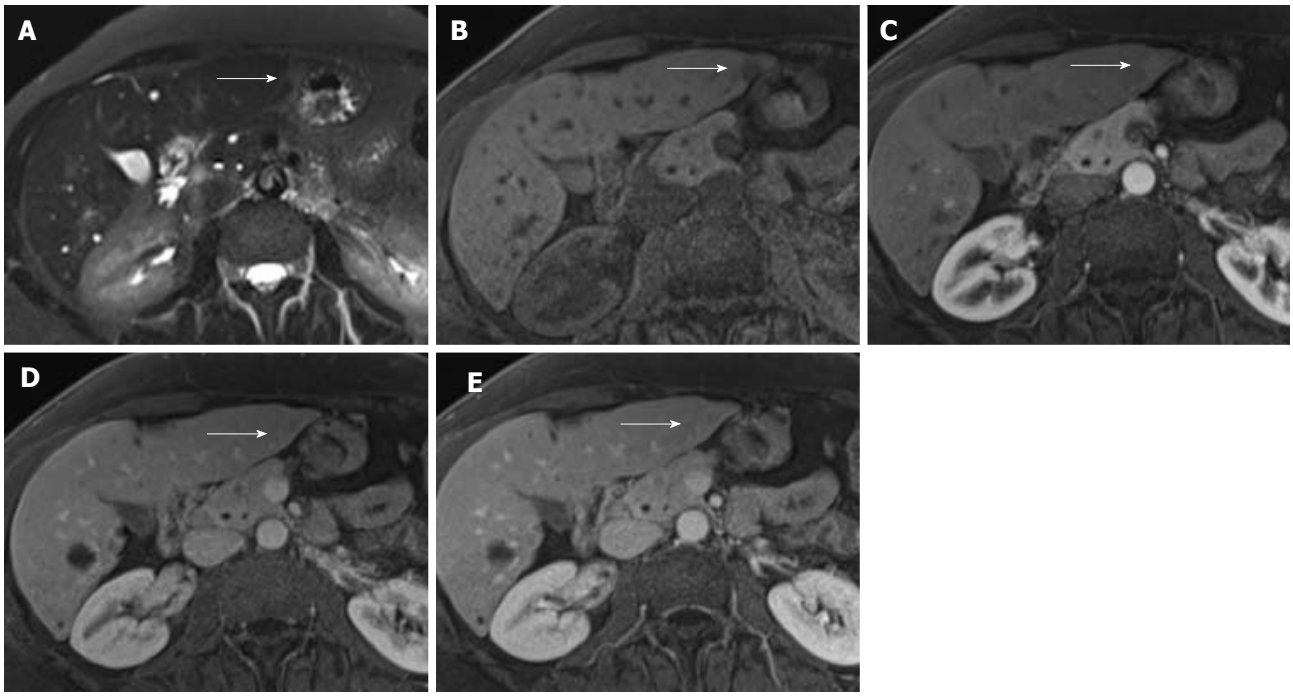

Figure 3 Imaging findings of case 2. A: T2-weighted fat-saturated image showing subtle focal mass (indicated by arrow) in subcapsular portion of segment 2 with increased signal intensity as compared to background liver; B: T1-weighted fat-saturated image showing small focal mass with decreased signal intensity as compared to background liver; C: T1-weighted fat-saturated image after contrast infusion in the late arterial phase. The mass shows subtle hypervascular enhancement on this phase; D: T1-weighted fat-saturated image after contrast infusion in the portal venous phase. The mass shows faint wash-out on this phase; E: T1-weighted fat-saturated image after contrast infusion in 3 min delayed post contrast phase. The mass shows faint filling in.

thus, lymphoma workup was performed including flow cytometry, immunohistochemistry and PCR analysis. The pathologic examination showed a 1 cm relatively well-circumscribed tumoral nodule containing reactive lymphoid follicles and interfollicular plasma cells within liver parenchyma surrounded by regenerative nodules (Figure 4A). The flow cytometric analysis showed mixed population of polyclonal B cells and T cells with a CD4/CD8 ratio of 2.5/1 and with no abnormal immunophenotype consistent with a reactive process. The immunohistochemical analysis showed numerous CD20 positive B cells mostly confined to follicles, without abnormal immunophenotype, numerous T cells and polyclonal plasma cells with kappa/lambda ratio 3/1 within the lesion area. CD21 immunostain showed round follicular dendritic networks in follicles (Figure 4B). The results were consistent with a reactive process. PCR was also performed, which was negative for monoclonal immunoglobulin heavy chain gene rearrangement. The sections of the adjacent liver parenchyma showed bridging fibrosis, portal and focal lobular lymphoid aggregates with plasma cells and focal nodule formation (Figure 4C and D). A diagnosis of RLH of liver in a background of autoimmune hepatitis was made.

DISCUSSION

Hepatic RLH is very rare presumably benign condition that may simulate malignancy on imaging studies[6,15] and low grade lymphoma especially extranodal marginal zone lymphoma on histology[16]. The mean age of hepatic RLH cases was 58 years with a marked female predominance with a male to female ratio of greater than 1:7[6]. The majority of cases were asymptomatic and diagnosed incidentally, and more than half of the cases were associated with an underlying inflammatory or autoimmune condition like viral hepatitis, primary biliary cirrhosis or autoimmune thyroiditis[6]. The majority of cases, 81%, had a solitary tumor at presentation[11]. The average size was 15.4 mm with range 4 to 55 mm[6].

With the use of intravenous gadolinium-enhanced MRI, RLH lesion may resemble HCC and cholangiocarcinoma. In patients at risk for HCC, a lesion with imaging features of arterial phase enhancement and washout on later phase images, or presence of a capsule, is highly worrisome for HCC[13,14].

In our first case, the conventional contrast-enhanced MRI showed a non-cirrhotic liver with a single lesion with arterial enhancement and washout, and presence of a capsule. This lesion showed lack of uptake on hepatocellular phase of Eovist-enhanced MR images, which may be seen in HCC, hepatic adenoma, and argues against focal nodular hyperplasia. Given the normal background liver morphology, hepatic adenoma was the favored diagnosis. On PET scan, the lesion was PET positive; PET positivity has been previously reported one case[11]. Hypointensity on the hepatocellular phase of Eovist is consistent with findings previously reported[17].

In our second case, the background liver was noted to be cirrhotic, and a small lesion in segment 2 showed

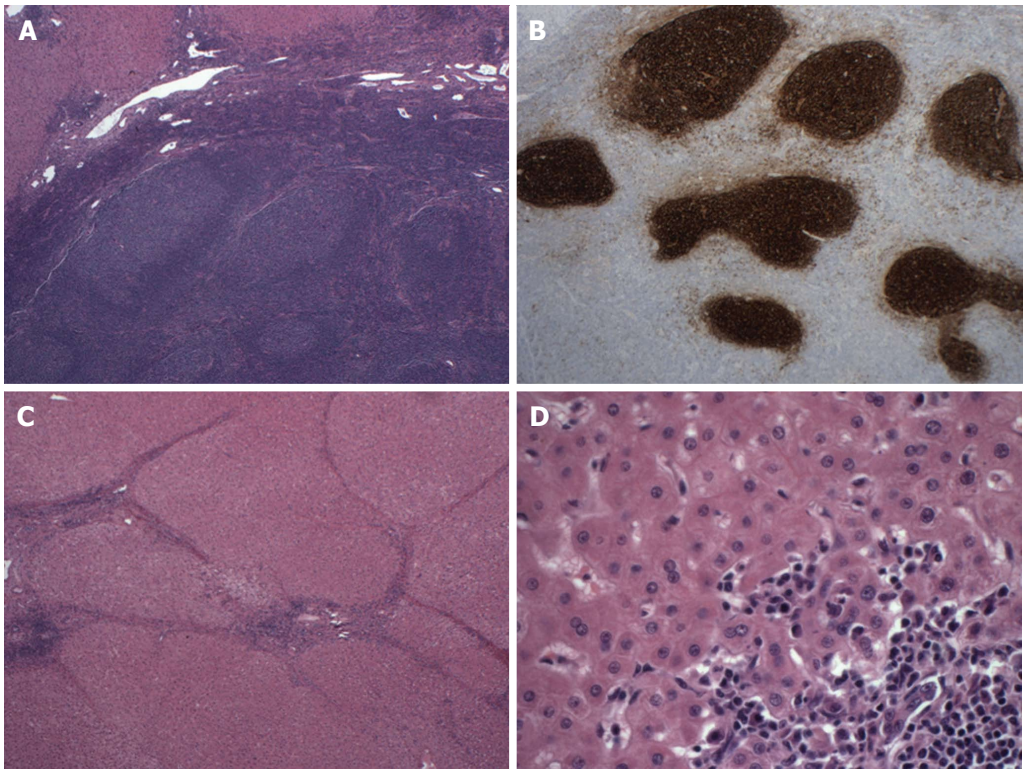

Figure 4 Histopathological findings of case 2. A: Tumoral nodule within liver parenchyma; B: CD21 immunostain showing round follicular dendritic networks in follicles; C: Sections of adjacent liver parenchyma showing bridging fibrosis; D: Portal and focal lobular lymphoid aggregates with plasma cells and focal nodule formation.

some features of arterial enhancement and faint washout, but a suggestion of subtle filling in on later delayed phase images. Abnormal increased signal intensity was also seen on T2 weighted images. These features were most consistent with malignancy, and with a differential diagnosis of HCC and cholangiocarcinoma or biphenotypic tumors[13,14].

Natural history of hepatic RLH is yet to be defined due to its rare occurrence. Although hepatic RLH is presumed to be a benign liver lesion, malignant transformation of RLH into lymphoma in other organs such as lung, stomach and skin has been well reported previously[18-20]. In liver, there is one case report by Sato *et al*[21] in 1999 where a hepatic RLH transformed into a low grade lymphoma in a 55-year-old patient with primary biliary cirrhosis and Sjogren's syndrome. To our best knowledge, there are no other reports of malignant transformation or local or distant recurrence of RLH from various follow-up periods ranging from 3 mo to 15 years[6,22].

Although majority of the reported cases in literature were treated with surgical resection due to uncertain diagnosis, three cases were treated with liver transplantation due to associated liver disease[4,23]. Since this lesion occurs with pre-existing liver disease, it's very important to consider this lesion in differential diagnosis before considering patients for transplants especially for oncological indication.

In conclusion, hepatic RLH will continue to present to clinicians as conundrum for correct diagnosis. Not much is known about this hepatic lesion, but up to 30% of the reported cases are associated with various autoimmune diseases[24]. Preoperative definitive diagnosis of hepatic RLH using various imaging modalities including MRI with hepatocellular agents such as Eovist is extremely difficult. Percutaneous needle aspiration or core biopsy may be helpful in differentiating hepatic RLH from metastatic carcinoma and primary liver tumors such as HCC. However, this approach may be inadequate in differentiating low-grade malignant lymphoma, particularly extranodal marginal zone lymphoma from RLH. Although extremely rare, one case of malignant transformation of hepatic RLH has been reported, and in other organs, RLH may undergo malignant transformation. Therefore, any patient with hepatic RLH should have close follow up. Based on limitations of imaging and pathology, for definitive diagnosis and treatment, surgical excision is the advised course.

COMMENTS

Case characteristics

Hepatic reactive lymphoid hyperplasia (RLH) is an extremely rare condition, which is often misdiagnosed.

Clinical diagnosis

RLH is often associated with various autoimmune diseases, and the authors' two patients had underlying autoimmune hepatitis.

Differential diagnosis

Hepatocellular carcinoma (HCC), cholangiocarcinoma, and extranodal marginal zone lymphoma.

Laboratory diagnosis
No specific lab values are associated with hepatic RLH.

Imaging diagnosis
With the use of intravenous gadolinium-enhanced magnetic resonance imaging, hepatic RLH lesion may resemble HCC and cholangiocarcinoma, often leading to misdiagnosis.

Pathological diagnosis
Pathologic examination of hepatic RLH shows lymphoid proliferation characterized by reactive lymphoid follicles and interfollicular plasma cells, often leading to misdiagnosis of lymphoma on initial evaluation.

Treatment
Although hepatic RLH is presumably benign condition, surgical excision is the advised for definitive diagnosis and as a definitive treatment.

Related reports
Misdiagnosis as HCC, cholangiocarcinoma or hepatic lymphoma will results in radically different treatment course, which may include liver transplant, major hepatic resection or chemotherapy.

Term explanation
Hepatic RLH is a presumably benign condition, which is associated with an underlying inflammatory or autoimmune condition like viral hepatitis, primary biliary cirrhosis or autoimmune thyroiditis.

Experiences and lessons
Since 1981, 51 cases of hepatic RLH have been reported to date. Hepatic RLH should on the differential diagnosis especially when facing with hepatic lesion with underlying inflammatory or autoimmune condition without clear risk factors for HCC and cholangiocarcioma.

Peer-review
Well written case report on two patients with hepatic RLH and on work-up for the correct diagnosis.

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