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Contents

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Jin-Lei Wang, Director

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World Journal of Hepatology
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-59080039
Fax: +86-10-85381893
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EDITORIAL

Hepatic metastatic disease in pediatric and adolescent solid tumors

Israel Fernandez-Pineda, John A Sandoval, Andrew M Davidoff

Israel Fernandez-Pineda, John A Sandoval, Andrew M Davidoff, Department of Surgery, St. Jude Children's Research Hospital, Memphis, TN 38105, United States

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Correspondence to: Israel Fernandez-Pineda, MD, Department of Surgery, St. Jude Children's Research Hospital, 262 Danny Thomas Place, Memphis, TN 38105, United States. israel.fernandez-pineda@stjude.org
Telephone: +1-901-5952315
Fax: +1-901-5952207

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Abstract

The management of hepatic metastatic disease from solid tumors in adults has been extensively described and resection of metastatic liver lesions from colorectal adenocarcinoma, renal adenocarcinoma, breast cancer, testicular cancer, and neuroendocrine tumors (NET) have demonstrated therapeutic benefits in select patients. However, there are few reports in the literature on the management of hepatic metastatic disease in the pediatric and adolescent populations and the effectiveness of hepatic metastasectomy. This may be due to the much lower incidence of pediatric malignancies and the higher chemosensitivity of childhood tumors which make hepatic metastasectomy less likely to be required. We review liver involvement with metastatic disease from the main pediatric solid tumors, including neuroblastoma and Wilms tumor focusing on the management and treatment options. We also review other solid malignant tumors which may have liver metastases including germ cell tumors, gastrointestinal stromal tumors, osteosarcoma, desmoplastic small round cell tumors and NET. However, these histological subtypes are so rare in the pediatric and adolescent populations that the exact incidence and best management of hepatic metastatic disease are unknown and can only be extrapolated from adult series.

Key words: Hepatic metastatic disease; Pediatric and adolescent solid tumors; Neuroblastoma; Wilms tumor

Core tip: Management of hepatic metastatic disease in pediatric and adolescent cancer patients is not as well delineated as for adults due to the lower incidence of pediatric malignancies and the higher chemosensitivity of childhood tumors. We review liver involvement by metastatic disease from the main pediatric and adolescent solid tumors focusing on management and treatment options.

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INTRODUCTION

Cancer is the most common cause of disease-related mortality for children and adolescents 1-19 years of age[1]. More than 12000 children and adolescents younger than 20 years of age are diagnosed with cancer every year in United States with approximately 2300 deaths in this age group[2,3]. Primary liver malignancies are uncommon in children (annual incidence rate of 1.5 per million) and account for only 0.5% to 2% of all pediatric neoplasms (100-150 new cases/year in United States). The two main histologies are hepatoblastoma and hepatocellular carcinoma[1,4]. Hepatoblastoma is the most common malignant tumor of the liver in children with a higher incidence during the first year of life. Hepatocellular carcinoma is the second most common hepatic malignancy and occurrs primarily in adolescents[5]. The most common site of origin of liver metastases in children with solid tumors is neuroblastoma (NB) followed by Wilms tumor (WT). Other solid malignant tumors which may give liver metastases are germ cell tumors (GCT), gastrointestinal stromal tumors (GIST), osteosarcoma (OS), desmoplastic small round cell tumors and neuroendocrine tumors (NET)[6,7]. Table 1 summarizes liver involvement from pediatric solid tumors. Some histological subtypes are so rare in the pediatric population that the exact incidence of hepatic metastatic disease is unknown and extrapolated from series of adult patients. Although hepatic metastatic disease in adults is often associated with abnormal liver function tests, including a decreased serum albumin and elevated serum levels of transaminases, bilirubin and alkaline phosphatase, these findings are rarely seen in pediatric patients with hepatic tumor involvement. While the exact mechanisms underlying hepatic metastasis in children remain unclear, we briefly summarize tumor biology concepts underlying liver metastatic disease.

Different treatment modalities have been used in the management of liver metastases in childhood including systemic chemotherapy, radiation therapy (RT), surgical resection, ablation techniques and image-guided interventional procedures (Table 1). Surgical resection of liver metastases from colorectal adenocarcinoma, renal adenocarcinoma, breast cancer, testicular cancer, and NET is feasible and has demonstrated therapeutic benefits in select adult patients[8-13]. The role of surgery for hepatic metastatic disease in pediatric malignancies is not as well described as for adults. This may be due to the lower incidence of malignancies in children and the higher chemosensitivity of pediatric histological subtypes. The decision to perform resection of liver metastases in pediatric cancer patients should be highly individualized with a clear understanding of tumor biology and chemosensitivity.

Patients whose primary tumor is under control and have adequate hepatic reserve for resection may be good candidates for liver metastasectomies. Some other patients may not be good surgical candidates but they may benefit from surgical relief of tumor biliary obstruction to improve liver function tests and permit the continuation of chemotherapy. Herein, we review liver involvement by metastatic disease from the main pediatric and adolescent solid tumors focusing on management and treatment options.

TUMOR BIOLOGY IN HEPATIC METASTASIS

As dissemination of systemic metastasis to the liver in advanced stage pediatric solid neoplasms is limited, the liver remains a select host to pediatric solid cancers, particularly NB and WT. While the exact mechanisms underlying hepatic metastases remain unclear in these particular tumors, the general understanding of the interactions between metastatic tumor cells and the liver microenvironment involves a reciprocal dynamic between primary tumor and the hepatic microenvironment[14]. Metastatic cells arriving at the liver *via* the bloodstream encounter the microenvironment of the hepatic sinusoid. The interactions of the tumor cells with hepatic sinusoidal and extrasinusoidal cells (endothelial, Kupffer, stellate, and inflammatory cells) determine their fate. The sinusoidal cells may play a dual role, sometimes killing the tumor cells but also facilitating their survival and growth. Adhesion molecules participate in these interactions and may affect their outcome. In NB and WT, for instance, the association of various growth factors, cell adhesion molecules, and extracellular matrix proteins have been described for these tumors and have been shown to be involved in metastases[15,16]. Lastly, bone marrow-derived cells and chemokines play a part in the early struggle for survival of the metastases. Once the tumor cells have arrested and survived the initial onslaught, tumors can grow within the liver in 3 distinct patterns, reflecting differing host responses, mechanisms of vascularization, and proteolytic activity. While much has been accomplished in the understanding of the complex biology of liver metastases, in the following sections, we emphasize recent progress in the clinical management and treatment of hepatic metastases in advanced childhood tumors. We refer the reader to the references[17-19] for reviews on the current understanding of the biology of liver metastases.

NB

NB is the most common extracranial malignant solid tumor in the pediatric population, representing approximately 8%-10% of total cancer cases in children younger than 15 years of age[20]. More than 650 cases are diagnosed each year in North America (incidence of 10.54 cases per 1 million per year). The prognosis of NB is dependent on age at diagnosis, stage of disease, histology and molecular biologic characteristics of the tumor (*e.g.,* amplification of MYCN-oncogene)[21-24]. Specifically, age less than 1 year is associated with a favourable prognosis, while MYCN-oncogene-ampli-

Table 1 Metastatic disease, liver metastases and treatment options in pediatric solid tumors

Primary malignancy	Metastatic disease at diagnosis	Hepatic metastatic disease	Treatment options
NB	50%-60%	20%-30%	Surgery, chemotherapy and radiation therapy
WT	10%-20%	10%-15%	Surgery, chemotherapy and radiation therapy
GCT	20%-30%	15%-20%[1]	Surgery and chemotherapy
GIST	30%-40%[1]	15%-20%[1]	Surgery and imatinib
OS	15%-20%[1]	1%-3%[1]	Surgery
DSRCT	30%-50%[1]	30%-40%[1]	HIPEC and surgery
NET	30%-45%[1]	30%-45%[1]	Surgery, HAE, cryoablation, radiofrequency ablation, liver transplant and radionuclides therapy

[1]Data from adult populations. DSRCT: Desmoplastic small round cell tumor; GCT: Germ cell tumor; GIST: Gastrointestinal stromal tumor; NB: Neuroblastoma; NET: Neuroendocrine tumor; OS: Osteosarcoma; WT: Wilms tumor; HAE: Hepatic artery embolization; HIPEC: Hyperthermic peritoneal perfusion with chemotherapy.

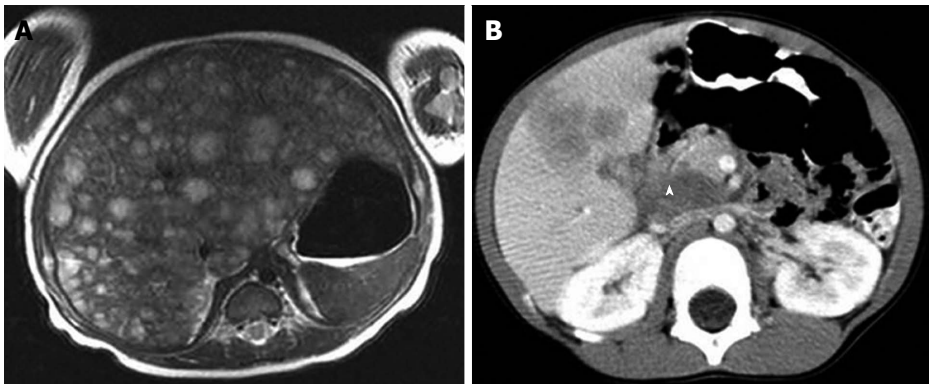

Figure 1 Hepatic involvement from (A) stage 4S *vs* (B) stage 4 neuroblastoma. Primary neuroblastoma (arrowhead).

fication confers a poor prognosis[25-27]. Since NB is the most common pediatric extracranial solid tumor and the most frequent tumor which metastasizes to liver in children, knowledge of the management of hepatic metastatic disease from NB is particularly important. Approximately, 30% of NB patients with metastatic disease have liver involvement and two distinct clinical entities can be differentiated: stage 4S (or metastatic which metastases are confined to skin, liver and/or bone marrow in children younger than 18 mo, according to the International Neuroblastoma Risk Group Staging System) and stage 4 (or metastatic)[28]. Liver involvement is seen in approximately 80% of patients with stage 4S NB, whereas 10%-50% of stage 4 NB patients have liver metastasis[29]. Differences have been observed in the initial presentation of hepatic metastatic disease from NB in these 2 stages. Stage 4S NB is reported to usually present with multiple ill-defined nodules or diffuse liver involvement where stage 4 NB hepatic involvement presents more frequently with discrete liver nodules (Figure 1).

Stage 4S NB

Stage 4S NB is defined by a localized primary tumor with dissemination limited to skin, liver, and/or bone marrow (involvement < 10%) in infants younger than 12 mo[30]. Bone marrow involvement > 10% or bone involvement is considered stage 4 disease. The first description of stage 4S NB by D'Angio *et al*[31] in 1971, reported frequent spontaneous tumor regression without adjuvant therapy. More recent reports have confirmed that stage 4S NB, with or without liver involvement, resolved in up to 50% of the cases without requiring therapy[32,33]. Overall survival is approximately 85%-92% in this group of patients and mortality is generally secondary to massive hepatic tumor infiltration causing respiratory compromise.

DuBois *et al*[34] reported the incidence of metastatic sites in stage 4 and 4S NB and the extent to which metastatic sites correlate with age, tumor biology, and survival. With regards to hepatic involvement, they showed that liver metastases were associated with a more favorable outcome overall, but in infants predicted a slightly greater event-free survival (EFS) and were associated with non-amplified MYCN and favorable histology tumors, while in children > 1 year at diagnosis, liver metastases were an unfavorable prognostic marker and associated with MYCN-amplified tumors. Although excellent outcome in stage 4S NB is common, there are subsets of infants with massive infiltration of the liver by tumor who experience significant morbidity and mortality secondary to respiratory compromise and symptoms of abdominal compartment syndrome with decreased venous return, renal impairment and coagulation disorders[35,36]. Nickerson *et al*[30] from the Children's Cancer Study Group reported six deaths, five

of which were in infants younger than 2 mo of age at diagnosis and were due to complications of extensive abdominal involvement with respiratory compromise or disseminated intravascular coagulation. Schleiermacher *et al*[37] reported that patients with stage 4S NB and progressive disease had a 20% mortality rate and suggested that the combination of etoposide and carboplatin may be more effective in these infants than radiation or vincristine and cyclophosphamide.

Multimodality therapy with surgery, radiation and chemotherapy has been used but the outcome of this approach has not yet been ascertained. In 2004, Weintraub *et al*[38] reported the first successful case of hepatic intra-arterial chemoembolization (HACE) in a neonate and 8 years later, they published a sequential treatment algorithm for infants with stage 4S NB and massive hepatomegaly based on initial observation without treatment, intravenous chemotherapy for those who have progressive disease and HACE for patients with progression despite chemotherapy[39]. Surgical management by partial hepatic resection or abdominal decompression with mesh placement in case of abdominal compartment syndrome has a high rate of associated complications and has rarely been shown to be effective[40].

Stage 4 NB

Stage 4 NB patients under 1 year of age have an overall survival ranging from 70% to 93%, in contrast to overall survival between 35% and 60% for patients greater than 1 year. Patients with isolated liver metastases may benefit from resection of these lesions, resulting in prolonged survival and/or treatment reductions[41,42], but these clinical circumstances are rare and several factors including histological tumor characteristics and close evaluation of extrahepatic metastatic disease should be discussed before considering hepatic metastasectomies. There are some reports of stage 4S NB that recurs after initial regression or progresses to stage 4 with bone metastases. There are no guidelines for patients with responsive extrahepatic metastatic disease to therapy and persistent liver disease. The biology of the tumor may lead the therapeutic approach and tumors without MYC-N amplification may have a chance of survival and no indication for major liver resections[43-45].

French *et al*[46] investigated the long-term hepatic outcomes in infants with stage 4S and 4 NB, with a special focus on the impact of liver involvement and abdominal radiation. They reviewed 38 patients with available follow-up 5 years following diagnosis, assessing hepatic imaging and function (transaminases, bilirubin, alkaline phosphatase). For stage 4S, benign hepatic changes on imaging studies in patients treated with hepatic radiation as well as those who had hepatic involvement at diagnosis but did not receive radiation were observed. For infants with stage 4 and hepatic metastasis at diagnosis, none was found to have late hepatic imaging changes. Blood work was normal in both groups. They concluded that adverse hepatic outcomes after liver involvement or radiation in infants with stage 4S or 4 NB are rare and when they do occur, often resolve over time. Also, infants with NB and metastatic hepatic disease seem to be a specific risk-group for the development of focal nodular hyperplasia (FNH) of the liver, especially if they underwent chemotherapy and/or hepatic RT during treatment and it should be considered in patients with persistent late imaging changes[47]. Although FNH is a benign lesion that is typically managed conservatively in adults, most children with FNH undergo biopsy or resection because of increasing size, concerning symptoms or inability to rule out malignancy, especially in pediatric cancer survivors[48,49].

Long-term follow-up guidelines from the Children's Oncology Group (COG) recommend yearly hepatic bloodwork screening (aspartate aminotransferase, alanine aminotransferase, and bilirubin) upon entry to the long-term follow-up clinic with repeat bloodwork only if clinically indicated in a patient that has received greater than 20-30 Gy to the liver. Bloodwork to check liver function is recommended by COG if there is an abnormality on screening bloodwork[50,51].

In summary, although stage 4 NB patients with isolated liver metastases may benefit from resection of these lesions, this is a rare clinical situation. Careful patient selection is indicated focusing on the histological tumor characteristics, evaluation of extrahepatic metastatic disease and tumor chemosensitivity. The role of surgery by partial liver resection or abdominal decompression with mesh or silo placement in stage 4S patients with massive hepatomegaly who are not responsive to chemotherapy/RT is also controversial and it has rarely been shown to be effective.

WT

WT or nephroblastoma is the most common malignant renal tumor in children, representing approximately 6% of total cancer diagnoses among children younger than 15 years with 500 new cases in United States each year[1]. Overall survival for children with WT has been consistently above 90% since the 1980s. Prognosis depends on the stage of disease at diagnosis and histopathologic and molecular features of the tumor. According to the staging criteria, the primary renal tumor is assigned a local stage (1-3), which determines local therapy with or without RT[52-55]. Stage 4 WT is defined by hematogenous metastases or lymph node metastases outside the abdominopelvic region and it represents 10% of the patients[56]. The most common sites of metastatic spread of WT are the lungs, regional lymph nodes and liver. In the National Wilms Tumor Study Group (NWTSG), the lung was the only metastatic site in approximately 80% of patients presenting with stage 4 disease at diagnosis, whereas metastases were present in the liver with or without lung involvement in 15% of the patients[56-60].

Metastatic disease is recognized as a poor prognostic factor with a lower overall survival rate that ranges from 30%-50% for diffuse anaplastic WT to 85% for favorable

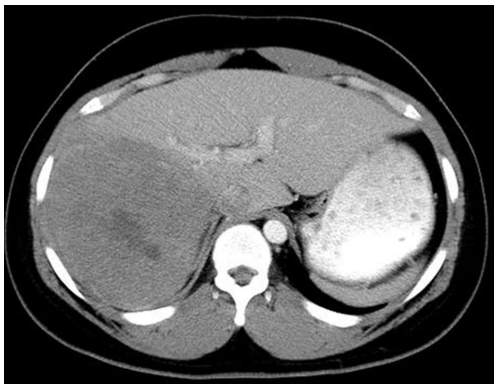

Figure 2 Patient with stage 3 favourable histology Wilms tumor who recurred eight years after therapy with an isolated liver metastasis. As part of the therapy for this disease recurrence, he underwent right hepatectomy and is currently disease free six years later.

histology WT[61]. Varan *et al*[62] reported results from 1971 to 2002 on 18 patients with liver metastases who were noted to have a lower overall survival than patients with pulmonary disease (16.6% *vs* 50.2%). These authors recommended a more intensive chemotherapy and more aggressive surgical treatment for patients with hepatic metastatic disease. Breslow *et al*[63] in the past have given a detailed analysis on the metastatic pattern of children with stage 4 WT from the NWTSG which showed no difference in survival according to metastatic site (liver and/or lung *vs* lung only). Szavay *et al*[64] observed a less favorable outcome in 29 patients with WT complicated by metastases of the liver primarily enrolled in the International Society of Pediatric Oncology (SIOP) and the German Pediatric Oncology Group studies, SIOP 93-01/GPOH study and the SIOP 2001/GPOH study. Two years later, Fuchs *et al*[65] published a series of a total of 45 patients enrolled in these two studies that corroborated the previous findings and suggested that successful complete surgical resection of the primary tumor and of liver metastases in children with WT improves survival.

Ehrlich *et al*[66] reported the largest series about the treatment and outcomes of patients with WT metastatic to the liver. They reviewed patients with favorable histology WT and hepatic metastasis at diagnosis treated on NWTS 4 and 5 to ascertain if they had a worse prognosis than other stage 4 disease. A total of 96 patients were identified. Twenty-two patients (22.9%) had a primary liver resection; 13 patients (13.5%) underwent liver resection after chemotherapy and/or RT. Seventy-one patients (67%) did not undergo surgery for their liver disease. In 14 patients, the liver disease disappeared with chemotherapy only. Eighty-two patients received abdominal RT. EFS for the patients with liver only metastatic favorable histology WT was 76% (95%CI: 58%, 87%) compared to 70% for patients with liver and lung involvement. EFS (95%CI) for the patients with primary resection of the liver metastases was 86% compared with 68% (P = 0.09) for the patients who did not have primary resection of liver metastases. This improved outcome may be the result of having limited hepatic disease and being a more appropriate surgical candidate. There was no significant difference in EFS for patients treated with chemotherapy compared with that of patients treated with chemotherapy and RT (P = 0.63). The EFS (95%CI) for patients who did not receive abdominal RT was 64% compared to 77% for patients who received abdominal RT without boost and 72% for patients who received abdominal RT with boost (P = 0.05). They concluded that liver metastases was not an independent adverse prognostic factor for children with stage 4 favorable histology WT. Although a more aggressive initial surgical approach for a child with WT and liver metastasis is not supported by this report, patients with residual liver disease after treatment with chemotherapy and/or RT that could be completely resected did well, suggesting there may be a role for complete surgical resection of residual metastases after adjuvant therapy. Furthermore, the impact of boost radiation to liver metastases on survival was not clear. The current approach for hepatic only metastatic disease WT patients depends on the tumor histology and type of protocol. Patients with favorable histology WT enrolled on the NWTSG protocol will undergo nephrectomy and lymph node sampling, followed by abdominal RT (planned according to local stage of renal tumor) and RT to sites of metastases and regimen DD-4A (vincristine, dactinomycin, doxorubicin × 24 wk)[61].

In conclusion, a role exists for complete surgical resection of residual metastases after adjuvant therapy in children with WT (Figure 2), a tumor that generally is very sensitive to chemotherapy.

OTHER MALIGNANCIES

GCT

GCT represent 7% of cancer diagnoses among children younger than 20% and 3.5% of cancer diagnoses for children younger than 15 with approximately 900 new cases under 20 years of age each year in United States[1]. After the introduction of cisplatin-based chemotherapy in the 1970s, the survival of children with GCTs substantially improved. For gonadal GCT, the 5-year survival rate has increased from 89% to 98% for children younger than 15 years and from 70% to 95% for adolescents aged 15 to 19 years. Extragonadal GCT 5-year survival rate has increased from 42% to 83% for children younger than 15 years[67-69]. The effectiveness of chemotherapy is monitored by decreases in serum tumor markers (alpha-fetoprotein and beta-HCG) which are produced by malignant GCT. Stage 4 disease includes distant metastases to liver, brain, bone, or lung. The presence of liver metastases represents an independent poor prognostic factor and one of the strongest indicators of a poor long-term outcome in adult patients with advanced GCTs. The literature suggests that liver resection in this age group age is feasible and safe[70]. Rivoire *et al*[71] reported 37 patients with a median age of 26 years (range, 14-47 years) who underwent liver resection

for the treatment of metastatic GCT. Their results were favorable with a median survival of 54 mo and an overall 5-year survival rate of 62% which appeared to justify an aggressive surgical approach for treatment of patients with postchemotherapy residual hepatic metastatic disease. Interestingly, time to appearance of liver metastases, lesion distribution within the liver, timing of liver surgery, extent of resection, and size of resection margins were not of additional predictive value. They recommended close follow-up for patients with residual liver metastases measuring < 10 mm regardless of the primary tumor type and patient gender; close follow-up for male patients with residual metastases measuring > 30 mm regardless of the primary tumor type and delayed surgery for surviving patients with growing lesions even if they are teratomas; liver resection for male patients with metastases measuring 10-29 mm, particularly in the absence of embryonal carcinoma in the primary or mixed tumor; and liver resection for female patients with metastases measuring > 10 mm.

The differences between children and adults regarding the location of the primary GCT site, pattern of metastatic dissemination and the biology of childhood GCTs may limit the applicability of adult therapeutic approaches to children. A report from the Children's Cancer Study Group showed that patients with malignant GCTs, (excluding dysgerminoma and tumors of the testis or brain) with more than one structure or organ involved at diagnosis increased the risk for adverse event[72,73]. In another study[74] from the Pediatric Oncology Group that aimed to investigate prognostic factors for pediatric extragonadal malignant GCT, patients older than 12 years of age with thoracic tumors had six times the risk of death compared with patients younger than 12 years of age with tumors at other sites. Metastatic disease at diagnosis was not a statistically significant prognostic factor for EFS. The role of postchemotherapy surgical exeresis of all residual hepatic metastatic disease may be justified for evaluation of the effectiveness of chemotherapy and resection of refractory disease, but this needs to be individualized.

GIST

GIST is a mesenchymal neoplasm of the gastrointestinal tract that originates from intestinal pacemaker cells, also known as interstitial cells of Cajal. It is typically seen in adults over the age of 40 and children are rarely affected. It has been estimated that there are 3300 to 6000 new GIST cases per year in the United States[75,76]. Of all GISTs, 1.4% to 2.7% occur in children and adolescents in large series[77]. A minority of GIST in pediatric patients (10%) can arise within the context of tumor predisposition syndromes such as Carney triad and Carney-Stratakis syndrome[78,79]. Pediatric GIST is commonly located in the stomach (gastric antrum) and usually occurs in adolescent females[80]. Histology in children is characterized by a predominance of epithelioid or epithelioid/spindle cell morphology and, unlike adult GIST, their mitotic rate does not appear to accurately predict clinical behavior[81]. Multifocal tumors and nodal metastases are common, which account for the high incidence of local recurrence seen in the pediatric population[82]. Pathogenesis in children and young adults may also differ from that of adult GIST, because activating mutations of KIT and platelet-derived growth factor receptor (PDGFR), which are seen in 90% of adult GIST, are present in only 11% of pediatric GIST. This fact is important in terms of therapeutic management. The administration of adjuvant imatinib mesylate, a selective tyrosine kinase inhibitor, has been shown to improve EFS in adult patients with GIST but this benefit is restricted to those with KIT and PDGFR mutations, and thus the use of this agent in pediatric GIST cannot be recommended if the mutation is not present[83-85]. Responses to imatinib in pediatric patients are uncommon and consist mainly of disease stabilization[86]. At presentation, approximately half of adult GISTs have already metastasized with the liver being the most frequent site of metastases. In this age group, gastric tumors of large size (> 5 cm) or arising from small intestine, colon, mesentery and omentum have a high frequency of recurrence and liver metastases. Few pediatric GISTs with hepatic metastatic disease have been reported[87].

The only definitive treatment for GIST is surgical resection, since it is highly resistant to conventional systemic chemotherapy and RT. The mainstay of surgical resection is to achieve a complete resection with negative margins in the primary and/or the metastatic disease[88]. Treatment varies based on whether a mutation is detected or not. For most pediatric patients with GIST and absence of KIT and PDGFR mutations, complete surgical resection of localized disease is recommended as long as it can be accomplished without significant morbidity. Since lymph node involvement is relatively common in younger patients, searching for overt or occult nodal involvement should be encouraged. Given the indolent course of the disease in pediatric patients, it is reasonable to withhold extensive and mutilative surgeries and to carefully observe children with locally recurrent or unresectable asymptomatic disease[89]. The few pediatric patients with KIT or PDGFR mutations should be managed according to adult guidelines and for those patients, resection of hepatic metastases following imatinib treatment may be curative when the primary disease has been eradicated and negative surgical resection margins are attained. Patients with solitary or limited hepatic metastases may be potential surgical candidates. However, a large tumor burden in the hepatic parenchyma may prohibit resection given the risk of insufficient remaining liver tissue and subsequent postoperative liver failure. Other treatment options may include thermal ablation (radiofrequency, laser, microwave, cryoablation), hepatic artery embolization and hepatic artery chemoembolization, but no experience has been reported in pediatric GIST patients[90,91].

In conclusion, the few pediatric patients with KIT or PDGFR mutations who present with solitary or limited hepatic metastases may be potential surgical candidates.

Given the indolent course of GIST in pediatric patients with absence of KIT and PDGFR mutations, it is reasonable to withhold extensive hepatic resections, but further investigations are needed.

OS

OS is the most common malignant bone tumor arising in children and adolescents. In the United States, 400 children and adolescents younger than 20 years of age are diagnosed with OS each year[1]. At diagnosis, 20% of patients will have radiographically detectable metastases, with the lung being the most common site. With improved survival of OS patients with pulmonary metastatic disease owing to a more aggressive treatment with surgery and intensified chemotherapy, the pattern of metastatic disease may be changing[92-95]. Moreover, new imaging modalities which are more sensitive at discovering new metastatic lesions are being incorporated in the tumor protocols.

Although extrapulmonary metastatic disease from OS is considered rare and generally occurs after the diagnosis of pulmonary metastases, a few studies also report some cases with presentation of isolated extrapulmonary metastases and no signs of lung invasion[96]. Hepatic metastatic disease from OS is extremely rare and few cases with or without simultaneous pulmonary metastases have been reported, although it is more commonly found at autopsy[97]. Daw *et al*[98] reported a case of ossified hepatic metastases detected at the time of diagnosis of a secondary OS. Complete resection of all disease is required for cure in patients with OS. Whereas pulmonary metastasectomy for OS has been shown to improve survival, surgical resection of hepatic metastases for this disease has been less well characterized[99,100]. Despite multimodal therapy including different chemotherapeutic agents, surgical resection and radiofrequency ablation, OS patients with hepatic metastatic disease have a poor prognosis and selection of surgical candidates must be individualized.

Desmoplastic small round cell tumor

Desmoplastic small round cell tumor (DSRCT) is a rare malignant abdominal tumor with less than 300 cases reported in the literature. It typically arises in adolescents and young adult men and has a strong tendency to spread within the peritoneum but also to the liver and lungs[101]. It is classified as a small round cell tumor and it is characterized by a distinct immunohistochemical pattern and a recurrent, specific, chromosomal translocation [t (11; 22) (p13; p12)] which results in a chimeric *EWS-WT1* fusion gene[102]. Most of the patients present with disseminated disease at diagnosis and the primary site of origin is frequently unknown. Because of this, it is associated with a very poor prognosis. Although surgery, chemotherapy, RT, radiofrequency ablation, hyperthermic intraperitoneal perfusion with chemotherapy (HIPEC) and combined therapy have been used in the treatment of DSRCT, no single therapy has been accepted as the standard strategy. Honoré *et al*[103] have recently published the largest series with a multimodal management of abdominal DSRCT. They reported on 38 patients with a median age of 27 years (range 13-57 years), but some adolescents were included. Nearly half of the patients at the time of diagnosis had extraperitoneal metastases with the liver involved in 78% of the cases. Different treatment modalities were used including systemic chemotherapy, surgery, HIPEC and RT. They concluded that the factors predictive of 3-year overall survival were the absence of extraperitoneal disease, complete surgical resection, postoperative whole abdominopelvic RT and postoperative chemotherapy. Patients with synchronous liver metastases treated with peritoneal cytoreductive surgery had an overall survival (14.8 mo) similar to patients treated with systemic chemotherapy alone. Therefore, no benefit of surgery was demonstrated in this group of patients. HIPEC had no impact on overall survival. In contrast, Hayes-Jordan *et al*[104,105] published the first report on the use of HIPEC in young children and showed that patients with disease limited to the abdominal cavity, including those with resectable liver metastases, were good candidates for HIPEC with good outcomes.

Therefore, conclusions about the best management of hepatic metastatic disease in DSRCT are difficult to draw. More studies in children and adolescents are necessary to elucidate if a different clinical behavior is documented in this age group.

NET

NET are rarely seen in the pediatric population with an incidence rate of 2.8 per million[106]. Overall, appendiceal NET (carcinoids) are the most common subtype and they are usually found incidentally upon final histopathologic analysis in cases of a suspected appendicitis[107]. This tumor location is rarely associated with metastatic disease in children. Extra-appendiceal carcinoid tumors and neuroendocrine carcinomas are more poorly characterized and have a greater chance for metastatic spread compared with carcinoids arising in the appendix[108]. Broaddus *et al*[109] published 5 of 13 cases that were initially diagnosed in the liver, with no other primary sites identified. They concluded that it is not known if these tumors represent true primary hepatic neoplasms or metastases from asymptomatic, occult gastrointestinal, pancreatic, or pulmonary primary tumors. Although the definitive role of surgery in children with metastatic disease from NET has not been established, the management of hepatic metastases may include surgical resection[110]. In adults, cytoreductive surgery for hepatic metastases from gastrointestinal NETs has resulted in prolonged survival rates[111]. Other treatment options may include hepatic artery embolization, cryoablation, radiofrequency ablation, orthotopic liver transplantation and radionuclides therapy such as 131I-MIBG and 177Lu-octreotate[112-114].

In summary, there may be a potential role for surgical resection of liver metastases in pediatric patients with

NET, but more experience is needed.

CONCLUSION

Hepatic metastatic disease and the benefit of hepatic metastasectomies in pediatric cancer patients are not as well delineated as for adults due to the lower incidence of pediatric malignancies and the higher chemosensitivity of childhood tumors. Patients with residual localized hepatic disease after neoadjuvant therapy and non-chemosensitive tumors may benefit from surgical resection, but careful patient selection remains critical.

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EDITORIAL

Spontaneous bleeding or thrombosis in cirrhosis: What should be feared the most?

Kryssia Isabel Rodríguez-Castro, Alessandro Antonello, Alberto Ferrarese

Kryssia Isabel Rodríguez-Castro, Alberto Ferrarese, Department of Surgery, Oncology and Gastroenterology, Padua University Hospital, 35128 Padua, Italy

Alessandro Antonello, Veneto Oncological Institute (IOV-IRCCS), 35128 Padua, Italy

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Correspondence to: Dr. Kryssia Isabel Rodríguez-Castro, MD, PhD, Department of Surgery, Oncology and Gastroenterology, Padua University Hospital, Via Giustiniani 2, 35128 Padua, Italy. kryssiarodriguez@yahoo.com
Telephone: +39-33-36167592
Fax: +39-49-8218727

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Abstract

The more modern and accurate concept of a rebalanced hemostatic status in cirrhosis is slowly replacing the traditional belief of patients with cirrhosis being "auto-anticoagulated", prone only to bleeding complications, and protected from thrombotic events. With greater attention to clinical thrombotic events, their impact on the natural history of cirrhosis, and with the emergence and increased use of point-of-care and global assays, it is now understood that cirrhosis results in profound hemostatic alterations that can lead to thrombosis as well as to bleeding complications. Although many clinical decisions are still based on traditional coagulation parameters such as prothrombin (PT), PT, and international normalized ratio, it is increasingly recognized that these tests do not adequately predict the risk of bleeding, nor they should guide pre-emptive interventions. Moreover, altered coagulation tests should not be considered as a contraindication to the use of anticoagulation, although this therapeutic or prophylactic approach is not at present routinely undertaken. Gastroesophageal variceal bleeding continues to be one of the most feared and deadly complications of cirrhosis and portal hypertension, but great progresses have been made in prevention and treatment strategies. Other bleeding sites that are frequently part of end-stage liver disease are similar to clinical manifestations of thrombocytopenia, with gum bleeding and epistaxis being very common but fortunately only rarely a cause of life-threatening bleeding. On the contrary, manifestations of coagulation factor deficiencies like soft tissue bleeding and hemartrosis are rare in patients with cirrhosis. As far as thrombotic complications are concerned, portal vein thrombosis is the most common event in patients with cirrhosis, but venous thromboembolism is not infrequent, and results in important morbidity and mortality in patients with cirrhosis, especially those with decompensated disease. Future studies and the more widespread use of point-of-care tests in evaluating hemostasis will aid the clinician in decision making when facing the patient with bleeding or with thrombotic complications, with both ends of a continuum being potentially fatal.

Key words: Bleeding; Hemorrhage; Thromboembolism; Portal vein thrombosis; Coagulation; Cirrhosis

Core tip: The two-faced, dynamic, and fragile hemostatic and coagulation system of patients with cirrhosis is of increasing interest. Thrombotic complications, and not only the well-known bleeding complications such as gastroesophageal bleeding, are now recognized complications of cirrhosis. Whether confined to the portal vein, due to venous stasis but also to other yet poorly characterized local as well as systemic factors, or in the presence venous thromboembolism, these complications warrant prevention and treatment with anticoagulation. Future clinical studies, as well as the broader implementation of point-of-care instruments and results from studies using global coagulation assays will outline the best strategies, tailored to each patient according to the severity of liver disease and the particular hemostatic alterations present at a given timepoint.

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INTRODUCTION

The traditional concept of an "auto-anticoagulated patient" has given way to the modern, and more accurate notion of a rebalanced hemostatic status in patients with cirrhosis. It is now accepted that classic determinations of the coagulation status such as prothrombin (PT) time, international normalized ratio (INR), and activated partial thromboplastin time (aPTT), although useful in the non-cirrhosis setting, are of much less value in patients with advanced chronic liver disease, firstly because they describe only a fraction of what is actually occurring in the hemostatic system, secondly because this system is fragile and dynamic, and thirdly because they do not predict neither thrombotic nor bleeding events.

Hand in hand with this new bulk of knowledge regarding both the pre-clinical as well as the clinical picture of hemostasis and coagulation in cirrhosis, therapeutic and preventive strategies that were routinely used in the non-cirrhotic population and rigorously avoided in the cirrhotic population, are being used with increasing frequency and confidence.

SPECIFIC ALTERATIONS OF THE HEMOSTATIC AND COAGULATION SYSTEM

Although the clinician might be misled to judging the state of a patient with cirrhosis as pro-hemorrhagic due to an alteration of traditional coagulation parameters, in cirrhosis actually both pro- as well as anti-coagulation factors are affected, the latter of which are not adequately reflected in these tests. Typical of cirrhosis are reduced levels of factors Ⅱ, Ⅸ, Ⅺ, and Ⅻ, and the magnitude of the reduction correlates with the severity of liver disease. However, levels of anticoagulant factors including protein S, protein C, and antithrombin, are also decreased in cirrhosis, and procoagulant factor Ⅷ is notably increased. Magnifying the complexity of hemostatic and coagulation abnormalities in cirrhosis, studies have demonstrated that liver damage increases plasminogen activator inhibitor (PAI-1) expression[1,2]. Increased to a greater extent than PAI-1, tissue plasminogen activator is elevated both due to reduced hepatic clearance and to enhanced release[3], which has been interpreted as a hyperfibrinolytic state in cirrhosis[4]. Moreover, levels of plasminogen and anti-plasmin antiplasmin (α 2-antiplasmin) are reduced, as well as levels of thrombin-activatable fibrinolysis inhibitor (TAFI). Whether observed alterations such as elevated fibrin degradation products[5-7], abnormalities in thromboelastography (TEG) tracings[8], and a decrease in TAFI[9] actually correspond to a state of hyperfibrinolysis which would hypothetically be frequent even in compensated cirrhosis is still controversial, however. Other studies have suggested that actually fibrinolysis is not enhanced in cirrhosis, with a balanced reduction of both pro- as well as anti-fibrinolytic agents[10], and a lack of association between TAFI reduction and actual hyperfibrinolysis[11,12]. Moreover, elevated levels of D-dimer may be a consequence of the activation of the coagulation cascade, which might accumulate in the presence of diminished hepatic clearance[13-15].

Responsible for stabilization of the fibrin clot and its resistance to lysis, factor XIII (FXIII) correlates with the liver's biosynthetic capacity, and has been shown to be diminished in nearly half of patients with advanced stages of cirrhosis (Child C); FXIII levels < 50% significantly correlated with an increased risk of severe upper gastrointestinal bleeding and mortality in a 6-year follow-up period[16]. Although this could be a reflection of the severity of liver disease, and despite reduced FXIII activity by itself is probably not sufficient to cause bleeding, the addition of this alteration upon the underlying multiple coagulation and hemostatic defects, might increase the risk of hemorrhage[16,17]. As the only method of detecting FXIII deficiency is at present measuring the factor itself, it is probably reasonable to perform this test in the event of uncontrolled bleeding in the presence of regular rotation thromboelastometry (ROTEM) patterns, and when bleeding cannot be explained by platelet count and serum fibrinogen within the normal ranges[18]. The combination of these events results in the establishment of a new - fragile and dynamic - thrombotic/hemostatic balance[10,11].

Regarding primary hemostasis, chronic liver disease is characterized by a variable degree of thrombocytopenia due to increased platelet destruction, increased splenic and/or hepatic sequestration, and to reduced levels of

thrombopoietin. Moreover, not only platelet number, but also platelet function has been shown to be compromised due to defective thromboxane A2 synthesis, storage pool deficiency and abnormalities of the platelet glycoprotein Ⅰb[19-22]. Different mechanisms compensate for reduced platelet number and function: von Willebrand factor is notably elevated in cirrhosis, probably as a result of its reduced clearance resulting from diminished levels of its cleaver ADAMTS13 and as a reflection of high levels of FⅧ, to which it is bound when circulating in plasma[23].

In addition to these acquired hemostatic and coagulation defects, superimposed (or rather, underlying) genetic thrombophilias may play an important role in tilting the balance towards thrombosis. In a study by Amitrano *et al*[24], the frequencies of factor Ⅴ Leiden and of PT A20210 polymorphism were reportedly 13% and 34.8% in cirrhotic patients with portal vein thrombosis (PVT), whereas frequencies were 7.5% and 2.5% in cirrhotic patients without PVT.

The actual hemostatic and coagulation changes in cirrhosis are not adequately reflected by traditional tests including the INR, aPTT, bleeding time, and platelet count, and are also imprecise in predicting bleeding episodes[25]. These tests are not able to detect natural anticoagulant deficiencies, nor do they reveal other pro-thrombotic alterations such as the elevation of von Willebrand factor. In addition, other aspects related to the risk of bleeding or thrombosis, such as clot formation, firmness, and degradation, are not assessed by conventional tests. Likewise, the determination of the individual factors does not provide a complete picture of hemostatic alterations occurring *in vivo*, either, since the intricate system strongly depends on the balance of pro- and anti-fibrinolytic as well as coagulation factors.

A test that is used ever less frequently, bleeding time correlates with platelet count[26], and is prolonged in nearly half of patients with cirrhosis, without, however a certain relationship with bleeding risk[27].

Whereas traditional coagulation tests measure only the initial 5% of thrombin that is generated and are insensible to detecting deficiencies in the anticoagulation mechanisms, global assays such as the thrombin generation test analyze more components of the hemostatic status and therefore offer a view that is closer to what is actually going on *in vivo*. When performed in the presence of thrombomodulin, which enables the activation of protein C, the amount of thrombin generated in plasma from patients with cirrhosis is at least equal to - even increased with respect to - that of healthy subjects[28,29]. Despite this test yields a more approximate view regarding generation as well as degradation of thrombin, this *in vitro* technique, apart from being impractical and complex, has the drawback of excluding platelets, which serve not only as a scaffold for coagulation, but play an active role in the process.

The "newcomers" in the field of bedside coagulation monitoring, which have actually been around for quite a while in other clinical scenarios, provide a more complete picture of what is going on *in vivo*. Point-of-care coagulation monitoring devices which assessing the viscoelastic properties of whole blood include TEG (Haemonetics Corporation, Braintree, MA, United States), ROTEM™ (Tem International, Munich, Germany), and theSonoclot coagulation and platelet function analyzer or Sonoclot (Sienco Inc., Arvada, CO, United States)[30]. The fact that analyses are performed in whole blood allow for platelets and red cells to be accurately reflected[31] and the interactions between plasmatic and cellular components of hemostasis to be analyzed. The rate of fibrin formation, clot strength, and clot lysis[32,33] can be determined by all three instruments. Moreover, TEG provides a more adequate characterization of hypofibrinogenemia and hyperfibrinolysis[34] than the clot lysis time and global fibrinolysis capacity[35].

At present, ROTEM™ or TEG™ are valuable tools that aid in decision making in the context of direct therapeutic interventions in the actual case of bleeding[18]. TEG is in fact currently employed to guide therapy during liver transplantation in many centers[36-38] and is gaining importance in the assessment of liver-disease associated hemostasis alterations[39,40], with a possible role in predicting variceal rebleeding[41] and guiding pre-procedural transfusions[42]. Intense correction of coagulation abnormalities should be avoided, and rather transfusions and other therapeutic interventions should be tailored to each patient's specific case, hopefully guided by point-of-care testing. This is very important in order to avoid risks associated with transfusions (acute lung injury, increase in portal pressure, *etc.*) and the increased risk of thromboembolism with, for example, the use of recombinant factor ⅦⅡa[43].

GASTROESOPHAGEAL VARICEAL BLEEDING

Gastroesophageal variceal bleeding (GEVB) constitutes a landmark in the natural history of a patient with cirrhosis, represents decompensated disease, and is one of the most feared complications. Mortality reaches 15%-20% during the 6 wk that follow an episode of variceal bleeding and is closely related to the severity of the underlying liver disease, ranging from 0% in patients in Child-Pugh class A to 40% in Child-Pugh class C patients[44,45]. Mortality significantly correlates with the presence of ascites or encephalopathy (OR = 4.18, 95%CI: 1.58-11.06; P = 0.004), the finding of fresh blood in the upper gastrointestinal tract at endoscopy (OR = 2.40, 95%CI: 1.28-4.51; P = 0.01), the presence of INR > 1.5 and/or PT prolonged > 3 s (OR = 3.06, 95%CI: 1.29-7.26; P = 0.01), in-patient status at the time of bleeding (OR = 7.14, 95%CI: 3.45-14.3; P < 0.001), and the presentation with hemodynamic shock (OR = 2.10, 95%CI: 1.07-4.13; P = 0.03), as demonstrated in a large United Kingdom study[46]. The principal determinants of GEVB are the severity of liver disease - as expressed by a Child Pugh class B

or C, the presence of portal hypertension, variceal wall tension, and the characteristics of the varix wall[47-50]. In fact, anticoagulants at a prophylactic dose do not seem to increase the risk of GEVB, even in patients with advanced stages of liver disease, while actually preventing thrombotic events and decompensation[51].

Rebleeding occurs in approximately 26% of cases and results in a dramatic increase in mortality of up to 39%. This event correlates with the presence of INR > 1.5 and/or PT prolonged > 3 s (OR = 2.23, 95%CI: 1.22-4.07; P = 0.01), as well as with the presence of high risk endoscopic stigmata (OR = 1.74, 95%CI: 1.02-2.99, P = 0.04)[46]. Moreover, an underlying bacterial infection, followed by the circulatory release of endogenous heparin-like substances with established anti-Xa activity[52] and abnormal thromboelastographic curves, appears to be an important trigger for bleeding, for the persistence of bleeding, and correlates with the impossibility of controlling bleeding[41,53-56]. Supporting this concept, a consistent reduction of both mortality and frequency of early rebleeding has been achieved with the use of antibiotics following GEVB[57].

The risk of bleeding from variceal ulcers following endoscopic band ligation seems to depend exclusively on the severity of liver disease, and not the hemostatic status, as demonstrated by thromboelastographic parameters and traditional coagulation tests[58]. As in the occurrence of a spontaneous event of GEVB, the use of anticoagulants - may it be vitamin k antagonists or heparins - does not seem to increase the risk further[59,60].

NON-VARICEAL SPONTANEOUS BLEEDING

Upper non-variceal gastrointestinal bleeding

Non-variceal upper gastrointestinal bleeding is not an infrequent cause of morbidity and mortality in patients with cirrhosis. In a recently published cross-sectional nationwide study conducted in the United States, of 96887 hospital discharges for peptic ulcer bleeding, 3574 (3.69%) occurred in patients with cirrhosis[61]. Mortality of peptic ulcer bleeding was significantly higher in patients with cirrhosis (5.5%) *vs* in the group without cirrhosis (2%, P = 0.01), and decompensated cirrhosis was associated with a significantly higher mortality than that of patients with compensated cirrhosis (6.6% *vs* 3.9%; P = 0.01). Moreover, multivariate analysis demonstrated that the presence of cirrhosis independently increased mortality (adjusted odds ratio) 3.3; 95%CI: 2.2-4.9)[61]. A prospective, 10-year study analyzing patients admitted for non-variceal upper gastrointestinal bleeding showed that of 2217 patients with upper gastrointestinal bleeding, 1077 patients had non-variceal bleeding (48.7%) patients, and amongst these, 160 (14.8%) were patients with cirrhosis[62]. Of note, within the group of cirrhosis patients with non-variceal upper gastrointestinal bleeding, rebleeding occurred in 3 patients (1.9%), and in-hospital mortality was 13.75% (22 of 160 patients). Although deaths were due to reasons other than hypovolemia in 12 patients, and other causes of death included renal, hepatic, or respiratory failure, amongst others, the initial reason for hospitalization had been the bleeding episode[62].

Portal hypertensive gastropathy, which has been described in as many as 80%-90% of patients with cirrhosis[63,64], has been shown to correlate with severity of liver disease and to hepatic venous portal gradient in patients with cirrhosis[63,65]. Bleeding from portal hypertensive gastropathy most often leads to chronic anemia, but can also cause important blood losses over a short period of time. In a multi-center Italian study published on behalf of the New Italian Endoscopic Club for the Study and Treatment of Esophageal Varices, the prevalence of portal hypertensive gastropathy was 80% and was associated to the duration of liver disease, past medical history of endoscopic variceal slerotherapy, and with the presence and size of esophagogastric varices. During the follow-up period of 18 mo (± 8 mo), acute bleeding from portal hypertensive gastropathy was observed in 2.5% of patients (8 of 315 patients), with bleeding-related mortality rate of 12.5%, and chronic bleeding in 10.8% (34 patients)[64]. Treatment and prevention consist primarily in reducing portal pressure, principally with the use of non-selective beta-blockers[66], although treatment with other vasoactive drugs such as long-acting somatostatin, TIPS placement[67], argon plasma coagulation[68], and newer therapies such as hemospray[69] are increasingly being used.

Lower gastrointestinal bleeding

According to the study design, including the population analyzed, portal hypertensive colopathy has been reported to occur in 50%-80% of patients with cirrhosis, and is apparently more frequent in patients with ascites[70-72]. In a study analyzing 60 cirrhosis patients who underwent colonoscopy before undergoing upper endoscopic variceal band ligation, hemorrhoids, anorectal varices, and portal hypertensive colopathy were found in 37%, 40%, and 57% of patients, respectively[73]. A higher prevalence (66%) of portal hypertensive colopathy was found in a Japanese study analyzing endoscopic findings in 47 patients with cirrhosis who underwent colonoscopy for positive fecal occult blood (34%), melena (23%), iron deficiency anemia (10%), diarrhea (4%), abdominal pain (4%), and screening (10%), amongst other causes[74]. Although large, prospective studies are lacking, the presence of portal hypertensive colopathy appears to correlate with severity of liver disease, and an increase in portal hypertension, as that induced by endoscopic esophageal variceal band ligation, does not seem to worsen preexisting colopathy or induce the appearance of new lesions[73]. Whether portal hypertensive colopathy is associated with the degree of portal hypertension as determined by hepatic vein pressure gradient, is yet controversial, however[74,75].

Regarding the ano-rectal tract, rectal varices

have been reported in 8% to 56% of patients with cirrhosis and portal hypertension[72,76,77]. Although hemorrhoids and polyps do not seem to occur more frequently in cirrhotics with respect to non-cirrhotic subjects undergoing colonoscopic evaluation[76], others hypothesize that the degree of portal hypertension and/or disease severity seems to be associated with hemorrhoids but not with rectal varices[78,79]. However, the improvement of bleeding rectal varices seems to point out a role for portal hypertension[80]. Moreover, although hematochezia has been reported[79], and a few cases of massive, fatal bleeding[81], life-endangering hemorrhage from the lower gastrointestinal tract due to complications of cirrhosis is relatively infrequent. Large, prospective studies are warranted in order to accurately determine the incidence and prevalence of these clinical entities, as well as their associated morbidity. Although studies which evaluate the best treatment options are lacking, reduction of portal hypertension with the use of non-selective beta-blockers and the employment of vasoactive agents such as somatostatin, octreotide and terlipressin, have demonstrated some benefit[82,83]. More recently, the use of argon plasma coagulation and hemospray have also been advocated[69,84].

Other bleeding sites

Minor but frequent bleeding in patients with cirrhosis seems to be more akin to that observed in patients with platelet defects than that observed in patients with hemophilia or other disorders that affect coagulation. Thus, aside from variceal bleeding, in which local factors, portal pressure and severity of liver disease play preponderant roles, manifestations of primary hemostasis defects are most frequently encountered in patients with cirrhosis: recurrent and prolonged epistaxis, gingivorrhagia, purpuric skin lesions, menometrorrhagia, and excessive bleeding after dental extractions or other surgical procedures. On the contrary, coagulation-related clinical manifestations such as intracerebral bleeding, deep muscle bleeding, and hemartrosis, are no more frequent in cirrhosis that they are in the general population. Although only very rarely epistaxis[85] and oral cavity bleeding[86] (gum bleeding and dental root bleeding) have been reported to be the cause of bleeding that endangers life, minor but repeated episodes are commonly encountered in cirrhosis.

PVT

PVT is the most common thrombotic event in patients with cirrhosis, and although its frequency is higher in patients with hepatic malignancy (approximately 35%[87], with reportedly 40% of these cases having histological confirmation of neoplastic thrombosis[88]), it is also common in patients with cirrhosis and without malignancy, with a prevalence of reportedly 0.6% to 26%. Moreover, a systematic review analyzing PVT in patients with cirrhosis who underwent liver transplantation found that of 25753 liver transplants, 2004 were performed in patients with PVT, for a prevalence of 9.7% ± 4.5%[89].

The most important risk factor for the development of PVT seems to be the severity of liver disease[90,91], with "paradoxically" a greater frequency of PVT when coagulation factors are lowest, as shown by traditional coagulation tests. Locally, venous stasis favors the development of thrombosis, and a prospective study revealed that reduced portal flow velocity was the only independent variable that correlated with the risk of developing PVT at 1 year follow-up[92], but this finding has not been univocally confirmed[93]. Elevated levels of FVIII have been correlated with PVT both in the presence and in the absence of concomitant cirrhosis[94,95], finding which was confirmed in a larger cohort study demonstrating that the odds ratio for PVT was 6.0 for patients with cirrhosis in whom FVIII levels were above 129 UI/dL[96]. Moreover, genetic thrombophilias have been found in up to 34% of patients with cirrhosis and PVT[24], which is why every patient who present this complication warrants complete thrombophilic screening.

Not only is this complication frequent, but its clinical presentation can be deadly in some cases; in a study analyzing newly diagnosed PVT in 79 patients with cirrhosis, in 39% the initial presentation was gastrointestinal bleeding (from esophagogastric varices or portal hypertensive gastropathy), and abdominal pain was the cardinal symptom in 18% of cases, amongst which 70% had intestinal infarction due to the extension of the thrombosis into the superior mesenteric vein[97]. Although recently it has been reported that PVT, when diagnosed during routine imaging screening in patients with cirrhosis, may not cause clinical deterioration and may even resolve spontaneously[98,99], a recently published systematic review revealed that the presence of non-neoplastic PVT at liver transplant entails a greater 30-d mortality after surgery when compared to patients without PVT (10.5% *vs* 7.7%, respectively (P = 0.01)[89]. Moreover, the presence of PVT at liver transplantation also increases the one-year mortality with respect to that of patients with patent portal vein (18.8% *vs* 15.3%, respectively (P < 0.001), and this is especially true for cases in which PVT is complete and extends into the superior mesenteric vein and the splenic vein.

DEEP VEIN THROMBOSIS AND VENOUS THROMBOEMBOLISM

It has been some time now since the publication of Northup and collaborators' important study demonstrating that not only "coagulopathy" does not protect cirrhosis patients from life-threatening venous thromboembolic events, but that these patients are actually at a greater risk for these events[100]. Low albumin, surrogate of a greater severity of liver disease, was associated with the greatest risk. Although large,

prospective population studies considering out-patient subjects with cirrhosis are needed, it seems that compared to the general population, the incidence of unprovoked deep vein thrombosis and pulmonary embolism (DVT/PE) is increased. In a large, prospective cohort study with case-control analysis of 6550 patients with venous thromboembolism, the presence of chronic liver disease was associated with PE (OR = 1.75, 95%CI: 0.91-3.36) and with DVT/PE combined (OR = 1.65, 95%CI: 0.97-2.82)[101]. Moreover, a large Danish population-based study showed that cirrhosis and liver disease were associated with a greater risk of venous thromboembolism (OR = 2.10) amongst 99000 patients with thromboembolism[102]. Thus, the incidence of DVT/PE in patients with cirrhosis has been reported to be between 0.5% to 8.1%[101-105].

Already deadly in the non-cirrhotic population, venous thromboembolism is associated with increased mortality in patients with compensated cirrhosis (OR = 2.16, 95%CI: 1.96-2.38) and those with decompensated cirrhosis (OR = 1.66, 95%CI: 1.47-1.87), with an in-hospital mortality for patients with venous thromboembolism of 16.8% and 18.6% for patients with compensated and decompensated cirrhosis, respectively[106]. Moreover, although the risk of venous thromboembolism is reduced with prophylactic anticoagulation, it is not annulled, as demonstrated in a recent study in which a higher than expected rate of venous thromboembolism occurred while on prophylaxis with unfractioned heparin or low molecular weight heparin[107].

Although guidelines do not yet provide recommendations regarding anticoagulation neither as prophylaxis nor as therapy, evidence has been accumulating supporting the efficacy and safety of such interventions[108]. Future studies, including the use of new anticoagulants such as direct thrombin inhibitors are warranted to establish which patients will benefit most from treatment, the time after which the risk-benefit ratio becomes inclined towards a greater risk, the most adequate dose, the choice of anticoagulant, and the means of monitoring of anticoagulation[109].

In conclusion, the pro-hemorrhagic and pro-thrombotic alterations of patients with cirrhosis correlate principally with the severity of liver disease, that determine a reduction in both pro- and anti-coagulant factors and an increased derangement of physiological blood flow causing portal hypertension and localized venous stasis. Routine laboratory tests do not reliably predict the risk of bleeding and there is yet no optimal management strategy to foretell potential bleeding complications. Although point-of-care testing is slowly being introduced to avoid intensive correction of coagulation parameters and better guide therapeutic decisions tailored to each patient's clinical and hemostatic status, more studies are clearly needed to determine the actual role of these new tools. A myriad of both thrombotic and bleeding complications can aggravate the clinical course of cirrhosis, but as for frequency and gravity, GEVB remains probably the most feared event. However, thrombotic complications should also be considered, especially in more advanced stages of disease, when anticoagulation prophylaxis and therapy might represent the less traveled, but proper, road to follow.

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EDITORIAL

Voriconazole and the liver

Romeo-Gabriel Mihăilă

Romeo-Gabriel Mihăilă, Faculty of Medicine, Lucian Blaga University of Sibiu, 550169 Sibiu, Romania

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Correspondence to: Romeo-Gabriel Mihăilă, MD, PhD, Faculty of Medicine, Lucian Blaga University of Sibiu, Str Lucian Blaga, 2A, 550169 Sibiu,
Romania. romeomihaila@yahoo.com
Telephone: +40-269-215050
Fax: +40-269-218365

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Abstract

Voriconazole is an azole useful for the prophylaxis and the treatment of aspergillosis and other fungal infections in immunosuppressed subjects, as those found in aplasia after aggressive polychemotherapy treatments, after hematopoietic stem cell, liver or lung transplantation. Its administration in therapeutic doses lead to extremely varied serum levels from patient to patient and even to the same patient. The explanations are varied: nonlinear pharmacokinetics, certain patient-related factors, including genetic polymorphisms in the cytochrome *P450 2C19* gene, the kidney and liver function, simultaneous administration with other drugs metabolised by the same cytochrome. It is recommended to maintain the serum concentrations of voriconazole between 1.5 and 4 μg/mL. At lower values its efficacy decreases and at higher values the risk of neurological toxicity increases. Even at these concentrations it is not excluded the possible appearance of a variety of toxic effects, including on the liver, manifested by cholestasis, hepatocytolisis, or their combination. It is recommended to monitor the clinical and laboratory evolution of all patients treated with voriconazole, and of the serum levels of the drug of those who belong to risk groups, even if there is still no consensus on this issue, given the lack of correlation between the serum level and the occurrence of adverse effects in many patients.

Key words: *CYP2C19*; Pharmacokinetics; Liver toxicity; Therapeutic drug monitoring; Voriconazole

Core tip: Voriconazole is an azole useful for the prophylaxis and the treatment of aspergillosis and other fungal infections in immunosuppressed subjects. Its administration in therapeutic doses lead to extremely varied serum levels from patient to patient and even to the same patient. It is recommended to maintain the serum concentrations of voriconazole between 1.5 and 4 μg/mL. At lower values its efficacy decreases and at higher values the risk of neurological toxicity increases. Even at these concentrations it is not excluded the possible appearance of a variety of toxic effects, including on the liver, manifested by cholestasis, hepatocytolisis, or their combination.

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INTRODUCTION

This article is written for clinicians who use voriconazole. Starting from pharmacological data, the aim of this literature synthesis is to find explanations and to establish correlations with clinical manifestations that may occur during its use.

Voriconazole is a usual antifungal drug with a good bioavailability, which is bound to plasma protein in a high percentage[1]. It is considered to be the drug of choice for the treatment of invasive aspergillosis[2]. Voriconazole was approved by the United States Food and Drug Administration in 2002[3]. Its metabolisation takes place in the liver, at the level of P450 CYP2C19[1,2], CYP2C9, and CYP3A4, and its products of metabolism are excreted by the kidneys[2]. Only 2% of the dose excreted in urine is unchanged. There is a variety of drug-drug interactions that must be considered. The ratio between the area under the time-concentration profile and the minimal inhibitory concentration is the main pharmacokinetic/pharmacodynamic parameter for voriconazole[2]. It is active against a large variety of fungi, but it also can have adverse effects, including the liver toxicity[1]. This aspect deserves to be good understood, as it is commonly administered to patients with various associated diseases, and sometime immunocompromised, together with other drugs, for serious infections.

LIVER TOXICITY

Liver toxicity can sometimes be present. From a total of 68115 regarded liver injuries, 2.9% of them (1964 cases, including 112 with acute liver failure) were related to antimycotics, during a period of 8 years, as was found in FAERS database. All antimycotics with systemic action or absorption can induce such toxicity, including voriconazole[4]. This was shown in 2 of 21 patients with chronic pulmonary aspergillosis treated with oral voriconazole, which imposed the drug discontinuation, in a recent published study[5]. In a prospective study, which included 95 patients, 6.3% of them developed liver toxicity under voriconazole[6]. In a prospective, open-label, multicenter study in which voriconazole was given to 48 patients, as first-line treatment for chronic pulmonary aspergillosis, 16.9% of treated patients had abnormal liver function tests. Liver serious adverse events were noted at 2.8% of them and imposed to stop the drug administration. The authors did not show any association between voriconazole serum levels and the appearance of adverse effects[7]. In a retrospective study made on a group of 105 patients who received a lung transplant and were treated with voriconazole, it was found that 51% of them developed liver toxicity and 34% of them had to discontinue the treatment for this reason. The univariate statistical analysis established that the following factors were associated with liver toxicity: age younger as 40 years, azathioprine treatment, presence of cystic fibrosis, history of hepatic disease and early start of voriconazole. The authors have developed an algorithm predictive model to predict liver toxicity, that has an accuracy of 70%, using the above risk factors. The start of voriconazole in the first 30 d since the date of transplantation was the sole factor independently associated with liver toxicity in the multivariable logistic regression analysis[8]. Patients aged $\geqslant$ 60 years were significantly more likely to have higher initial voriconazole plasma levels, while those with cystic fibrosis - higher ones, in a prospective study on 93 lung transplant recipients who received prophylaxis with voriconazole. Voriconazole serum levels were not correlated with the presence or absence of liver toxicity, neither here[9].

A worsening of liver function tests was shown at 69% of patients with severe liver dysfunction who received at least 4 doses of voriconazole, in an observational study. Most of them had a combination of liver cytolysis and cholestasis (45%). An increase of transaminases was shown in 35%, and an isolated cholestasis was present in 15% of them. All of them had a severe reaction. There was noted a correlation between the initial dose of more than 300 mg (4.5 mg/kg) and the risk of liver toxicity. These patients represent a special category that needs a frequent monitoring of liver function tests[10].

Two percent of patients with liver transplantation who received different antifungal drugs as targeted prophylaxis (including voriconazole in 54% of 145 subjects) or universal voriconazole prophylaxis (237 patients) developed toxicity which required drug discontinuation, after a median treatment of 11, and, respectively, 6 d[11]. It was shown that liver dysfunction had greater rates under voriconazole, as fluconazole or itraconazole, but voriconazole decreased transplant mortality in a meta-analysis which included 5122 patients who received antifungal prophylaxis[12].

Prophylaxis with voriconazole was better tolerated by patients who received allogeneic hematopoietic stem-cell transplant as those with itraconazole. It was shown that 12.9% of those who received voriconazole had liver toxicity/dysfunction, which was the most frequent adverse effect[13].

A recently published method uses unlabeled or deuterated methanol, followed by GC-MS analysis in order to speed up the metabolic profiling of fatty acids, useful for establishing the presence and the mechanism of voriconazole induced liver toxicity, and the possibility to use fatty acids as markers of toxicity[14]. Frechen *et al*[15] developed a coupled dynamic model useful to study the interaction of voriconazole (a CYP3A inhibitor) and midazolam (a CYP3A substrate). Thus, it is possible to maximize the information obtained from clinical drug-drug interactions studies[15].

Therefore, among the liver side effects of voriconazole, jaundice, including the cholestatic one, is more common while hepatomegaly or hepatitis occurs

less frequently. It rarely induces liver failure and very rarely hepatic coma. So the liver damage produced by voriconazole is mainly due to cholestasis, and more rarely can be cytotoxic or mixed.

Voriconazole is not involved in the appearance of autoimmune hepatitis, but an increase in the levels of immunosuppressive drugs (cyclosporine or tacrolimus) used in patients after solid organ transplants or with autoimmune disorders was reported during coadministration with this antifungal drug. One of five such patients developed a moderate increase in hepatic enzymes. This combination of drugs requires immunosuppressive dose adjustment (by its reducing)[16].

IS THERAPEUTIC DRUG MONITORING NECESSARY?

Serum levels

Voriconazole serum levels are important in relation to efficacy and toxicity: a lack of clinical response has been seen at below 1 or 2 μg/mL and toxic effects appear frequently at above 5 μg/mL[1]. But, it was shown that voriconazole steady-state concentrations had a large variability from patient to patient, with values between 0 and 16.6 μg/mL, in a study that included 69 patients with primarily acute leukemia under intensive chemotherapy. In this study, about 20% of patients had concentrations < 1 μg/mL[17], therefore, ineffective. This variability was even larger (< 0.10-20 mg/L) in a study made on 108 patients of whom 77.8% had a hematologic cancer, who were treated for a presumed fungal infection[18].

There may be several explanations for this inter-patient variability: the nonlinear pharmacokinetics and some patient-related data, as gender, age, weight, a possible liver disease, and genetic polymorphisms in the cytochrome *P450 2C19* gene (*CYP2C19*), so that the knowledge of *CYP2C19* genotype can be used to establish initial voriconazole dose and the modality of therapeutic drug monitoring, in order to obtain the therapeutic effects without toxicity[3]. But, contrary to theoretical considerations, it was observed that *CYP2C19* and *CYP2C9* genotypes had only a minor influence on the serum levels of voriconazole, though the 4 patients who were homozygous for the *2C19*2* genotype had higher average serum levels of the drug, in a prospective study which included 95 patients. It is notable that patients who had hallucinations had higher average voriconazole levels[6], which highlights the importance of careful clinical monitoring of patients. But in the study of Chu *et al*[18], mentioned above, where was no relationship between therapeutic drug levels of voriconazole and the response to this antifungal treatment, and unlike other studies, there was no association with an increase of hepatotoxicity at voriconazole levels > 5.5 mg/L. This observation suggests that therapeutic drug monitoring could be limited to a subset of high-risk patients[18]. In another multicenter study which included 264 patients with hematological diseases, who received voriconazole for prophylaxis or treatment of invasive aspergillosis, a large range of plasma concentration (between < 0.20 and 13.47 μg/mL) was also seen. The authors found only an association between voriconazole plasma troughs and the use of omeprazole. They did not find any correlation with other parameters, as age, gender, dose or route of voriconazole administration (including those by nasogastric tube), *CYP2C19*2* genotype, possible abnormalities of gastrointestinal tract, serum levels of liver enzymes or creatinine, with treatment outcome of patients with invasive aspergillosis, or with the cases of reported toxicity to this drug. This study also raises the question of the utility of routine clinical monitoring of voriconazole plasma concentrations[19].

It was shown that 40 immunocompromised children had higher average exposure to voriconazole at steady state during oral treatment with 200 mg q12h than adults, so that a weight-based oral dose could be more appropriate for them[20].

Given the accumulated clinical and laboratory experience, in order to obtain therapeutic efficacy and to avoid toxicity, most authors suggest a serum voriconazole concentrations between 1.5 and 4 μg/mL[9,21]. The lower limit was set considering the significantly higher success rate of fungal infections treatment over this level, and the superior limit - due to the fact that above its it was shown that adverse neurological effects appeared significantly more frequent. The metaanalysis included 12 studies and also found an increase of liver adverse effects at higher blood concentrations, but obtained data did not allow the formulation of a limit above which the risk increases significantly[19,21].

Arguments for clinical monitoring

The following categories of patients should be carefully monitored, although there is no consensus in this respect: the children, the patients with cystic fibrosis, liver or renal failure (including those under chronic hemodialysis), and those who are concomitant under treatment with other drugs, which voriconazole could interfere with[1]. There was found no relationship between plasma voriconazole concentrations and age, gender, genotype or concomitant administration of proton pump inhibitors, but there was a correlation between the drug concentration and higher serum levels of serum alkaline phosphatase, aspartate aminotransferase, and bilirubin, in the study published by Saini *et al*[17].

A case of triple drug combination was published in the literature: lansoprazole, voriconazole (400 mg/d) and tactolimus. It was found that the concentration of voriconazole became half of the initial one (from 5.0 ng/mL) and those of tactolimus also fell after reducing the dose of lansoprazole [from 60 mg/d intravenous (*iv*) to 15 mg/d per os] in a patient with *CYP2C19* and *CYP3A5* heterozygous mutations. This is an example of drug interaction: the lowering of lansoprazole dose decreased voriconazole concentration and this seems

to be the explanation for the decrease of those of tacrolimus[22]. This drug monitorization needs future controlled studies for its validation and for a personalized treatment, with dose adjusted according to the patient[1].

Another explanation for high voriconazole levels consists in the presence of inflammation, due to the fact that inflammatory stimuli can modify the activities and expression levels of cytochrome P450 isoenzymes. Indeed, in a retrospective chart review which included 128 patients found under voriconazole treatment, higher drug trough concentrations were present in those with severe inflammation (C-reactive protein about 6.2 mg/L), although the dose of the drug was similar reported on mg/kg body weight. Every increase of C-reactive protein with 1-mg/L contributed to an elevation of voriconazole trough concentration with 0.015 mg/L[23]. It was found that lipopolysaccharide did not exacerbate the effect of voriconazole, which induces non-idiosyncratic liver injury, unlike clozapine and ketoconazole[24].

An association between the use of voriconazole and a higher survival probability in liver transplant recipients with invasive aspergillosis was found, comparing to other antifungal drugs[25]. In another retrospective study, 39 patients with acute-on-chronic liver failure developed invasive pulmonary aspergillosis; it was shown that those treated with voriconazole had better prognosis comparing with those without antifungal drug. Although the dose of voriconazole was not adjusted, there was no negative impact on renal or hepatic function, but this does not preclude the need for careful monitoring the function of vital organs for the metabolism of voriconazole[26]. Multiple logistic regression analysis made on 39 patients established that persistent high trough concentration of this drug may increase the risk of liver toxicity; this fact may be avoided by reducing the trough concentration to < 4 μg/mL[27].

An important drug association that may be found in oncohematology is between voriconazole and tacrolimus, especially in patients who received allogeneic bone marrow transplantation. Voriconazole at clinically relevant concentrations will increase more than two-fold the serum levels of tacrolimus, by the inhibition of its hepatic metabolism[28].

Baseline liver impairment of patients with renal dysfunction was found to be a significant predictor of worsening renal function in multivariate analyses in the seventh day of voriconazole treatment[29].

HOW TO REDUCE THE TOXICITY OF VORICONAZOLE?

It would be ideal to have the possibility to measure serum concentrations of voriconazole in all patients and to ensure that during treatment they are between the recommended limits, although we know that it is not a way able to prevent the possible occurrence of adverse effects in all cases.

But lowering the dose is another way to reduce the likelihood of adverse effects induced by voriconazole administration, especially when we are not able to monitor the serum drug level. That was done in a study that included 83 patients who were subjected to allogeneic hematopoietic stem cell transplantation, and who received *iv* voriconazole (only 100 mg two times per day) since the time of conditioning regimen until their neutrophils exceeded 0.5×10^9/L. This led to a rate of invasive fungal infections of only 8.43%, compared to 18.06% in the group treated with oral fluconazole (200 mg/d). This difference was statistically significant, and, very important, the frequency of functional liver abnormalities was similar under the two drugs[30]. The question which remains is whether the fungal infection rate would have not been lower under a normal dose of voriconazole, and also whether the benefit gained by lowering the rate of fungal infections would not have been canceled by the increased toxicity induced by voriconazole.

Other ways to prevent the potential drug toxicity, including those on the liver, are avoiding the use of this drug in patients with severe liver disease or advanced renal failure, and clinical (for the detection of the first events that might suggest toxicity, *e.g.*, hallucinations) and laboratory monitoring (transaminases, bilirubin, cholestatic enzymes, serum creatinine), especially in patients who are in treatment with other drugs that are metabolized by cytochrome P450. Voriconazole is recommended for patients with liver disease only if the benefit outweighs the potential risk.

Another way to reduce the adverse effects (including those on the liver) induced by azols consists in the development of new antifungal agents that are more selective for the target fungal enzyme CYP51, in comparison with the human CYP enzymes CYP3A4. These newly created agents have less avid metal-binding groups and molecular changes in order to enhance their potency. Such an oral agent is 7 d (VT-1161), found in phase 2 of clinical trials[31].

CONCLUSION

Voriconazole serum levels are not correlated with the presence or absence of liver toxicity in many studies, but in others an increase of liver adverse effects at higher blood concentrations was shown, although obtained data did not allow the formulation of a limit above which the risk increases significantly.

Patients who have hallucinations have, probably, higher average voriconazole levels.

Most authors suggest a steady-state serum voriconazole concentrations between 1.5 and 4 μg/mL.

A correlation between the drug concentration and higher serum levels of serum alkaline phosphatase, aspartate aminotransferase, and bilirubin was found.

Therapeutic drug monitoring could be limited to a subset of high-risk patients, as those with severe liver dysfunction, who are prone to develop liver toxicity under voriconazole, especially if the initial dose is more than 300 mg (4.5 mg/kg). The following categories of

high-risk patients should also be carefully monitored, although there is no consensus in this respect: the children, the patients with cystic fibrosis, liver or renal failure (including those under chronic hemodialysis), and those who have severe inflammation or are concomitant under treatment with other drugs, which voriconazole could interfere with.

A weight-based oral dose could be more appropriate for children.

In order to reduce the likelihood of its adverse effects, lowering the *iv* dose of voriconazole was able to reduce the fungal infection rate comparing with oral fluconazole, but there is no study to prove that its efficiency is the same with that of normal dose.

A way to avoid its potential toxicity is the development of new antifungal agents that are more selective for the target fungal enzyme CYP51, in comparison with the human CYP enzymes CYP3A4.

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REVIEW

Circulating biomarkers of hepatocellular carcinoma response after locoregional treatments: New insights

Maria Tampaki, Polyxeni P Doumba, Melanie Deutsch, John Koskinas

Maria Tampaki, Polyxeni P Doumba, Melanie Deutsch, John Koskinas, Academic Department of Medicine, Medical School of Athens, Hippokration General Hospital, 11527 Athens, Greece

Author contributions: Tampaki M, Doumba PP, Deutsch M and Koskinas J contributed to this paper.

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Correspondence to: John Koskinas, MD, PhD, Academic Department of Medicine, Medical School of Athens, Hippokration General Hospital, Vas. Sofias 114, 11527 Athens, Greece. koskinasj@yahoo.gr
Telephone: +30-21-32088641
Fax: +30-21-32088641

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Abstract

Hepatocellular cancer is the 5th most common cancer in the world and the third cause of death by malignant disease. Locoregional therapies are the most usual treatment of choice for patients with early or intermediate stage of disease. The main diagnostic tools for the detection of recurrence are the radiological techniques such as 4-phase computed tomography or dynamic contrast enhanced magnetic resonance imaging. However, in order to achieve best evaluation of treatment outcome and recurrence rates, there is a great need for the identification of specific and easily measured circulating biomarkers. The aim of this review is to analyze the existing data considering the prognostic significance of changes of serum diagnostic markers such as alpha-fetoprotein, des-gamma-carboxy prothrombin, alpha-fetoprotein-L3, angiogenetic factors (vascular endothelial growth factor, hypoxia inducible factor-1a) and immune parameters before and after radiofrequency ablation or transarterial chemoembolization.

Key words: Radiofrequency ablation; Transarterial chemoembolization; Hepatocellular cancer; Circulating biomarkers; Prognosis

Core tip: Hepatocellular cancer is the 5th most common cancer in the world and the third cause of death by malignant disease. Locoregional therapies are available for patients with early or intermediate stage of disease. However even with these techniques recurrence rates are high. The use of accurate prognostic biomarkers is of great importance in order to select the most suitable-personalised treatment. The aim of this review is to analyze the existing data regarding circulating biomarkers measured before and after locoregional therapies and their effect on treatment outcome.

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INTRODUCTION

Hepatocellular cancer (HCC) is the 5th most common cancer in the world and the third cause of death by malignant disease[1]. Despite its high occurrence, the survival rates are not yet satisfying. The therapeutic tools that we possess are variable depending on the stage of HCC and the severity of the underlying liver disease. However, the only one that provides complete cure is liver transplantation. Unfortunately, due to the specific selection criteria and the low availability of liver transplants, this treatment is implemented in a limited number of patients[2]. Moreover, surgical resection is applicable to a confined number of patients, as over 70% of them have advanced hepatic disease often combined with portal hypertension or multifocal disease, conditions that preclude any possibility of hepatic surgery[3,4]. Therefore, locoregional therapies have been developed in order to treat patients that did not fulfill the criteria for surgical interventions. Transarterial chemoembolization (TACE) is a minimally invasive technique combining the impact of chemotherapy and obstruction of blood supply on the tumor area without systematic effects. The patients that benefit mostly are those with intermediate stage of HCC[5,6]. On the other hand, radiofrequency ablation (RFA) is a technique suitable for small tumors (< 3 cm) and early stage disease[7]. Its action is based on the direct destruction of the tumor by radiofrequency waves[7]. Despite their efficacy, both these techniques have high rates of local and distant recurrence[8,9] (Figure 1). The main diagnostic tools for the early detection of recurrence are the radiological techniques such as 4-phase computed tomography or dynamic contrast enhanced magnetic resonance imaging based on the mRECIST criteria[10]. The tumor response is evaluated according to target lesions' diameters after locoregional treatment. Complete response indicates disappearance of targeted lesions, while partial response indicates that the the sum of the greatest one-dimensional diameters is decreased more than 30%. When the targeted lesions' diameters are increased more than 20% according to baseline the tumor is characterized as progressive. Finally in cases that do not qualify for partial response or progressive disease the term "stable disease" is used[10]. However, these tools are not adequate for the exact estimation of treatment response, the immediate diagnosis of disease recurrence and the patients' prognosis. For the above reasons, there is a current need for biomarkers that may provide the possibility to predict the course of the disease post-treatment and to recognize certain groups of patients with different prognosis. In other words, there is a need for biomarkers that could allow the personalization of therapy aiming to better treatment results and reduction of recurrence rates. To achieve this goal, a number of traditional as well as recently discovered serum biomarkers have been measured before and after locoregional therapies, in order to identify patterns that could be indicative of treatment efficacy and prognosis. For example, the prognostic value of diagnostic markers' post-treatment changes such as alpha-fetoprotein (AFP)[11-13], lens culinaris agglutinin A-reactive fraction of AFP (AFP-L3)[14] and des-gamma-carboxy prothrombin (DCP)[15,16] has been put under investigation. At the same time, the response of vascular endothelial growth factor (VEGF) and other angiogenetic factors to hypoxic conditions caused by TACE or RF was evaluated according to tumor response[17-21]. Another intriguing observation was the immunomodulatory effect of loco-regional techniques, affecting $CD4^+$, $CD8^+$ T cells and T regulatory cells (Treg)[22-24] and its potential impact on patients' survival. Finally, the post-treatment behavior of various new serum HCC markers such as nucleosomes, osteopontin (OPN)[25], soluble receptor of advanced glycation end products (sRAGE)[26] and heat shock proteins[27,28] has been examined along with its role in treatment outcome (Figure 2). The aim of this review is to present the results of these studies and analyze the emerging conclusions.

AFP

AFP is one of the first markers used for the detection and prognosis of HCC. It has been used in clinical practice for many years despite its limitations in the diagnosis of small tumors and the fact that other diseases apart from HCC may cause a mild to moderate rise of its levels[12]. The diagnostic values of this marker vary according to the chosen cut-off values. In cirrhotic patients, when the cut-off value is 20 ng/mL, its sensitivity and specificity are 60% and 90% respectively[12]. Lately, the use of AFP as a screening test for HCC is not strongly recommended as curable tumors smaller than 3 cm may not cause a detectable rise and thus may not be immediately diagnosed. However, AFP is regarded as a reliable prognostic marker of recurrence[13].

It has been shown that high levels of AFP in the serum of patients 24 h after TACE are a strong independent prognostic factor for poor survival[29]. Additionally, patients with poor response of AFP levels after TACE (decrease less than 50% of baseline) had a hazard ratio for progression free survival up to 4.2 (95%CI: 2.4 to 7.2) in comparison to patients with a higher response[30]. According to another study, reduction of AFP less than 20% from the baseline level was correlated to the progression free survival as well as the overall survival of treatment naïve patients after TACE ($P = 0.009$)[31]. The decrease of AFP is also predictive one month post-treatment as the patients without strong AFP response at that point of time have lower overall survival (34.9 mo *vs* 13.2 mo; $P = 0.002$)[32] and are more likely to have extrahepatic metastasis six months of TACE initiation ($P < 0.001$)[33]. Consequently, the impact of TACE on AFP levels may reflect the efficacy of the method and the patients' overall survival. Therefore, AFP could be used as a possible tool for further treatment choices.

As far as RFA is concerned, pre and post-treatment levels of AFP have been also associated with the

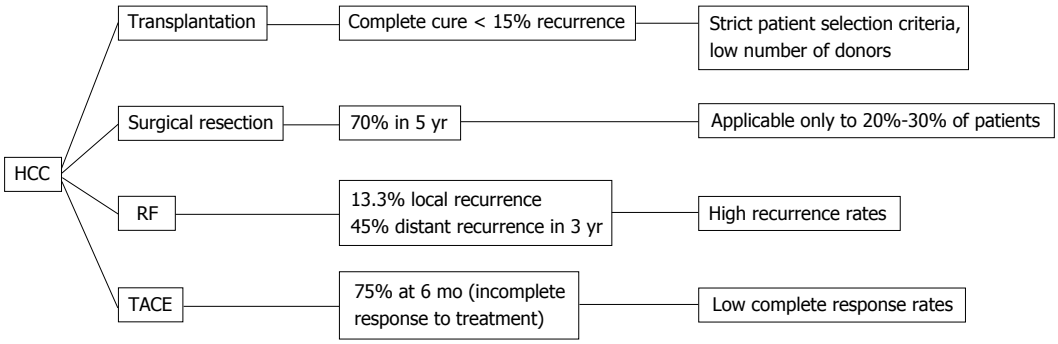

Figure 1 Hepatocellular cancer therapeutic methods. HCC: Hepatocellular cancer; TACE: Transarterial chemoembolization; RF: Radiofrequency.

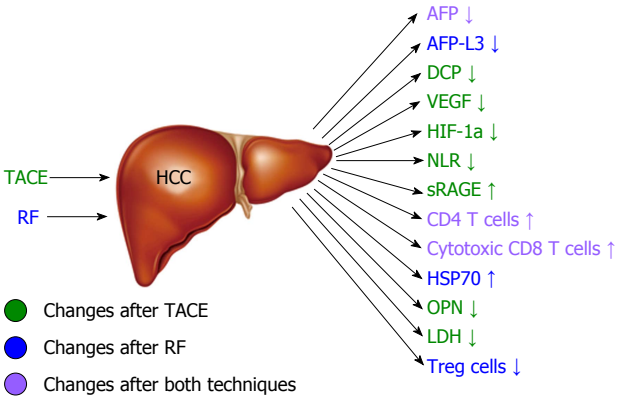

Figure 2 Changes of circulating markers and immune parameters associated with good hepatocellular cancer response after radiofrequency or transarterial chemoembolization. HCC: Hepatocellular cancer; TACE: Transarterial chemoembolization; RF: Radiofrequency; AFP: Alpha-fetoprotein; DCP: Des-gamma-carboxy prothrombin; VEGF: Vascular endothelial growth factor; HIF-1a: Hypoxia inducible factor-1a; NLR: Neutrophil-to-lymphocyte ratio; sRAGE: Soluble receptor of advanced glycation end products; HSP70: Heat shock protein 70; OPN: Osteopontin; LDH: Lactate dehydrogenase.

response as well. It is believed that patients without an adequate decrease of AFP after RFA (AFP half-life less than 7 d) have not a complete response to treatment and thus have a lower disease free survival even if the radiological findings show successful outcome (P = 0.003)[34]. Moreover, high post treatment AFP levels (less than 20% reduction from baseline) one month after RFA are an independent risk factor for tumor recurrence (P < 0.001) as well as for low overall survival (P = 0.023) according to multivariative analysis[35].

AFP-L3

AFP-L3 is a biomarker detected in the serum of HCC patients even in cases of small tumors (35% of patients with tumors < 3 cm)[36]. Its sensitivity varies from 45% for tumors < 2 cm to 90% for tumors > 5 cm and its specificity reaches 95%. It is considered to be a marker of poor prognosis as it is often combined with tumor size, higher possibility of early metastasis and limited liver function[37]. It is believed that in combination with AFP or other markers may enhance the sensitivity and specificity of HCC diagnostic tools[12]. However, the utility of this biomarker is not yet adequately established and AFP-L3 is not currently integrated in clinical practice.

Positive pre-treatment values of AFP-L3 (> 24.4%) along with tumor size were found to be the two statistically significant predicting factors of treatment response[14]. Additionally, AFP-L3 positivity before TACE was significantly associated with 2-year survival rates (P = 0.01)[14]. Recently, it has been shown that An AFP-L3 decrease > 20% after 2 cycles of TACE is indicative of median overall survival (P < 0.0001)[38]. Therefore, the evaluation and the surveillance of its values in the course of therapy could be useful for the estimation of disease progression.

In contrast to TACE, the role of AFP-L3 changes in the serum of patients before and after RFA has been more thoroughly investigated. AFP-L3 fragment positivity (> 15%) before and 2 mo after ablation was found to be indicative of high risk of recurrence (P = 0.0096) and possibly a marker of residual HCC that cannot be depicted by radiological techniques[39]. On the other hand, the patients who had positive pre-treatment values of AFP-L3 and became negative post-treatment, did not show significantly higher rates of recurrence[39]. In another study, AFP-L3 was the only significant predictor of disease free and overall survival in comparison to AFP and DCP when measured before and after RFA[40].

DCP

DCP is an abnormal form of prothrombin produced by malignant hepatocytes. It has been used as a diagnostic marker (sensitivity 72%, specificity 90%) mainly in Japan and is associated with microvascular invasion of tumor cells[41-44]. Due to its correlation with HCC angiogenesis[41], it is thought to be indicative of high recurrence incidence. Like AFP, the elevation of its levels may be induced by chronic hepatitis C or advanced cirrhosis[41] and consequently DCP is not suitable for surveillance protocols. However, if combined with AFP, the sensitivity of the screening test may be increased. As a marker of tumor invasiveness, it is another potential biomarker for the effectiveness of locoregional treatments for HCC.

As suggested by a recent study, the response of DCP values to TACE may be useful for the estimation of treatment outcome. Decrease of DCP values greater than

50% was significantly associated with radiologic response to therapy ($P < 0.001$) as well as higher disease free and overall survival ($P < 0.001$)[45]. Moreover, DCP levels trend before and after TACE is considered to correlate both with treatment response ($P = 0.009$) (according to Response Evaluation Criteria in Solid Tumors) and overall survival[15].

On the other hand, it has not been proved so far that the change of DCP levels before and after RFA reflects the progress of the disease. Despite the fact that high DCP pre-treatment values are considered as a marker of high possibility of local recurrence, no study has yet proposed that the response of DCP levels after RFA has a statistically significant correlation with recurrence free survival or overall survival[46]. However, in a study examining the vascular invasion predictive factors after RFA in 1057 HCC patients, DCP was found to be the most significant predictor[16].

ANGIOGENETIC FACTORS

Angiogenesis is a key process for the expansion of hepatocellular carcinoma involving a number of factors stimulating, inhibiting or regulating various cellular pathways[47]. VEGF for example, is an angiogenetic factor often up regulated in the serum of HCC patients. The VEGF serum levels are proven to reflect the tumor mass size as well as the tumor potential to infiltrate nearby tissues. Moreover, VEGF elevated values are associated with portal vein invasion and extended disease[48]. Therefore, it is a possible tool to predict HCC prognosis and metastasis. Nevertheless, more extended investigation is needed to prove its value in HCC patients' monitoring. Hypoxia inducible factor-1a (HIF-1a) plays also an important role in the angiogenetic process[49]. The survival of malignant cells under hypoxic conditions is mainly regulated by this factor, as it is responsible for the expression of a number of proteins including VEGF[49]. As a result, the evaluation of its behavior in HCC patients is really interesting.

Especially for TACE, a technique that induces hypoxic conditions in the tumor environment, the response of factors such as VEGF and HIF-1a has been put under investigation in a number of studies. Patients with complete response to TACE were found to have lower pretreatment levels of serum VEGF ($P < 0.001$) than patients with partial or no response[50]. Serum VEGF, when measured after TACE, seems to reach the highest value 1 d after treatment and then it is gradually reduced[17]. It has been shown that patients with higher levels of VEGF 7 d after TACE had a rapid progression of HCC in a 3-mo period ($P < 0.05$)[18]. Additionally, decreased levels of serum VEGF receptor-2 4 wk after TACE are predictive of higher survival rates (19.0 mo *vs* 9.8 mo, $P < 0.001$)[19]. Moreover, HIF-1a, a factor responsible for the regulation of the expression of VEGF[20], showed the same response as VEGF in the serum of patients 1 d, 1 wk and 1 mo after TACE[21]. The above support the correlation between the two angiogenetic factors. Additionally, patients with complete response to TACE had lower levels of both HIF-1a and VEGF 1 mo after TACE than those with partial response, stable or progressive disease (all $P < 0.01$)[21]. Recently, the changes of the two angiogenetic markers in the serum of 22 patients 30 to 40 d after TACE were correlated with tumor's hepatic artery perfusion (HAP) and hepatic artery perfusion index (HPI). According to the study, VEGF and HIF-1a post-treatment levels were higher while HAP and HPI were lower in patients with recurrent disease comparing to the baseline values ($P < 0.05$)[51]. As a result, VEFG and HIF-1a should be further studied as potential markers of response to this therapeutic technique.

The levels of VEGF in the serum of patients treated with RFA are also considered to be a potential prognostic factor. High pre-treatment levels (> 240 pg/mL) are an independent prognostic factor of the recurrence free survival as well as the overall survival after RFA ($P = 0.005$ and 0.002, respectively)[52]. However, when serum VEGF levels were measured 2 and 5 d after RFA, there was no significant change between the pre- and post-treatment levels[53]. This is probably explained by the mechanism of action of RFA. In other words, this treatment method is based on the direct necrosis of the tumor tissue and has not as a clear antiangiogenetic effect as TACE. Therefore the impact of RFA on the levels of angiogenetic factors and tumor angiogenesis is not yet adequately delineated and should be investigated in future studies.

OSTEOPONTIN

OPN is an integrin-binding glycophosphoprotein with an important role in bone metabolism, immune responses and vascular remodeling. It is produced by various tissues including macrophages, activated lymphocytes and Kupffer cells and is considered to have a cytokine's action[54,55]. Additionally, OPN due to its immunogenic function is involved in the pathogenesis of alcoholic and nonalcoholic liver disease and T cell mediated hepatitis. Its expression has been found to be up regulated in HCC tumors and especially in metastatic HCC tumors. As a result, OPN has been associated with advanced disease, portal vein and lymph node invasion and early metastasis[55]. However, its value as a prognostic HCC biomarker is not yet proven by a large study in broader HCC populations. The above OPN functions though, could explain the fact that low baseline OPN levels and their decrease (> 10%) 4 wk after TACE, have been correlated to better response to treatment and better cumulative survival[25]. Nevertheless, when evaluated in a multivariative analysis, this relationship was not statistically significant[25].

IMMUNE RESPONSE CIRCULATING PARAMETERS

Locoregional therapies for HCC cause tumoral necrosis resulting in an immunomodulatory effect. Necrotic cell

death provides a source of antigens that stimulate a strong immune response firstly mediated by antigen presenting cells[56].

The outcome of TACE has been associated to the blood neutrophil-to-lymphocyte ratio (NLR), a marker of immune activity often up regulated in patients with HCC[25,57]. In a recent study 42 patients with an elevated pre-treatment NLR (> 1.85) had a median survival of 8 mo while 136 patients with normal NLR had a median survival of 17.5 mo ($P < 0.001$)[58]. In addition, the down regulation of NLR after TACE is indicative of higher overall survival and thus an independent prognostic factor with possible clinical value ($P = 0.006$)[59]. TACE may also influence the levels of sRAGE, a biomarker still under investigation, related to immunogenic cell death with a possible role in stimulation of immune response and angiogenesis[60]. Patients that exhibited higher levels of sRAGE before and 24 h after treatment had a better treatment response[26]. Moreover, TACE induces a specific $CD4^+$ T cell response targeting the tumor tissue. It is possible that the acute inflammation caused by the necrosis of the tumor sensitizes the previously tolerant immune system and promotes the activation of AFP- specific $CD4^+$ T Cells[22]. These cells through the production of interferon-gamma (IFN-γ) further promote the destruction of tumor cells by cytotoxic $CD8^+$ T cells[22]. Finally, according to a recent paper, higher levels of Th17 cells 30 d post-TACE were found to be significantly associated with elevated overall survival ($P = 0.007$)[23]. Th17 cells through the production of interleukin-17 (IL-17) are responsible for the accumulation of neutrophils after acute tissue injury[24]. Consequently it is possible that TACE due to its hypoxic effect on HCC tissue promotes the activation of Th17 cells resulting it the recruitment of neutrophils in the damaged area.

RFA causes direct destruction of the targeted HCC lesion, resulting in the induction of acute inflammation and extended immune response. It has been shown *in vitro* that the ablated malignant tissue promotes intensely the maturation of antigen presenting cells in comparison to the non-ablated malignant tissue or normal liver tissue. This possibly happens due to the release of previously "hidden" intracellular antigens of malignant cells[61]. Additionally, the dendritic cells (DCs) that were activated in the presence of ablated HCC tissue produce higher amounts of IL-12, a cytokine promoting T helper 1 cells responses, while DCs activated by non-ablated HCC tissue produce mainly IL-10 resulting in the induction of T helper 2 cells responses[61]. The post-RFA activated DCs were also found to secrete high amounts of IL-1 and tumor necrosis factor-a[61]. In another study, RFA caused a significant 5-6 fold rise ($P < 0.0001$) of HCC specific $CD4^+$ T cells, cytotoxic T cells and IFN-γ 8 wk after treatment[62]. Interestingly, this strong anti-tumor T cell immune response has been proven to be mediated mainly by $CD4^+$ T cells. However, the pool of circulating lymphocytes when measured 1 mo after RFA, presented an elevation of CD56 differentiation antigens of T cells and natural killer cells. In other words, RFA caused the rise of circulating effector cytotoxic cells. Despite this fact, the tumor recurrence rate was not correlated to the extent of the immune response. In fact, the antigens extracted from the recurrent tumor tissue did not initiate an intense response of DCs and T cells that was produced by the ablated tumor tissue[62]. The effect of RFA on the levels of cytotoxic T cells was further confirmed recently, as 5 out of 9 patients presented with elevated glypican-3 specific cytotoxic T cells after treatment[63]. On the contrary, only 1 of 9 patients treated with surgical resection had increased levels of this specific type of cells[63]. Moreover, the number of tumor-associated antigen - specific T cells produced after RFA has been correlated to HCC recurrence rates[64].

Additionally, a recent study focusing on the role of $CD4^+CD25^+Foxp3^+$ Treg cells in the prognosis of HCC after cryoablation has produced interesting results[65]. This type of cells is known to affect the immune response against malignant cells through the production of specific cytokines such as transforming growth factor-β or by direct cell contact. The above functions result in the suppression of APCs maturation and T cell differentiation and the apoptosis of effector cells[66]. According to the study, patients with higher levels of circulating $CD4^+CD25^+FoxP3^+$ Treg had significantly higher rates of reccurence after cryoablation ($P = 0.026$). Moreover, among 31 patients subjected to cryoablation, those who presented with tumor progression during the follow-up period (12-48 wk after treatment), had elevated Treg frequency. In fact, the Treg cells isolated from 6 patients with recurrent HCC had increased immunosuppressive effect against PBMCs isolated from healthy controls. On the contrary, Treg cells extracted from 6 patients with good tumor response did not have such a strong immunosuppressive effect[65].

Finally, as suggested by studies on animal models and patients with HCC as well, RFA enhances the production of heat shock proteins such as heat shock protein 70 (HSP70)[67,68]. In HCC tissue extracted immediately before and 24 h after RFA the expression of HSP70 and HSP90 was found to be increased 8-fold and 1.2-fold respectively[27]. In a study with a limited number of patients with liver, kidney or lung malignancies, the levels of serum HSP70 were significantly higher 1 d after RFA (paired *t* test, $P = 0.001$)[28]. Moreover the patients with the higher increase tended to have lower recurrence rates than those without detectable increase post-RFA[28]. Heat shock proteins are thought to play an important role in the activation of dendritic cells and may be the local stimuli for the strong immune response taking part after RFA[69].

All the above mechanisms could be the basis of new treatment strategies combining immunotherapy with locoregional therapies in order to enhance the therapeutic effect of these techniques.

CELL DEATH PARAMETERS

As mentioned above, due to the induction of tumor

necrosis by local therapies, a number of cell death products are released into the circulation directly after treatment[61]. Some of these products could be put under investigation in order to acquire useful markers for the evaluation of early treatment response.

Recently, the kinetics of serum cell death products, such as nucleosomes, cytokeratine-19 fragments (CYFRA 21-1) and lactate dehydrogenase (LDH) pre- and post-TACE, have been studied along with their correlation with treatment response[70]. The results showed that all three parameters were increased 24 h after TACE. However, the levels of nucleosomes were the only marker that was significantly different between the group of responders and the group of non-responders ($P < 0.001$)[70]. In other words, higher percentage changes between the baseline levels and the levels of serum nucleosomes 24 h post-treatment were correlated with disease progression. Interestingly, in the multivariative analysis the combination of nucleosomes (24 h), alkaline phosphatase (24 h) and number of TACE was the best prognostic model for treatment response[70]. LDH is considered to be another potential prognostic marker for patients treated with TACE. It has been shown that patients with increased post-treatment LDH values had lower disease free and overall survival in comparison to patients with decreased values within 1 mo after TACE[71]. This difference was found to be statistically significant both for disease free and overall survival ($P < 0.0087$ and $P < 0.0001$ respectively)[71]. Nevertheless, due to the limited patient number in this study, the exploitation of cell death markers needs to be extensively studied by further clinical trials.

DISCUSSION

In comparison to other types of cancer, HCC is characterized by the ability to produce a significant variety of potential biomarkers. This aspect is an important advantage for the amelioration of the existing diagnostic and prognostic tools as well as the development of new ones. As mentioned above, the immediate estimation of treatment response and disease prognosis plays a pivotal role for the clinical outcome of patients treated with locoregional therapies, due to the high rates of recurrence. Towards this direction, the evaluation of circulating biomarkers' values after TACE or RFA may reflect the tumor behavior and the possibility of disease progression. According to the available studies, there is a correlation between the changes of classical or newer biomarkers such as AFP, AFP-L3 or DCP and the patients' survival rates. Although each of the above biomarkers is not established as a separate, useful diagnostic or prognostic tool, their combination in a prognostic model could prove beneficial. Moreover, since angiogenesis plays a key role in the pathophysiology of HCC, the response of angiogenetic factors' levels to therapy could be indicative of treatment efficacy and future outcome. This is of great importance especially for TACE, a therapeutic technique with anti-angiogenetic mechanism of action. Another interesting development is the measurement of cell death products caused by the destruction of the malignant tissue. The extent of tumor necrosis is indicative of treatment efficiency. Therefore, the quantification of tumor necrosis with the use of circulating cell death parameters provides a direct measure of treatment outcome even more specific than mRECIST criteria. Finally, the unique ability of locoregional therapies to induce a major immune response could be also exploited either by associating the intensity of specific HCC-targeting cell production with treatment outcome or by the implementation of a new combination of treatment strategies.

CONCLUSION

As locoregional therapies are currently the most common treatment choice for patients with early or intermediate stage of HCC, the discovery of prognostic models for patients stratification is undoubtedly of great importance. Apart from the above biomarkers, there is a need for new molecular parameters that could enhance the understanding of HCC behavior and susceptibility to different therapeutic techniques. In other words, the identification of more specific molecular markers for HCC may permit the generation of specific HCC molecular profiles resulting in more targeted treatment strategies or perhaps new combinations of them. In order to achieve this, a number of clinical trials should be conducted in the future.

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MINIREVIEWS

Hepatitis C cirrhosis: New perspectives for diagnosis and treatment

Vikas Khullar, Roberto J Firpi

Vikas Khullar, Roberto J Firpi, Department of Medicine, University of Florida College of Medicine, Gainesville, FL 32608, United States

Roberto J Firpi, Section of Hepatobiliary Diseases and Transplantation, University of Florida College of Medicine, Gainesville, FL 32608, United States

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Correspondence to: Roberto J Firpi, MD, MS, AGAF, Section of Hepatobiliary Diseases and Transplantation, University of Florida College of Medicine, 1600 S.W. Archer Road, MSB- 440, Gainesville, FL 32608,
United States. roberto.firpi@medicine.ufl.edu
Telephone: +1-352-2739466
Fax: +1-352-3927393

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Abstract

Chronic hepatitis C infection is the leading cause of chronic liver disease, cirrhosis, hepatocellular carcinoma as well as the primary indication for liver transplantation in the United States. Despite recent advances in drugs for the treatment of hepatitis C, predictive models estimate the incidence of cirrhosis due to hepatitis C infection will to continue to rise for the next two decades. There is currently an immense interest in the treatment of patients with fibrosis and early-stage cirrhosis as treatment can lead to decrease in the rates of decompensated cirrhosis, hepatocellular carcinoma and need for liver transplantation in these patients. The goal of this paper is to provide clinicians and health care professionals further information about the treatment of patients with hepatitis C infection and cirrhosis. Additionally, the paper focuses on the disease burden, epidemiology, diagnosis and the disease course from infection to treatment. We provide an overview of multiple studies for the treatment of chronic hepatitis C infection that have included patients with cirrhosis. We also discuss the advantages and disadvantages of treatment in cirrhotic patients and focus on the most up to date guidelines available for treatment.

Key words: Cirrhosis; Diagnosis; Treatment; Simeprevir; Sofosbuvir; Ledipasvir; Liver transplantation; Hepatitis C virus

Core tip: The treatment of chronic hepatitis C infection has undergone a revolution with the introduction of new and highly-effective therapies allowing for high rates of cure and relatively low adverse effects. While there is strong evidence for the treatment of patients without cirrhosis, limited studies and numbers are available for patients with cirrhosis; yet this is the group likely to benefit most from treatment. This paper focuses on the current evidence and regimens for the treatment of patients with cirrhosis and addresses the advantages and disadvantages of pursuing treatment.

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INTRODUCTION

Hepatitis C virus (HCV) infection is the leading cause of chronic liver disease, cirrhosis, hepatocellular carcinoma (HCC) and currently the primary indication for liver transplantation in the United States[1]. As per most recent estimates from the World Health Organization, chronic HCV infection is estimated to have a prevalence between 130 to 150 million worldwide[2]. Chronic HCV infection is defined as the persistence of HCV viremia for greater than 6 mo. While the estimated prevalence is low in developed countries (1%-2%), the less developed countries may carry a prevalence as high as 5%-10% of the adult population[3]. In United States alone, the most conservative estimates suggest the prevalence of people infected with chronic HCV to be around 2.7-5.2 million[4,5]. Amongst those who are infected with chronic HCV, studies evaluating the natural course of the disease suggest that around 55%-85% would progress to chronic liver disease, 15%-30% would progress to cirrhosis and 1%-5% are expected to die due to decompensated cirrhosis and HCC[2]. Worldwide, there are an estimated 350000 to 500000 deaths per year due to HCV related liver disease[2]. Hence, identifying the patients infected with chronic HCV infection and treating them with newly available treatments provides a unique opportunity to help decrease the morbidity and mortality from the disease. Based on these potential benefits, the center for disease control (CDC) in the United States recommends one time birth cohort screening of the population born between 1945-1965 (defined as "baby boomers") with a HCV antibody test[6]. However, despite recent advancements in the treatment of chronic HCV infection, predictive models estimate that the prevalence of HCV cirrhosis will continue to increase through the next decade and is projected to reach 45% in 2030 of chronically infected persons[7]. The incidence of hepatic decompensation and HCC is also expected to continue to increase for an additional 10 to 13 years prior to seeing a decline due to the wider application of antiviral treatment and better responses with newer agents[7]. Currently those with cirrhosis due to chronic HCV infection are considered difficult-to-treat however may be the group that is likely to benefit most from treatment as virus eradication can potential reduce morbidity and mortality in this population.

In this manuscript, we provide an overview of chronic HCV infection in the context of disease burden, epidemiology, diagnosis and the disease course of HCV infection in the United States population. We present the current treatment regimens and trials which have included patients with cirrhosis and provide information for physicians who may be interested in learning further or pursing treatment for chronic HCV infection in patients with cirrhosis. Additionally, as cirrhotic patients represent a challenge among those with chronic HCV infection, we also discuss the advantages and disadvantages of providing treatment to patients in this pathologic stage of disease.

DISEASE BURDEN AND EPIDEMIOLOGY

Many patients with chronic HCV infection are asymptomatic and it is estimated that 45%-85% are unaware they are even infected[6]. Large population studies testing for positivity of anti-HCV antibody in non-institutionalized population in the United States have shown the prevalence to be approximately 1.8% in the general population[8]. In these studies, the strongest risk factors predicting a positive HCV infection were illegal drug use, blood transfusions prior to 1992 and high risk sexual behavior with high number of lifetime sexual partners. Other risk factors associated with a positive HCV infection included poverty, having less than twelve years of education and having been divorced or separated[8]. Surprisingly the study also showed that 15%-30% of infected patients' reported no risk factors for the transmission of HCV infection. Additional studies examining the burden of HCV infection in the United States, show that by 2007, HCV had superseded human immunodeficiency virus (HIV) as a cause of death in the United States[9]. Several additional United States studies have also predicted a two-fold increase in HCV related deaths with direct medical expenditure exceeding $6.7 billion USD between 2010 and 2019[10] and without intervention, suggest that morbidity and mortality from HCV will peak between 2030 and 2035 forecasting for 38600 incident cases of end-stage liver disease, 3200 referrals per year for liver transplant and 36100 deaths[11].

DIAGNOSIS AND DISEASE COURSE FOR CHRONIC HCV INFECTION

HCV infection is rarely diagnosed during in the acute phase of infection. Although a variety of host-factors play a role in eradication of HCV, only 15%-25% of adults spontaneously clear the infection[12]. The remaining proportion of patients continue to have persistent viremia[8] and retrospective studies on the natural history of HCV infection, have found that about 15%-30% of people with chronic infection would progress to cirrhosis over the duration of two to three decades[13]. Progression to cirrhosis has been shown to occur at an accelerated pace in those with concomitant alcohol use (> 50 g/d), co-infection with HIV and hepatitis B virus (HBV), as well as male sex, and older age at time of infection (Figure 1)[13,14]. In the patients' that develop HCV related cirrhosis, the risk of development of HCC has been shown to be 1%-4% per year and warrant surveillance for complications[15].

The first step in the diagnosis of HCV infection is

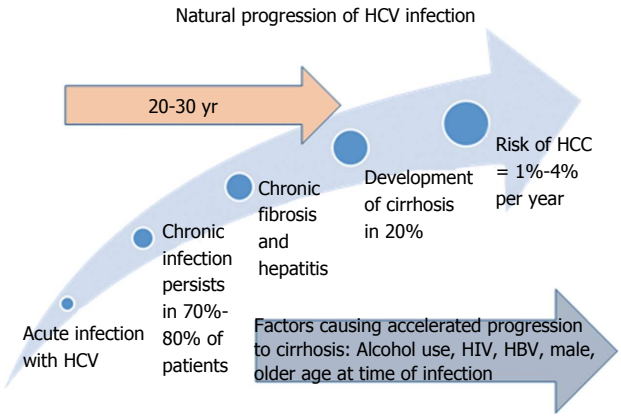

Figure 1 Natural progression of hepatitis C virus infection in the United States. HBV: Hepatitis B virus; HCV: Hepatitis C virus; HCC: Hepatocellular carcinoma; HIV: Human immunodeficiency virus.

testing for anti-HCV antibody. Currently in the United States, HCV testing is recommended at least once for persons born between 1945 and 1965[6]. A positive test result for anti-HCV antibody indicates either current infection - acute or chronic, previous infection that may have resolved, or a false positive test result[16]. For individuals with a positive test result, confirmatory test (HCV RNA) to confirm viremia should be performed. In certain individuals who are negative for anti-HCV antibody, however are either immunocompromised or who might have been exposed to HCV within the last 6 mo further testing with HCV RNA test is recommended. A negative test to HCV RNA indicates that patient has no evidence of current HCV infection and further HCV testing is unnecessary. Quantitative HCV RNA testing is also recommended prior to the initiation of antiviral therapy to document the level of baseline viral load[17]. Table 1 highlights current CDC recommendations on testing of the general population based on risk and non-risk factors for HCV infection in the United States. People with risk factors of exposure to HCV should be periodically tested, although the evidence regarding the frequency of testing is lacking. Hence, physicians should determine the periodicity of testing depending upon the risk of re-infection and risk factors.

Interventions at the time of diagnosis are aimed at reducing the progression to liver cirrhosis as well as educating the patient to prevent the transmission to others. Multiple studies have documented the detrimental effects of alcohol on the liver and the association between alcohol intake and development of liver fibrosis and cirrhosis, including the development of HCC[13,18,19]. HBV and HIV co-infections have been associated with an accelerated fibrosis in patients with chronic HCV infection[13] and testing patients for both HIV and HBV infection may be beneficial. Obesity and metabolic syndrome have also been associated with development of non-alcoholic fatty liver disease and there is some evidence that obesity may be associated with rapid disease progression to cirrhosis[20]. Therefore, weight loss should be advised to any persons infected with chronic HCV infection due to its beneficial potential.

Table 1 Centers of disease control recommendations on hepatitis C virus infection screening in the general population[17]

Birth between 1945-1965 without identifiable risk factors
History of illegal drug use
Receipient for clotting factors before 1987
Receipients for blood transfusion or solid organ transplantation before 1992
Received hemodialysis
Health-care workers after needle sticks
All HIV-positive individuals
Signs and symptoms of liver disease
Children born to HCV positive mothers
Elevated liver function tests

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

Once a person is diagnosed with chronic HCV infection, the decision on when to start treatment is controversial, however, generally depends on the level of fibrosis and staging. Liver biopsy is the "gold standard" for the evaluation of the level of fibrosis and can be a key factor in determining follow-up evaluation in patients. Although multiple scoring systems exist for the evaluation of the stage of liver fibrosis (Table 2)[21], a general recommendation is to initiative treatment in those with stage $\geqslant$ 3 as this stage in an important predictor of future progression to cirrhosis[22]. However, a liver biopsy carries potential risk such as excessive bleeding and injury to the liver and less invasive methods can also be utilized for determination of inflammation and fibrosis. Many clinicians use the Aspartate aminotransferase-to-platelet ratio index to determine the degree of fibrosis and studies have validated this index to be sensitive in detecting minimal fibrosis or cirrhosis in patients with HCV infection[23]. Liver elastography is also increasingly being used to determine liver stiffness; however, can only reliably distinguish cirrhosis from non-cirrhosis at this time[24]. The decision to pursue a liver biopsy over currently available non-invasive tests should be based on both the clinician and patient's wish to gain useful information regarding fibrosis stage for prognostic purposes as well as to determine the urgency for treatment[21].

NEW PERSPECTIVE ON TREATMENTS FOR PATIENTS WITH HCV INFECTION AND CIRRHOSIS

The goal of treatment for HCV infection early in the disease process is to reduce all-cause mortality and prevent development of liver complications. Immediate benefits of treatment include decrease in liver inflammation as reflected by improvement in aminotransferase levels and reduction in the rate of liver fibrosis[25]. Long-term benefits include a more than 70% reduction in the risk of HCC[26] and a 90% reduction in the risk of liver related mortality and need for liver transplantation[26,27].

Table 2 Various scoring system for the histological staging for liver fibrosis

Stage	IASL score	Bats-Ludwig score	Metavir	Ishak score
0	No fibrosis	No fibrosis	No fibrosis	No fibrosis
1	Mild fibrosis	Fibrous portal expansion	Presence of periportal fibrotic expansion	Fibrous expansion of some portal areas with or without short fibrous septae
2	Moderate fibrosis	Rare bridges or septae	Periportal septae 1 (septum)	Fibrous expansion of most portal areas with or without short fibrous septae
3	Severe fibrosis	Numerous bridges or septae	Porto-central septae	Fibrous expansion of most portal areas with occasional portal to portal bridging
4	Cirrhosis	Cirrhosis	Cirrhosis	Fibrous expansion of most portal areas with marked bridging (portal to portal and portal to central)
5				Marked bridging (portal to portal and portal to central) with occasional nodules (incomplete cirrhosis)
6				Cirrhosis

Adapted from Ghany *et al*[21].

Achievement of virologic cure is determined by achieving undetectable HCV RNA levels defined as sustained virologic response (SVR) at 12 wk or more following treatment completion[21,22]. SVR has been shown in multiple studies to be a good marker for cure of chronic HCV infection in patients followed for greater than five years[28] and corresponds with presence of anti-HCV antibodies but without detectable HCV RNA in the serum, in liver tissue and mononuclear cells[29]. SVR at 12 wk (SVR12) has generally been accepted as primary efficacy end-point and a marker for "virologic cure"[22]. Although previously SVR at 24 wk (SVR 24) was used as a marker for "virologic cure", multiple new studies show high concordance rate between SVR24 and SVR12 hence allowing for its use in multiple studies for effectiveness of treatment[30].

Multiple studies have evaluated SVR rates in patients with and without cirrhosis, and all studies have concluded that patients with cirrhosis have lower SVR rates. Previous studies have provided ranges of SVR between 40%-50% in patients with Child-Pugh (CP) class A and 7%-26% in patients with CP class C[31-33]. Additionally, genotype also shown to have an influence on the treatment of patients with HCV cirrhosis with patients with genotype 1 and 4 having suboptimal SVR rates compared with those with genotype 2 and 3. A study by Bruno *et al*[34] showed that in patient treated with pegylated interferon alfa-2a (peg IFN) plus ribavirin those with genotype 1 and 4 had SVR rates of 51% if they had advanced fibrosis and 33% if they had cirrhosis. Same study also showed that patients with genotype 2 and 3 had SVR rates of 61% in those with advanced fibrosis and 57% if they had cirrhosis. These studies hence show us that patients without advanced fibrosis are more likely to have an earlier response to treatment and higher rates of SVR and if affordable treatments are available, should undergo treatment prior to development of fibrosis and cirrhosis[34].

NEW TREATMENTS FOR HCV INFECTION IN CIRRHOTIC PATIENTS

The treatment of HCV infection has evolved over the past decades and many more changes are anticipated in the treatment of patients in the coming years. The focus of this paper is to discuss treatment regimens based on recent clinical trials that have included patients with cirrhosis and discuss their success rates in achieving SVR. Although many changes are anticipated in the coming months, currently the American Association for the Study of Liver Disease (AASLD) guidelines for the treatment of cirrhotic patients recommend that treatment-naïve patients with compensated cirrhosis, including those with HCC, may be treated with the same regimen as patients without cirrhosis[22]. Tables 3 and 4 provide AASLD recommendations for treatment based on genotype and peg IFN eligibility. For patients who are decompensated (moderate to severe hepatic impairment or CP-B or CP-C) who may or may not be candidates for liver transplantation including HCC, AASLD recommends referral to an experienced treatment center ideally with liver transplantation capabilities. In this paper, we present the current treatment regimens and trials which have included patients with compensated cirrhosis and provide information for physicians who may be interested in learning further or pursing treatment for chronic HCV infection in patients with cirrhosis.

SOFOSBUVIR BASED TRIALS

Sofosbuvir (SOF) is a nucleotide analogue HCV non-structural protein (NS)5B polymerase inhibitor which has shown to have *in-vitro* activity against all HCV genotypes[35]. When incorporated as a substrate for viral RNA polymerase in the HCV-RNA genome, SOF leads to inhibition of viral replication. Studies have also shown pan-genotype antiviral activity against HCV and a high barrier to resistance. SOF is administered once daily *via* oral tablets (400 mg) with no restrictions on food intake. It enters the hepatic circulation as a pro-drug and undergoes phosphorylation to its active form in hepatocytes. While studies have shown that variables such as age, sex, body mass index (BMI), race, common concomitant medications and cirrhosis have less influence on the metabolism of the drug, it is cleared by the renal system and dose adjustment may

Table 3 The recommended for treatment of hepatitis C virus infection by genotype in treatment-naïve patients and in treatment naïve patients with compensated cirrhosis[22]

Genotype	Recommended regimen and duration	Recommended regimen for compensated cirrhosis (CP-A) and duration
1a	Three options with similar efficacy: (1) Daily fixed dose LDP (90 mg)/SOF (400 mg) for 12 wk (2) Daily fixed-dose combination of paritaprevir (150 mg)/ritonavir (100 mg)/ombitasvir (25 mg) plus twice-daily dosed dasabuvir (250 mg) and weight-based RBV [1000 mg (< 75 kg) to 1200 mg (≥ 75 kg)] for 12 wk (3) Daily SOF (400 mg) plus SMV (150 mg) with or without weight-based RBV [1000 mg (< 75 kg) to 1200 mg (≥ 75 kg)] for 12 wk	Three options with similar efficacy: (1) Daily fixed dose LDP (90 mg)/SOF (400 mg) for 12 wk (2) Daily fixed-dose combination of paritaprevir (150 mg)/ritonavir (100 mg)/ombitasvir (25 mg) plus twice-daily dosed dasabuvir (250 mg) and weight-based RBV [1000 mg (< 75 kg) to 1200 mg (≥ 75 kg)] for 12 wk (3) Daily SOF (400 mg) plus sMV (150 mg) with or without weight-based RBV [1000 mg (< 75 kg) to 1200 mg (≥ 75 kg)] for 24 wk
1b	Three options with similar efficacy: (1) Daily fixed dose LDP (90 mg)/SOF (400 mg) for 12 wk (2) Daily fixed-dose combination of paritaprevir (150 mg)/ritonavir (100 mg)/ombitasvir (25 mg) plus twice-daily dosed dasabuvir (250 mg) for 12 wk (3) Daily SOF (400 mg) plus SMV (150 mg) with or without weight-based RBV [1000 mg (< 75 kg) to 1200 mg (≥ 75 kg)] for12 wk	Three options with similar efficacy: (1) Daily fixed dose LDP (90 mg)/SOF (400 mg) for 12 wk (2) Daily fixed-dose combination of paritaprevir (150 mg)/ritonavir (100 mg)/ombitasvir (25 mg) plus twice-daily dosed dasabuvir (250 mg) and weight-based RBV [1000 mg (< 75 kg) to 1200 mg (≥ 75 kg)] for 12 wk (3) Daily SOF (400 mg) plus SMV (150 mg) with or without weight-based RBV [1000 mg (< 75 kg) to 1200 mg (≥ 75 kg)] for 24 wk
2	SOF (400 mg) and weight-based RBV [1000 mg (< 75 kg) to 1200 mg (≥ 75 kg)] for 12 wk	SOF (400 mg) and weight-based RBV [1000 mg (< 75 kg) to 1200 mg (≥ 75 kg)] for 16 wk
3	(1) SOF (400 mg) and weight-based RBV [1000 mg (< 75 kg) to 1200 mg (≥ 75 kg)] for 24 wk (2) Alternative for IFN eligible: SOF (400 mg) and weight-based RBV [1000 mg (< 75 kg) to 1200 mg (≥ 75 kg)] plus weekly peg IFN for 12 wk	
4	Three options with similar efficacy and 2 alternatives available: (1) Daily fixed dose LDP (90 mg)/SOF (400 mg) for 12 wk (2) Daily fixed-dose combination of paritaprevir (150 mg)/ritonavir (100 mg)/ombitasvir (25 mg) and weight-based RBV [1000 mg (< 75 kg) to 1200 mg (≥ 75 kg)] for 12 wk (3) Daily SOF (400 mg) and weight-based RBV [1000 mg (< 75 kg) to 1200 mg (≥ 75 kg)] for 24 wk (4) Alternative 1 for IFN eligible: Daily SOF (400 mg) and weight-based RBV [1000 mg (< 75 kg) to 1200 mg (≥ 75 kg)] plus weekly peg IFN for 12 wk (5) Alternative 2 for IFN eligible: Daily SOF (400 mg) plus SMV (150 mg) and weight-based RBV [1000 mg (< 75 kg) to 1200 mg (≥ 75 kg)] for 12 wk	
5	(1) Daily SOF (400 mg) and weight-based RBV [1000 mg (< 75 kg) to 1200 mg (≥ 75 kg)] plus weekly peg IFN for 12 wk (2) Alternative 1 for IFN eligible: Weight-based RBV [1000 mg (< 75 kg) to 1200 mg (≥ 75 kg)] plus weekly peg IFN for 48 wk	
6	(1) Daily fixed dose LDP (90 mg)/SOF (400 mg) for 12 wk (2) Alternative 1 for IFN eligible: Daily SOF (400 mg) and weight-based RBV [1000 mg (< 75 kg) to 1200 mg (≥ 75 kg)] plus weekly peg IFN for 12 wk	

LDP: Ledipasvir; SOF: Sofosbuvir; SMV: Simeprevir; Peg IFN: Pegylated interferon alfa-2a; RBV: Ribavirin; CP-A: Child-Pugh class A.

Table 4 Factors that determine ineligibility to interferon based regimens for treatment[22]

Intolerance to IFN in the past
Autoimmune hepatitis or other autoimmune disorders
Hypersensitivity to PEG or any of its components
Decompensated hepatic disease
Major uncontrolled depression
A baseline neutrophil count below 1500/μL, a baseline platelet count below 90000/μL or baseline hemoglobin below 10 g/dL
A history of pre-existing heart disease

IFN: Interferon; PEG: Percutaneous endoscopic gastrostomy.

be needed in patients with creatinine clearance less than 30 mL/min. Studies have also shown that despite being metabolized in the hepatocytes, no dose adjustment is recommended in patients with mild or severe hepatic impairment. The following studies evaluated the use of SOF in cirrhotic patients (summarized in Table 5).

NEUTRINO trial[36]

The NEUTRINO trial was a phase Ⅲ single-group, open label study of SOF with peg IFN plus ribavirin in 327 treatment naïve patients infected with HCV genotype 1, 4, 5 and 6. All patients received a 12-wk treatment with SOF plus peg IFN plus ribavirin. SOF was administered once daily at a dose of 400 mg orally, with daily weight-based ribavirin (1000 mg if body weight < 75 kg

Table 5 Summary of sofosbuvir trials and enrollment of cirrhotic patients

Trial	Regimen	Duration (wk)	Patient population (patients with cirrhosis in treatment group)	SVR and additional findings	SVR for cirrhotic patients
NEUTRINO[36]	SOF + peg IFN + RBV	12	327 treatment naïve (54) with G1, 4-6	90% overall	80%
			G1: 292	89%	
			G4: 28	96%	
			G5-6: 7	100%	
FISSION[36]	SOF + RBV	12	253/499 treatment naïve with G2, G3 (49 cirrhotic) assigned to treatment arm	67%	47%
			G2: 70/253	97%	91%
			G3: 183/253	56%	34%
POSITRON[37]	SOF + RBV	12	207/278 IFN intolerant or ineligible with G2, G3 (31 cirrhotic) assigned to treatment group	78%	61%
			G2: 109	93%	94%
FUSION[37]	SOF + RBV	12	G3: 98	61%	21%
			100 treatment experienced with G2, G3 (26)	50%	42%
			G2: 36	86%	60%
	SOF + RBV	16	G3: 64	30%	19%
			95 treatment experienced with G2, G3 (32)	73%	66%
			G2: 32	94%	78%
VALENCE[38]	SOF + RBV	12	G3: 63	62%	61%
			73 patients with G2 (10)	93%	90%
			Treatment naïve G2: 32	97%	100%
	SOF + RBV	24	Treatment experienced G2: 41	90%	88%
			250 patients with G3: (58)	85%	67%
			Treatment naïve G3: 105	93%	92%
			Treatment experienced G3: 145	79%	60%

SOF: Sofosbuvir; Peg IFN: Pegylated interferon alfa-2a; RBV: Ribavirin; G: Hepatitis C virus genotype; SVR: Sustained virologic response.

and 1200 mg if body weight ⩾ 75 kg) and peg IFN administered subcutaneously once weekly at dose of 180 μg. Of the 327 patients who underwent treatment, 89% had HCV genotype 1; 9% had genotype 4, and 2% had genotype 5 or 6. Black patients represented 17% of the patients, and 17% of the patients had cirrhosis. At the end of the study, 90% of the patients overall (295/327) achieved SVR. It should be noted however that the SVR was 92% for genotype 1a and 82% for genotype 1b). When comparing patients who were cirrhotic, SVR rates were lower (80% or 43/54 patients in cirrhotic cohort compared with 92% or 252/273 patients in non-cirrhotic cohort) (Table 5).

FISSION trial[36]

The FISSION trial was a phase Ⅲ randomized, open label active-control study of SOF plus ribavirin in 499 treatment naïve patients infected with HCV genotype 2 or 3. Patients were enrolled in an approximately 1:3 ratio and patients were further assigned in a 1:1 ratio to receive either 12 wk of SOF plus ribavirin or 24 wk of peg IFN plus ribavirin. HCV genotype 3 accounted for 72% of the patients and 20% of the patients in this study had cirrhosis. SOF was dosed at 400 mg daily while ribavirin was dosed daily based on weight (1000 mg if body weight < 75 kg and 1200 mg if body weight ⩾ 75 kg) in group receiving SOF plus ribavirin however, in patients receiving peg IFN plus ribavirin it was dosed at 800 mg in two divided doses as per product labeling. Peg IFN was administered subcutaneously once weekly at dose of 180 μg. There were 253 patients in the treatment group with SOF plus ribavirin while there were 243 patients in the peg IFN plus ribavirin group.

At the end of the study, SOF plus ribavirin was shown to be non-inferior to peg IFN plus ribavirin and both groups had overall similar SVR of 67%. However, significant differences were present between the two genotypes. Patients with genotype 2 achieved a 93% SVR while only 56% SVR was achieved in genotype 3 patients. Liver fibrosis was one of the strongest predictors of treatment failure in the multivariate analysis and showed that presence of cirrhosis was associated with an SVR of 34% in genotype 3 patients, while did not influence SVR rates in genotype 2 patients. This trial would suggest the patients with HCV genotype 3 with advanced liver fibrosis or cirrhosis would be the "difficult to treat" patient group despite advancements in treatment regimen (Table 5).

POSITRON trial[37]

The goal of the POSITRON trial was to evaluate for tolerability of the drug SOF. It was a blinded, placebo controlled trial which compared 12 wk of treatment with SOF plus ribavirin with matching placebo in patients who had previously discontinued IFN therapy due to adverse events or had a contraindication to IFN treatment. These patients had either HCV genotype 2 or genotype 3 infections. In this study, 207/278 patients were assigned to the treatment group, out of which 31 (15%) of the patients had evidence of cirrhosis. Findings of this trial showed that genotype 3 infection was associated with a lower SVR compared with those infected with HCV genotype 2. Presence of cirrhosis was associated with a lower SVR. Patients without cirrhosis achieved an SVR of

93% for HCV genotype 2 and 61% for HCV genotype 3 while patients with cirrhosis achieved an SVR of 94% for HCV genotype 2 and 21% for HCV genotype 3 infection. The trial also showed that the combination of SOF plus ribavirin was an optimal regimen with better tolerability. Most frequent adverse effects included fatigue (44%), nausea (22%), headache (21%), insomnia (19%) and pruritus (11%) with these symptoms likely from ribavirin than SOF. The drop in hemoglobin to < 10 g/dL occurred in only 7% of the patients and no reduction in platelets or neutrophil values were reported. Additionally, the discontinuation rate due to adverse effects was low at only 2%.

FUSION trial[37]

The FUSION trial evaluated the efficacy of SOF plus ribavirin in patients with mainly HCV genotype 2 and 3 who had failed prior treatment with Peg IFN plus ribavirin. Two hundred and one patients with HCV genotype 2 and 3 were included in the FUSION trial with 76% of patients having prior relapse. Treatment was continued for either 12 or 16 wk. Approximately 35% of the patients had compensated cirrhosis although the majority of them had HCV genotype 3 (62%). The results of the study showed an overall SVR in treatment experienced patients with SOF plus ribavirin to be significantly lower in the 12 wk (100 patients included in analysis with SVR of 50%) when compared with 16 wk arm (95 patients included in analysis with SVR of 73%). HCV genotype 2 patients had SVR of 86% with 12-wk treatment regimen and 94% for 16-wk treatment regimen, however HCV genotype 3 had SVR of only 30% with 12-wk and 62% with 16-wk regimen. Cirrhosis was associated with poor SVR rates with only 60% (12-wk regimen) and 78% (16-wk regimen) in patients with HCV genotype 2 and with 19% (12-wk regimen) and 61% (16-wk regimen) in patients with HCV genotype 3. Although the trial demonstrated efficacy in HCV genotype 2 treatment with a shorter and all oral regimen in patients with prior treatment failure, it identified both cirrhosis and HCV genotype 3 as a major predictors of SVR failures. The trials also showed that extension to a 16 wk regimen was associated with higher SVR and further studies may be needed to evaluate for a longer treatment regimen for treatment in HCV genotype 3 patients.

VALENCE trial[38]

The VALENCE trial was a multi-center phase 3 clinical trial with European patients with genotype 2 and 3 HCV infection who were randomly assigned in a 4:1 ratio to either receive SOF plus ribavirin or matching placebo. Randomization was stratified according to status with respect to prior therapy (defined either a previous therapy or no previous therapy), and the presence or absence of cirrhosis. Although initially planned to treat patients with only a 12-wk regimen of SOF plus ribavirin, results of the FUSION trial led to an amendment of the protocol to allow for extending treatment beyond 12 wk. The study protocol was amended to allow for study-group assignment such that they were unblended and the placebo group was removed and only patients with HCV genotype 3 were extended treatment to 24 wk. Patient with HCV genotype 3 who had finished 12 wk of treatment before the amendment were not candidates to receive additional duration of treatment. Subgroup analysis in this trial showed that among patients with HCV genotype 2, the response were consistently high across subgroups as seen in previous studies (SVR rates of 93% after 12 wk of treatment) (Table 5). Rates of SVR for HCV genotype 3 patients (identified as the "difficult-to-treat") however depended on treatment history, cirrhosis status and length of treatment. Patients with HCV genotype 3 who received 24 wk of treatment, 213/250 (85%) achieved SVR 12 after cessation of treatment. At 24 wk however 2 patients had virologic relapse while 4 were lost to follow-up and 1 patient had invalid HCV RNA result. Among patients who had not received prior treatment who were treated for 24 wk, the rates of SVR were 92% among those with cirrhosis and 93% among those without cirrhosis. However, if patient had received prior treatment, the rates SVR were 60% among those with cirrhosis compared with 79% among those without cirrhosis. The presence of cirrhosis had an overall lower SVR (67%) compared with non-cirrhotic patients who had higher SVR (85%).

SIMEPREVIR BASED TRIALS

Simeprevir (SMV) is an oral, reversible HCV NS3/4A protease inhibitor which has been shown to have *in-vivo* activity against all genotype except for HCV genotype 3[39]. Studies show that SMV is extensively metabolized in the liver and intestinal tract and has bioavailability of 44% after a single oral administration. It is a CYP3A4 substrate and hence its concentration is significantly affected based on drugs that are either inhibitors or inducers of the CYP3A4. Additionally, its efficacy is decreased in patients with certain mutations, most concerning *in-vivo* studies being the Q80K polymorphism at baseline in patients with genotype 1a who are now advised to seek alternative therapy. The following studies evaluated the use of SMV in cirrhotic patients.

QUEST trials

Two trials evaluated the use of SMV in phase Ⅲ clinical trials for genotype 1 infection. Both QUEST-1 and QUEST-2 were global phase Ⅲ, randomized, double blind, placebo controlled clinical trials which were designed to assess the safety, efficacy and tolerability of SMV with combination with peg IFN and ribavirin in treatment naïve patients with genotype 1 HCV infection with compensated liver disease.

In QUEST-1 trial[40], 394 patients with chronic HCV genotype 1 who were treatment naïve were stratified

Table 6 Sustained virologic response achieved in the COSMOS study[43]

Cohort	Regimen	Duration (wk)	SVR12
Cohort 1: Prior non-responder HCV patients with METAVIR scores (F0-F2)	SMV/SOF + RBV	24	79%
	SMV/SOF	24	93%
	SMV/SOF + RBV	12	96%
	SMV/SOF	12	93%
Cohort 2: Prior non-responder and treatment naïve HCV patients with METAVIR scores (F3-F4)	SMV/SOF + RBV	24	93%
	SMV/SOF	24	100%
	SMV/SOF + RBV	12	93%
	SMV/SOF	12	93%

SOF: Sofosbuvir; SMV: Simeprevir; RBV: Ribavirin; SVR12: Sustained virologic response at 12 wk; HCV: Hepatitis C virus.

by HCV subtype and interleukin-28B genotype and were randomly assigned in a 2:1 ratio to received SMV (150 mg orally once daily) with peg IFN plus ribavirin for 12 wk followed by peg IFN plus ribavirin for 12 or 36 wk (SMV group) or placebo orally plus peg IFN with ribavirin for 12 wk, followed by peg IFN plus ribavirin for 36 wk (placebo group). In this randomized double-blind multicenter trial undertaken in 13 countries, the treatment duration was 24 wk or 48 wk in the SMV group based on criteria for response. Treatment was stopped at week 24 if HCV RNA was less than 25 IU/mL (detectable or undetectable) at week 4 and undetectable at week 12, otherwise continued with peg IFN plus ribavirin until week 48. Both groups were followed up to 72 wk after the start of treatment. This study included 48 patients with cirrhosis (defined as METAVIR score of F4), in whom SVR12 was achieved in 58% (18/31) in the SMV group while only 29% (5/17) in the placebo group. For comparison, in the same trial, 82% (188/229) of the non-cirrhotic patients treated in the SMV group achieved SVR12 while 53% (60/113) of non-cirrhotic patients in the placebo group achieved SVR12. Similar treatment criteria was used in QUEST-2 trial[41], which included 32 patients with cirrhosis (METAVIR score F4) of which 17 were in the SMV group and 15 in the placebo group. In the SMV group, 11/17 patients (65%) achieved SVR12 compared with 6/15 (40%) in the placebo group. In comparison, 209/257 (81%) of non-cirrhotic patients treated in the SMV group achieved SVR12 while 67/134 (50%) of non-cirrhotic patients in the placebo group achieved SVR12.

The most common adverse events seen in patients receiving SMV in QUEST-1 were fatigue (42% *vs* 41% for placebo), itching (26% *vs* 16% for placebo), and headache (33% *vs* 39% for placebo). The most common adverse events seen in patients receiving SMV in QUEST-2 were fatigue (37% *vs* 42% for placebo), itch (25% *vs* 25% for placebo), headache (39% *vs* 37% for placebo), fever (31% *vs* 40% for placebo), and influenza-like illness (26% *vs* 26% for placebo). In QUEST-1, in both the SMV and placebo arms, 3% of patients discontinued treatment due to an adverse event. In QUEST-2, 2% of patients in the SMV arm and 1% of patients in the placebo arm discontinued treatment due to an adverse event[42].

SMV PLUS SOF: COSMOS TRIAL[43]

The COSMOS study evaluated the efficacy of combined SOF plus SMV in patients with HCV genotype 1 infection who had previously not responded to peg IFN and ribavirin or were treatment naïve. Patients in this study were assigned in a 2:2:1:1 ratio to receive 150 mg SMV and 400 mg SOF orally and once daily for 12 or 24 wk with ribavirin or without ribavirin in two cohorts - Cohort 1 (non-cirrhotic - METAVIR score F0-F2) and Cohort 2 (previous non-responders and treatment naïve patients with METAVIR scores F3-F4). Table 6 shows the results of the COSMOS study demonstrating SVR in patients in each cohort. The most common side effects in the pooled groups were fatigue [*n* = 52 (31%)], headache [*n* = 33 (20%)], and nausea [*n* = 26 (16%)]. This study also showed that the combination of SOF plus SMV achieved excellent SVR rates in all subgroups regardless of duration of therapy (12 or 24 wk) or co-administration of ribavirin in difficult to treat patients. Although, it should be noted that this study was not powered to show non-inferiority of ribavirin (RBV)-free regimens and hence benefit from RBV is not apparent from the results of the study.

SOF PLUS LEDIPASVIR ± RBV

Ledipasvir is a NS5A inhibitor with potent antiviral activity against HCV genotype 1a and 1b[44]. Inhibition of NS5A viral phosphoprotein leads to disruption in viral replication, assembly and secretion. Most drug interactions with ledipasvir involve drugs that are Pgp-inducers such as rifampin or St. John's wort. The following studies evaluated the use of ledipasvir in combination with SOF.

ION-1 trial[45]

The ION-1 study was a phase 3 open label study with previously untreated patients with HCV genotype 1 infection and randomly assigned patients in a 1:1:1:1 ratio to receive either 12- or 24-wk of SOF/ledipasvir (400/90 mg daily) with or without RBV. Up to 16% of patients had cirrhosis, 12% were black and 67% had HCV genotype 1a infection. Overall the rates of SVR12 were 99% in the group that received 12 wk of

Table 7 Summary of sofosbuvir and ledipasvir trials and enrollment of cirrhotic patients

Trial	Regimen	Patient population (% with cirrhosis)	Duration (wk)	SVR12
ION-1[45]	SOF + LDP	212 naïve (16%)	12	99%
	SOF + LDP + RBV	211 naïve (15%)	12	97%
	SOF + LDP	214 naïve (15%)	24	98%
	SOF + LDP + RBV	215 naïve (17%)	24	99%
ION-2[46]	SOF + LDP	109 treatment experienced (20%)	12	94%
	SOF + LDP + RBV	111 treatment experienced (20%)	12	96%
	SOF + LDP	109 treatment experienced (20%)	24	99%
	SOF + LDP + RBV	111 treatment experienced (20%)	24	99%
ION-3[47]	SOF + LDP	215 naïve (0%)	8	94%
	SOF + LDP + RBV	216 naïve (0%)	8	93%
	SOF + LDP	216 naïve (0%)	12	95%

SOF: Sofosbuvir; LDP: Ledipasvir; RBV: Ribavirin; SVR12: Sustained virologic response at 12 wk.

ledipasvir/SOF and 97% in the group that received 12 wk of ledipasvir-SOF with RBV. The SVR was 98% in the group that received 24 wk of ledipasvir-SOF and 99% in the group that received 24 wk of ledipasvir-SOF with RBV. Adverse reactions commonly included fatigue, headache, insomnia and nausea and were tolerable by most patients. Presence of cirrhosis was associated with slightly reduced SVR but rates were still 94%-100% within each treatment group. ION-1 trial has been summarized in Table 7.

ION -2 trial[46]

ION-2 study was a phase 3 randomized control trial which involved patients with HCV genotype 1 infection who had not achieved SVR after treatment with peg IFN and ribavirin with or without protease inhibitor. Similar to the ION-1, the study randomly assigned patients in a 1:1:1:1 ratio to receive either 12- or 24-wk of SOF/ledipasvir (400/90 mg daily) with or without RBV. In the study, 20% of the patients had cirrhosis and 79% were HCV genotype 1a. Overall rates of SVR were 94% in the group that received 12 wk of ledipasvir/SOF and increased to 96% in the group that received 12 wk of ledipasvir/SOF with RBV. SVR rates were 99% with 24 wk of ledipasvir/SOF and 99% in the group with 24 wk of ledipasvir/SOF with RBV. No patient in the study discontinued the drug due to adverse event. Among patients' with cirrhosis who were assigned to 12 wk of treatment, rates of SVR were 86% for those receiving ledipasvir/SOF and 82% with those receiving ledipasvir/SOF with RBV for 12 wk. For the patients in the 24 wk arm of treatment, the response rates were similar among cirrhotic and non-cirrhotic patients. However in patients with cirrhosis those who received 12 wk of treatment compared with those who received 24 wk of treatment, the difference in SVR was significant (P = 0.007). ION-2 trial has been summarized in Table 7.

ION -3 trial[47]

ION-3 study was a phase 3 open label trial that evaluated treatment of patients with HCV genotype 1 infection without cirrhosis who had not received any prior treatment. Although the study did not include any patients with cirrhosis, the aim of the study was to evaluate shorter duration of treatment with achievement of SVR. The study included 647 previously untreated patients who were randomized to receive ledipasvir/SOF for 8 wk, ledipasvir/SOF plus ribavirin for 8 wk or ledipasvir/SOF for 12 wk. The rates of SVR12 were 94% in ledipasvir/SOF for 8 wk group, 93% in ledipasvir/SOF plus ribavirin for 8 wk and 95% in ledipasvir/SOF for 12 wk. The trial confirmed that non-inferiority of the 8 wk regimen when compared with 12 wk of ledipasvir/SOF. ION-3 trial has been summarized in Table 7 and allow treatment regimens to be shortened to 8 wk in non-cirrhotic patients based on clinician's judgement and patient situation.

Based on these studies, the Food and Drug Administration (FDA) in the United States approved the first combination pill to treat HCV genotype 1 infection which is a blend of SOF and ledipasvir. It is also the first approved regimen that does not require administration with interferon or ribavirin for the treatment of HCV genotype 1 infection.

ABT-450/R (PARITAPREVIR/RITONAVIR)-OMBITASVIR AND DASABUVIR

ABT-450 (Paritaprevir) is an inhibitor of NS3/4A protease and is administered with ritonavir (ABT-450/r). Addition of ritonavir leads to inhibition of ABT-450 metabolism increasing drug levels and allowing for once daily dosing, however, ritonavir by itself does not have any activity against HCV. Ombitasvir on the other hand is a NS5A inhibitor and dasabuvir is a non-nucleoside inhibitor of the HCV NS5B RNA polymerase. Although trials have evaluated the efficacy of this regimen in HCV genotype 1 patients without cirrhosis (SAPPHIRE-Ⅰ[48], SAPPHIRE-Ⅱ[49], PEARL-Ⅲ and Ⅳ[50]), the trial that included cirrhotic patients was the TURQUOISE-Ⅱ[51] trial which evaluated treatment-naïve and treatment experienced patients with CP-A cirrhosis. The trial included 380 patients with CP-A cirrhosis and randomized them to either a 12 or 24 wk of treatment with ABT-450/r-Ombitasvir and Dasabuvir + RBV according to body weight. SVR12 rates were 91.8% (191/208) in the 12

wk group and 95.9% (165/172) in the 24-wk group. Based on this trial, the FDA has approved this drug regimen for patients with compensated cirrhosis as an alternative to other regimens.

As per most recent guidelines, the first line recommended treatment for patients with decompensated HCV genotype 1 and genotype 4 cirrhosis (defined as CP-B or C) who may or may not be candidates for liver transplantation, including those with HCC, includes a daily fixed-dose combination of ledipasvir/SOF and ribavirin for 12 wk. If the patient has anemia or is ribavirin intolerant, the recommended regimen is fixed combination of ledipasvir/SOF for 24 wk. For patients with HCV genotype 2 and 3 cirrhosis (defined as CP-B or C) who may or may not be candidates for liver transplantation, including those with HCC, the AASLD recommends daily SOF and weight-based ribavirin for up to 48 wk. Treatment of patients with decompensated cirrhosis is recommended only by highly experienced HCV practitioners, ideally in a center with liver transplantation capabilities. Table 2 includes the current recommendations by AASLD for treatment of non-cirrhotic and compensated cirrhotic patients with chronic HCV infection.

ADVANTAGES TO TREATMENT OF HCV INFECTION IN CIRRHOTIC PATIENTS

There are multiple advantages to treating HCV infection in the cirrhotic liver and in those with advanced fibrosis. Studies have shown that treatment of patients with CP-A and CP-B can result in slowing of disease progression, decrease all-cause mortality, prevent the formation of esophageal varices, decrease the risk of development of HCC as well as prevent the need for liver transplantation[27,52-55]. Although there are numerous studies on the benefit of treatment of patients with compensated HCV cirrhosis who achieve SVR, limited data is available for the treatment of patients' with decompensated cirrhosis. A study of seventy-five decompensated HCV cirrhosis patients treated with peg IFN and ribavirin demonstrated significant lower rates of decompensation events and hospitalizations[56], however, this regimen needs to be used with extreme caution given the high incidence of serious adverse effects including life-threatening infection, worsening hepatic decompensation and death[57]. With the new treatment regimens which are peg IFN free, it is important to note that many studies exclude patients with decompensated cirrhosis or have a limited number. Metabolism of the drugs is significantly different in those with cirrhosis and hence caution needs to be exercised when prescribing certain regimens. For example, SMV has not been studied in patients with decompensated cirrhosis (CP-B or CP-C) and it is unclear how hepatic impairment would affect its drug metabolism. On the other hand although limited data is available for treatment with SOF and ribavirin, it appears to be well-tolerated in patients with advanced liver disease[58].

HCV infection is the leading indication of liver transplantation in the United States and recurrence of the graft liver post-transplantation is nearly universal[59]. Studies show that the patients who undergo liver transplantation and have HCV-RNA viral titers $\geqslant 1 \times 10^6$ copies/mL had a five year survival of 57% *vs* 84% for patients with lower viral RNA titers ($P = 0.0001$)[59]. Additionally, studies indicate that pre-transplant treatment prevents post-transplant recurrence in selected patients and efficacy is higher with > 16 wk between treatment and transplantation[60]. A recent phase 2, open-label study evaluated if SOF and ribavirin treatment before liver transplantation can prevent recurrence post-transplantation. This study had 61 patients with chronic HCV infection with any genotype and cirrhosis who were on wait-list for liver transplantation for HCC and were treated with 48 wk of SOF and ribavirin prior to transplantation. Forty-six received liver transplantation and forty-three patients had HCV-RNA level of less than 25 IU/mL. Of these forty-three patients, 30 (70%) had a post-transplantation SVR at 12 wk, 10 (23%) had recurrent infection and 3 (7%) died from complications of transplantation. Recurrence was related inversely to the number of consecutive days of undetectable HCV RNA before transplantation and among 26 patients with undetectable HCV RNA for at least 30 d prior to transplantation, only one had recurrence post-transplant[61]. Hence treatment of patients with liver cirrhosis prior to transplantation should be considered especially given its advantage of prolonged graft survival, decreased mortality and need for re-transplantation[62].

DISADVANTAGES TO TREATMENT IN HCV CIRRHOSIS PATIENTS

The treatment of patients with HCV cirrhosis has shown to have lower SVR rates than in patients who are non-cirrhotic. Studies show that treatment with peg IFN plus ribavirin in patients with advanced fibrosis or cirrhosis leads to a significantly lower SVR when compared with patients with mild to moderate fibrosis[34]. Additionally, previous studies evaluating the use of triple therapy (peg IFN plus ribavirin with either boceprevir or telaprevir) in patients with cirrhosis showed not only a lower SVR but also a high incidence of significant adverse events including worsening of liver disease, severe infection and difficult to manage anemia[57]. Hence due to the risk of adverse effects, treatment of these patients requires significant oversight and should be considered only at experienced centers with transplantation capabilities leading to increasing cost and accessibility issues. Unfortunately, the treatment in some transplant centers is also controversial. There may be a tendency in some liver transplant centers to wait until transplantation and pursue treatment post-transplant. Additionally, having positive HCV infection in a cirrhotic liver may also provide access to HCV positive liver transplant options in such patients given the paucity of available organs.

CONCLUSION

With the availability of newer, shorter duration and simpler therapies with high SVR rates, HCV infection today has become a curable disease. Although the costs of treatment are still prohibitive for many patients, those with cirrhosis are likely to derive the most benefit from treatment. Earlier eradication of HCV viremia in those with cirrhosis can potentially reduce the need for liver transplantation, risk of development of HCC and reduce HCV associated morbidity and mortality both pre-and post-transplantation. Treatment in this patient population should be considered especially given the emergence of newer and safer therapies. Due to the rapid advances and new therapies being available, the Infectious Disease Society of America and AASLD have jointly developed a clinical guidance tool[17] that should be considered by clinicians as a reference tool for treatment of patients with HCV infection(www.hcvguidelines.org).

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MINIREVIEWS

Spectrum of biliary complications following live donor liver transplantation

Priya Simoes, Varun Kesar, Jawad Ahmad

Priya Simoes, Varun Kesar, Jawad Ahmad, Division of Liver Diseases, Icahn School of Medicine at Mount Sinai, New York, NY 10029, United States

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Correspondence to: Jawad Ahmad, MD, FRCP, Division of Liver Diseases, Icahn School of Medicine at Mount Sinai, 1428 Madison Ave, New York, NY 10029, United States. javbob@hotmail.com
Telephone: +1-212-2418035
Fax: +1-212-7317340

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Abstract

Liver transplantation is the optimal treatment for many patients with advanced liver disease, including decompensated cirrhosis, hepatocellular carcinoma and acute liver failure. Organ shortage is the main determinant of death on the waiting list and hence living donor liver transplantation (LDLT) assumes importance. Biliary complications are the most common post operative morbidity after LDLT and occur due to anatomical and technical reasons. They include biliary leaks, strictures and cast formation and occur in the recipient as well as the donor. The types of biliary complications after LDLT along with their etiology, presenting features, diagnosis and endoscopic and surgical management are discussed.

Key words: Liver transplantation; Biliary stricture; Bile leak

Core tip: Living donor liver transplantation (LDLT) is associated with increased risk of post transplant biliary complications in recipients and donors, namely bile leaks and biliary strictures. Large bile leaks present early after LDLT and are treated with endoscopic stenting. Ischemic injury to cholangiocytes is the main cause of stricture formation. These may present early or late and are managed with endoscopic dilation followed by stent placement. Occasionally, surgical repair may be required. Cast formation may complicate biliary strictures, requiring endocopic extraction and frequent replacement of stents with cleaning of biliary sludge and debris.

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INTRODUCTION

Liver transplantation (LT) is the optimal treatment for

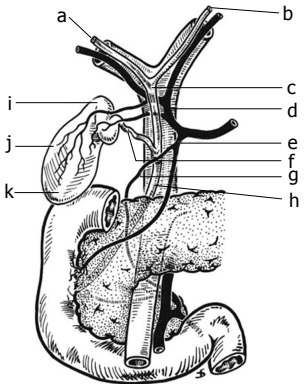
many patients with advanced liver disease, including decompensated cirrhosis, hepatocellular carcinoma and acute liver failure. The vast majority of LT involves the use of organs from deceased donors but despite strategies to increase the supply of deceased donors, organ shortage continues to be the main determinant of death on the waiting list[1]. Due to the lack of organs and also cultural and societal beliefs against the use of deceased donors, living donor LT (LDLT) with split liver grafts was developed in the late 1980s[2,3]. LDLT has potential benefits over deceased donor LT (DDLT) including lower overall costs with elective transplantation, better graft viability and reduced cold ischemia time, and theoretical immunological advantages suggested by the lower incidence of steroid resistant rejection[4-6]. Recipient survival is higher in LDLT but this has to be tempered against the risk of donor complications. Recipient morbidity in LDLT is primarily related to the risk of biliary complications which are twice as common as seen with DDLT[7]. Hospitalization rates and duration of hospital stay post LDLT are also significantly higher than after DDLT even in experienced centers and this is primarily attributed to the higher incidence of biliary complications[8].

The incidence of biliary complications after orthotopic liver transplantation varies between 11%-35%[7,9], with a decreasing trend in recent years. These include strictures, leaks, casts, sludge, stones and Sphincter of Oddi dysfunction of which strictures, bile leaks and cast formation are the commonest, affecting patient and graft survival as well as re transplantation rates. Biliary complications occur because of several anatomical and technical reasons and the management depends on a multi-disciplinary approach involving surgery, hepatology and radiology.

ANATOMICAL CONSIDERATIONS IN THE BILIARY TRACT

It is important to have an understanding of hepatic vascular anatomy as it explains the high incidence of biliary complications after LT. The liver parenchyma has a dual blood supply *via* the hepatic artery and portal vein, but the biliary system is only supplied arterially. The biliary epithelium is more liable to ischemic injury than hepatocytes. While bile ducts are relatively more tolerant than hepatocytes to anoxic injury, they are more susceptible to reoxygenation/reperfusion injury[10]. This in part explains the biliary complication rate as does the higher incidence of ischemic cholangiopathy in donation after cardiac death (DCD) organs compared to donation after brain death organs[11,12].

The biliary tree is divided into 3 segments: the hilar segment consisting of the right and left hepatic ducts, the supra-duodenal segment consisting of the common hepatic duct (CHD) and the upper common bile duct (CBD) and the retro-pancreatic segment consisting of the lower CBD. The supra-duodenal duct receives its blood



Anterior aspect of the biliary anatomy and of the head of the pancreas: (a) right hepatic duct; (b) left hepatic duct; (c) common hepatic duct; (d) hepatic artery; (e) gastroduodenal artery; (f) cystic duct; (g) retroduodenal artery; (h) common bile duct; (i) neck of the gallbladder; (j) body of the gallbladder; (k) fundus of the gallbladder. Note particulary the position of the hepatic bile duct confluence anterior to the right branch of the portal vein, the posterior course of the cystic artery behind the common hepatic duct, and the relationship of the neck of the gallbladder to the right branch of the hepatic artery. Note also the relationship of the major vessels (portal vein, superior mesenteric vein, and artery) to the head of the pancreas.

Figure 1 Arterial supply of the biliary tree[19] (reprinted with permission from Elsevier).

supply in the form of a plexus of many small arteries, mainly the 3 o'clock artery and the 9 o'clock artery running along the lateral borders of the duct arising from the retro-portal, retro-duodenal artery, gastro-duodenal artery, right branch of the hepatic artery, and/or cystic artery. Around 60% of the arterial supply runs superiorly, mainly from the gastro-duodenal artery, around 40% runs inferiorly from the common hepatic artery with a tiny fraction coming off the main trunk of the middle hepatic artery[13,14] (Figure 1).

The hilar and intrahepatic ducts are supplied by the peri-biliary vascular plexus, a network of capillaries arising from the terminal arterial branches of the right and left hepatic artery which also connects with the peri-ductal plexus supplying the supra-duodenal bile duct. A communicating arcade of blood vessels connecting the right and left arterial system of the liver is located within the hilar plate originating from the segment 4 artery and the right branch of the middle hepatic artery. This communicating arcade is spared during LDLT to provide adequate blood supply to the donor duct[15].

Most of the arterial supply of the middle portion of the CBD comes from the retro-duodenal and retro-portal arteries below, and less comes from the right hepatic artery above. During surgery, when these are dissected the middle part of the CBD is prone to ischemic injury. The nature of the arterial supply is the basis for why ischemia chiefly affects the middle third of the CBD, followed by the hepatic duct confluence, with intrahepatic involvement being the least common. Segment 4 and the central portion of the left hepatic duct are often supplied by the right arterial system which is generally transected while performing a right hepatectomy compromising the blood supply to the donor biliary system contributing to

Table 1 Clavien system for classification of complications in general surgery and solid organ transplantation

Grade 1	Any alteration from the ideal postoperative course, with complete recovery or which can be easily controlled and which fulfills the following general characteristics: (1) Not life threatening (2) Not requiring use of drugs other than immunosuppressants, analgesics, antipyretics, anti-inflammatory agents, antiemetic, drugs required for urinary retention or lower urinary tract infection, arterial hypertension, hyperlipidemia or transient hyperglycemia (3) Requiring only therapeutic procedures that can be performed at the bedside (4) Postoperative bleeding requiring ⩽ 3 units of blood transfusion (5) Never associated with a prolongation of ICU stay or total hospital stay to more than twice the median stay for the procedure in the population of the study
Grade 2	Any complication that is potentially life threatening or results in ICU stay > 5 d, hospital stay > 4 wk for the recipient, but which does not result in residual disability or persistent disease
Grade 3	Any complication with residual or lasting functional disability or development of malignant disease
Grade 4	Complications that lead to re transplantation (grade 4a) or death (grade 4b)

ICU: Intensive care unit.

Table 2 Biliary complications in recipients after live donor liver transplantation

Ref.	Year	Country	Grafts (*n*)		Biliary complications (%)		
			Right	Left	Leaks	Strictures	Overall rate
Ghobrial *et al*[22]	2001	United States	20		25	-	-
Gondolesi *et al*[23]	2004	United States	96	0	21.9	22.9	40.6
Liu *et al*[24]	2004	China	41	0	7.3	24.3	24.3
Giacomoni *et al*[25]	2006	Italy	23	0	21.7	21.7	34.8
Soejima *et al*[26]	2006	Japan	50	132	11.5	25.3	36.8
Shah *et al*[27]	2007	Canada	128	0	14.8	17.1	26.0
Mita *et al*[28]	2008	Japan	5	226	-	9.5	-
Freise *et al*[7]	2008	United States (A2ALL)	384	0	27.2	18	35.5
Marubashi *et al*[29]	2009	Japan	57	26	1.2	7.2	8.4
Lin *et al*[30]	2009	China	-	-	-	-	8.9
Wadhawan *et al*[31]	2010	India	338	0	8.8	10.3	19
Kim *et al*[32]	2010	South Korea	22	0	0	9.1	9.1
Soin *et al*[14]	2010	India	218	26	2	3.7	5

donor morbidity[11,13,14].

Generally the stump of the donor bile duct is divided away from the confluence of bile ducts to avoid a stricture of the bile duct remaining in the donor liver resulting in a higher incidence of multiple ducts in the right liver graft[14,16]. Two or more ductal anastomoses has been shown to be a risk factor for developing biliary complications[17,18]. However, studies published[19] since 2008 have shown a considerable drop in overall incidence of biliary complications in recipients owing to more experience and better technique of the biliary anastomosis.

Biliary complications occurring after LDLT are classified according to the Clavien system described below[20,21] (Table 1).

RECIPIENT BILIARY COMPLICATIONS

The incidence of biliary complications after LDLT is very variable but can be divided into two main categories: bile leaks and strictures of the biliary tree (Table 2). The type of graft used in LDLT affects the complication rate, depending on whether the right or the left lobe is used. To try and ensure adequate graft function and prevent small for size syndrome, the graft size required is dependent on the weight of the recipient (typically at least 0.8%-1% of the recipient weight). Hence, in adult to adult liver transplantation the larger right lobe is almost always used. This typically increases the complication rate but the management strategies remain similar.

Bile leaks

Bile leaks are a common biliary complication after LDLT compared to DDLT. In the United States, the multicenter A2ALL study reported two thirds of biliary complications after LDLT were due to bile leaks compared to less than a third after DDLT. Studies have reported a 6%-27% overall incidence of bile leaks after LDLT[31,33-35]. Most of these bile leaks were Clavien grade 2 or 3 complications resulting in prolonged hospital stay or permanent disability while a few resulted in graft failure, re transplantation and occasionally death of the recipient, though grade 4 complications in LDLT were less common than in DDLT. Anastomoses involving three or more donor bile ducts were associated with an increased risk while hepatitis C virus cirrhosis as the indication for LT and greater surgical expertise were associated with a lower risk for developing bile leaks[7].

There are two main types of bile leak after LDLT-anastomotic leaks, and cut surface leaks[36]. Anastomotic leaks are the more common type and occur more frequently with Roux-en-Y anastomoses than with duct

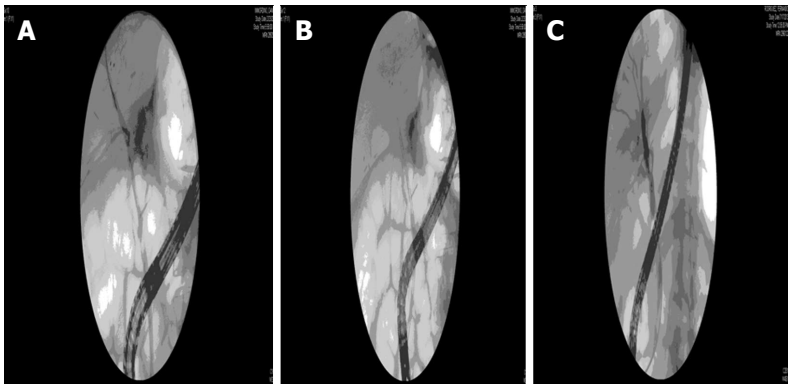


Figure 2 Endoscopic retrograde cholangiograms from a patient with an anastomotic leak after live donor liver transplantation. A: Cholangiogram demonstrating leak (extravasation of contrast) coming off the anastomosis after right lobe live donor liver transplant; B: Cholangiogram with plastic stent deployed across the anastomosis to heal the leak; C: Cholangiogram showing resolution of the leak several months later.

to duct anastomoses[23]. Cut surface bile leaks usually originate from small bile ducts in the caudate lobe that are transected during surgery and are usually detected incidentally at reoperation[37].

Bile leaks have been shown to decrease overall survival and graft survival post transplant[23,38] and are also a significant risk for subsequent development of a stricture[23,27,31,38].

Bile leaks usually present within 2 mo after LDLT, with most presenting 2-14 d post transplant[7,23,39]. They may present as bilious ascites, biloma or persistent post-operative bile drainage or can be incidentally discovered during computed tomography or endoscopic retrograde cholangiopancreatography (ERCP) performed for other reasons[27].

Treatment is often not required for small leaks as they usually heal spontaneously. Larger leaks can be managed with endoscopic treatment by transpapillary stenting (with or without sphincterotomy) which typically seals the leak. Endoscopic methods have shown excellent success in the management of bile leaks, with a reported resolution rate of 82%-92%[31,34]. Percutaneous drainage and stenting by endoscopic retrograde cholangiography (ERC) may be performed simultaneously if there is a significant collection. If copious biliary drainage persists or if there is simultaneous stricture development, surgical reconstruction or conversion of the anastomoses may be performed[27,34]. Most bile leaks in recipients of LDLT resolve within 3 mo after presentation, with a median time to resolution of 1 mo[38]. Figure 2 demonstrates a leak at the anastomosis in a right lobe recipient which was successfully treated with stent placement.

Biliary strictures

Biliary strictures are also common after LDLT. The A2ALL study reported the incidence of biliary strictures to be 18%-21%[7] with other studies reporting an incidence of 13%[31]. Most biliary strictures described in these studies were Clavien grade 2 or 3 complications. Biliary strictures are of 2 types - anastomotic strictures (AS) and non-AS (NAS).

Anastomotic strictures: Anastomotic strictures occur at the site of duct to duct anastomosis and are typically isolated and shorter in length.

The development of AS is associated with multiple operative factors such as biliary ischemia, cold ischemia time, type of anastomosis (duct to duct *vs* hepatico-jejunostomy), single *vs* double duct anastomosis, surgical expertise, prior bile leak and donor factors such as age, gender, weight, blood type and liver steatosis. In DDLT, transplantation in the post MELD era and the use of DCD organs also appears to influence AS formation[18,31,40-44].

The incidence of AS is reported to be around 8%-31% after LDLT[23,24,34], with a cumulative incidence of 6.6%, 10.6% and 12.3% after 1, 5 and 10 years respectively after DDLT[41].

Anastomotic strictures may present either early or late post-transplant. The median time to presentation reported varies between 2.5-9 mo post-transplant[23], with most presenting within 6 mo[18].

The most common presentation is an asymptomatic patient with elevated cholestatic liver enzymes. Abdominal pain, jaundice, fever, increased liver enzymes and recurrent cholangitis may also be presenting features and if present, warrant further investigation for an AS.

If an AS is suspected, liver ultrasound with Doppler imaging or computed tomography angiography to rule out hepatic artery thrombosis should be performed. Ultrasound alone has poor sensitivity for detecting a stricture and is generally followed by magnetic resonance cholangiopancreatography which is a non-invasive diagnostic test with 94.9% sensitivity and 88.9% specificity. The gold standard for diagnosing biliary strictures remains ERCP.

Serial endoscopic balloon dilatation with stenting is the main treatment for an AS. Balloon dilatation followed by plastic stent placement has shown better results than stenting alone. In general, stents are changed every few months, and if the stricture is adequately treated, they are removed between 3-12 mo[45]. Verdonk *et al*[41] showed that 75% of AS could be successfully stented by ERCP, with a median of 3 ERCP sessions for diagnosis and

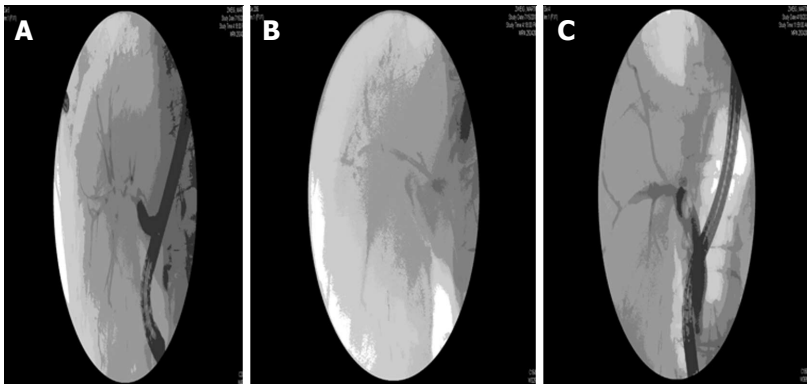


Figure 3 Endoscopic retrograde cholangiograms from a patient with an anastomotic stricture after live donor liver transplantation. A: Cholangiogram demonstrating complex anastomotic stricture after right lobe live donor liver transplant; B: Cholangiogram with plastic stent deployed across the stricture; C: Cholangiogram showing marked improvement in stricture after multiple dilation and stenting.

successful treatment of the stricture. They also showed a higher number of ERCP sessions and greater number of stents were required to treat strictures presenting after 6 mo compared with those presenting earlier. The success of endoscopic treatment varies between 53%-88% depending on center experience[23,31,34,46] and is preferred as the initial method of treatment. In cases where endoscopic management has failed, percutaneous trans hepatic biliary dilatation and stenting of strictures may be attempted, however the success rate of this method is lower and has a higher complication rate[23,41].

Surgical management may be attempted if both endoscopic and percutaneous treatment fails, especially if there are concomitant bile leaks. This may involve surgical repair or revision of the anastomosis from a duct to duct anastomosis to hepaticojejunostomy[47].

The most common complications of endoscopic, percutaneous and surgical methods of treating biliary strictures are recurrent cholangitis, post procedural bleeding, post ERCP pancreatitis, peritonitis and rarely death[45,48].

Figure 3 illustrates a typical anastomotic stricture which was treated with multiple dilations and stent placement.

Non anastomotic strictures: Non anastomotic strictures usually occur in the hilar region but may occur diffusely in the recipient biliary tract. They are thought to be related to ischemic and /or immune injury to the biliary mucosa during LT.

A number of operative factors such as total ischemia time, hepatic artery thrombosis, total operative time, type of bile duct anastomosis, and recipient factors such as pre transplant liver disease especially primary sclerosing cholangitis (PSC), bile salt composition and chronic ductopenic rejection as well as donor factors like ABO incompatibility, cytomegalovirus (CMV) infection, donor and recipient gender matching and miscellaneous factors like preservation techniques have all been variably associated with development of NAS[49-51].

Studies by Moench *et al*[52] and Buis *et al*[53] attempted to classify NAS into those caused by macro-angiopathy (hepatic artery thrombosis), micro-angiopathy (prolonged ischemia times and preservation injury) and immunological causes (ABO incompatibility, CMV infection, autoimmune hepatitis, or PSC and rejection).

In DDLT early NAS are found more often at the bifurcation of the CHD common hepatic duct or around the CBD common bile duct while late NAS are more often peripherally located within the liver[54].

Hepatic artery thrombosis and prolonged ischemia times both result in ischemic injury to the biliary endothelium, which heals by fibrosis and stricture formation. Previous studies have shown that the biliary epithelium is exquisitely sensitive to ischemia[10]. During LT, the blood supply to the bile ducts *via* the pancreatic head and gastro duodenal artery is interrupted, making the bile ducts solely dependent on the hepatic artery for perfusion, and thus more susceptible to ischemic injury. In LDLT cold ischemia time is short so interruption to hepatic artery flow is the main concern. Immunologically mediated injury of the biliary epithelium may be from direct cytokine mediated activation of inflammatory cells and thus more often affects the peripheral bile ducts.

The reported incidence of NAS varies between 9%-32%[49,55]. Guichelaar *et al*[49] found the mean duration to presentation varies between 23.6 ± 34.2 wk to after LDLT. Other studies have described the presentation being between 3.3-5.9 mo, with a median of 4.1 mo[55,56].

Non-anastomotic strictures present in the same way as AS with elevated cholestatic liver enzymes, abdominal pain, pruritus or cholangitis. Biliary ductal dilatation may also be seen incidentally on imaging. Studies have suggested a variation in the time to presentation with NAS secondary to ischemic causes presenting before 1 year and NAS secondary to immunological causes presenting after 1 year[55].

Initial evaluation may include liver United States with Doppler examination of the vasculature. However, this method has only 33%-66% sensitivity and may not be suitable for detecting biliary complications in liver transplant recipients. Magnetic resonance cholangiogram has good sensitivity and specificity and is the best initial non-invasive diagnostic test. Endoscopic

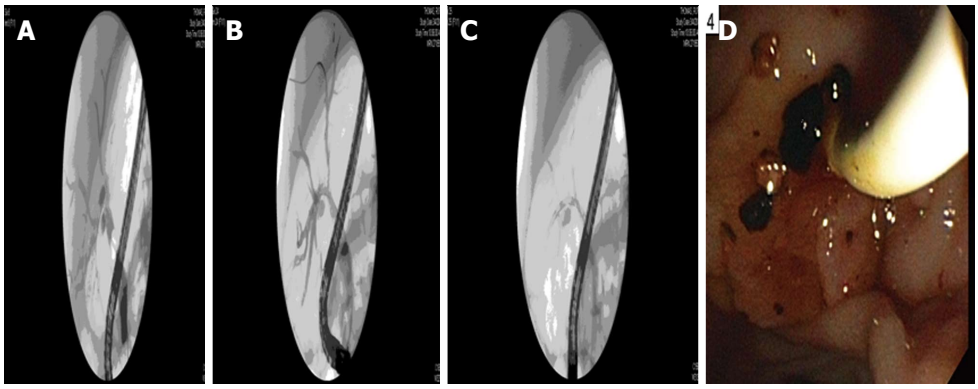


Figure 4 Endoscopic retrograde cholangiograms from a patient with a non-anastomotic stricture after live donor liver transplantation complicated by biliary cast formation (endoscopic image). A: Cholangiogram demonstrating non-anastomotic stricture after right lobe live donor liver transplant with irregular filling defects (casts) in a dilated segment (running at 8 o'clock in the image); B: Cholangiogram demonstrating clearance of the filling defects; C: Cholangiogram demonstrating two plastic stents deployed into the right anterior and right posterior systems after the casts were removed. Note how well the biliary tree has drained; D: Endoscopic image of the cast material being removed through the ampulla.

retrograde cholangiogram allows simultaneous diagnosis and intervention and is less invasive than percutaneous cholangiography[57]. Rarely, a liver biopsy may be required to differentiate rejection or CMV infection in a patient with elevated cholestatic enzymes and concern for biliary obstruction due to NAS. The ERCP findings in NAS include pre-stenotic dilatation and mucosal narrowing. Non-anastomotic strictures tend to be multiple and longer than AS and less amenable to endoscopic treatment.

Endoscopic management for NAS includes balloon dilatation of all accessible strictures with plastic stent placement and replacement every few months and cleaning out of biliary sludge and casts that may be present in the damaged duct. The success of endoscopic management of NAS in LDLT has been disappointing, with a success rate of 25%-30%[34,57-59] which is below that for NAS seen with DDLT or for AS in LDLT. Percutaneous interventions have a success rate of 40%-85%, but are more invasive and associated with hemorrhagic complications and bile leaks. Non-anastomotic strictures require regular surveillance. Long term outcomes of NAS include recurrent cholangitis, development of biliary cirrhosis and decreased graft survival. Endoscopic and percutaneous methods are often only a temporary solution and re-transplantation has to be considered[27,49,56].

Biliary complications after LDLT are less likely to respond to endoscopic therapy than in DDLT, so preventive strategies to avoid these are important[60]. In right lobe LDLT, high hilar dissection to create a short donor stump and a long recipient stump and ductoplasty to ensure adequate vascularization of the duct ends and intraoperative cholangiogram to early identify biliary leaks are being examined as strategies to reduce the incidence of both AS and NAS[27,47,61]. However, leaving too long a common hepatic duct remnant in the recipient also poses a risk, as some part of this may develop ischemia and later develop a stricture[62].

Other techniques like side to side duct anastomosis and use of interrupted *vs* uninterrupted biliary sutures have shown minimal benefit[63,64]. Generally, good perfusion of the biliary end and avoidance of vascular injury is the best way to prevent biliary complications[57,59]

Another potential complication of NAS is the formation of casts that deposit in the biliary tree, typically in the setting of ischemic injury. There are 2 main types, composed of either collagen from sloughed off necrotic biliary epithelium or precipitated bile with high bilirubin content. The second type are more frequently seen with biliary strictures[31,65,66] and lead to obstruction and an increased incidence of cholangitis.

Biliary casts develop in 4%-18% of LDLT recipients and are associated with an increased morbidity in the recipient[67-69]. Ischemic events, hepatic artery thrombosis and the presence of biliary strictures are all independently associated with the development of biliary casts[62]. Recurrent cholangitis, prolonged cold ischemia time and acute cellular rejection have also been hypothesized as risk factors.

Biliary cast syndrome presents within a year of transplant, usually within 16 wk, though some delayed cases have been described[67,70]. Elevated cholestatic liver enzymes or incidental bile duct dilatation with echogenic material filling the bile duct may be seen on ultrasound. However, ultrasound has low sensitivity and biliary casts can only reliably be detected by ERC or percutaneous transhepatic cholangiography (PTC), where they may appear as irregular filling defects within the biliary tree[65].

Endoscopic or percutaneous removal of casts with the use of basket or balloon devices, irrigation and hydraulic or mechanical lithotripsy is the typical management strategy. The success of endoscopic and percutaneous methods is reported at 25%-70%[67,70].

A complex NAS is shown in Figure 4 with biliary cast formation. Several ERCPs and dilation, cast extraction and stenting were required over several years with preservation of graft function.

DONOR COMPLICATIONS

Adult to adult LDLT also carries some risks to the

Table 3 Incidence of biliary complications in donors after live donor liver transplantation

Ref.	*n*	Graft type	Leak (%)	Stricture (%)	Overall rate (%)
Iida *et al*[76]	500	Right	10.6	1.6	12.2
	762	Left	4.7	0.3	4.9
El-Meteini *et al*[77]	207	Right	22	1.6	13.04
Taketomi *et al*[78]	69	Right	-	-	10.1
	137	Left	-	-	2.9
Lo *et al*[79]	561	Right	6.1	1.1	7.1
	939	Left			
Shio *et al*[75]	434	Right	9.9	2.1	11.1
	297	Left	1.7	1	2.4
Ghobrial *et al*[72]	393	Right	9	0.5	9.6
Ozgor *et al*[74]	500	Right	-	-	10.8
		Left			
European Liver Transplant Registry[80]	276	Right	5	3	8
		Left			

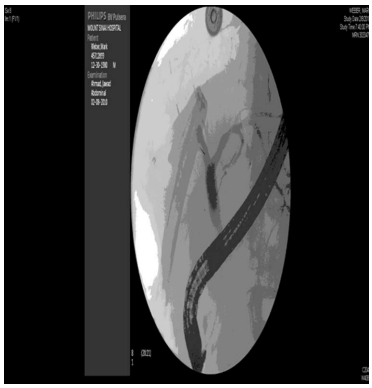


Figure 5 Endoscopic retrograde cholangiogram from a patient with a leak from the remnant right common hepatic duct a few days after right lobe live donor liver transplantation. The drain to the left can be seen filling when contrast is injected into the right common hepatic duct. This was managed successfully by a transpapillary stent.

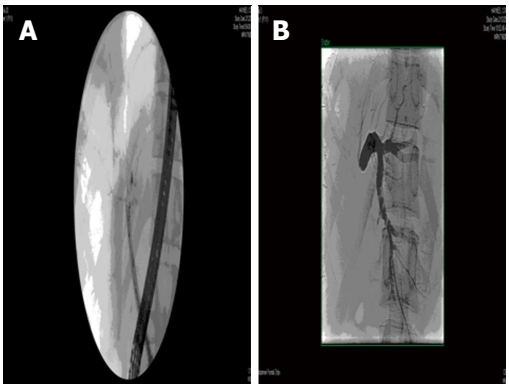


Figure 6 Stricture in donor after right lobe live donor liver transplantation. A: Endoscopic retrograde cholangiogram showing minimal filling of the left system a few weeks after right lobe live donor liver transplantation; B: Percutaneous transhepatic cholangiogram from the same patient in Figure 4A demonstrating a tight stricture at the take off the left common hepatic duct.

donor beyond the typical complications associated with abdominal surgery. Various studies (Table 3) have reported a 6%-18% incidence of donor biliary complications[39,71-73]. Most of these complications were classified as Clavien grade 3 or 4.

In contrast to the recipient, bile leaks and biliary fistulas are more common in the donor than strictures. The A2ALL study in the United States reported on almost 400 patients who donated the right lobe and found an incidence of 9% of bile leak or biloma, with a 0.5%-1.5% incidence of post-operative biliary strictures since no biliary anastomosis is required in the donor[72,74]. Due to larger graft size, complications are most after right lobe donation and least with left lateral grafts[74-76]. Factors associated with developing bile leaks include elevated pre-operative alkaline phosphatase levels to > 86 IU/L and requiring a blood transfusion during surgery but center experience was not a factor in donor biliary complications[72]. Figure 5 demonstrates a bile leak from the right common hepatic duct stump a few days after right lobe donation.

Donor biliary complications generally present within 2 wk of surgery. Bile leaks can be noted from bilious drain output or present with pain or suspicion for an intra-abdominal collection. Imaging can also be helpful. As in the recipient, strictures present with elevated cholestatic liver enzymes or jaundice.

Management of bile leaks and strictures is similar to the recipient with ERCP and stent placement the mainstay. Almost 80% of leaks were successfully treated by ERCP or percutaneous drainage, though a few required surgical revision or repair[75]. Strictures can be more difficult to manage after right lobe donation as they form as the liver regenerates and wire access to the remaining left lobe biliary tree can be very difficult either endoscopically or percutaneously. Surgical revision is then required. Figure 6 shows a stricture that developed at the takeoff of the left common hepatic duct a few weeks after right lobe donation which could not be treated at ERCP or PTC. The patient was asymptomatic but presented with rising cholestatic enzymes and was successfully treated with biliary bypass surgery.

CONCLUSION

The development of LDLT with split liver grafts has

allowed for elective liver transplants with shortened wait times. It offers several advantages over DDLT but carries an increased risk of biliary complications, mainly bile leaks and strictures. These present within a few weeks to months post transplant. They are usually managed endoscopically, with stenting for bile leaks and dilatation followed by stenting for strictures. Occasionally, endoscopic methods fail and surgical repair or even re transplantation may be required. Strategies to avoid vascular injury and ischemia of the biliary tree are important in preventing these complications.

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MINIREVIEWS

Usefulness of contrast enhanced ultrasound in monitoring therapeutic response after hepatocellular carcinoma treatment

Davide Roccarina, Matteo Garcovich, Maria Elena Ainora, Laura Riccardi, Maurizio Pompili, Antonio Gasbarrini, Maria Assunta Zocco

Davide Roccarina, Matteo Garcovich, Maria Elena Ainora, Laura Riccardi, Maurizio Pompili, Antonio Gasbarrini, Maria Assunta Zocco, Department of Internal Medicine, Catholic University of Sacred Heart, 00168 Rome, Italy

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Correspondence to: Dr. Davide Roccarina, Department of Internal Medicine, Catholic University of Sacred Heart, Largo A. Gemelli, 8, 00168 Rome, Italy. davideroccarina@gmail.com

Telephone: +39-06-30156018
Fax: +39-06-35502775

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Abstract

In the last years, the development in the oncology field has been huge and rapid. In particular, the evaluation of response to anti-tumour treatments has been being object of intense research, producing significant changes. Response assessment after therapy in solid neoplasias has always used radiological imaging techniques, with tumour size reduction representing a presumed therapeutic efficacy. However, with the introduction of anti-angiogenetic drugs the evaluation of tumour size has become unsuitable because some tumours, under treatment, show only tumour perfusion changes rather than lesion shrinkage. Between different imaging techniques with contrast-enhancement, contrast-enhanced ultrasound (CEUS) and, in particular, dynamic CEUS have arisen as a promising and non-invasive device for monitoring cancer treatments. Moreover, the introduction of perfusion software has even more refined the technique since it is able to provide quantitative parameters related to blood flow and blood volume that can be associated with tumour response and clinical outcome such as the progression free survival and the overall survival. Here, we give an overview of the current status of CEUS in monitoring hepatocellular carcinoma response to different kind of treatments.

Key words: Dynamic contrast-enhanced ultrasound; Hepatocellular carcinoma; Ablative treatment; Anti-angiogenetic drugs; Time-intensitive curve

Core tip: Hereby we present a literature revision about the current status of contrast enhanced ultrasound in monitoring hepatocellular carcinoma response to different kind of treatments. This is a very important topic because of the rapid development in the oncology field due to the introduction of novel anti-cancer therapies. Among different contrast enhanced imaging techniques, dynamic contrast-enhanced ultrasound has emerged as a versatile tool as standard radiological imaging has become unsatisfactory.

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INTRODUCTION

The potential applications of ultrasound (US) imaging in the oncology field are vast, ranging from early cancer detection and tumour characterisation to treatment response monitoring[1]. In the last years, the evaluation of response to anti-tumour treatments has been being object of intense investigations and changes, since a number of new anti-cancer agents are progressively becoming available[2-4]. In this setting a proper evaluation of tumour response is very important in the achievement of therapeutic decisions.

Until now the classical response assessment criteria in solid cancers were based on tumour size measurement by radiological imaging techniques and a reduction in tumour size during treatment was associated with therapeutic and clinical benefit. However, with the recent development of molecularly targeted therapies it has become necessary to introduce different methods to evaluate treatment efficacy. To achieve this goal, the traditional response criteria based on tumour size [Response Evaluation Criteria In Solid Tumours (RECIST)] were lately modified introducing new criteria that evaluate changes in tumour vascularisation[5].

Among different contrast-enhanced imaging techniques, contrast-enhanced US (CEUS) and dynamic CEUS (D-CEUS) have arisen as a promising, non-invasive and cost-effective device for monitoring cancer treatments. Moreover, the introduction of perfusion software has refined the technique even more since it is able to provide quantitative parameters related to blood flow and blood volume[5-8].

The present review focused on the current standards and perspectives of application of both CEUS and D-CEUS in the evaluation of treatment response in patients affected from hepatocellular carcinoma (HCC).

HCC AND CEUS

Liver cancer is the sixth most common cancer, the third cause of cancer related death, and accounts for 7% of all cancers. HCC represents more than 90% of primary liver cancer, is a major global health problem and its worldwide incidence is growing up[9].

Diagnosis of HCC can be done using histopathology or by identifying the typical vascular hallmark (hypervascular in the arterial phase with washout in the portal venous or delayed phases) using contrast-enhanced imaging techniques.

The treatment depends on the tumour stage at the moment of the diagnosis. Liver resection, liver transplantation and ablative procedures such as radiofrequency ablation (RFA) and percutaneous ethanol injection (PEI) are curative. Trans-catheter arterial chemo-embolisation (TACE) and systemic therapies such as anti-angiogenetic drugs and chemotherapies represent palliative treatments[10].

The advent of microbubble US contrast agents (UCA) has allowed the display of parenchyma microvasculature, impossible with B-mode and color-Doppler method[11]. The enhancement patterns of the tumours can be studied during arterial, portal venous, late and post-vascular phases, in real time and with a higher temporal resolution compared to other imaging modalities, allowing a deeper study of the lesion enhancement behavior. Moreover, the good safety profiles of UCA make possible to administer repeated boluses during the same exam, if necessary.

Recent European Federation of Societies for Ultrasound in Medicine and Biology (EFSUMB) guidelines highlighted the role of CEUS, as a cost-effective technique with a good safety profile, not only in the characterisation and detection of focal liver lesion but also in monitoring tumour response after curative, loco-regional and systemic HCC treatments[12,13].

CEUS AND TUMOUR RESPONSE

An accurate evaluation of treatment efficacy is fundamental both for phases Ⅱ and Ⅲ clinical trials and for clinician as a guide for therapeutic decisions.

When we evaluate the role of CEUS in monitoring tumour response it is important to distinguish between morphological and functional response.

In the first case vascular changes produced by the treatment are evaluated according modified-RECIST (mRECIST) by a qualitative or semi-quantitative CEUS. On the contrary, functional response can be assessed by D-CEUS that combines morphological and functional data leading to a more accurate measurement of tumour characteristics. The kinetics of microbubble flow through the tumour is evaluated by mathematical models applied

to signal intensity *vs* time able to provide quantitative parameters associated to blood flow and blood volume. This application has encouraging clinical potential for delineating changes in tumour vascularisation secondary to anti-angiogenetic treatment[5-8].

CEUS AND ABLATIVE TREATMENTS: RFA AND PEI

All ablative procedures cause the destruction of both the tumour itself and its vasculature by alterations in the target lesions such as coagulative necrosis, apoptosis and tissue granulation. Contrast-enhanced computed tomography (CE-CT) performed 4-6 wk after the treatment is currently considered the "gold standard" for the evaluation of tumour response.

Tumour necrosis is identified according to the absence of hyper-enhancement areas in the context of the treated lesion[10].

Due to the ability in representing HCC micro-vessels, CEUS has been utilised to evaluate intra-tumoral vascularisation after ablative treatments. Response is complete when the tumour treatment has determined a coagulative and vascular necrosis of the entire lesion and in this case no contrast enhancement is detected during all contrastographic phases of the dynamic study. On the contrary, when the therapy has failed zones of well-perfused residual tumour remain in the target lesion and focal contrast enhancement is detected in these areas. The residual unablated tumour appears like an irregular, eccentric or nodular peripheral enhancement[14-17]. Sometimes, especially in HCC treated with PEI, septa enhancement as well as a vessel passing through the nodule may be detected.

In large tumours, incomplete ablations might look as zones with contrast up-taking, which usually are localised nearby the periphery of the lesions. In these cases, an accurate comparison between the pre- and post-ablation images is necessary to achieve a correct evaluation of treatment efficacy[17].

Timing strategy

No unanimous strategy exists concerning the most appropriate timing schedule for the performance of CEUS. In fact, in the currently available studies on this topic CEUS has been performed at very heterogeneous time-points after the ablative treatments. In particular, tumour response can be evaluated in the immediate post-treatment, after 1 d, 1 mo or later during the follow-up[18].

Immediate post-treatment assessment

The possibility to detect the residual enhancing tumour immediately after ablation by means of CEUS could be a tempting approach in the interventional setting as it may lead to a prompt retreatment in the same session[19]. In fact, when CEUS is carried out within 60 min after PEI or RFA, there is a fair agreement with standard radiological imaging performed 2 or 4 wk later. However, despite its high specificity (94%), CEUS is characterised by only 40% of sensitivity in the detection of viable remnant tumour, due to false negative results. This high number of false negative cases could be related to the difficult interpretation of the images obtained immediately after the procedure and, especially, to the presence of a thin marginal area of hyper-vascularity in the arterial phase not followed by a proper washout in the portal/venous phase[15]. More specifically, the differentiation between the hyper-vascularity produced by a localised tissue response (hyperemia) or arteriovenous shunting and the residual hypervascular tumour in the periphery of the ablated area may be challenging. Reactive hyperemia usually shows a diffuse and homogeneous peripheral enhancement, with uniform and ring-like thickness, no more than 4-5 mm thick, followed by iso-enhancement in the portal and late phase. In contrast, residual tumour shows a local, heterogeneous or irregular peripheral enhancement, a thickness greater than 7-8 mm and the pattern of enhancement is characterised by hyper-enhancement in the arterial phase, followed by hypo-enhancement in the portal and late phase. However, in some cases viable tumour could be associated with arterial enhancement without complete washout in the portal and late phase, usually likewise to the enhancement pattern of the tumour before treatment[20,21].

Other reasons justifying the high number of false negative results could be a scan plane not including the residual tumour, uncooperative patients under conscious sedation or general anesthesia and, finally, an incorrect scanning time. In fact, intra-lesional gas developing during RFA or PEI may hinder a proper evaluation in the immediate post-procedural follow-up. These artefacts may persist for 15-180 min[22], but a delay of at least 20 to 40 min after the procedure would help to adjust visibility minimizing gas development.

Based on the results of different studies the positive predictive value (PPV) and negative predictive value of immediate post-procedural CEUS in detecting viable tumour tissue are around 82% and 50%, respectively[15].

Accordingly, the only significant role of CEUS performed within 60 min after treatment is to detect viable tumour during the same ablative session and allow an immediate retreatment, thereby lowering the rate of unsuccessful treatment, improving the cost-effectiveness ratio and optimising patient care[23].

24 h follow-up

Some authors suggest that CEUS should be performed at least 24 h after RFA or PEI. However, this strategy seems to be less attractive than the immediate post-treatment assessment, not permitting an ablation refinement in the same session if required. Moreover, as immediate post-procedural CEUS may not be available in all clinical settings, a delayed CEUS should overcome some of the aforementioned technical issues of intra-operative CEUS.

Good concordance between CEUS and CE-CT performed at the same time point has been reported[24,25]. However, a recent study shows that 1 d after the procedure gas bubbles could be displayed within half of the tumour, with a reported sensitivity in detecting residual viable tumour only equal to 27%. In addition, one patient with suspected residual disease at this time point was finally classified as a false positive result. Thus, CEUS performed at 24 h after ablative treatment may show both false negative and false positive results hampering its routine application in clinical practice[24].

These results were confirmed by Meloni *et al*[26], who found that the sensitivity and specificity of CEUS performed at 24 h were 33% and 98%, respectively.

Overall, these data indicate that CE-CT and CEUS at 24 h are not always helpful in the evaluation of percutaneous ablation response, having only poor sensitivity and a specificity not equal to 100%.

These unsatisfactory results might be related to the gas persistence in the context of the tumour, as well as to the frequent post-treatment peritumoral inflammation. Both these conditions may be still detectable several days after treatment and, in some cases, may persist up to 1 or 2 mo[24].

One month follow-up

Several studies evaluated the usefulness of CEUS performed 1 mo after ablative therapies compared to the CE-CT at the same time-point. These studies demonstrated almost the same diagnostic accuracy between CEUS and CE-CT. In particular, Vilana *et al*[24] found a sensitivity and specificity of 91% and 97%, respectively. Similarly, Pompili *et al*[27] reported a sensitivity of 87% and a specificity of 98.4%, with a good diagnostic agreement with CE-CT (94.6%).

Based on these results CEUS performed 1 mo after the procedure can be considered an appropriate, reliable, comparatively inexpensive and safe alternative technique to CE-CT in the assessment of therapeutic response after RFA or PEI[24,27,28].

Long term follow-up

A 2 years follow-up with an imaging technique is mandatory to detect HCC recurrence, satellites or seeding[29,30]. The ability of CEUS in detecting local tumour progression or new intrahepatic recurrence during follow-up has been evaluated in different studies. In all cases the sensitivity and the PPV of CEUS compared to CE-CT were unsatisfactory[31]. These results could be related to the short duration of the arterial phase that makes difficult to scan the whole liver or to the intrinsic shortcomings of US technique (small lesion, unfavorable location, *etc.*).

Thus, in the long time follow-up, CE-CT or contrast-enhanced magnetic resonance (CE-MRI) are the mainstay for the imaging of treated patients and the detection of local or remote intra-hepatic and extra-hepatic relapse[23].

However, even though several studies has been published regarding the role of CEUS *vs* CE-CT after ablative treatments, the results remain still controversial and an ideal imaging follow-up scheme is not yet available. Where CEUS is available both techniques should be recommended in order to combine the virtues and to reduce the limits of both modalities. Anyway, further studies are still needed to evaluate the efficacy of this approach[29].

CEUS AND TACE

Since TACE has been introduced as a palliative treatment in patients with unresectable HCC it has become one of the most common form of interventional therapies, although in many cases it is difficult to achieve complete necrosis of the tumour. Intratumoral vascularity after TACE has been shown to correlate with tumour viability and is used as the major criterion to assess treatment efficacy and to plan additional treatment.

Similarly as previously described for RFA and PEI, CEUS has been proved to be efficient in differentiating residual from necrotic tumour after TACE.

Moschouris *et al*[32] reported that the early assessment of treatment response by CEUS performed 48 h after drug-eluting bed TACE could underestimate the degree of necrosis in comparison with delayed evaluation (35-40 d after the procedure) with a percentage of tumour necrosis of 43.5% and 52.3%, respectively. The same authors found a good agreement between delayed post-TACE CEUS and CE-CT[32].

In another study CEUS resulted even more sensitive than CE-CT in the detection of residual vascular enhancement after TACE using angiography as reference standard. In fact, CE-CT performed 1 mo after treatment detected 20 of 23 incomplete responses whereas CEUS performed at the same time point detected all cases of incomplete response. Results of CEUS and CE-CT agreed with those of the reference standard (angiography) in 38/38 (100%) and in 35/38 (92.1%) nodules, respectively[33]. Another recent study from a Chinese group suggests a leading role of CEUS compared to CE-CT for detecting residual tumour after lipiodol-based TACE. Liu *et al*[34] evaluated treatment response in 130 HCC patients who underwent CEUS 15 to 90 d after procedure. The sensitivity and accuracy of detecting residual tumour by CEUS *vs* CE-CT were 95.9% *vs* 76.2% and 96.2% *vs* 77.7% respectively, thus recommending CEUS as an optional procedure for assessing the tumour response after TACE[34].

Based on these results, CEUS performed at 1-mo with second generation contrast agents can be regarded as a valid alternative technique to CE-CT in the assessment of therapeutic response after TACE for HCC (Figure 1).

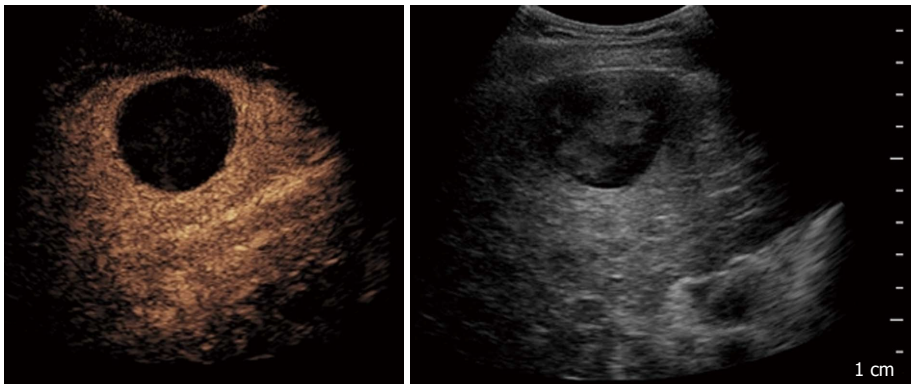

Figure 1 Contrast-enhanced ultrasound performed after 1 mo in a 71-year-old man treated with trans-catheter arterial chemo-embolisation: On the left side complete necrosis is depicted as an avascular area; on the right side B-mode imaging of the treated area.

CEUS AND QUANTITATIVE ASSESSMENT OF FUNCTIONAL RESPONSE

Tumour angiogenesis

Angiogenesis plays a critical role in the growth and spread of tumour. Cancer cells are able to produce some biochemical signals stimulating angiogenesis and to enhance the production of angiogenesis signaling molecules by the surrounding normal cells. Fed by new blood vessels cancer cells proliferate and progressively lose their differentiation, invading the around tissues, going in the blood and lymphatic vessels and forming new colonies of cancer cells far from the primitive cancer, called metastases[35].

The "gold-standard" to assess the angiogenesis is the histological evaluation of the average number of micro-vessels [microvascular density (MVD)][36]. However, biopsy is invasive and sampling bias may happen due to tumours heterogeneity, producing a possibly under- or overestimation of the angiogenesis grade[37].

Furthermore, MVD is not able to give information about changes of blood flow or vascular bed hyper-permeability. On the contrary, functional imaging is able to quantify these changes above all as an early consequence of the anti-angiogenesis therapy[38,39].

Anti-angiogenetic agents

One of the most important recent steps in the oncology field is the development of anti-angiogenetic drugs. These agents act interfering with various steps in the angiogenesis process. Usually, they bind to receptors on the surface of endothelial cells or to other proteins in the downstream signaling pathways, inhibiting factors needed for new blood vessels arrangement.

The United States Food and Drug Administration approved different drugs showing anti-angiogenetic activity including sorafenib, sunitinib and bevacizumab. To date sorafenib is the only anti-angiogenetic approved for HCC treatment but other drugs with similar activity are under investigation in many phase Ⅱ and Ⅲ trials.

Angiogenesis inhibitors interfere with various steps in this process. In particular, sorafenib acts by inhibiting the serine-threonine kinases and the receptor tyrosine kinase activity of vascular endothelial growth factor receptors (VEGFRs) and platelet-derived growth factor receptor β, which have been implicated in the molecular pathogenesis of HCC[40-45].

The different mechanism of action of these new agents from classical cytostatic drugs requires a shift from standard efficacy evaluation criteria to new imaging modalities that assesses changes in tumour vascularisation. Although progression free survival (PFS) and overall survival (OS) represent the most significant efficacy end-points in the medium and long term, early assessment of tumour angiogenesis remains a crucial aim in this area as it allows optimisation of individualised treatment[46]. Especially, early evaluation of failed response allows a tailored therapy, avoiding needless toxicity, psychological burden and costs.

D-CEUS

D-CEUS is a new functional technique enabling a quantitative assessment of solid tumour perfusion. This is achieved by a quantitative analysis performed on contrast uptake curves which are built up from raw linear data after automatic modelisation. The robustness of this approach relies on the fact that signal intensity is proportional to the microbubble concentration in the region of interest. Raw linear data are used to quantify parameters such as peak intensity (PI), time to PI, mean transit time, slope coefficient of wash-in (T_P), total area under the curve (AUC), AUC of wash-in and AUC of wash-out. All these parameters provide information about blood flow and volume, but an optimal parameter has not been clearly identified yet[47].

D-CEUS is supported by the French National Cancer Institute (INCa), which is currently evaluating such technique in different malignancy as well as in primary HCC to establish the reliable perfusion parameters and timing for quantitative anticancer efficacy assessments[48].

Reduction in tumour vascularisation can easily be detected in responders after 1 or 2 wk and is correlated

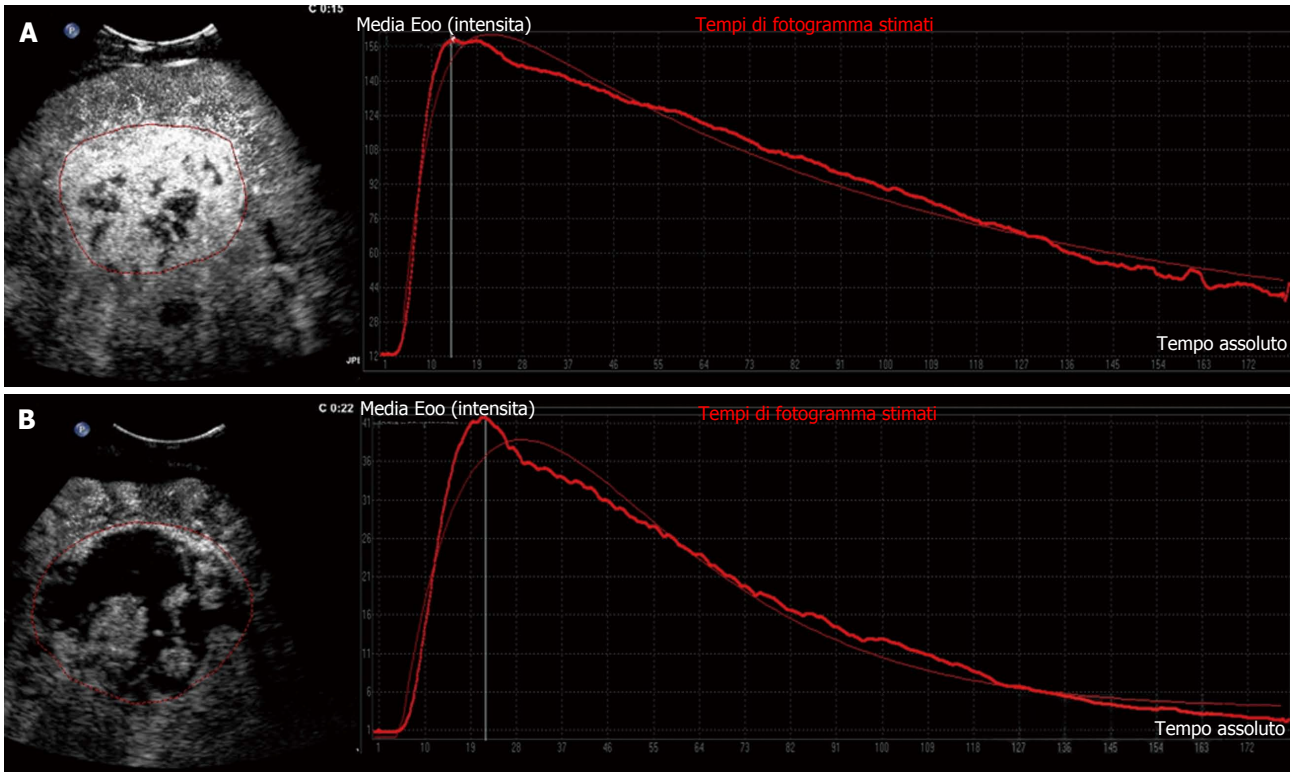

Figure 2 Target hepatic lesion in a 65-year-old man treated with sorafenib: Clinical example of responder on dynamic contrast-enhanced ultrasound. A: Contrast-enhanced ultrasound with corresponding time-intensity curve at baseline; B: Fifteen days after onset of sorafenib therapy, contrast-enhanced ultrasound revealed an increase in tumour necrosis with drastic reduction of tumour perfusion parameters shown by contrast enhancement pattern and corresponding time-intensity curve.

with mRECIST response, PFS and OS in renal cell carcinoma and HCC[6,47].

D-CEUS in monitoring anti-angiogenetic treatment for HCC

As already said, VEGF plays a critical role in mediating angiogenesis in HCC, and the tumour expression of VEGF correlates with vascular density, tumour invasiveness and prognosis[49-51]. Several studies demonstrated the utility of D-CEUS for the quantification of tumour perfusion as a prognostic tool in patients with advanced HCC treated with anti-angiogenetics and identified quantitative parameters correlated with standard efficacy endpoints such as tumour response, PFS and OS.

In a recent experimental study D-CEUS was able to detect a reduction in tumour vascularisation as early as 3 d after bevacizumab therapy for HCC, with a good agreement with CE-CT performed at 2 mo in the identification of responders and non-responders patients[52].

Another study from our group corroborated that D-CEUS is a reliable method to identify early reduction in tumour vascularisation in patients undergoing treatment with sorafenib. Changes in selected quantitative parameters, detected after 14 d of therapy, agreed with tumour response evaluated by means of standard criteria at 2 mo. Between the parameters analysed PI, AUC and T_p showed a significant reduction soon after the beginning of therapy with sorafenib in most of the patients reaching long-term stable disease (Figure 2).

Moreover, a relationship was found between D-CEUS variables and improved clinical outcome such as prolonged OS and PFS[53].

Some researchers evaluated the usefulness of D-CEUS in the quantification of liver parenchymal perfusion for the early detection of major adverse events in patients with advanced HCC treated with sorafenib. The decrease in functional parameters related to blood volume (AUC and PI) between baseline and day 7 after the initiation of treatment was strongly associated with changes in laboratory data related to liver function and was able to predict the occurrence of major adverse events such as liver failure[54].

The dynamic enhancement parameters of D-CEUS can provide important references for clinical pathological factors in prognosis prediction such as VEGF expression and MVD. In fact, a recent study reported a good correlation between VEGF and CD34 expression, (evaluated by immune-histo-chemistry), MVD and some D-CEUS parameters (enhanced time, washout time and AUC)[55].

CONCLUSION

Over the past decades, different locoregional and systemic therapies have emerged as a suitable alternative to surgery in patients with HCC. An accurate assessment of therapeutic response is mandatory, as complete

tumour necrosis significantly increases patient survival, whereas residual viable tumour requires additional treatment. CEUS suggests an effective procedure when a previously enhancing, hyper-vascularised HCC tumour shows lack of contrast enhancement after treatment, whereas still viable tumoral tissue is usually visualised as an arterial-enhancing area with subsequent washout[6].

Several studies demonstrated the usefulness of CEUS and D-CEUS in monitoring tumour response after HCC treatment. In fact, it is able to provide both morphological and functional data associated with low cost and good safety profile.

CEUS performed within 60 min after RFA or PEI with a correct timing scan seems to be reliable for the immediate post-treatment assessing, allowing an immediate retreatment during the same session, if necessary[23]. As concerning recently introduced devices for ablative treatment such as cryoablation and irreversible electroporation, the usefulness of CEUS was investigated only in few studies showing preliminary and inconclusive results[56]. One important information stemming from these studies is that CEUS pattern after cryoablation appears different compared to that after RFA because the margins of the lesions are less well defined and shrink significantly faster than RFA lesions, explaining why it is often difficult to identify them on B-mode or even CEUS more than 1 year after the procedure[57]. Overall, CEUS can be considered a reliable and safe alternative technique to CE-CT in the assessment of therapeutic response to ablative treatment and TACE after 1 mo[28].

Finally, CEUS associated with perfusion software and time intensity curves can be used as a new functional technique enabling a quantitative assessment of solid tumour perfusion by means of a quantitative analysis. This is very important in the early assessment of tumour vascularisation in HCC treated with vascular targeting agents since it would enable an optimisation of individualised treatment. Especially, early evaluation of failed response allows a tailored therapy, avoiding unnecessary toxicity, psychological burden and costs.

The effective application of CEUS and D-CEUS in clinical practice has been recently highlighted by EFSUMB guidelines. For example this panel of experts recognised the important role of CEUS in the very early evaluation of ablative treatment as a guidance for immediate re-treatment of residual unablated tumour[12].

Novel CEUS-based techniques may even exploit the advantages of this imaging modality in evaluating tumour response after HCC treatment. For instance, a technical development based on real-time fusion of CEUS with CE-CT or CE-MRI enables a precise mapping of tumour lesions in CEUS. This new technique allows a multi-plane display of tumour lesions and also shows small lesions which are normally hard to display in standard US. In a pilot study by Ross *et al*[58] the fusion of pre-interventional CE-CT or CE-MRI with post-interventional CEUS performed immediately after treatment showed an improved visualisation of microcirculation and residual tumour perfusion after TACE. A high correlation between early fusion study (CEUS with CE-CT or CE-MRI) and CE-CT performed 6 wk after TACE granted an early assessment of therapeutic success[58]. More recently, three-dimensional CEUS technique (3D CEUS) has been reported to improve the study of tumour vascularity, thus allowing the response evaluation of HCC treatments in the three orthogonal planes. Nevertheless, it has been suggested that the spatial resolution of the current 3D probes may be limited as 3D CEUS provided similar diagnostic performance compared to conventional CEUS in the assessment of therapeutic response of HCC treated with ablative treatments[59].

In conclusion, the perspectives about a large diffusion of CEUS and D-CEUS in clinical practice are very positive and promising, although further studies are warranted to determine the still unclear aspects such as the best timing and the best quantitative dynamic parameter for the assessment of response to HCC treatment.

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ORIGINAL ARTICLE

Basic Study

Cartilage oligomeric matrix protein: A novel non-invasive marker for assessing cirrhosis and risk of hepatocellular carcinoma

Gary L Norman, Nikolaos K Gatselis, Zakera Shums, Christos Liaskos, Dimitrios P Bogdanos, George K Koukoulis, George N Dalekos

Gary L Norman, Zakera Shums, Inova Diagnostics, Inc., San Diego, CA 92131, United States

Nikolaos K Gatselis, Christos Liaskos, George N Dalekos, Department of Medicine and Research Laboratory of Internal Medicine, School of Medicine, University of Thessaly, 41110 Larissa, Thessaly, Greece

Dimitrios P Bogdanos, Division of Rheumatology, School of Medicine, University of Thessaly, 41110 Larissa, Thessaly, Greece

Dimitrios P Bogdanos, Division of Transplantation Immunology and Mucosal Biology, Institute of Liver Studies, King's College London School of Medicine, Denmark Hill Campus, London SE5 9RJ, United Kingdom

George K Koukoulis, Department of Pathology, School of Medicine, University of Thessaly, 41110 Larissa, Thessaly, Greece

Author contributions: Norman GL, Gatselis NK, Bogdanos DP and Dalekos GN had the original idea, designed the study and wrote the first draft of the paper; Shums Z, Liaskos C and Gatselis NK performed the laboratory analysis, collect the data and did the statistical analysis; Koukoulis GK did the interpretation of the histological data of the patients and along with Dalekos GN and Norman GL made the final critical revision of the manuscript for important intellectual content; all authors have seen and approved the final draft of the paper.

Institutional review board statement: The study was reviewed and approved by the Ethical Committee Review Board of the School of Medicine of Thessaly University. All study participants provided informed written consent prior to study enrollment.

Conflict-of-interest statement: Norman GL and Shums Z are employees of Inova Diagnostics Inc. They have a pending patent application US 2012/0183981 relevant to the present study. Inova Diagnostics Inc. provided funds to Norman GL and Shums Z (ELISA kits) for the support of this study. All other authors have no disclosures relevant to this manuscript. Other authors have declared that no competing interest exists.

Data sharing statement: No additional data are available (all relevant data are within the paper).

Correspondence to: George N Dalekos, MD, PhD, Professor, Head, Department of Medicine and Research Laboratory of Internal Medicine, School of Medicine, University of Thessaly, Biopolis, 41110 Larissa, Greece. dalekos@med.uth.gr
Telephone: +30-24-13502285
Fax: +30-24-13501557

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Abstract

AIM: To assess serum cartilage oligomeric matrix protein (COMP) as a marker of cirrhosis and risk of progression to hepatocellular carcinoma (HCC).

METHODS: A COMP enzyme-linked immunosorbent

assay was used to test 187 patients with chronic liver diseases at the time point of first evaluation. The selected patients included 72 with chronic hepatitis B infection, 75 with chronic hepatitis C infection, 22 with primary biliary cirrhosis, 7 with autoimmune hepatitis type 1, and 11 with alcoholic liver disease. Demographic, biochemical, histological and clinical characteristics of the patients were recorded at the first evaluation. One hundred and forty-seven patients were followed for a median [interquartile range (IQR)] duration of 96.5 (102) mo. The clinical, biochemical and histological data, as well as the development of cirrhosis, HCC according to internationally accepted criteria and in case of death, a liver-related cause during the follow-up period, were recorded at the electronic database of our clinic. COMP determination was also performed in 43 healthy individuals who served as the control study group.

RESULTS: COMP positivity (> 15 U/L) was detected in 22%-36% among chronic liver disease groups. Strikingly, almost 83% of COMP-positive patients were cirrhotic at baseline, independently of cause of liver disease. Among the patients who developed HCC during follow-up, 73.7% (14/19) were COMP positive at baseline. COMP positivity was significantly associated with older age ($P < 0.001$), advanced fibrosis ($P = 0.001$) and necroinflammatory activity ($P = 0.001$), higher aspartate aminotransferase ($P < 0.001$), alanine aminotransferase ($P < 0.02$), γ-glutamyl transpeptidase ($P = 0.003$), alkaline phosphatase ($P = 0.001$), bilirubin ($P < 0.05$), international normalized ratio ($P = 0.002$) and alpha-fetoprotein levels ($P < 0.02$), and lower albumin ($P < 0.001$), and platelet count ($P = 0.008$). COMP levels [median (IQR)] were significantly higher in cirrhotics compared to non-cirrhotics [13.8 (7.9) U/L *vs* 9.8 (4.6) U/L, respectively; $P < 0.001$]. On multivariate logistic regression analysis, COMP-positivity was independently associated only with cirrhosis (OR = 4.40, 95%CI: 1.33-14.69, $P = 0.015$). Kaplan-Meier analysis showed that COMP positivity was significantly associated with HCC development ($P = 0.007$) and higher incidence of liver-related death ($P < 0.001$).

CONCLUSION: Elevated COMP levels are strongly associated with cirrhosis and HCC progression. Serum COMP is a new promising non-invasive biomarker for HCC risk assessment in surveillance programs.

Key words: Hepatic fibrosis; Hepatocellular carcinoma; Viral hepatitis; Enzyme-linked immunosorbent assay; Biomarker; Cirrhosis

Core tip: We report our first results regarding the utility of serum cartilage oligomeric matrix protein (COMP), an antigen over-expressed in developing liver, as a novel non-invasive marker of liver fibrosis and risk of progression to hepatocellular carcinoma (HCC). HCC is the third leading cause of cancer deaths worldwide, therefore non-invasive tests of fibrosis, as well as tests that can predict which patients are at high risk to develop HCC are needed. Our results suggest that COMP levels are associated with cirrhosis and a worse prognosis, thus serum COMP may assist clinicians as a non-invasive biomarker for risk assessment in surveillance programs.

Norman GL, Gatselis NK, Shums Z, Liaskos C, Bogdanos DP, Koukoulis GK, Dalekos GN. Cartilage oligomeric matrix protein: A novel non-invasive marker for assessing cirrhosis and risk of hepatocellular carcinoma. *World J Hepatol* 2015; 7(14): 1875-1883 Available from: URL: http://www.wjgnet.com/1948-5182/full/v7/i14/1875.htm DOI: http://dx.doi.org/10.4254/wjh.v7.i14.1875

INTRODUCTION

Hepatocellular carcinoma (HCC) accounts for almost 90% of all primary liver cancer cases, being the third most common cause of tumor-related death among males and the sixth among females[1-4]. The major causes of HCC are hepatitis B virus (HBV)- or hepatitis C virus (HCV)-related cirrhosis and alcoholic cirrhosis[1-5]. In Greece, data from the HEPNET-GREECE Study Group has shown a cumulative 5-year incidence of HCC approaching 20% in decompensated and 10% in compensated patients with HBV-related cirrhosis. The incidence in non-cirrhotic HBV infected patients is less than 4%. This is in contrast to a lower incidence (1.4%) in HCV patients[4-7].

Prompt diagnosis of early or very early stage HCC is difficult due to the lack of specific symptoms and the relatively limited prognostic value of the serological and radiological approaches currently used for surveillance. The prognosis of HCC is generally poor, as a result of the aggressive nature of the disease, concurrent liver decompensation and the sometimes limited availability of potential treatment options[2,3,8-13]. Screening using determinations of serum alpha-fetoprotein (AFP) levels and ultrasonography every 6 mo appear to identify only a minority of cases with early stage HCC and therefore its use is not recommended by several international authorities[9-14].

Increasing evidence suggests that fibrosis progression is a key parameter in estimating the risk of HCC development[12,14]. Therefore, there is a need for non-invasive tests of fibrosis, as well as tests that can predict which patients are at high risk to develop HCC. Non-invasive markers currently reported are not sufficiently accurate, largely because they can only identify non-cirrhotic patients or those with advanced cirrhosis and are least useful in the early stages of HCC, when detection could be life-saving[14,15]. In this context, despite considerable controversy, AFP continues to be used extensively because it is inexpensive and there is a long clinical history supporting its use[14].

Progressive damage of the liver leading to fibrosis, cirrhosis, and eventually HCC, is associated with a remodeling of the liver as a consequence of both the

Table 1 Baseline characteristics of the patients (*n* = 187)

Sex (male/female), *n* (%)	95 (50.8%)/92 (49.2%)
Age (yr), mean ± SD	53.7 ± 15.2
HBV/HCV/PBC/AIH/ALD, *n*	72/75/22/7/11
Duration of follow-up (mo), median (IQR)	96.5 (102)
INR, median (IQR), (normal range: 0.85-1.15)	1.04 (0.24)
Platelets (× 10^3/μL), median (IQR), (normal range: 140-440)	190 (116)
AST (U/L), median (IQR), (UNL: 40 U/L)	38 (45)
ALT (U/L), median (IQR), (UNL: 40 U/L)	40 (46)
γ-GT (U/L), median (IQR), (UNL: 37 U/L)	33 (52)
ALP (U/L), median (IQR), (UNL: 104 U/L)	90 (74)
Bilirubin (mg/dL), median (IQR), (UNL: 1.1 mg/dL)	0.8 (0.8)
Albumin (g/dL), median (IQR), (normal range: 3.5-5.2 g/dL)	4.3 (1.0)
IgG (mg/dL), mean ± SD, (UNL: 1650 mg/dL)	1583 ± 508
IgM (mg/dL), median (IQR), (UNL: 200 mg/dL)	135 (147)
IgA (mg/dL), mean ± SD, (UNL: 300 mg/dL)	312 ± 192
AFP (ng/mL), median (IQR), (UNL: 10 ng/mL)	4.6 (6.4)
Cirrhosis (yes/no), *n* (%)	98 (52.4%)/89 (47.6%)
Decompensation of cirrhosis[1] (yes/no), *n* (%)	27 (27.6%)/71 (72.4%)
HCC (yes/no), *n* (%)	12 (6.4%)/175 (93.6%)
Histological grade (none/minimal/mild *vs* moderate/severe), *n* (%)	61 (55.5%)/49 (44.5%)
Histological stage (none/minimal/mild *vs* moderate/severe/cirrhosis), *n* (%)	62 (56.4%)/48 (43.6%)

[1]Decompensation of cirrhosis denotes the development of at least one of the following: variceal bleeding, hepatic encephalopathy or ascites. *n*: Number of patients in each respective group; SD: Standard deviation; HBV: Hepatitis B virus; HCV: Hepatitis C virus; PBC: Primary biliary cirrhosis; AIH: Autoimmune hepatitis; ALD: Alcoholic liver disease; INR: International normalized ratio; AST: Aspartate aminotransferase; UNL: Upper normal limit; ALT: Alanine aminotransferase; γ-GT: γ-glutamyl transpeptidase; ALP: Alkaline phosphatase; IgG: Immunoglobulin G; IgM: Immunoglobulin M; IgA: Immunoglobulin A; AFP: Alpha fetoprotein; HCC: Hepatocellular carcinoma.

degradation of the extracellular matrix and the accumulation of fibrotic scar tissue. This led us to consider that cartilage oligomeric matrix protein (COMP) could be a potential marker of liver fibrosis and early HCC. COMP, the fifth member of the thrombospondin family, is a pentameric extracellular, calcium-binding glycoprotein that modulates the cellular phenotype during tissue genesis and remodeling[16]. This glycoprotein is predominantly expressed in articular cartilage, but also in other tissues, including the developing liver[17-20]. Diseases that cause damage to the cartilage lead to the release of COMP into the blood[21] and thus it is reasonable that changes in serum COMP levels may reflect alterations in cartilage breakdown[22]. Hence, measurement of serum COMP levels has been used diagnostically in the non-invasive estimation of the degree of cartilage damage in patients with inflammatory joint diseases such as rheumatoid arthritis (RA) and osteoarthritis (OA)[23-26].

In the present study, we speculated that serum COMP could be an early marker of fibrosis, and that increased serum COMP levels could reflect the degree of cartilage breakdown during liver destruction and remodeling. Our assumption is supported by data showing an over-expression of COMP in liver tissue specimens from patients with viral hepatitis-related HCCs[27]. These data have led researchers to speculate that COMP may have a central role early in the development of cirrhosis and liver carcinogenesis[27]. If this holds true, the presence of COMP could be a non-invasive tool to assist in selectively identifying individuals at significantly increased risk of progressing to cirrhosis and hepatocellular carcinogenesis.

To investigate this hypothesis, we measured COMP levels in serum samples from cirrhotic patients, including patients who developed HCC over time, as well as non-cirrhotic patients with chronic HBV and HCV infections, autoimmune hepatitis (AIH), primary biliary cirrhosis (PBC), and alcoholic liver disease (ALD).

MATERIALS AND METHODS

Patients

Serum samples from 187 Caucasian patients with chronic liver diseases followed at the outpatient clinic of the Department of Medicine, Medical School, University of Thessaly, Larissa, Greece during the period 2000-2013, were chosen at the time point of first evaluation from available specimens in the biobanking facility of the Research Laboratory of Internal Medicine and were stratified according to the presence (*n* = 98) or absence of cirrhosis (*n* = 89). The serum samples were randomly selected in order to avoid any potential bias selection and were stored at -80 ℃ (never-thawed) until the determination of COMP levels. The demographic, biochemical, histological and clinical baseline characteristics of patients are shown in Table 1. All patients had negative history of RA and OA or other autoimmune rheumatic diseases at the time of investigation and during follow-up. The selected patients included 72 with chronic HBV infection (45 males, mean age 55 ± 12 years); 75 with chronic HCV infection (34 males, mean age 50 ± 16 years); 22 with PBC (4 males, mean age 57 ± 17 years); 7 with AIH-type 1 (1 male, mean age 67 ± 21 years); and 11 with ALD (11 males, mean age 54 ± 12 years).

Histological data were available for 110 patients. The histologic evaluation for inflammation and fibrosis was assessed using the Knodell histologic activity index[28]. According to previous publications of our group[29-31], for statistical reasons the patients were divided into two groups: (1) according to inflammation: minimal/mild (score 0-8) and moderate/severe (score 9-18); and (2) according to fibrosis: none/mild (score 0-1) and moderate/severe/cirrhosis (score 2-3).

Ninety-eight patients (52.4%) were classified as cirrhotic at initial presentation (Table 1) based on histological findings where available and/or ultrasonographic findings (nodules in the hepatic parenchyma, spleen > 12 cm, portal vein > 16 mm) and/or endoscopic findings of cirrhosis (varices, portal gastropathy) and/or clinical findings of decompensation (ascites, variceal bleeding, encephalopathy)[32,33]; 38/72 with HBV, 36/75 HCV, 6/22 PBC, 7/7 AIH-1 and 11/11 ALD. Among the 98 patients

with cirrhosis, 12 had developed HCC at the time of serum collection. One hundred and forty-seven patients were followed for a median [interquartile range (IQR)] duration of 96.5 (102) mo. Ultrasonography and AFP measurements were performed every 6 mo in cirrhotic patients and every 12 mo approximately in the non-cirrhotics. The clinical, biochemical and histological data, as well as the development of cirrhosis, HCC according to internationally accepted criteria for its diagnosis[10,12,13] and in case of death, a liver-related cause during the follow-up period, was recorded in the electronic database of our clinic. COMP determination was also performed in 43 healthy individuals who served as the control group of the study.

Methods

COMP levels in serum were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (AnaMar Diagnostics, Sweden). The COMP ELISA is a solid-phase, two-site enzyme immunoassay. It is based on the direct sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants on the COMP molecule. During incubation, COMP in the sample reacts with peroxidase-conjugated anti-COMP antibodies and anti-COMP antibodies bound to the well of the microwell plate. A washing step removes unbound enzyme-labeled antibody and the bound conjugate is detected by a reaction with 3,3',5,5'-tetramethylbenzidine. The reaction is stopped by adding acid to give a colorimetric endpoint that is read spectrophotometrically. A calibration curve is obtained using 5 calibrators corresponding to 0.4, 0.7, 1.2, 1.8 and 3.2 U/L. According to the manufacturer, patients with inflammatory joint disease and serum COMP levels lower than 12 U/L have lower risk of joint destruction in the future compared to those with 12-15 U/L, who have an increasing risk, and those with more than 15 U/L, who have a higher risk for aggressive joint destruction. Using these values for guidance, we evaluated the effectiveness of two different cut-offs (12 and 15 U/L) for assessing positivity in our cohort of patients with chronic liver diseases. Based on studies of normal as well as disease controls, it was determined that the more rigorous and specific cut-off of 15 U/L was more appropriate for patients with chronic liver diseases.

Levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (γ-GT), alkaline phosphatase (ALP), bilirubin, albumin, international normalized ratio (INR), serum immunoglobulin IgA, IgG and IgM, and AFP were determined using standard techniques. Serological markers of HBV infection (HBsAg, anti-HBs, anti-HBc, HBeAg and anti-HBe) and antibodies to HCV were determined by the AxSYM system using the respective MEIA kits (Abbott Laboratories, Diagnostics Division, 100 Abbott Park Road, Abbott Park, IL).

All subjects provided written informed consent to participate in the study. The ethical committee of Thessaly University Medical School approved the study protocol.

Statistical analysis

Kolmogorov-Smirnov test was used to assess the normality of the distribution of variables. Normally distributed values are expressed as mean ± SD, while non-normally distributed values as median (IQR). Data were analyzed by *t*-test, Mann-Whitney *U* test, χ^2 test (two by two with Yate's correction), Fischer's exact test and Spearman's rho correlation where applicable. The parameters that were significant in the univariate analysis entered a binary logistic regression model, in order to identify independent risk factors. Survival analysis was carried out using the Kaplan-Meier plot for COMP-positive or COMP-negative patients up to the time patients reached the following study end-points: development of cirrhosis, decompensation, HCC, or death due to liver disease. The comparisons were done by log-rank test. Two-sided *P* values less than 0.05 were considered statistically significant.

RESULTS

Significance of serum COMP antigen detection in relation to the baseline characteristics of patients

COMP antigen levels (> 15 U/L) were detected in 52 of the total cohort of 187 (27.8%) patients. The frequency of COMP positivity was comparable among patients with various chronic liver diseases (30.6% in HBV, 25.3% in HCV, 22.7% in PBC, 28.6% in AIH-1 and 36.4% in ALD; $P = 0.880$). Strikingly however, 82.6% (43/52) of the COMP positive sera originated from patients with cirrhosis (Figure 1 and Table 2). The increased frequency of COMP levels in patients with cirrhosis was similar when patients were stratified according to disease group (Figure 1). All 43 healthy controls had serum levels of COMP less than 15 U/L.

The association of the presence of COMP antigen with the demographic, clinical, laboratory, and histological parameters of patients at baseline are shown in Table 2. COMP positivity was significantly associated with older age ($P < 0.001$), higher levels of AST ($P < 0.001$) and ALT ($P < 0.02$), higher levels of γ-GT ($P = 0.003$), ALP ($P = 0.001$), bilirubin ($P < 0.05$) and INR ($P = 0.002$) and lower levels of albumin ($P < 0.001$) and platelet count ($P = 0.008$) (Table 2). Moreover, COMP positivity was significantly correlated with advanced fibrosis ($P = 0.001$), necroinflammatory activity ($P = 0.001$), higher levels of AFP ($P < 0.02$), the presence of cirrhosis ($P < 0.001$), and the presence of HCC ($P < 0.05$) (Table 2). Moreover, COMP levels were positively correlated with age ($r = 0.417$; $P < 0.001$), AST ($r = 0.474$, $P < 0.001$), ALT ($r = 0.324$, $P < 0.001$), γ-GT ($r = 0.268$; $P < 0.001$), ALP ($r = 0.212$; $P = 0.005$), bilirubin ($r = 0.192$; $P = 0.02$), INR ($r = 0.275$; $P = 0.002$) and AFP ($r = 0.261$; $P = 0.003$), while were negatively correlated with platelet count ($r = -0.192$, $P < 0.02$) and albumin ($r = -0.343$, $P < 0.001$).

Table 2 Demographic, clinical, laboratory and histological baseline characteristics of 187 patients according to cartilage oligomeric matrix protein antigen positivity

	COMP-positive (*n* = 52)	COMP-negative (*n* = 135)	*P* value
Sex (male/female), *n* (%)	26/26 (50%/50%)	69/66 (51.1%/48.9%)	1.000
Age (yr), mean ± SD	61.7 ± 11.9	50.9 ± 15.3	< 0.001
Duration of follow-up (mo)	45 (91)	107 (95)	< 0.01
INR	1.15 (0.22)	1 (0.17)	0.002
Platelets (× 10^3/μL)	166 (125)	199 (104)	0.008
AST (U/L)	59 (57)	31 (33)	< 0.001
ALT (U/L)	46 (50)	38 (39)	< 0.02
γ-GT (U/L)	50 (65)	27 (48)	0.003
ALP (U/L)	115 (102)	83 (63)	0.001
Bilirubin (mg/dL)	1.1 (1.5)	0.7 (0.6)	< 0.05
Albumin (g/dL)	3.9 (1)	4.4 (1)	< 0.001
IgG (mg/dL), mean ± SD	1724 ± 614	1533 ± 461	0.151
IgM (mg/dL)	134 (180)	135 (120)	0.265
IgA (mg/dL), mean ± SD	359 ± 196	295 ± 190	0.196
AFP (ng/mL)	6.9 (10.5)	3.8 (5.5)	< 0.02
Cirrhosis (yes/no), *n* (%)	43/9 (82.7%/17.3%)	55/80 (40.7%/59.3%)	< 0.001
Decompensation of cirrhosis[1] (yes/no), *n* (%)	11/32 (25.6%/74.4%)	16/39 (29.1%/70.9%)	0.874
HCC (yes/no), *n* (%)	7/45 (13.5%/86.5%)	5/130 (3.7%/96.3%)	< 0.05
Histological grade (none/minimal/mild vs moderate/severe), *n* (%)	5/17 (22.7%/77.3%)	56/32 (63.6%/36.4%)	0.001
Histological stage (none/minimal/mild vs moderate/severe/cirrhosis), *n* (%)	5/17 (22.7%/77.3%)	57/31 (64.8%/35.2%)	0.001

[1]Decompensation of cirrhosis denotes the development of at least one of the following: variceal bleeding, hepatic encephalopathy or ascites. Data are present as median (interquartile range) unless other indicated; *n*: Number of patients in each respective group; COMP: Cartilage oligomeric matrix protein; SD: Standard deviation; INR: International normalized ratio; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; γ-GT: γ-glutamyl transpeptidase; ALP: Alkaline phosphatase; IgG: Immunoglobulin G; IgM: Immunoglobulin M; IgA: Immunoglobulin A; AFP: Alpha fetoprotein; HCC: Hepatocellular carcinoma.

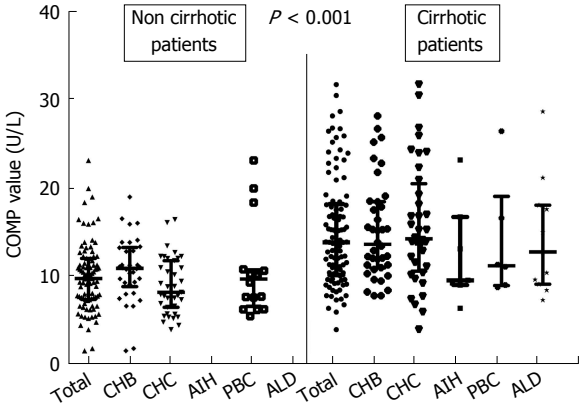

Figure 1 Cartilage oligomeric matrix protein antigen values in the non-cirrhotic and cirrhotic patients in total and according to the etiology of liver disease. Cartilage oligomeric matrix protein (COMP) antigen titers were significantly higher compared to non-cirrhotic patients irrespectively of the etiology of liver disease. Bars indicate median values with interquartile range. CHB: Chronic hepatitis B; CHC: Chronic hepatitis C; AIH: Autoimmune hepatitis; PBC: Primary biliary cirrhosis; ALD: Alcoholic liver disease.

Restricting the analysis in the subgroup of patients with available histological data (*n* = 110), showed that COMP positivity was similarly associated with older age (*P* < 0.001), higher levels of AST (*P* < 0.001) and ALT (*P* < 0.02), γ-GT (*P* = 0.005), ALP (*P* < 0.001), bilirubin (*P* = 0.005), INR (*P* < 0.001), lower levels of albumin (*P* < 0.001), the presence of cirrhosis (*P* < 0.001) and the presence of HCC (*P* = 0.001) (data not shown).

All parameters that were univariately associated with COMP positivity were entered in a multivariate logistic regression model. COMP antigen positivity was independently associated only with the presence of cirrhosis (OR = 4.40, 95%CI: 1.33-14.69, *P* = 0.015). Of note, COMP antigen titers [median (IQR)] in cirrhotic patients [13.8 (7.9) U/L] were significantly higher compared to non-cirrhotic patients [9.8 (4.6) U/L; *P* < 0.001, Figure 1].

Significance of serum COMP antigen detection in the outcome of the patients

As shown in Figure 2, 147 patients had a long-term follow-up of 96.5 (102) mo. Seventy-eight of these 147 patients were cirrhotic at the baseline visit, while 3 out of the remaining 69 non-cirrhotic patients developed cirrhosis during the follow-up period (Figure 3A). Twenty-seven cirrhotic patients had decompensated cirrhosis at baseline visit, while 16 subjects out of the remaining 51 cirrhotic patients developed decompensation during follow-up. HCC was diagnosed in 12 patients at baseline visit, including 11 with long-term follow-up and one lost in follow-up (7/12, 58.3% were COMP positive), whereas HCC developed in other 19 patients during follow-up at least 6 mo after baseline visit. After excluding the 11 HCC cases diagnosed at baseline, the remaining 136 patients were evaluated for HCC development during long-term follow-up. Of interest, development of HCC was observed in 14/34 (41.2%) of the patients positive for COMP at baseline compared to only 5/102 (4.9%) of the patients negative for COMP at baseline (*P* = 0.008). In addition, 14 out of the 19 (73.7%) patients who developed HCC on follow-up had tested positive

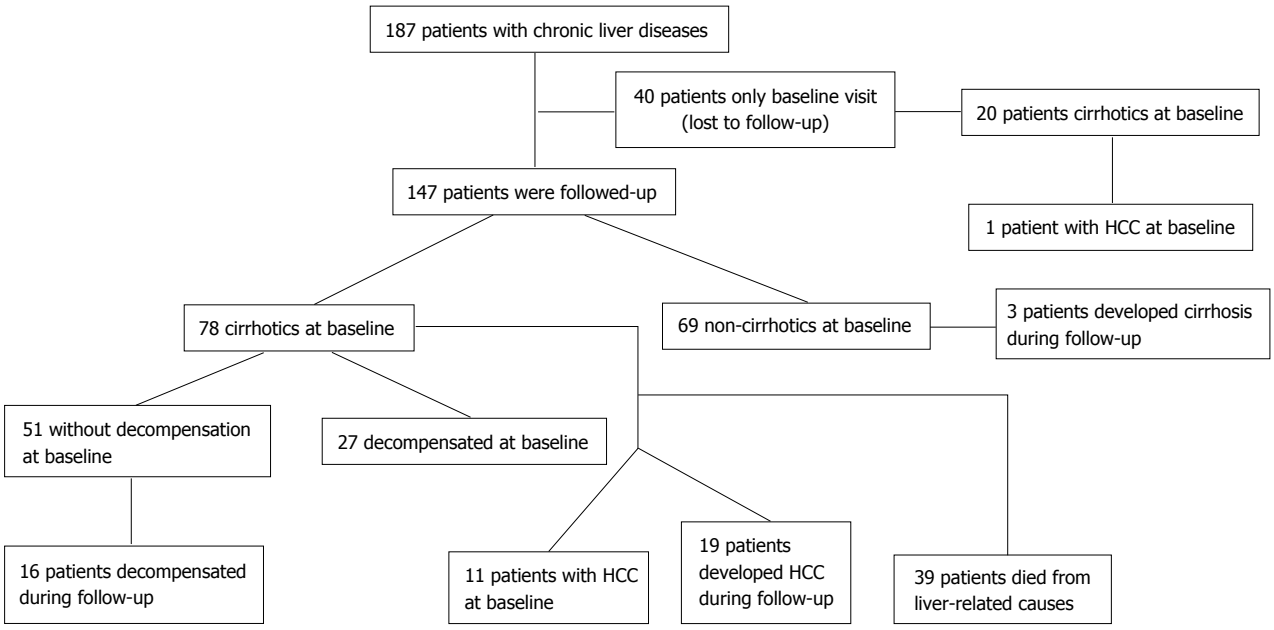

Figure 2 The follow-up schedule diagram of the 187 patients enrolled in the study. HCC: Hepatocellular carcinoma.

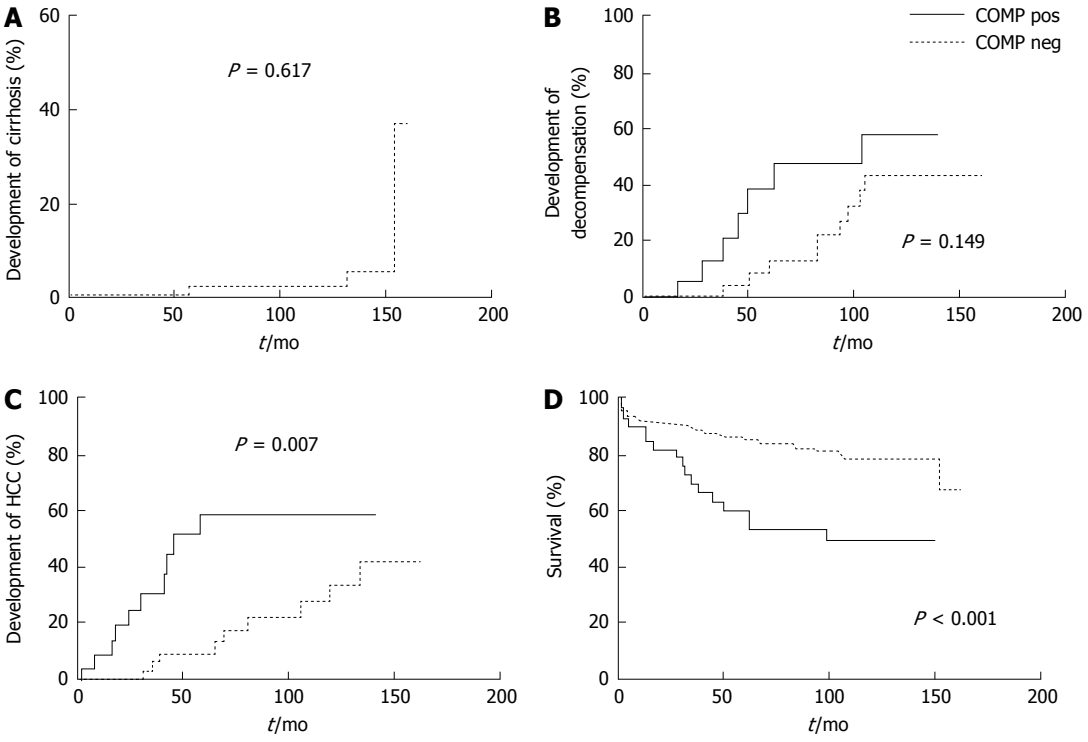

Figure 3 Kaplan-Meier analysis of patients according to cartilage oligomeric matrix protein antigen values (cartilage oligomeric matrix protein-positive *vs* cartilage oligomeric matrix protein-negative). Only, three patients, that were cartilage oligomeric matrix protein (COMP)-negative, developed cirrhosis during follow-up (A). A trend for more rapid development of decompensation in COMP-positive patients was noticed (B). The rates of HCC development (C) and liver-related mortality (D) are significantly higher in COMP-positive group compared to COMP-negative group by log-rank test. HCC: Hepatocellular carcinoma.

for COMP prior to the diagnosis of HCC. Similarly, in the subgroup of non-HCC patients with advanced fibrosis (moderate, severe fibrosis or cirrhosis) as it was determined by liver biopsy (n = 39), development of HCC was observed in 6/11 (54.5%) of COMP positive patients at baseline compared to 4/28 (14.3%) of COMP negative patients at baseline (P < 0.02).

While there was a trend for more rapid development of decompensation in COMP positive compared to COMP negative patients (Figure 3B), this difference did not reach statistical significance (P = 0.149). In contrast, the Kaplan-Meier analysis in cirrhotic non-HCC patients during long-term follow-up (n = 70) revealed a significant difference regarding the development of

HCC between COMP-positive (14/25; 56%) and COMP-negative (5/45; 11.1%) cirrhotic patients (Figure 3C). Moreover, COMP-positive patients demonstrated a statistically higher incidence of liver-related deaths (17/39; 43.6%) compared to COMP-negative patients (22/108; 20.4%) (Figure 3D). Similarly, Kaplan-Meier analysis in the sub-group of patients with available histological data at baseline that were followed-up (n = 84), revealed that COMP-positive patients at baseline had a higher incidence of development of HCC (P = 0.001) and liver-related deaths (P < 0.001) during follow up (data not shown).

DISCUSSION

In the present study we have demonstrated for the first time that the presence of COMP in the sera of patients with chronic liver diseases is strongly associated with liver cirrhosis and that increased COMP levels appear to identify a subgroup of patients who are at an increased risk of progressing to HCC and liver-related mortality. In fact, after multivariate logistic regression analysis, COMP antigen positivity was independently associated only with the presence of cirrhosis. In addition, Kaplan-Meier analysis showed significantly higher rates of HCC development and liver-related mortality during follow-up in COMP-positive patients compared to those with a negative test.

The COMP assay measures peptides released during the breakdown of cartilage[17-19]. Clinically COMP has been primarily used to assess the destruction of cartilage in patients with RA and OA[21-26]. Our hypothesis that during liver remodeling, COMP fragments could be detected in patients' sera has been proven valid, and the amount of COMP likely indicates the level of fibrogenic activity. As we postulated, the frequency of COMP positivity was clearly increased in patients with chronic liver diseases compared to healthy controls. Indeed, a dramatic increase of COMP was largely seen in patients with cirrhosis, regardless of the etiology of liver disease. Thus, 44% (43/98) of the cirrhotic patients were positive for COMP, compared to just 10% (9/89) of the non-cirrhotic patients. Notably, the great majority of patients with a positive COMP result (43/52, 82.7%) had well-documented cirrhosis. Furthermore, 73.7% (14/19) of patients who developed HCC during follow-up were COMP positive prior to the diagnosis of HCC. Although the presence of cirrhosis is clearly associated with an increased risk of disease progression, our findings suggest the detection of COMP in cirrhotic patients is a potentially useful marker to identify a subgroup of cirrhotic patients with a higher likelihood of development of HCC.

Cirrhosis represents a critical milestone in the decline of liver function and the progression of individuals towards decompensation and HCC[34-36]. The absence of fibrosis, as well as the presence of advanced fibrosis, can be established by physical examination and current non-invasive techniques. However, early fibrosis, as well as the identification of patients with a higher likelihood of progressing to cirrhosis, cannot be identified with certainty, leading to significant delay in implementing proper surveillance and rigorous management[34-36]. Currently, several markers have been considered diagnostically meaningful for assessing the development and extent of liver fibrosis[14]. These include costly profiles consisting of biochemical markers and physical measurement techniques such as ultrasonography, fibroscan, and magnetic resonance imaging. Despite the ever-increasing number of described fibrosis markers, most are used for research purposes only and have not been incorporated in routine clinical practice[37-41].

According to our data, COMP levels above 15 U/L are associated with an increased likelihood of cirrhosis, development of HCC, and liver-related death. Although transient expression of megakaryocyte-derived protein immunoreactive with antiserum to COMP in the developing rat liver has been described[42], it is generally accepted that COMP is not expressed in normal liver tissue[16-18]. Only one small study of 30 patients with cirrhosis or HCC investigated the presence of COMP in liver diseases[27]. Consistent with our results, these authors documented an increased COMP mRNA expression in HCC tissue samples, suggesting that COMP is upregulated and overexpressed in HCC tissues[27]. They also showed that COMP was only weakly expressed in cirrhotic liver tissues, indicating that this gene might have a function early in the course of liver carcinogenesis, and this was further supported by their findings that COMP expression was not associated with the stage of HCC[27]. Taking into account that tumors often express genes that are normally restricted to the development of an organ, the observation of overexpression in the liver of a COMP-like protein during embryogenesis, but not shortly after birth[42], may explain the findings of the former study[27].

In conclusion, our novel results support the notion that determination of serum COMP levels may assist clinicians in identifying patients with cirrhosis and those at an increased risk of liver-related death and the development of HCC. Single measurement of COMP shows utility on its own, but it will be certainly of greater diagnostic value with serial determinations obtained during follow-up visits or in combination with other tests, by casting the net wider. The present exploratory study has provided intriguing results and may assist enhanced management of hepatic fibrosis, in particular the assessment of regression or progression of fibrosis before and after specific therapeutic treatments. While larger studies of prospectively collected serum samples will be needed to better address these possibilities, COMP appears to be a promising, simple, non-invasive serological biomarker that may help guide the management of patients with chronic liver disease.

COMMENTS

Background

Hepatocellular carcinoma (HCC) is one of the most frequent cancers worldwide

and accounts approximately one-third of all malignancies. Early diagnosis is a prerequisite for radical treatment, such as surgical resection or liver transplantation.

Research frontiers

The study of novel biomarkers is an increasingly important field in the early detection of HCC, taking account that currently prompt diagnosis is difficult due to the lack of specific symptoms and the relatively limited prognostic value of the available serological and radiological methods used for surveillance.

Innovations and breakthroughs

In the present study, the authors demonstrate for the first time that the presence of cartilage oligomeric matrix protein (COMP) in the sera of patients with chronic liver diseases is strongly associated with liver cirrhosis and that increased COMP levels appear to identify a subgroup of patients at increased risk of progressing to HCC and liver-related mortality.

Applications

The study results suggest that COMP is a new promising, non-invasive biomarker for risk-assessment and surveillance of patients with chronic liver diseases at risk to develop HCC.

Terminology

Extracellular matrix degradation is closely associated with fibrosis, cirrhosis and cancer development. COMP is an antigen expressed in articular cartilage, but also in other tissues, including the developing liver.

Peer-review

Starting part of this paper is excellent, specially the abstract. It is concise and organized. The study is a timely research. Objectives are consistent with literature review and analysis.

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ORIGINAL ARTICLE

Retrospective Study

Utility of liver biopsy in predicting clinical outcomes after percutaneous angioplasty for hepatic venous obstruction in liver transplant patients

Ammar Sarwar, Edward Ahn, Ian Brennan, Olga R Brook, Salomao Faintuch, Raza Malik, Khalid Khwaja, Muneeb Ahmed

Ammar Sarwar, Edward Ahn, Ian Brennan, Olga R Brook, Salomao Faintuch, Muneeb Ahmed, Divison of Vascular and Interventional Radiology, Beth Israel Deaconess Medical Center/Harvard Medical School, Boston, MA 02215, United States

Raza Malik, Division of Gastroenterology and Hepatology, Beth Israel Deaconess Medical Center/Harvard Medical School, Boston, MA 02215, United States

Khalid Khwaja, Division of Transplant Surgery, Beth Israel Deaconess Medical Center/Harvard Medical School, Boston, MA 02215, United States

Author contributions: Sarwar A performed research study, collected data, analyzed data, wrote paper; Ahn E collected data, analyzed data, wrote paper; Brennan I designed research study, wrote paper; Brook OR analyzed data, wrote paper; Faintuch S, Malik R and Khwaja K performed research study, wrote paper; Ahmed M designed research study, performed research study, wrote paper.

Institutional review board statement: The study was reviewed and approved by the Institution Review Board at Beth Israel Deaconess Medical Center.

Informed consent statement: Since this was a retrospective report involving analysis of existing medical records and no investigator-initiated patient contact was performed, our institutional review board approved a waiver for informed consent.

Conflict-of-interest statement: None of the authors report and conflict of interest pertaining to this study.

Data sharing statement: No additional data are available.

Correspondence to: Ammar Sarwar, MD, Divison of Vascular and Interventional Radiology, Beth Israel Deaconess Medical Center/Harvard Medical School, 1 Deaconess Road, Boston, MA 02215, United States. asarwar@bidmc.harvard.edu
Telephone: +1-617-7542523

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Abstract

AIM: To determine utility of transplant liver biopsy in evaluating efficacy of percutaneous transluminal angioplasty (PTA) for hepatic venous obstruction (HVOO).

METHODS: Adult liver transplant patients treated with PTA for HVOO (2003-2013) at a single institution were reviewed for pre/post-PTA imaging findings, manometry (gradient with right atrium), presence of HVOO on pre-PTA and post-PTA early and late biopsy (EB and LB, < or > 60 d after PTA), and clinical outcome, defined as good (no clinical issues, non-HVOO-related death) or poor (surgical correction, recurrent HVOO, or HVOO-related death).

RESULTS: Fifteen patients meeting inclusion criteria underwent 21 PTA, 658 ± 1293 d after transplant.

In procedures with pre-PTA biopsy (n = 19), no difference was seen between pre-PTA gradient in 13/19 procedures with HVOO on biopsy and 6/19 procedures without HVOO (8 ± 2.4 mmHg *vs* 6.8 ± 4.3 mmHg; P = 0.35). Post-PTA, 10/21 livers had EB (29 ± 21 d) and 9/21 livers had LB (153 ± 81 d). On clinical follow-up (392 ± 773 d), HVOO on LB resulted in poor outcomes and absence of HVOO on LB resulted good outcomes. Patients with HVOO on EB (3/7 good, 4/7 poor) and no HVOO on EB (2/3 good, 1/3 poor) had mixed outcomes.

CONCLUSION: Negative liver biopsy greater than 60 d after PTA accurately identifies patients with good clinical outcomes.

Key words: Hepatic venous outflow obstruction; Liver transplantation; Post-transplant biopsy; Angioplasty

Core tip: Percutaneous angioplasty and/or stent placement is the first-line of treatment in patients with hepatic venous obstruction (HVOO) after liver transplantation. Recognizing recurrence of HVOO after percutaneous treatment solely based on clinical, laboratory or imaging findings is difficult, and there is not a clear consensus regarding which measure provides the best or "gold standard" assessment for response to treatment. We report the utility of biopsy in predicting outcomes of percutaneous transluminal angioplasty (PTA) in patients with HVOO after liver transplantation. Specifically, we have found that patients without HVOO on a liver biopsy 60 d or more after PTA had no recurrence of HVOO on long-term follow-up.

Sarwar A, Ahn E, Brennan I, Brook OR, Faintuch S, Malik R, Khwaja K, Ahmed M. Utility of liver biopsy in predicting clinical outcomes after percutaneous angioplasty for hepatic venous obstruction in liver transplant patients. *World J Hepatol* 2015; 7(14): 1884-1893 Available from: URL: http://www.wjgnet.com/1948-5182/full/v7/i14/1884.htm DOI: http://dx.doi.org/10.4254/wjh.v7.i14.1884

INTRODUCTION

Hepatic venous outflow obstruction (HVOO) is an uncommon complication after liver transplantation, occurring in 1.5%-2.5% of patients with orthotropic liver transplantation using the piggyback technique and up to 9.5% of patients with living donor liver transplantation[1-3]. HVOO can be treated either by percutaneous transluminal angioplasty (PTA) (*i.e.*, using an inflatable balloon to treat a luminal stenosis; PTA), hepatic venous stenting, or when percutaneous revascularization is unsuccessful, by surgical revision[3-7]. Primary patency rates of PTA for HVOO at 1 year range from 51%-67% and recurrent HVOO after PTA occurs in 20%-50% of patients[3,4,6-9].

Response of HVOO to treatment may be determined based upon improvements in clinical symptoms, laboratory findings or imaging findings[10]. However, there is significant overlap of the clinical symptoms of HVOO and other causes for early and late allograft dysfunction such as rejection, drug toxicity or biliary complications[11]. Similarly, liver function tests do not always show significant change after successful PTA[8,12]. Finally, while an appropriate imaging response can be useful in determining effectiveness of PTA, imaging assessment can be subjective and may be operator dependent[6,13].

As such, liver biopsies are frequently performed to assess HVOO response to endovascular intervention and to distinguish persistent HVOO from other diseases in liver transplants, either at regular intervals or in response to change in the clinical or laboratory status[14]. Patients with HVOO usually have biopsy findings of zone 3 hepatocyte necrosis, sinusoidal congestion and hemorrhage in the space of Disse[15,16]. Correlation of histologic findings of HVOO with clinical findings such as pressure gradients between the hepatic vein and right atrium on manometry is not well studied. Furthermore, change in histologic findings following successful endovascular treatment is currently unknown. Therefore, the purpose of our study was to evaluate histologic findings at liver biopsy after PTA for HVOO in liver transplant patients and correlate these to treatment response and long-term outcome.

MATERIALS AND METHODS

Study population

Institutional Review Board approval was obtained prior to initiation of the study. As this was a retrospective medical record review, the review board waived the need to obtain informed consent. We performed a retrospective, HIPAA-compliant electronic medical records review of all consecutive patients who underwent endovascular revascularization after liver transplantation. Between July 3, 2003 and September 12, 2013, 15 patients known or suspected to have HVOO after liver transplantation were referred for a total of 21 PTA procedures. Patients were suspected of having HVOO due to one or more of the following: core biopsy histology suggesting outflow obstruction [8/15 patients (53%), clinical symptoms (5/15 patients 33%), or imaging findings of outflow obstruction (2/15 patients)]. Biopsies prior to venograms were performed due to abnormal laboratory findings [7/10 (70%)] or clinical symptoms [3/10 (30%)]. Clinical symptoms suggestive of HVOO included lower extremity edema, ascites, and abdominal pain. Abnormal laboratory findings included elevated transaminases and elevated total bilirubin. Core biopsy findings consistent with HVOO were centrivenular or perivenular congestion, hemorrhage, and zone 3 hepatocyte atrophy (Figure 1)[15].

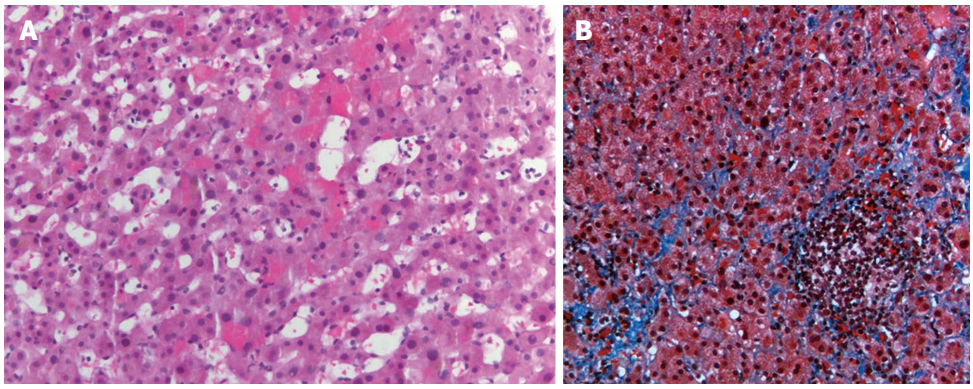

Figure 1 Hemotoxylin-eosin stains of a core liver biopsy from a patient with venous outflow obstruction shows hemorrhage within sinusoidal spaces (A) as well as evidence of sinusoidal fibrosis on a trichrome stain (B).

Liver transplantation

For patients in the study group, liver transplantation was performed between February 1998 and August 2013. Liver transplantation was performed due to hepatitis C virus induced cirrhosis [11/15 (73%)], primary sclerosing cholangitis [2/15 (13%)], alcoholic cirrhosis [1/15 (7%)] and autoimmune hepatitis induced cirrhosis [1/15 (7%)]. Two patients received living donor right liver grafts and the remaining patients received deceased donor transplants. Living donor transplant hepatic venous anastomoses were performed with either donor right hepatic vein (RHV) to recipient vena cava anastomosis or donor RHV to recipient RHV. Ten deceased donor transplant hepatic venous anastomoses were performed with piggyback technique, one was performed with side-to-side anastomosis, and two donor transplant hepatic venous anastomoses were unspecified (operative records not available in those two cases).

Venography and angioplasty

Informed consent regarding percutaneous hepatic venography, PTA and percutaneous or transjugular liver biopsy was obtained from the patient or the patient's health care proxy during routine clinical care. Percutaneous venography and subsequent PTA was performed in the same session in all procedures. The procedure was primarily performed *via* right internal jugular (IJ) venous puncture [16/21 (76%) procedures]. In patients with unfavorable anatomy, other routes were used [right IJ and right common femoral vein 2/21 (10%), right common femoral vein only 2/21 (10%), left common femoral vein 1/21 (5%)].

After obtaining access to the infra-renal IVC and/or hepatic veins, pressure gradient between the hepatic vein/IVC and right atrium were determined using manometry. A decision to proceed to PTA was made by the operator based on one or more of the following criteria: the presence of pressure gradient > 2-3 mmHg (20), imaging findings of stenosis (> 50% narrowing in the hepatic vein outflow or IVC or both relative to pre- and post-vessel caliber), persistent clinical findings, or a combination of the above.

PTA was performed matching the balloon diameter to that of the vein on the hepatic side of the stenosis. In case of poor response to initial PTA, a larger diameter or higher pressure balloon was used. After balloon dilatation, venography and manometry were repeated to evaluate the effectiveness of PTA. No anticoagulation was prescribed after PTA. One patient, who underwent hepatic vein stenting, was placed on oral Coumadin for anticoagulation.

Post-PTA liver biopsy

Liver core biopsies were performed after 13/21 (62%) PTA procedures. Post-PTA biopsies were performed once in 6 patients and multiple times in 9 patients for a total of 42 post-PTA biopsies. Of these biopsies, 35 (83%) were transhepatic and 7 (17%) were transjugular. Eighteen of 42 (43%) core biopsies were obtained using an 18 gauge needle, 3 (7%) were obtained using a 16 gauge needle, and the core biopsy needle caliber of the remaining procedures was not specified. The samples were sent to pathology placed in a formalin container. Early biopsy was defined as a biopsy performed within 2 mo ($\leqslant$ 60 d) of PTA and late biopsy was defined as a biopsy performed greater than 2 mo (> 60 d) after PTA.

Clinical follow-up

Medical records were accessed and available for all 15 patients. Medical records were reviewed for mortality, HVOO-related morbidity (*e.g.*, lower extremity edema, recurrent ascites), repeat interventional procedures to relieve HVOO, persistent biopsy proven HVOO, surgical correction, re-transplantation, or HVOO-related death.

Data collection

As part of our retrospective review, a single observer recorded the following data points: patient demographics, date and type of transplant, dates of all percutaneous revascularization procedures for the transplant hepatic veins, and dates and results of all transplant liver biopsies. For each percutaneous revascularization procedure, the name of the vessel, the luminal and

vessel diameter at the point of maximal narrowing and the pre-PTA and post-PTA venous pressure gradients were recorded. For each patient, medical records were reviewed and clinical outcomes were recorded as persistent HVOO or no HVOO-related symptoms up to death, re-transplantation or loss to follow-up.

Definitions

Technical success and patency rates were calculated. Technical success of PTA was defined as successful traversal of the stenosis with a catheter and completion of PTA. Clinical success was defined as resolution or improvement in presenting signs, symptoms or laboratory data or no further need for revascularization. Complications were defined by the Society of Interventional Radiology classification system[17]. Minor complications were defined as those requiring nominal or no additional treatment. Major complications were defined as those requiring significant additional treatment or hospitalization or those causing permanent sequelae up to death.

Primary patency was defined as the interval between initial PTA and first instance of a repeat hepatic venogram necessitated by adverse clinical status. Primary assisted patency was defined as patency after initial PTA until treatment with percutaneous intervention was abandoned.

"Good outcomes" were defined as resolution of clinical signs, symptoms, laboratory and/or imaging findings, resolution of venous congestion on biopsy findings and/or death due to non-HVOO related reasons. "Poor outcomes" were defined as unresolved clinical signs, symptoms, laboratory and/or imaging findings, re-transplantation, surgical correction and/or HVOO-related death.

Statistical analysis

Statistical analysis of pressure gradients before and after PTA was performed using a paired students *t*-test. Kaplan-Meier analysis was used to determine primary patency and primary-assisted patency rates[3,4,6-9]. Patency rates were calculated for patients with sufficient clinical documentation during follow-up intervals. Patients were censored if they expired, underwent retransplantation for non-HVOO related causes or were lost to follow-up during the study interval. Fisher's exact test was used to calculate correlation between biopsy findings and clinical outcomes. $P < 0.05$ was considered to be significant. Data processing and analysis was performed using Microsoft Excel (Microsoft, Seattle, WA) and online statistical calculators (www.vassarstats.net).

RESULTS

Fifteen patients (10 males, 5 females, 54 ± 8 years) consecutive patients underwent 21 PTAs, 94 ± 184 wk (range 4-652 wk) after transplantation for treatment of HVOO.

Procedure outcomes

All patients had successful traversal of the stenosis and PTA of the lesion resulting in a technical success rate of 100%. The gradient between stenosed vein and the right atrium was 7.5 ± 4 mmHg prior to PTA and 3.8 ± 3 mmHg after PTA ($P = 0.001$). The luminal diameter as a percentage of vessel diameters at the point of maximal narrowing was 50% ± 19% prior to PTA and 58% ± 22% after PTA ($P = 0.21$). PTA was performed once in 10 (66%) patients, twice in 4 (27%) patients and thrice in 1 (7%) patient. Primary patency was 79% at 30 d, 79% at 3 mo, 63% at 6 mo, 63% at 1 year and 52% at 3 years. Primary-assisted patency was 80% at 30 d and 79% at 3 mo, 6 mo, 1 year and 3 years. There were no minor or major complications.

Biopsy findings

Liver biopsies were performed prior to PTA in 19/21 (90%) procedures (Table 1). In patients with pre-PTA biopsy findings consistent with HVOO [13/19 (68%)], the maximum gradient ranged from 2-17 mmHg (8 ± 2.4 mmHg). In patients with no evidence of HVOO on pre-PTA biopsy [6/19 (32%)], the maximum gradient ranged from 5-11 mmHg (6.8 ± 4.3 mmHg). There was no significant difference in the pre-PTA pressure gradient between patients with evidence of HVOO on pre-PTA biopsy *vs* patients without evidence of HVOO on pre-PTA biopsy ($P = 0.35$).

In the 13 patients with pre-PTA biopsy showing hepatic venous congestion, 9/13 (70%) had clinical findings of venous stenosis (ascites, lower extremity edema, hepatomegaly, *etc*.) but only 4/13 (30%) had imaging findings of venous stenosis (on computed tomography, magnetic resonance imaging or ultrasound). In the 6 patients with pre-PTA biopsy showing no hepatic venous congestion 5/6 (83%) had clinical findings of venous stenosis and only 2/6 (33%) had imaging findings of venous stenosis.

Post-PTA liver core biopsies were performed after 13/21 (61%) PTA procedures. Early biopsy was performed after 10/21 (48%) PTA (mean 29 ± 21 d, range 2-48 d); late biopsy was performed after 9/21 (43%) PTA (mean 153 ± 81 d, range 62-304 d) and 8/21 (38%) patients had no biopsy after PTA. Of patients with late biopsy, 6/9 (67%) had both early and late post-PTA biopsy, 3/9 (33%) patients had only late biopsy after PTA.

Patients with evidence of HVOO on early biopsy ($n = 7$) included 3/7 patients (43%) with no HVOO-related complications on follow-up (205-3096 d) and 4/7 patients (57%) with HVOO related complications requiring repeat PTA or surgical revascularization (1-47 d). Patients without evidence of HVOO on early biopsy ($n = 3$) included 2/3 patients (67%) with no HVOO related complications on follow-up (62-964 d) and 1/3 patients (23%) requiring repeat PTA (at 66 d).

Table 1 Pre- and post-procedure gradients, biopsy findings and clinical outcomes

Patient No.	Procedure No.	Gradient (mmHg)		Biopsy findings indicating HVOO			Clinical outcome
		Pre	Post	Pre-	< 60 d	> 60 d	
1	1	8	NA	+	+	-	Good
2	1	7	NA	-	-	-	Good
3	1	8	4	+	+	-	Good
4	1	5	8	-	+	-	Good
5	1	9	9	NA	NA	-	Good
6	1	2	1	NA	NA	NA	Good
	2	5	4	-	NA	NA	Poor
7	1	15	17	+	+	NA	Poor
	2	17	12	+	NA	NA	Poor
8	1	7	2	+	NA	NA	Poor
9	1	NA	NA	+	NA	+	Poor
	2	6	1	+	NA	NA	Good
10	1	5	NA	-	NA	NA	Good
11	1	9	2	+	-	+	Poor
	2	8	1	-	NA	-	Good
12	1	2	2	+	NA	NA	Good
13	1	4	NA	+	-	-	Good
14	1	8	6	+	+	NA	Poor
	2	5	4	+	+	NA	Poor
	3	11	5	+	NA	NA	Poor
15	1	11	8	-	+	NA	Poor

HVOO: Hepatic venous obstruction; NA: Not available.

Table 2 Clinical outcomes in patients with both early (< 60 d) and late (> 60 d) biopsy after percutaneous transluminal angioplasty for hepatic venous obstruction

Early biopsy findings	Late biopsy findings	Clinical outcome (days post-PTA)
HVOO (3 patients)	No HVOO (3/3)	Non-HVOO related death (205 d) Non-HVOO related death (242 d) Doing well (3096 d)
No HVOO (3 patients)	No HVOO (2/3) HVOO (1/3)	Doing well (62, 964 d) Needed repeat PTA (66 d)

PTA: Percutaneous transluminal angioplasty; HVOO: Hepatic venous obstruction.

Two patients with evidence of HVOO on late biopsy [2/9 (22%)] underwent additional revascularization. Patients without evidence of HVOO on late biopsy [7/9 (78%)] did not need revascularization [4/7 (57%)] with no clinical issues and 3/7 (43%) with non-HVOO related death in 205-964 d.

Patients with both early and late biopsy (n = 6) showed late biopsy findings to be more predictive of clinical outcomes (Table 2).

In patients with no biopsy after PTA (n = 8), 2 (25%) patients died of non-HVOO related causes (12 and 86 d post-PTA), 4 (50%) needed revascularization (8, 22, 43, 138 d post-PTA) and 2 (25%) are doing well (215 and 223 d post-PTA).

The correlation of histologic findings on early *vs* late biopsy with clinical outcomes is outlined in Table 2.

Clinical outcomes

Clinical follow-up was available in all patients (mean: 56 ± 110 wk; Figure 2). Eight/15 (53%) patients had no recurrence of HVOO after a single PTA. Of the 7/15 (47%) patients with recurrence, 5 (71%) underwent repeat PTA, 1 (14%) underwent surgical revascularization and 1 (14%) needs further percutaneous treatment. Of the 5 patients undergoing repeat PTA 3/5 patients (60%) had no recurrence, 1/5 patients (20%) with recurrence required repeat PTA and 1/5 patients (20%) with recurrence resulting in re-transplantation (Table 1).

Importantly, of the 5 patients with clinical symptoms of HVOO without histological evidence of HVOO on pre-PTA biopsy, 60% (3/5) had a good outcome with resolution of clinical symptoms. Separately, in the 4/13 (30%) patients with histological evidence of HVOO without clinical symptoms of HVOO, 100% (4/4) had resolution of venous congestion on post-PTA biopsy.

Five out of 15 patients (33%) died of non-HVOO related causes (mean: 43 ± 54 wk, median 29 wk). Causes of death included cholestatic hepatitis, methicillin resistant staphylococcus aureus bacteremia and sepsis, recurrent hepatitis C virus infection in the transplant liver and disseminated intravascular coagulation in 2 patients. In the remaining 10 patients, 6 (60%) are alive with no signs or symptoms of HVOO (mean follow-up 142 ± 181 wk, median 40 wk). In the remaining

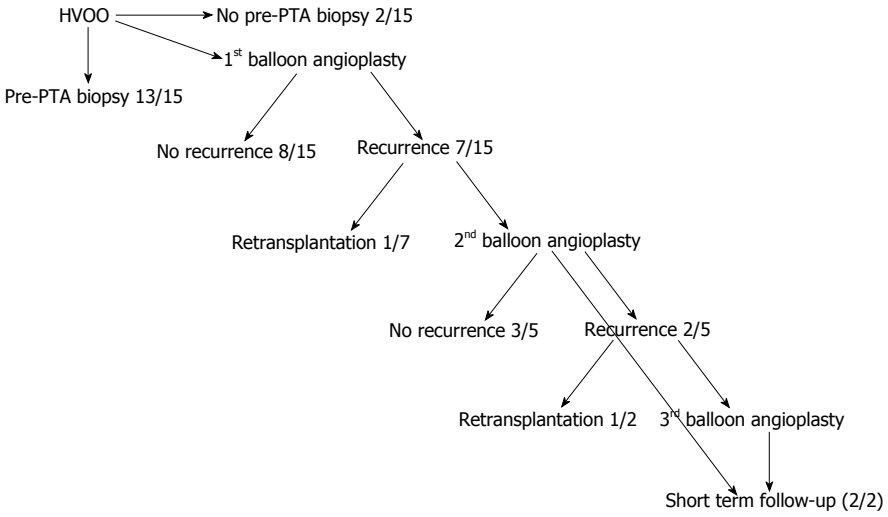

Figure 2 Flowchart presenting number of treatments and recurrence of hepatic venous obstruction in all patients. HVOO: Hepatic venous obstruction; PTA: Percutaneous transluminal angioplasty.

4 patients, 2 (50%) underwent surgical correction for HVOO and 2 (50%) still have signs and symptoms of HVOO (3 and 8 d post-PTA).

Overall, histological findings on biopsies < 60 d did not correlate with clinical outcomes (P = 0.99) whereas histological findings on biopsies > 60 d correlated with clinical outcomes (P = 0.02).

DISCUSSION

Percutaneous angioplasty and/or stent placement is the first-line of treatment in patients with HVOO after liver transplantation. Recognizing recurrence of HVOO after percutaneous treatment solely based on clinical, laboratory or imaging findings is difficult, and there is not a clear consensus regarding which measure provides the best or "gold standard" assessment for response to treatment. While liver biopsy is often utilized to determine response to therapy and need for repeated treatments, pathologic results after intervention have not been correlated to clinical symptoms nor long-term clinical outcomes.

Here, in a retrospective review of this cohort at our institution, we report the utility of biopsy in predicting outcomes of PTA in these patients with HVOO after liver transplantation. Specifically, we have found that patients without HVOO on a liver biopsy 60 d or more after PTA had no recurrence of HVOO on long term follow-up. Conversely patients with HVOO on a liver biopsy performed more than 60 d after PTA had recurrent stenosis or other adverse outcomes. On the other hand, liver biopsy findings of HVOO on early biopsy (less than 60 d after PTA) did not correlate with treatment durability or long term outcomes. This "latency" period of 60 d between percutaneous treatment of HVOO and resolution of histological changes may represent the time needed for the liver to recover following successful treatment of HVOO. In clinical terms, a patient with HVOO on biopsy less than 60 d after PTA may not need repeat PTA, if there are no associated clinical symptoms (*e.g.*, worsening ascites or lower extremity edema). On the other hand, patients with HVOO on biopsy more than 60 d after PTA represent an at-risk population and should undergo attempts at percutaneous or surgical revascularization.

In a recent study, Lorenz *et al*[9] reported the follow-up interval from PTA to the first biopsy demonstrating absence of HVOO. They found in 25 patients with primary inferior vena cava stenosis following liver transplantation, the interval from treatment to biopsy findings without evidence of HVOO was 37-4136 d. To our knowledge, no study has systematically investigated the ability of post-transplant liver biopsy to predict long-term response to percutaneous revascularization. However, features of HVOO on histology are known to overlap with features of other hepatic diseases such as chronic biliary disease or drug-induced reactions[15]. Therefore, the pathologist often recommends clinical correlation of histological findings suggestive of HVOO. While this may represent a limitation in our study, we used identical histological findings to categorize biopsy findings as HVOO in the two cohorts (biopsy less than and greater than 60 d after PTA) and found the latter to be more predictive of long-term outcomes.

Additionally, we found intra-procedural parameters such as pressure gradients or luminal diameter to be poor surrogate markers of existing histologic HVOO and poor predictors of histologic response to therapy (Figures 3 and 4). In our study, four patients with HVOO on pre-PTA biopsy had a gradient less than 6 and 1 patient had a gradient less than 3. Specifically, there was no significant difference in pre-PTA pressure gradients between patients with or without evidence of HVOO on a pre-PTA biopsy. Similarly, improvement in pressure gradients to < 3 mmHg or persistent pressure gradients > 3 mmHg were not always associated with

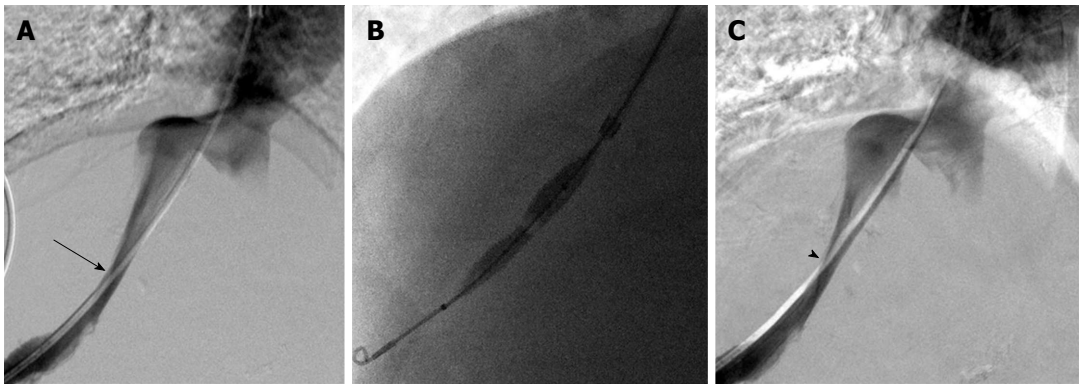

Figure 3 Fifty years old woman with elevated LFTs after liver transplantation and biopsy findings of venous outflow obstruction found to have a right hepatic vein stenosis (A, arrow); following angioplasty with a 10 mm × 4 cm balloon (B), there was decrease in pressure gradient from 8 mmHg to 1 mmHg, though the venographic appearance remained the same (C, arrowhead). Late biopsy demonstrated no evidence of venous outflow obstruction and the patient was doing well at 1 year follow-up.

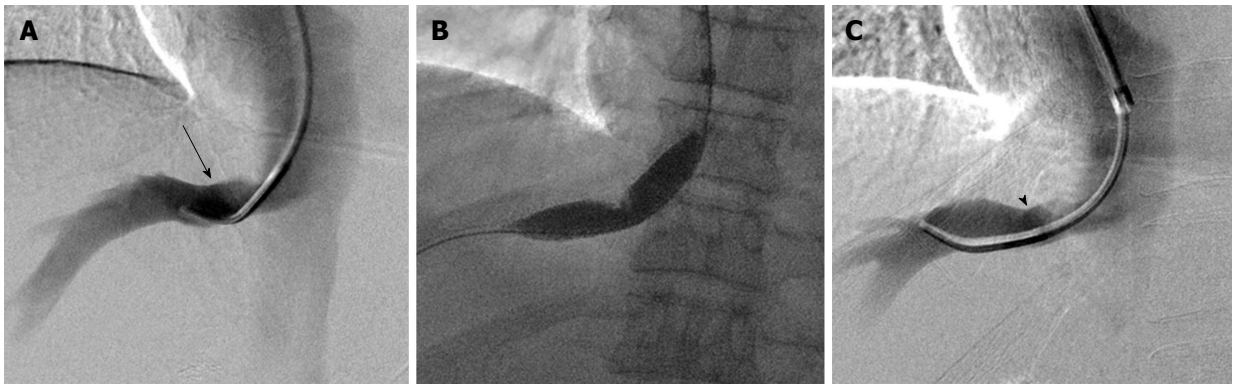

Figure 4 Sixty-six years old man with elevated LFTs after liver transplantation and biopsy consistent with venous outflow obstruction found with right hepatic vein stenosis (A, arrow); following angioplasty with 6, 8, 10, and 12 mm × 4 cm balloons (B), mild improvement was seen in luminal diameter on venography (C, arrowhead). On late biopsy, the patient had persistent evidence of venous outflow obstruction and repeat angioplasty was performed.

good or poor clinical response, respectively (Table 1, *e.g.*, patients 3, 4, 5, 8). While gradients (rather than degree of stenosis on venography) have been the primary intra-procedural measure in all of the available studies on HVOO, there is currently no consensus on what constitutes an abnormal gradient between the hepatic veins and the right atrium. Early reports of PTA for HVOO recommended using a gradient of 10 mmHg[18,19] however, a surgical evaluation of normal hepatic vein to right atrium gradient during liver transplantation found the mean gradient to be less than 3 mmHg[20]. Multiple reports in the literature corroborate our findings of patients where an elevated post-PTA gradient (> 3 mmHg) can still have good clinical outcomes[3,4,6-8,21]. These findings suggest that a combination of clinical symptoms and biopsy findings are more accurate than any gradient threshold in diagnosing HVOO and a good clinical outcome may be obtained despite a post-PTA gradient > 3 mmHg.

Similarly, we found that 70% of patients with histological evidence of HVOO also have clinical symptoms but more importantly, even the 30% of these patients who do not have clinical symptoms show resolution of their histological findings after PTA. We also found that 83% of patients can have clinical symptoms of HVOO without histological evidence of HVOO. These findings support the combined used of clinical symptoms and pre-PTA biopsy findings in diagnosing HVOO and pursuing interventional treatment.

Finally, we report primary patency rates of 53% at 3 years and primary assisted patency rates of 79% at 3 years after only PTA for HVOO. This is similar to post-PTA patency rates reported in the literature using Kaplan-Meier analysis (Table 3). Some authors advocate the use of primary stenting for HVOO when it occurs early in the post-transplant period or when dealing with primarily IVC stenosis[3,9]. The patency rates for these studies were similar to our results, however, the hepatic veins are technically challenging for stent deployment and stent migration is a rare but severe complication[3,8]. Therefore, we agree with the use of PTA as a primary treatment for HVOO and reserving use of hepatic venous stenting for patients refractory to multiple PTA sessions, as previously proposed[8,22].

There are some limitations to our study. This includes a small sample size and retrospective nature of the study as well as the absence of a control group. However, given the low frequency of HVOO in liver transplant patients

Table 3 Published studies of efficacy of percutaneous treatment of hepatic venous obstruction using the Kaplan-Meier method

Ref.	Transplant type	Procedure	*n*	Follow-up (converted to weeks)	Primary patency rates									Primary assisted patency rates									Outcomes	Complications
					1 mo	3 mo	6 mo	12 mo	18 mo	36 mo	60 mo	84 mo	120 mo	1 mo	3 mo	6 mo	12 mo	18 mo	36 mo	60 mo	84 mo	120 mo		
Kubo *et al*[4]	LDLT	19 PTA, 1 PTA + stent	20	20-334 wk (med: 129 wk)			80	65	60		60	60	NA			100	100	100		100	NA	NA	9/20 recurrence, 0% retransplanted, 0% HVOO death	*n* = 1, transient hypotension
Lorenz *et al*[7]	OLT 15 LDLT 1	12 PTA, 2 PTA + stent, 3 only stent	16	45-244 wk (mean: 161 wk)	100	75		67	67	60	NA	NA	NA	93	91	90	89	88	83	NA	NA	NA	3/12 recurrence, 3/12 retransplanted, 3/12 non-HVOO deaths, 1/12 HVOO death	*n* = 1, transient hypoxia
Ko *et al*[3]	LDLT	108 only stent	108	52-360 wk (mean: 39 mo)					82		75	72	NA	NR	NR	NR	NR	NR	NR	NR	NR	NR	22/108 recurrence, 2/108 retransplanted, 3 HVOO deaths	4.60%, stent migration, stent malposition
Ikeda *et al*[6]	LDLT	9 PTA, 2 PTA + stent	11	20-292 wk (med: 60 wk)	78	78	67	67	56			56	NA	100	100	100	100			100	NA	NA	6/10 recurrence, 1/10 retransplanted, 2/10 death, 2/2 hepatic	*n* = 1, abdominal pain
Yabuta *et al*[8]	LDLT	41 PTA, 7 PTA + stent	48	4-728 wk (med: 206 wk)				64		57	57		52				98		95	95		95	20/48 recurrence, 2/48 retransplanted, 0/48 HVOO-related deaths	*n* = 1, stent migration
Lorenz *et al*[9]	OLT 23 LDLT 2	14 PTA, 4 PTA + stent, 10 only stent	25	1-603 wk (mean: 192 wk)			57	51		51	51	51	NA			57	51		51	51	51	NA	6/14 recurrence, 1/14 retransplanted, 3/14 died of primary graft failure	*n* = 1, fractured stent

LDLT: Living donor liver transplant; OLT: Orthotropic liver transplant; PTA: Percutaneous transluminal angioplasty; NA: Not available; NR: Not reported.

as well as the consequences of untreated HVOO, it is difficult to perform a prospective study or include a control group. Existing literature faces similar issues, as available studies to date include a similar number of patients and study design (2-109 patients)[3-9,13,18,19,21,22]. Secondly, a number of patients (4/15) did not undergo biopsy after PTA, however even within this group 2/4 patients had good clinical outcomes. A post-PTA biopsy may have helped distinguish these two patients with good outcomes from the six patients with poor outcomes. Therefore, biopsy has a role in stratifying patients with HVOO. Additionally, a small number of patients underwent revascularization based solely on clinical symptoms. We also acknowledge that the timeframe of histologic response after intervention for HVOO is variable, poorly characterized in the literature and likely not known. Further studies to correlate histologic changes to our 60 d timeframe are required, though this may be difficult to achieve given the small patient numbers available. Future studies can, however, help delineate if a post-PTA biopsy adds prognostic value above and beyond clinical or laboratory findings in determining recurrence of HVOO.

In conclusion, our study suggests that liver biopsy performed more than 60 d after treatment may be used to predict long-term clinical outcomes after primary PTA for relieving HVOO after liver transplantation.

COMMENTS

Background

Hepatic venous outflow obstruction (HVOO) is an uncommon complication after liver transplantation, occurring in 1.5%-2.5% of patients with orthotropic liver transplantation using the piggyback technique and up to 9.5% of patients with living donor liver transplantation. HVOO can be treated either by percutaneous transluminal angioplasty (PTA) (*i.e.*, using an inflatable balloon to treat a luminal stenosis; PTA), hepatic venous stenting, or when percutaneous revascularization is unsuccessful, by surgical revision. As such, liver biopsies are frequently performed to assess HVOO response to endovascular intervention and to distinguish persistent HVOO from other diseases in liver transplants. Correlation of histologic findings of HVOO with clinical findings such as pressure gradients between the hepatic vein and right atrium on manometry is not well studied. Furthermore, change in histologic findings following successful endovascular treatment is currently unknown. Therefore, the purpose of the authors' study was to evaluate histologic findings at liver biopsy after PTA for HVOO in liver transplant patients and correlate these to treatment response and long-term outcome.

Research frontiers

The use of an appropriately timed biopsy to determine response to treatment after angioplasty of hepatic veins will improve patient outcomes and reduce uncertainity in treating these patients.

Innovations and breakthroughs

Although the techniques used to treat and assess these patients are well known. The precise correlation of histological findings with clinical findings, liver function tests and imaging findings as well as the effect of treatment on histological findings is not well known. This study shows the accuracy of post-angioplasty biopsy in determining prognosis.

Applications

These findings suggest that in patients who do not immediately respond to balloon angioplasty with improvement in clinical symptoms should undergo biopsy to determine histological response. However, the biopsy should be performed up to 60 d after endovascular treatment.

Peer-review

This is an interesting paper that assesses the utility of liver histopathology to predict the outcome of PTA after HVOO in transplant patients.

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