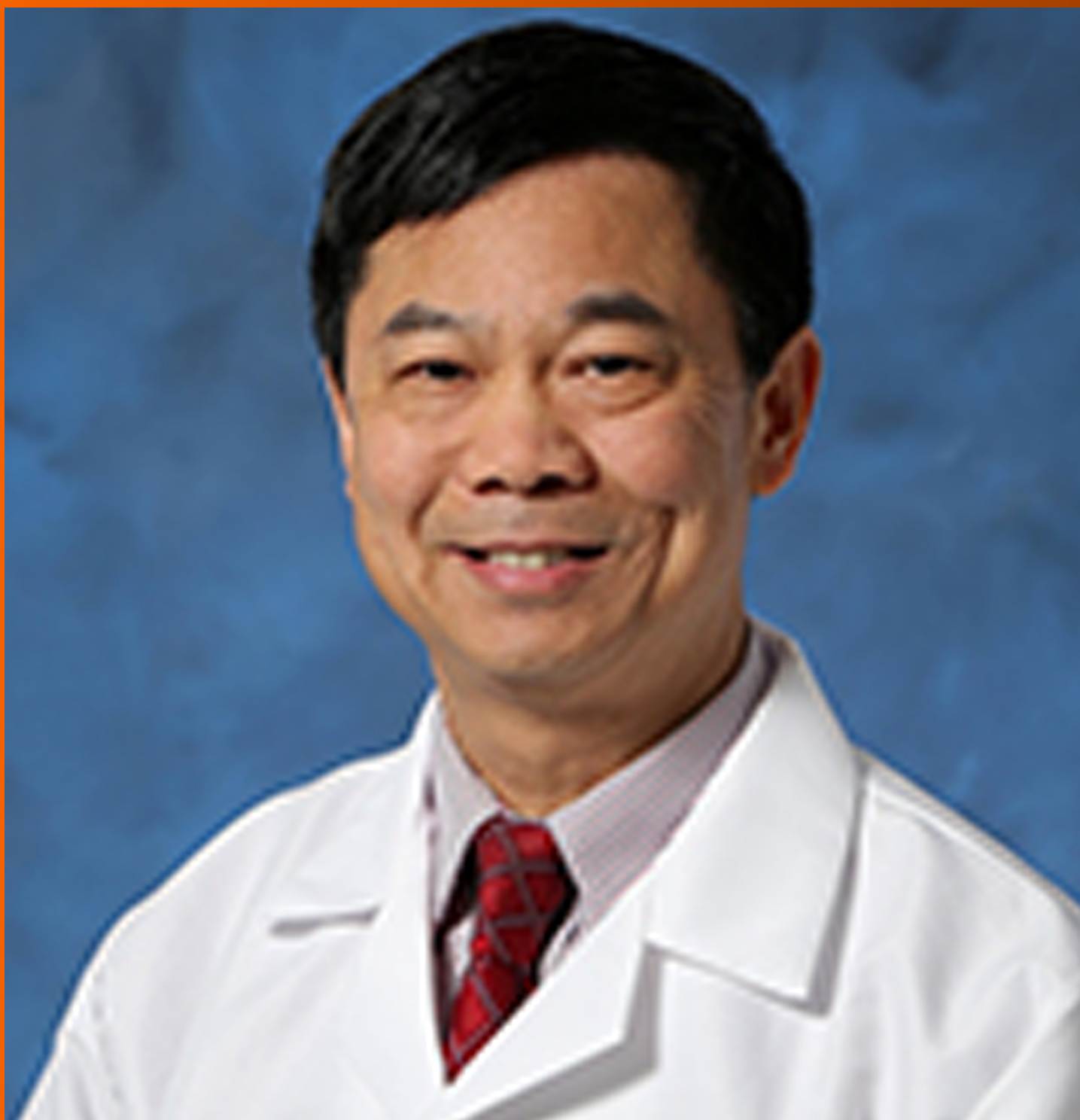


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Editor-in-Chief of *World Journal of Hepatology*, Ke-Qin Hu, FAASLD, MD, Director, Professor, Division of Gastroenterology and Hepatology, University of California, Irvine Medical Center, Orange, CA 92868, United States

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WJH covers topics concerning liver biology/pathology, cirrhosis and its complications, liver fibrosis, liver failure, portal hypertension, hepatitis B and C and inflammatory disorders, steatohepatitis and metabolic liver disease, hepatocellular carcinoma, etc. Priority publication will be given to articles concerning diagnosis and treatment of hepatology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, etc.

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Bariatric surgery in patients with non-alcoholic fatty liver disease - from pathophysiology to clinical effects

Tea L Laursen, Christoffer A Hagemann, Chunshan Wei, Konstantin Kazankov, Karen L Thomsen, Filip K Knop, Henning Grønbaek

ORCID number: Tea L Laursen (0000-0003-2494-0526); Christoffer A Hagemann (0000-0001-6917-3223); Chunshan Wei (0000-0002-3960-4069); Konstantin Kazankov (0000-0002-8111-213X); Karen L Thomsen (0000-0002-8118-4643); Filip K Knop (0000-0002-2495-5034); Henning Grønbaek (0000-0001-8998-7910).

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Tea L Laursen, Chunshan Wei, Konstantin Kazankov, Karen L Thomsen, Henning Grønbaek, Department of Hepatology and Gastroenterology, Aarhus University Hospital, Aarhus N DK-8200, Denmark

Christoffer A Hagemann, Gubra ApS, Hørsholm 2970, Denmark

Christoffer A Hagemann, Filip K Knop, Clinical Metabolic Physiology, Steno Diabetes Center Copenhagen, Gentofte Hospital, Hellerup 2900, Denmark

Christoffer A Hagemann, Filip K Knop, Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen 2200, Denmark

Chunshan Wei, Department of Hepatology, Shenzhen Traditional Chinese Medicine Hospital, the Fourth Clinical Medical College of Guangzhou University of Chinese Medicine, Shenzhen 518033, China

Filip K Knop, Novo Nordisk Foundation Center for Basic Metabolic Research, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen 2200, Denmark

Corresponding author: Henning Grønbaek, MD, PhD, Professor, Department of Hepatology and Gastroenterology, Aarhus University Hospital, 99 Palle Juul-Jensens Boulevard, Entrance C, Level 1, C116, Aarhus N DK-8200, Denmark. henngroe@rm.dk
Telephone: +45-21-679281

Abstract

Non-alcoholic fatty liver disease (NAFLD) is increasingly recognized as a significant liver disease, and it covers the disease spectrum from simple steatosis with a risk of development of non-alcoholic steatohepatitis (NASH) to fibrosis, subsequent cirrhosis, end-stage liver failure, and liver cancer with a potential need for liver transplantation. NAFLD and NASH are closely related to obesity, metabolic syndrome, and type 2 diabetes (T2D). The role of gut hormones, especially glucagon-like peptide 1 (GLP-1), is important in NAFLD. Bariatric surgery has the potential for inducing great weight loss and may improve the symptoms of metabolic syndrome and T2D. Recent data demonstrated significant effects of bariatric surgery on GLP-1 and other gut hormones and important lipid metabolic and inflammatory abnormalities in the pathophysiology of NAFLD. Therefore, bariatric surgery may reverse the pathological liver changes in NAFLD and NASH patients. In the present review, we describe NAFLD and NASH pathophysiology and the primary effects of bariatric surgery on metabolic pathways. We performed a systematic review of the beneficial and harmful

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effects and focused on changes in liver disease severity in NAFLD and NASH patients. The specific focus was liver histopathology as assessed by the invasive liver biopsy. Additionally, we reviewed several non-invasive methods used for the assessment of liver disease severity following bariatric surgery.

Key words: Non-alcoholic fatty liver disease; Non-alcoholic steatohepatitis; Bariatric surgery; Insulin resistance; Gut hormones; Glucagon-like peptide 1; Steatosis; Inflammation; Fibrosis

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Core tip: Non-alcoholic fatty liver disease (NAFLD) is a significant liver disease with risks of steatohepatitis (NASH), fibrosis, and cirrhosis. NAFLD and NASH are closely related to obesity, the metabolic syndrome, and type 2 diabetes (T2D). Bariatric surgery induces weight loss and improves the features of the metabolic syndrome and T2D. Surgery may reverse pathological liver changes. In the present review, we focus on the primary effects of bariatric surgery on metabolic pathways and systematically reviews the effects of bariatric surgery on changes in liver disease severity in NAFLD and NASH patients.

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INTRODUCTION

Alarming increases in obesity and diabetes coupled with changes towards unhealthy lifestyles and dietary habits have contributed to a dramatic increase in non-alcoholic fatty liver disease (NAFLD), which affects 25%-30% of the general population^[1]. NAFLD is most often asymptomatic and consists of a disease spectrum ranging from simple steatosis (NAFL) and steatohepatitis (NASH) to fibrosis and cirrhosis, with significant clinical consequences, including but not limited to ascites, varices, hepatic encephalopathy, liver cancer and liver transplantation or early death^[2]. NASH may develop with hepatic inflammation, hepatocellular injury, macrophage and hepatic stellate cell activation in patients with simple steatosis. If untreated, NASH may progress to cirrhosis. NASH-induced cirrhosis is fast becoming the most common indication for liver transplantation, which is strongly associated with poor quality of life^[3].

NAFLD is closely related to obesity and type 2 diabetes mellitus (T2D), and it is often termed the hepatic manifestation of metabolic syndrome^[4,5]. The prevalence of NASH increases with components of the metabolic syndrome in T2D^[6]. Available epidemiological data suggest a prevalence of NAFLD of 40%-70% in European T2D patients^[1]. Insulin resistance is more prevalent in NASH patients than patients with simple steatosis^[7], and patients with NAFLD without T2D exhibit decreased insulin sensitivity^[8].

Bariatric surgery is an efficient treatment of obesity and causes sustained weight loss with potential reductions in hepatic fat, inflammation and fibrosis^[9,10]. Roux-en-Y gastric bypass surgery (RYGB) is the most effective treatment for obesity^[11]. This procedure improves glycaemic control, and T2D patients experience a reduced need for antidiabetic medication within days after surgery^[9]. Sleeve gastrectomy (SG) and adjustable gastric banding (AGB) are alternative surgical approaches that significantly reduce gastric volume without changing the upper gastrointestinal tract anatomy. SG has gained popularity in recent years and been established as a comparable method to RYGB. In contrast, AGB is associated with less weight loss than RYGB surgery. The indication for bariatric surgery is severe obesity with or without T2D and/or other comorbidities^[9].

LITERATURE SEARCH

We searched the following databases: MEDLINE Ovid (1946 to June 2018), Science Citation Index Expanded (Web of Science; 1900 to June 2018), and PubMed [Bethesda (MD): National Library of Medicine (US) 1966 to June 2018]. The following search terms were used: “Non-alcoholic fatty liver disease” (MeSH, all fields) or “Non-alcoholic steatohepatitis” (all fields) and “bariatric surgery (MeSH, all fields). Only English language articles were selected, and case reports were excluded. Full-text evaluation was performed, and references from relevant manuscripts were reviewed manually for additional manuscripts. This search strategy identified 404 studies at the end of June 2018. Studies were included in our comprehensive review if they were published between January 2010 and June 2018, were prospective or retrospective observational studies and if they evaluated the effects of bariatric surgery on histopathological NAFLD. In total, we ended up with 13 studies.

PATHOGENESIS OF NAFLD

The pathogenic mechanisms for the development and progression of NAFLD are complex and multifactorial^[12] (Figure 1). Genetic and epigenetic factors affect the development of NAFLD and NASH progression and potentially influence or modify risk factors^[13-16]. Dietary sugars, fat, adipose tissue lipolysis, and *de novo* lipogenesis contribute to increased hepatic fat influx and accumulation in obese patients^[17]. Obese patients exhibit increased adipose tissue mass, which leads to adipocyte dysfunction, including insulin resistance, increased lipolysis and apoptosis, and results in local inflammation and cytokine release. Insulin resistance reduces insulin-induced inhibition of lipolysis, and negatively affects the ability of the adipose tissue to store fat, which results in increased free fatty acids in the blood. Insulin resistance induces further insulin secretion, which instigates high blood insulin levels^[18,19].

Hepatic *de novo* lipogenesis is also augmented in obese patients, partially due to enzyme upregulation induced by hyperinsulinaemia, elevated plasma glucose levels and endoplasmic reticulum (ER) stress^[20-22]. Lipid accumulation in the liver primarily consists of triglycerides, which may not be hepatotoxic *per se*, but reflects the general inability of hepatocytes to handle fatty acids and leads to the concurrent accumulation of toxic lipid metabolites^[23-25]. Long-chain saturated fatty acids resulting from *de novo* lipogenesis specifically harm liver cells via triggering the formation of reactive oxygen species, which highly contribute to hepatic lipotoxicity^[19].

Activation of death receptors and their ligands, induction of ER stress, the production of reactive oxygen species and mitochondrial stress and dysfunction lead to hepatocyte injury and death with subsequent release of proteins, debris, *etc.*, which are collectively defined as damage-associated molecular patterns (DAMPs)^[26,27]. Fatty acids, DAMPs and pathogen-associated molecular patterns (PAMPs), *e.g.*, bacteria and endotoxins, likely originating from a leaky gut, are the primary inducers of hepatic inflammation, which involves activation of resident and recruited macrophages in the liver^[28,29]. Macrophage activation results in pro-inflammatory cytokine secretion and the activation of hepatic stellate cells into myofibroblasts, which secrete the collagen that contributes to extracellular matrix formation. Myofibroblasts are also directly responsive to cytokines, DAMPs and PAMPs, thus further propagating fibrosis formation^[30].

METABOLIC EFFECTS OF BARIATRIC SURGERY

Bariatric surgery has tremendous effects on metabolic functions. Buchwald *et al.*^[31] performed a meta-analysis of 136 studies that assessed the impact of bariatric surgery on metabolic outcomes and reported a complete resolution of T2D in more than 75% of diabetic patients and an excessive weight loss of almost 60%. A review of key results from the Swedish Obese Subjects study reported a 72% remission rate of T2D two years post-bariatric surgery^[32]. Several other studies demonstrated that RYGB and SG were superior to conventional pharmacological therapy in achieving glycaemic control in T2D patients^[33-35].

NAFLD is closely associated with obesity and T2D, and the mechanisms implicated in improving obesity and T2D following bariatric surgery likely play important roles in the resolution of NAFLD. Several mechanisms independent of weight loss are involved in the initial metabolic responses to RYGB and SG procedures and maintaining these improvements over the long term, despite the obvious causality between weight loss and improvements in T2D and NAFLD.

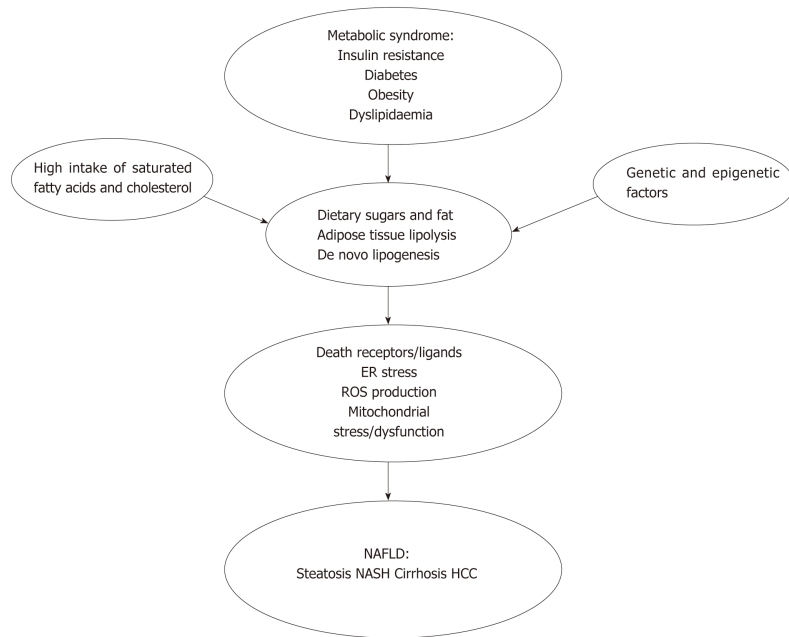


Figure 1 Risk factors and mechanisms associated with non-alcoholic fatty liver disease development and progression. NAFLD: Non-alcoholic fatty liver disease; NASH: Non-alcoholic steatohepatitis; ER: Endoplasmic reticulum; ROS: Reactive oxygen species; HCC: Hepatocellular carcinoma.

The almost immediate metabolic benefits of bariatric surgery, independent of any significant weight loss, have been known for decades^[36] but are striking nonetheless^[31,37,38]. Three primary mechanisms are involved in the improved glycaemic control associated with the RYGB and SG procedures: (1) early improved hepatic insulin sensitivity due to the post-surgery calorie restriction; (2) late improved peripheral insulin sensitivity due to weight loss; and (3) improved post-prandial insulin secretion due to a rise in glucagon-like peptide 1 (GLP-1) secretion (Figure 2). Several studies investigated the post-surgical metabolic changes, and whether the change in the release of gut hormones or surgery-induced restriction of food intake provides the essential effects on glycaemic control remains controversial. Jørgensen *et al.*^[38,39] found an increase in post-prandial GLP-1, insulin secretion and hepatic insulin sensitivity within days after RYGB, and this increase was sustained for at least 1 year in diabetic and non-diabetic-matched subjects, which is consistent with other RYGB and SG studies^[37,40,41]. The GLP-1 increase represents a powerful beta-cell stimulus and is explained by the accelerated entry of nutrients into the small intestine after RYGB^[42,43] and SG^[44], which increases the glucose absorption rate in the L cells responsible for the GLP-1 secretion^[45]. The accelerated transport of nutrients into more distal parts of the small intestine may further explain the exaggerated GLP-1 response because a higher density of L cells are found in this area^[46]. Notably, GLP-1 may have beneficial gene-regulatory effects on fatty acid oxidation and insulin sensitivity in hepatocytes^[47], but these findings require confirmation in humans. Postprandial glucagon responses also increase post-operatively^[38,48] despite the inhibitory effects of GLP-1 on glucagon secretion^[39]. This paradoxical effect (in the context of improved glucose metabolism) may represent gut-derived glucagon^[42] and may exert an attenuating effect on glycaemic control post-surgery.

Steven *et al.*^[49], among others^[50], demonstrated that the reduced liver fat content from calorie restriction explained the early improvement in hepatic insulin sensitivity, as illustrated using magnetic resonance imaging. These data suggest that significant caloric restriction explains the almost immediate metabolic benefits from bariatric surgery due to improved liver function^[49,51]. Vetter *et al.*^[48] demonstrated that the improvements in liver insulin sensitivity from RYGB exceeded lifestyle modifications. However, blockade of the GLP-1 receptor using the antagonist exendin9-39 consistently lowered insulin secretion after RYGB and SG^[39,52], and it reversed the postprandial hyperinsulinaemic hypoglycaemia observed post-operatively^[38], which confirmed the causative role of GLP-1 in beta-cell stimulation^[53-55]. Calorie restriction is the leading mechanism of the early metabolic changes after bariatric surgery, but the gut hormones, particular GLP-1, remain crucial for the fine-tuning of the glycaemic control and post-prandial insulin secretion^[56].

A hyperinsulinaemic clamp study of Bojsen-Møller *et al.*^[37] demonstrated that

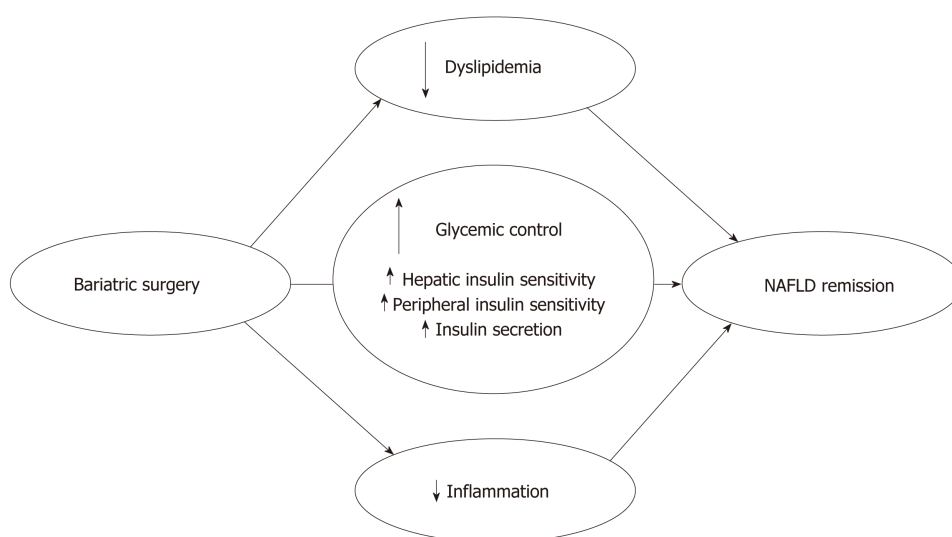


Figure 2 Main metabolic effects of bariatric surgery on remission. NAFLD: Non-alcoholic fatty liver disease.

peripheral insulin sensitivity, as assessed using glucose disposal and suppression of fatty acids, increased after three months in relation to the surgery-induced weight loss. Lifestyle modifications exert the same powerful effects on glycaemic control and NAFLD^[48,57], but this intervention generally fails to sustain the short-term weight loss^[32]. Notably, bariatric surgery is superior in maintaining calorie restriction and long-term weight loss, which is facilitated at least partially by the reduced appetite observed post-operatively^[58,59]. The long-term reduced appetite may be attributable to a favourable shift in the anorectic gut hormones GLP-1 and peptide-YY (PYY)^[58,60]. An antagonist study using exendin9–39 and a dipeptidyl peptidase 4 (DPP-4) inhibitor (blocking the DPP-4-mediated formation of active PYY from its precursor) in RYGB-operated subjects demonstrated a 20% increase in food intake^[61]. Several other factors are candidates for the long-term metabolic improvements of bariatric surgery, including ghrelin^[62], adiponectin^[63], increased plasma bile acids^[64] and changed intestinal microbiota^[65], but proof of causality for these factors remains to be established in humans.

HEPATIC EFFECTS OF BARIATRIC SURGERY

Bariatric surgery affects NAFLD not only through a rapid and substantial weight loss but also via simultaneous effects on important lipid metabolic and inflammatory pathways involved in NAFLD pathophysiology^[66,67]. Bariatric surgery promotes changes in three crucial metabolic areas influencing NAFLD: improved glucose homeostasis, improved lipid metabolism and reduced inflammatory activity (Figure 2). These effects are followed by significant effects on liver abnormalities in NAFLD and NASH patients.

Liver histology

A systematic review and meta-analysis^[68] in 2008 assessed the histological effects of bariatric surgery in NAFLD patients. This review concluded that the features of steatosis, steatohepatitis, and fibrosis improve or resolve in most patients following weight loss after bariatric surgery. A Cochrane review in 2010 reported more discrete conclusions^[69]. These authors were not able to identify any randomized clinical trials at that time and advised caution even though several reports demonstrated potential favourable effects of bariatric surgery. Most studies reported beneficial effects on steatosis, and more than half of the studies demonstrated significant improvements in histological inflammation. Six studies demonstrated improvement in fibrosis scores, but 4 studies^[70–73] reported some worsening of fibrosis.

Our literature search did not identify any randomized controlled trials that assessed the hepatic histological effects of bariatric surgery in NAFLD patients. We performed a comprehensive review of prospective and retrospective observational studies published since 2010 to evaluate the effects of bariatric surgery on histopathological NAFLD (Table 1). We identified 13 studies: Eight studies with prospective designs, two studies with retrospective designs and three studies in

which the design was not obvious. The types of surgery included RYGB, AGB and SG, and most studies assessed the effect of RYGB. The sample size ranged from 9 to 578 patients. The studies clustered into three categories based on participant numbers: Two large studies with more than 150 participants^[74,75], three studies with 50-150 participants^[10,76,77] and eight small studies with less than 50 participants^[78-85].

In the largest study by Caiazzo *et al.*^[74] including more than 500 patients, the effects of RYGB and AGB were compared. Improvement and resolution of steatosis, inflammation and fibrosis were observed one and five years after both types of surgery. Biopsies at all three time points (before and 1 and 5 years post-surgery) were available in 315 patients, and the authors did not describe any cases of worsening. The best effects on weight loss and liver histology were achieved in the RYGB patients, and the primary effect derived from a greater weight loss but additionally explained by a more positive influence on glucose and lipid metabolism.

The second largest study of 160 patients undergoing RYGB or ABG with a mean follow-up of 31 mo demonstrated resolution or improvement of steatosis and inflammation in most patients^[75]. Fibrosis resolved or improved in more than half of the patients. However, 8% of the patients progressed or developed steatosis *de novo* after surgery. Portal inflammation worsened in 10% and developed *de novo* in 27% of the patients, and 16% developed lobular inflammation after surgery. Fibrosis progressed in 12% of the patients with pre-surgery fibrosis and another 21% developed *de novo* fibrosis. Three patients developed NASH *de novo* after surgery.

In general, all of the smaller studies reported improvements in steatosis, inflammation and fibrosis. However, three of these studies found a worsening of some histological features, *e.g.*, inflammation^[79] and fibrosis^[77,81], and three other studies reported no worsening^[76,78,80]. The remaining studies did not describe worsening in any patients. The histological liver changes were accompanied with beneficial effects on metabolic syndrome^[78,79], hypertension, dyslipidaemia and obstructive sleep apnoea^[84]. Most studies performed follow-up biopsies after one year or later after surgery, but two studies performed follow-up at three^[83] and six^[80] months. Notably, the effects of surgery were visible at these time points, even for fibrosis.

Lassailly *et al.*^[10] investigated differences in patients with resolution of NASH one year after surgery and patients with persistent NASH and found that these patients had lost significantly less weight and were more frequently classified with a refractory IR profile, which suggests that the weight loss was of primary importance.

Non-invasive methods

Other studies used diverse non-invasive methods to examine the hepatic effects of bariatric surgery and found improvements in general. Several studies investigated how bariatric surgery affected the levels of circulating liver transaminases, in general reporting favourable effects, as summarized in a meta-analysis^[86]. However, transaminases exhibit limited accuracy for the prediction of NASH severity.

Several studies used non-invasive fibrosis scores. One study demonstrated decreases in NAFLD fibrosis scores, ratio of aminotransferase (AST) to alanine aminotransferase (ALT), AST-to-platelet ratio index (APRI), and BARD score (BMI, ASAT/ALAT ratio, and the presence of T2D) one year after surgery^[87]. Decreases in the NAFLD fibrosis score were confirmed in several other studies. Two studies of RYGB found a significant decrease 12 mo^[88] and 36 mo after surgery^[89], but higher levels in patients who regained weight after the initial weight loss. One study of 56 adolescents described a decrease in NAFLD fibrosis score one and two years after AGB^[90]. However, Simo *et al.*^[91] questioned the feasibility of the NAFLD fibrosis score in relation to RYGB because several patients were wrongly classified or ended in a group of indeterminate classification. None of these studies included pre- or post-surgery biopsies as golden standards for treatment effects.

Forty-two patients who underwent diverse types of surgery were followed up with liver stiffness measurements and controlled attenuation parameter (CAP) using transient elastography. Liver stiffness declined from 8.6 to 6.0 kPa one year after surgery. CAP values declined from 322 dB/m at baseline to 251 dB/m at one year. These changes paralleled the histological changes in 32 of the patients^[85]. The decrease in liver stiffness was consistent with a decrease from 6.95 to 5.37 kPa in another study of 38 patients after bariatric surgery^[92]. Another study of 100 prospectively included bariatric patients reported a decrease from 12.9 to 7.1 kPa^[87].

Magnetic resonance imaging (MRI) is increasingly used in the assessment of liver pathology. A prospective study of 31 obese patients undergoing RYGB demonstrated a significant reduction in hepatic fat content using MRI spectroscopy 12 mo after surgery^[93]. A decrease in the liver fat fraction using MRI was also observed six^[94] and 12 mo^[95] after surgery in other studies. These results were supported by a decreased or complete resolution of liver steatosis/NAFLD on ultrasonography one to five years

Table 1 Recent studies (2010-) with histological assessment of liver biopsies at follow-up

Ref.	Design	Patients with follow-up	Surgical intervention	Steatosis	Inflammation	Fibrosis	Any cases of worsening	Mean/median follow-up in months
Weiner <i>et al</i> ^[76] , 2010	Missing	116	RYGB, AGB, BPD-DS	Improved, 70% complete resolution	Improved, 86% complete resolution	Improved	No	19.4 (± 8.3)
Moretto <i>et al</i> ^[77] , 2012	Retrospective	78	Gastric bypass	Improved	Improved ballooning, 55% complete resolution of NASH	Trend for improvement	Yes	Unknown
Vargas <i>et al</i> ^[78] , 2012	Prospective	26	RYGB	Improved	Improved, 84% complete resolution of NASH	Improved	No	16 (± 3)
Tai <i>et al</i> ^[79] , 2012	Prospective	21	RYGB	Improved, 95% complete resolution	Improved, 100% complete resolution of NASH	Improved	Yes	12
Caiazzo <i>et al</i> ^[74] , 2014	Prospective	578 (1 yr), 413 (5 yr)	RYGB, AGB	Improved	Improved	Improved	NA	12 and 60
Lassailly <i>et al</i> ^[10] , 2015	Prospective	82	Gastric bypass, AGB, SG, PBD-DS	Improved	Improved, 85% complete resolution of NASH	Improved	NA	12
Praveen <i>et al</i> ^[80] , 2015	Prospective	30	RYGB, SG	Improved in 97%	Improved in 46%	Improved in 46%	No	6
Taitano <i>et al</i> ^[75] , 2015	Missing	160	RYGB, AGB	Improved, complete resolution in 73%	Improved, 88% complete resolution of NASH	Improved, 53% complete resolution	Yes	31 (± 26)
Schneck <i>et al</i> ^[81] , 2016	Missing	9	RYGB	Improved in all patients	Improved in all patients	Improved	Yes	55 (44-75)
Froylich <i>et al</i> ^[82] , 2016	Retrospective	25	RYGB, SG	Improved	Improved	Trend for improvement	NA	18
Aldoheyen <i>et al</i> ^[83] , 2017	Prospective	27	SG	Improved	Improved	Improved	NA	3
Manco <i>et al</i> ^[84] , 2017	Prospective	20	SG (<i>n</i> = 20) <i>vs</i> IGWLD, NSWL	Improved	Improved, 100% complete resolution of NASH	Improved	NA	12
Garg <i>et al</i> ^[85] , 2018	Prospective	32	RYGB, AGB, SG	Improved	Improved	Improved	NA	12

RYGB: Roux-en-Y gastric bypass; AGB: Adjustable gastric banding; BPD-DS: Biliopancreatic diversion with duodenal switch; SG: Sleeve gastrectomy; IGWLD: Intra-gastric weight loss device; NSWL: Nonsurgical weight loss; NA: Not assessed.

after SG^[96,97]. A decrease in hepatic left lobe volume on ultrasound was also observed after two years in 75 women who underwent laparoscopic AGB^[98]. Two small studies found improvements in liver damage and NAFLD using ultrasound imaging^[99] and CT^[100].

Additional exploratory results include decreased serum levels of the hepatocyte apoptosis marker cytokeratin (CK)-18 one year after surgery in nine patients, which was maintained approximately four years later with corresponding improvements in liver histology^[81]. Significant improvements in metabolic liver function capacity using LiMax, which is based on hepatic ¹³C-methacetin metabolism by cytochrome P450 1A2, were also observed six and 12 mo after surgery^[101].

Liver-related mortality

Overall, current studies reported no significant effects on liver-related mortality after bariatric surgery, but larger long-term follow-up studies are necessary to firmly establish the effect of bariatric surgery on liver-related mortality. A recent nation-wide study of patients after bariatric surgery observed no increase in all-cause mortality compared with the general population. However, there was an increased mortality rate ratio 2.01 (95% CI: 1.06-3.84) for gastrointestinal and liver diseases, including

peritonitis and intestinal obstruction^[102]. Mortality was significantly reduced in patients undergoing bariatric surgery compared to a propensity-score matched cohort of obese patients, but there was no difference in survival when the analysis was restricted to include NASH patients only^[103]. Liver cirrhosis is a relative contraindication to bariatric surgery. However, no increased risks of postoperative complications or cirrhosis-related complications were observed in 13 cirrhosis patients undergoing SG with a follow-up of 18 mo. Weight loss in the cirrhosis patients was comparable to the non-cirrhotic patients^[104].

CONCLUSION

In conclusion, bariatric surgery has the potential to induce great weight loss and improve the features of metabolic syndrome and T2D. Recent data demonstrate significant effects of bariatric surgery on GLP-1 and other gut hormones and important lipid metabolic and inflammatory abnormalities involved in the pathophysiology of NAFLD. Therefore, bariatric surgery may reverse the pathological liver changes in NAFLD and NASH patients. Several cohort studies demonstrated improvements in NASH histology, but some studies reported worsened liver histology after bariatric surgery. No studies demonstrated reduced liver-related mortality.

Large randomized clinical trials with long-term follow-up are needed to demonstrate the beneficial effects of bariatric surgery and identify a definitive role of bariatric surgery in NASH patients.

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Colorectal liver metastases: An update on multidisciplinary approach

Felix Che-Lok Chow, Kenneth Siu-Ho Chok

ORCID number: Felix Che-Lok Chow (0000-0001-8800-9525); Kenneth Siu-Ho Chok (0000-0001-7921-3807).

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Felix Che-Lok Chow, Department of Surgery, Queen Mary Hospital, Hong Kong, China

Kenneth Siu-Ho Chok, Department of Surgery and State Key Laboratory for Liver Research, the University of Hong Kong, Hong Kong, China

Corresponding author: Kenneth Siu-Ho Chok, FRCS(Ed), Associate Professor, Department of Surgery and State Key Laboratory for Liver Research, the University of Hong Kong, 102 Pok Fu Lam Road, Hong Kong, China. chok6275@hku.hk

Telephone: +852-22553025

Fax: +852-28165284

Abstract

Liver metastasis is the commonest form of distant metastasis in colorectal cancer. Selection criteria for surgery and liver-directed therapies have recently been extended. However, resectability remains poorly defined. Tumour biology is increasingly recognized as an important prognostic factor; hence molecular profiling has a growing role in risk stratification and management planning. Surgical resection is the only treatment modality for curative intent. The most appropriate surgical approach is yet to be established. The primary cancer and the hepatic metastasis can be removed simultaneously or in a two-step approach; these two strategies have comparable long-term outcomes. For patients with a limited future liver remnant, portal vein embolization, combined ablation and resection, and associating liver partition and portal vein ligation for staged hepatectomy have been advocated, and each has their pros and cons. The role of neoadjuvant and adjuvant chemotherapy is still debated. Targeted biological agents and loco-regional therapies (thermal ablation, intra-arterial chemo- or radio-embolization, and stereotactic radiotherapy) further improve the already favourable results. The recent debate about offering liver transplantation to highly selected patients needs validation from large clinical trials. Evidence-based protocols are missing, and therefore optimal management of hepatic metastasis should be personalized and determined by a multi-disciplinary team.

Key words: Colorectal cancer; Liver metastases; Hepatic resection; Neoadjuvant therapy; Adjuvant chemotherapy; Intra-arterial therapy; Precision medicine; Multidisciplinary approach

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Core tip: Surgery offers the only hope of cure in colorectal liver metastasis. It can be

performed if complete metastasectomy is attainable. There is no consensus on the ideal management strategy for synchronous disease. A subset of patients presenting with unresectable disease may become eligible for resection after liver remnant augmentation or conversion therapy (chemo-therapeutics +/- biological agents). Amid increasing application of loco-regional therapies to colorectal liver metastasis, their role in the treatment paradigm remains to be defined. Refined patient selection – with greater emphasis on tumour biology – is essential to improving treatment outcome. The multidisciplinary approach helps determine the optimal treatment strategy from an expanding armamentarium of therapeutic options for each patient.

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INTRODUCTION

Colorectal cancer (CRC) is a leading cause of tumour-related morbidity and mortality worldwide^[1]. Approximately 50% of patients develop liver metastases (LM) in their course of disease^[2]. Surgical resection is the only treatment that offers a chance of cure and long-term survival, with 5- and 10-year survival rates at around 40% and 25% respectively^[3]. In selected cohorts, up to 97% of ten-year survivors remained disease-free after resection of colorectal LM (CRLM)^[4]. However, only a minority of patients is suitable for upfront surgery. While improving surgical techniques and better adjuvant therapies are pushing forward the frontiers of resection, the importance of careful patient selection should not be overlooked. Not all patients undergoing resection enjoy long-term benefit – around 30% developed recurrence and 15% succumbed to their disease within a year after surgery^[5]. A personalized treatment approach – taking tumour biology, disease staging and patient condition into consideration – is the key to improving outcomes.

Management of stage IV CRC is optimized by grouping relevant specialists together under a multidisciplinary team (MDT) setting^[6]. A proficient MDT consists of at least a colorectal surgeon, a liver surgeon, medical and radiation oncologists, a radiologist, a pathologist and a case nurse^[7]. Better patient and disease evaluation, joint decision-making and optimization of multimodal therapy not only improve patient outcome, but also enhance consistency and coordination of care. The value of multidisciplinary approach in the management of gastrointestinal malignancies has been demonstrated in a prospective study. Despite 84% of clinicians being certain of their original plan before discussion, a change was recommended in 36% of cases, 72% of which were major^[8]. This review highlights current controversies and relevant evidence in the management of CRLM.

PATIENT AND TUMOUR EVALUATION

Accurate assessment of a patient's general health condition (comorbidities, performance status and liver function) and the extent of disease is important for treatment planning.

Radiological assessment

Guidelines recommend CT scan of thorax, abdomen and pelvis for initial workup. It is adequate for determining resectability in most cases. In cases of doubt, a second imaging modality such as MRI could be added^[9,10]. In patients planning for upfront resection, sensitivity of MRI and CT were similar (94% *vs* 91%)^[11]. However, in general, MRI was more sensitive than CT in detecting CRLM (91% *vs* 82%), particularly for sub-centimetre lesions or reassessment after neoadjuvant chemotherapy (when the sensitivity of CT dropped to 77%)^[12]. Gadoteric contrast further increased the diagnostic confidence of MRI to 98.3%, compared with 85.7% and 65.2% in conventional contrast MRI and CT^[13]. High-quality baseline imaging is essential before any chemotherapy, when lesions are more readily detectable; whereas comparison with post-chemotherapy films gauges treatment response and delineates tumour biology. MRI is useful when characterization is difficult *e.g.*, underlying fatty

liver, or multiple small nodules with uncertain nature.

Controversy 1: Role of positron emission tomography - computed tomography:

Whether positron emission tomography - computed tomography (PET-CT) offers additional information to CT and/ or magnetic resonance imaging (MRI) is controversial. An early randomized study showed PET (without CT) significantly reduced the number of futile operations (28% *vs* 45% in control) and prevented an unnecessary surgery in every 6 patients^[14]. In another randomized trial, however, PET-CT did not influence decision-making in patients with resectable CRLM – the PET-CT group had similar hepatic resection rate and survival as the controls; it only altered surgical management in 8% patients (2.7% did not undergo surgery and 3.4% underwent additional organ surgery)^[15]. Long-term follow-up of this trial concluded PET-CT did not improve disease-free or overall survival^[16]. According to a meta-analysis, PET-CT was less sensitive but more specific than CT or MRI in detecting CRLM – sensitivity 66% *vs* 79% *vs* 89%; specificity 86% *vs* 67 *vs* 81%^[17]. In our practice, we still perform PET-CT in the majority of patients to assess for extra-hepatic disease (EHD). As an adjunct to CT, its value in staging EHD was evident in 20% patients – preventing futile operations, guiding resection of loco-regional nodal disease, or clarifying indeterminate CT findings^[18].

Evaluation of future liver remnant (before major hepatectomy)

Accurate preoperative estimation of liver functional reserve is essential to prevent post-hepatectomy liver failure, especially in patients with extensive tumour load or highly compromised livers. CT volumetry is routinely used before major hepatectomy. In most centres, future liver remnant (FLR) volumes of 25% and 40% are accepted as adequate for normal and diseased liver respectively. However, FLR volume does not necessarily reflect its function, particularly as quality of liver tissue can be affected by pre-operative chemotherapy^[19].

Indocyanine green (ICG) clearance is a long-established functional test for selecting surgical candidates with adequate liver reserve; its determination by pulse dye densitometry and intra-operative application have attracted great interest^[20,21]. The use of ICG has limitations though: its uptake by hepatocytes can be impaired by hyperbilirubinemia, and it reflects the total liver function rather than specifically the FLR performance, failing to address regional variations within the liver^[19]. Segmental hepatic function can be measured by hepatobiliary scintigraphy; the commonest used agent being Technetium-99m (99mTc) labelled mebrofenin, which is taken up by hepatocytes and directly excreted into the biliary tree. Using a single cut-off value of 2.7%/min m² irrespective of the liver tissue quality, 99mTc-mebrofenin hepatobiliary scintigraphy has been shown to outperform CT volumetry in predicting the risk of post-hepatectomy liver failure^[22]. Nonetheless, further evidence is required to support its widespread use in clinical practice.

PATIENT SELECTION AND PROGNOSTIC PREDICTION

Although hepatic resection gives the best results on a population level, not everyone with technically operable disease benefits from surgery^[5]. Appropriate patient selection ensures the offered intervention, surgical or systemic, is optimal to each particular patient.

Resectability of a particular CRLM should be determined in a multidisciplinary setting, with input from hepatobiliary surgeons, oncologists, radiologists and pathologists. Apart from pure technical considerations, there is a growing emphasis placed on oncological resectability^[23]. The former focuses on whether a margin-negative (R0) resection can be achieved while preserving a liver remnant comprised of two contiguous segments with adequate volume, function, vascular inflow and outflow, as well as biliary drainage. Oncological/prognostic evaluation aims to select patients with higher likelihood of cure or sustained disease remission; taking tumour biology (in particular disease progression/remission during neoadjuvant therapy), mutation status, intra-hepatic tumour burden and extent of EHD into consideration. Resection criteria based on the number, maximal size and distribution of tumours no longer apply; instead resectability should be defined case-by-case based on different prognostic factors. With continued advancement in surgical technology, systemic therapies and multimodality treatment, the definition of resectability will continue to evolve and expand to cover advanced diseases once deemed non-resectable.

Traditional clinico-pathological prognostic factors include^[24-26]: (1) Characteristics of primary CRC, *e.g.*, advanced T stage, nodal status, location of tumour (right sided tumour associated with poorer outcome); (2) Factors related to CRLM *e.g.*, size of largest liver metastasis, number of lesions, grade of differentiation, margin status; (3)

Presence of extrahepatic disease; (4) Elevated serum carcinoembryonic antigen (CEA) level; and (5) Disease-free interval between primary CRC and metachronous CRLM

In clinical practice, the predictive value of each individual factor is limited. Several scoring systems were devised aiming to provide an overall risk assessment; the most widely quoted one is the Fong clinical risk score (assigning points to positive margin, EHD, node-positive primary, disease-free interval from primary to CRLM, more than one solitary LM, largest LM > 5 cm, and CEA > 200 ng/mL)^[27]. These systems, however, failed to demonstrate predictive accuracy for long-term survival or in the neoadjuvant setting across institutions^[28,29]. Their clinical utility remained uncertain.

Both radiological and pathological response to preoperative chemotherapy predict better survival after resection of CRLM^[30,31]. On CT or MRI scans, treatment response can be judged by degree of tumour shrinkage and morphological changes unrelated to size (*e.g.*, tumour density, tumour-liver interface). Due to the limitations of the Response Evaluation Criteria in Solid Tumours (RECIST), new parameters like early tumour shrinkage and depth of response have been proposed to aid in prognostication^[32,33]. 18-fluorodeoxyglucose (¹⁸FDG) PET/CT also has a role in prognostication—LM with high glucose metabolism [high pre-treatment standardized uptake value (SUV)] and poor metabolic response to systemic therapy had poorer overall and disease-free survivals^[34]. Complete pathological response, on the other hand, has been associated with an excellent 5-year survival of 76%^[35].

All these clinical, radiological and pathological characteristics are considered surrogate markers of the underlying tumour biology. There is growing interest in directly assessing tumour biology by molecular profiling and integrating biomarkers into prognostication systems. *KRAS* gene has been extensively studied. As there was a high concordance of *KRAS* status between primary CRC and CRLM, it can be evaluated on biopsy or resected specimens of the primary tumour^[36]. Apart from predicting poor response to anti-epidermal growth factor receptor (EGFR) therapies, *KRAS* mutation has been associated with higher rates of EHD, adverse response to chemotherapy, positive resection margin, worse overall and recurrence-free survivals after surgery irrespective of the chemotherapy regimen, and poorer survival after re-resection for recurrence^[25,37,38]. In view of the poor prognosis, some suggested aggressive treatment might not be worthwhile for *KRAS* mutant tumours with multiple risk factors (*e.g.*, node-positive primary, individual CRLM > 3 cm, more than 7 cycles of systemic chemotherapy given)^[39]. Incorporating *KRAS* mutation status to traditional risk scores might improve survival prediction^[40]. *BRAF*, another commonly tested gene in CRC, also predicted survival outcome in CRLM. *BRAF* mutated tumours were refractory to standard chemotherapy and anti-EGFR agents and had far inferior survival; resection should only be offered to those with limited disease, taking note of the high risk of recurrence^[41,42]. By using gene expression microarray on resected CRLM, a 20-gene molecular risk score was externally validated to be an independent prognosticator of overall and recurrence-free survival after liver metastasectomy; it was more accurate than traditional clinical risk scores^[43]. With further research, risk stratification and individualized therapy based on molecular profiling could be realized in the foreseeable future.

Nowadays we realize tumour heterogeneity exists not only amongst different patients, but also within individual tumours and among metastatic sites. Multiple cancer subclones coexist and evolve simultaneously, with treatment acting as selection pressure. Tumour biopsy at a single site at a particular time cannot reflect the entire disease throughout the treatment period^[25]. Serial imaging has the advantage of assessing multiple tumour locations in a longitudinal fashion, but is frequently limited by spatial resolution. The role of circulating liquid biomarkers is under investigation; its collection at several time points (*e.g.*, pre-treatment, after each cycle of chemotherapy, before and after resection) can offer insight into the evolving tumour biology. Presence of circulating tumour cells (CTCs) predicted impaired survival in CRLM; it could also be a source to detect *KRAS* and *BRAF* mutations to guide choice of targeted therapy^[44,45]. Plasma level of circulating cell-free DNA (cfDNA) has been proven a predictor of survival in metastatic CRC^[46]. Early work showed cfDNA sequencing allowed identification of gene mutation or micro-satellite instability^[47]. Its detection and analysis may improve diagnostic efficiency in both screening and surveillance settings^[48]. MicroRNA provides a molecular snapshot of intracellular activity within cancer; its signature has been shown to predict metastasis and prognosis in CRC^[49]. If microRNA profiling can be obtained from serum samples, tumour prognostication from blood tests is distinctly feasible.

NEOADJUVANT TREATMENT

Controversy 2: Role of neoadjuvant therapy in clearly R0 resectable CRLM

Theoretically, neoadjuvant therapy allows assessment of the natural history of disease before embarking on metastasectomy. It potentially shrinks the tumour and reduces the extent of liver resection, treats micro-metastases thereby lowering recurrence rate, as well as guides further therapeutic plan based on disease response to treatment. Its benefit is not proven from an evidenced-based point of view though.

Earlier studies confirmed an objective radiological response could be expected in two-thirds of treated patients^[50]. However, the majority of retrospective studies failed to demonstrate any overall survival (OS) benefits from neoadjuvant therapy – five-year OS rates ranged from 38.9% to 74% in patients who had pre-operative chemotherapy before liver resection, compared with 20.7 to 56% in patients who underwent upfront surgery^[51]. A landmark randomized controlled trial (the EORTC intergroup trial 40983) showed perioperative FOLFOX (folinic acid, fluorouracil, and oxaliplatin; 6 cycles before and 6 cycles after surgery) improved 3-year progression-free survival (PFS) modestly – 42.4% compared with 33.2% in surgery-only patients, an absolute 9.2% increase – at the cost of higher peri-operative morbidity (25% *vs* 16%). This did not translate into any overall survival benefit at a median follow-up of 8.5 years^[52,53]. A meta-analysis including 18 studies concurred neoadjuvant treatment, in general, did not offer PFS or OS advantage; however, it could improve survival in patients considered high risk of recurrence (pooled hazard ratio for 5-year OS = 0.69)^[54]. The CHARISMA randomized trial is underway to investigate whether neoadjuvant XELOX improves survival in high-risk CRLM patients^[55].

Based on current evidence, the European Society for Medical Oncology (ESMO) guidelines suggested the need for perioperative systemic therapy is defined by “technical criteria for resection and prognostic considerations”. Upfront surgery is justified in patients with clearly resectable disease and favourable prognosis; while peri-operative FOLFOX or XELOX should be considered when resectability or prognostic criteria is unclear or “not excellent”^[9]. In our centre, we favour the surgery-first approach as long as R0 resection can be attained, because the unclear survival benefit of neoadjuvant treatment carries with it the risk of chemotherapy-associated liver injury (discussed below). Future research should focus on accurately defining “high-risk” patients who will benefit most from preoperative therapy.

Regimens and potential risks

Current guidelines suggest oxaliplatin-based doublet chemotherapy (FOLFOX/XELOX) as the neoadjuvant treatment-of-choice for resectable CRLM, while FOLFIRI or FOLFOXIRI are alternatives^[9,10]. A meta-analysis showed the addition of molecular targeted therapy conferred a higher overall response rate than chemotherapy alone (68% *vs* 43%), but did not improve survival^[56]. A lack of PFS benefit was also observed in the New EPOC trial, which studied the effect of combining EGFR-inhibitor (cetuximab) to perioperative systemic chemotherapy; patients who received cetuximab actually experienced worse PFS (14.1 *vs* 20.5 months in control)^[57]. Given these results, cetuximab should not be added to standard perioperative chemotherapy regimens. Bevacizumab, an anti-vascular endothelial growth factor (VEGF), plus FOLFIRI in the neoadjuvant setting yielded a response rate of 66.7% in resectable CRLMs^[58]; whether this translates into any survival benefit remains to be investigated. The ongoing PERIMAX trial compares perioperative bevacizumab plus FOLFOXIRI with adjuvant FOLFOX; with the primary endpoint being failure-free survival^[59].

The potential risks of perioperative chemotherapy include disease progression during treatment and hepatotoxicity. Initially resectable CRLM may progress despite using the best available chemotherapeutics, and become unresectable or require a more extensive surgical approach; the rate was 7% in the EORTC trial^[52]. From another perspective, this small group of patients have very aggressive tumour and their disease would progress despite any treatment given; some argue neoadjuvant treatment only selects them out and prevents futile operations.

Chemotherapy-associated liver injury (CALI) can occur with commonly used regimens. Oxaliplatin was associated with sinusoidal obstruction syndrome in up to 38% patients, while steatosis and steatohepatitis complicated 9.3% patients receiving irinotecan^[60]. Patients with severe sinusoidal dilation (OR 1.73) or steatohepatitis (OR 2.08) were more likely to suffer from postoperative major morbidity and liver surgery-specific complications^[61]. It has been shown postoperative complication rate increases if the interval between chemotherapy completion and surgery is too short (< 4 wk) or too many cycles (>9) of preoperative chemotherapy are given^[62,63]; limiting the duration of neoadjuvant therapy and ensuring recovery of liver function before operation can reduce the impact of CALI. Future research should focus on identifying patients at risk of CALI and devising liver protective strategies for such patients.

Disappearing LM

A subset of CRLMs can totally vanish on imaging after neoadjuvant treatment; they were referred as disappearing LMs (DLMs). This phenomenon is related to the quality of imaging, particularly as chemotherapy can compromise radiological detection of CRLM^[64]. The key question is whether the lesions have been truly cured or just being missed by suboptimal post-neoadjuvant scans.

MRI has the highest sensitivity and is the preferred imaging modality in this setting^[65]. In existing literature, the percentage of patients with one or more DLMs ranged widely from 7% to 37%; however, this phenomenon may be over-reported as most studies only utilized CT and intraoperative ultrasound for reassessment^[66]. Complete disappearance of all initial CRLMs is rare, with an incidence of 0%-6%; therefore, most patients still undergo surgery. In laparotomy, macroscopic residual disease could be detected at 11%-67% DLM areas, highlighting the inadequacy of current post-neoadjuvant imaging. Importantly, there was clear difference between complete radiological and pathological responses – when areas of DLM were resected, microscopic residual disease was found in up to 80% of specimens^[67].

Only surgery potentially offers cure; the phenomenon of DLM should be prevented, albeit complete pathological response has been associated with good prognosis^[35]. To reduce the risks of DLMs, preoperative over-treatment has to be avoided – some proposed restaging after every 3 cycles of neoadjuvant therapy to enable earlier decision of surgery, and limiting the entire course of chemotherapy to 4 to 6 cycles^[66,68]. Using a combination of imaging modalities enhances the detection of residual metastases and lowers the incidence of false DLMs. As conservative management i.e. leaving DLMs in-situ resulted in a local recurrence rate of 19% to 74%^[66], DLMs should be resected whenever feasible. Some proposed even in patients with complete radiological resolution of all CRLMs, surgical exploration may be warranted for meticulous intra-operative assessment. Whether resection offers survival benefit is unclear though; patients with untreated DLMs could have comparable OS to those who underwent complete surgical treatment, in spite of a higher intrahepatic recurrence rate^[69]. In selected patients, leaving certain DLMs untreated may be reasonable but this decision should only be made in a multi-disciplinary setting^[23,68,70]. Ablation of DLM sites is an appealing alternative, but there is no evidence proving its efficacy to date. Initial experience with computer-guided resection of DLMs showed promising results. Augmented reality is a technology fusing reconstructed pre-treatment CT images with real-time patient images, thereby facilitates DLM localization and ensures clear resection^[71].

SYNCHRONOUS DISEASE

About one sixth of CRC patients had LM at presentation^[72]. Defined as CRLM detected concurrently or before the primary CRC, synchronous disease has been shown to have less favourable cancer biology and post-resection survival – 5-year survival was 39%, compared with 48% in metachronous CRLM^[7]. Western guidelines and expert consensus recommend neoadjuvant chemotherapy in this higher-risk setting, unless resection at both sites are considered easy^[7,9]. However, an analysis based on the Liver Met Survey International Registry showed neoadjuvant therapy offered no survival advantage in resectable synchronous CRLM, with a 5-year OS of 42% similar to 47% in the upfront surgery group^[73]. In Asian countries, preoperative chemotherapy is not a standard in resectable synchronous disease.

Controversy 3: Optimal surgical sequencing for resectable synchronous disease

To date, there has been no randomized trial on the surgical approach to synchronous disease. The optimal strategy remains controversial. Decision should be individualized taking into consideration of patient's fitness and tumour status – whether the primary CRC is symptomatic or obstructive (CRC needs to be resected first under these circumstances), as well as the extent of CRLM and magnitude of liver resection required. Treatment strategy is best determined in multi-disciplinary setting, which has been associated with greater likelihood of simultaneous resection^[74].

The conventional approach comprises of resection of the primary CRC followed by liver metastasectomy, commonly with chemotherapy applied between the two surgeries. This avoids potential complications of the primary tumour, but carries significant risk of CRLM progression beyond resectability; less than 30% patients completed the whole treatment and underwent liver resection^[75].

The liver-first approach, also known as the reversed strategy, may be more appropriate if an asymptomatic colon primary coexists with extensive CRLM or the primary tumour is a locally advanced rectal cancer (which needs neoadjuvant chemoradiation). After systemic chemotherapy, patients underwent liver

metastasectomy prior to removal of primary CRC. Given hepatic metastases rather than the primary tumour dictates these patients' prognosis, early administration of chemotherapy to control liver and systemic diseases optimizes the chance of potentially curative hepatic resection and long-standing survival^[76]. Compared to the classical approach, more patients (around three quarters) could complete the whole paradigm and underwent both liver and colorectal resection^[77]. In spite of a respectable 5-year OS of 33.1% and acceptable perioperative morbidity and mortality rates of 31.5% and 3.3%, a recent single-centre study reported a high recurrence rate of 51.4% after a median follow-up of 20.9 mo^[78]. Whether liver-first approach confers survival benefit remains undetermined.

Combined colorectal and liver resection in one single operation is generally reserved for patients with easy-to-resect primary tumours and limited hepatic disease^[7]. Traditionally this approach was associated with increased morbidity, including infectious liver and anastomotic complications. With technological advancement, outcomes of combined resection have improved. Provided resection at the other site is minor, incorporating a major liver or rectal resection into this approach is deemed safe^[79,80]. Cumulative morbidity and mortality rates were comparable to, or even better than, staged procedures; long-term oncological outcomes were similar – 1- and 5-year OS rates for one-stage operation were 90.5% and 38.5% respectively, while 5-year DFS was 25.3%^[81,82]. A recent meta-analysis of 30 studies confirmed simultaneous liver and colorectal resection was associated with shorter hospital stay compared with staged procedure, without adversely affecting perioperative morbidity or long-term survival^[83]. This approach is gaining popularity as more evidence proved its safety and efficacy.

A meta-analysis comparing the classical, synchronous and liver-first approaches did not show any difference in surgical outcomes or survival advantage^[84]. Because of the limited evidence available, the optimal treatment strategy is unclear. Choice of procedure should be personalized and based on expertise available in different institutions.

In the presence of unresectable metastases, the benefit of resecting an asymptomatic primary without liver surgery is debatable. A meta-analysis showed primary tumour resection conferred a survival benefit of 6.4 months compared with chemotherapy alone, however the result is questionable given the significant selection bias^[85]. To date, no randomized trial was completed to clarify this issue.

HEPATIC RESECTION

CRLM resection offered an overall median survival of 3.6 years; five- and 10-year survival ranged from 16% to 74% (median 38%) and 9% to 69% (median 26%) respectively^[3]. Following major hepatectomy for CRLM, large contemporary series reported perioperative mortality and major morbidity rates of 1%-5% and approximately 20% respectively.

Surgical approach

Despite the addition of systemic chemotherapy, intra-hepatic recurrence is common after surgery of curative intent and occurred in two-thirds of patients within 3 years^[6]. Increasing evidence showed re-resection of liver-limited recurrence is safe and can produce good long-term outcomes in selected patients^[86]. Compared with anatomical resection, parenchymal-sparing hepatectomy (PSH) enhances the likelihood of repeated resection in the event of intra-hepatic recurrence. Both approaches have equivalent safety profile and oncological outcomes (R0 resection rate, liver recurrence-free survival and OS) after the index operation. However, in case of recurrence, PSH has been associated with a better 5-year survival since more patients could undergo salvage surgery^[87,88]. Nowadays, PSH is considered the standard approach to CRLM unless it is precluded by the anatomy of the disease.

Amid growing interest in laparoscopic liver resection (LLR), the recently published OSLO-COMET randomized controlled trial demonstrated the safety and short-term efficacy of laparoscopic minor PSH for CRLM. Compared with the open-surgery group, the LLR group had less postoperative complications and a shorter hospital stay, while 90-d mortality and percentage of involved resection margins were similar^[89]. For oncological outcomes, both approaches had comparable tumour recurrence rate, DFS, and 5-year OS in a meta-analysis of propensity-score matched studies; the LLR group even had better 3-year OS^[90]. While there were evidence supporting the use of minimally invasive techniques for minor CRLM resection, its role in major liver surgery and challenging tumour locations needs further clarification. The advanced laparoscopic skills required can limit its widespread

application. There are ongoing efforts to facilitate the education and implementation of LLR worldwide^[91].

Width of resection margin

Contrary to the historic “1-cm rule”, several large series showed as long as R0 resection (≥ 1 mm) is achieved, extra margin width does not add DFS/OS advantage^[92-94]. From observations that (1) survivals were similar between R0 and R1 resections if tumour showed optimal radiological or pathological response to neoadjuvant chemotherapy^[95]; (2) most recurrences occurred outside the surgical margin in a disseminated manner; and (3) margin re-resection from R1 to R0 did not improve long-term outcomes^[96], positive margin status could just be a surrogate of aggressive tumour biology. The latter is increasingly recognized as the most important prognostic determinant. While an expected close margin (especially when tumour is close to vital vessels) is not a contraindication to surgery, surgeons should aim at wider margins to ensure a R0 resection^[97].

Controversy 4: Role of surgery in the presence of extrahepatic disease: Management of CRLM in this setting remains controversial. Albeit defined as a poor prognostic factor, limited extrahepatic disease (EHD) does not contraindicate liver surgery; the prerequisite is all diseases including the primary CRC, LM and EHD can be completely resected or controlled^[9,98]. Resection of CRLM together with concurrent EHD significantly improved the 5-year OS from 0% to 28%, with a median survival of 31 mo^[99].

The location of EHD matters – lung metastasis carries a more favourable prognosis than peritoneal or portal/para-aortic nodal metastases (5-year OS being 26%, 17% and 15% respectively after complete resection of CRLM and EHD)^[100]. According to data from the LiverMetSurgery registry, resection of concurrent liver and lung metastases was associated with similar OS as isolated liver metastasectomy^[101]. The relative indolent nature of most colorectal pulmonary metastases supports CRLM resection in the presence of unresectable lung disease – 5-year OS of 13.1% was better than 1.6% achieved by chemotherapy alone, but as expected much worse than the 56.9% after complete metastasectomy at both sites^[102].

Although long-term survival is possible, true definitive cure is rare after resecting concurrent CRLM and EHD. In a retrospective review, disease recurred in 90.2% patients at a median of 8 mo, 85% being systemic recurrence; 5-year recurrence-free survival was 5%^[103]. Effort was made to identify subgroups who benefit most from radical surgery. For example, in patients with synchronous LM and peritoneal carcinomatosis (PC) of colorectal origin, complete cytoreductive surgery and liver metastasectomy followed by intra-peritoneal chemotherapy achieved a median OS of 40 mo in those with limited PC (peritoneal cancer index, PCI < 12) and 1-2 LM. Outside these criteria, the survival benefit of radical surgery was significantly reduced – patients with PCI ≥ 12 or at least 3 LM had a median OS of 27 mo^[104]. Non-pulmonary EHD, EHD concomitant to LM recurrence, CEA ≥ 10 ng/mL, more than 5 LM and right-sided colonic primary all predict poor prognosis after surgery for concurrent CRLM and EHD; no patients with more than 3 factors achieved 5-year survival^[105]. However, this prognostication system has not been externally validated.

In summary, complete resection of all concurrent metastases can improve disease control and survival in selected patients, although cure is rare and recurrence is expected. Future research can help develop prognostic scores to better select patients for radical surgery.

Controversy 5 – Ablation or surgical resection? Thermal ablation involves destruction of cancer by heat, with radiofrequency ablation (RFA) and microwave ablation (MWA) being the most widely employed and studied modalities. Despite showing advantage of fewer complications and better post-procedural quality of life, RFA was inferior to hepatic resection in terms of survival (lower OS and DFS) and recurrence (higher rates of local, intra-hepatic and any recurrences)^[106-108]. Surgical resection is the choice of treatment in young, fit patients, based on its proven long-term efficacy and lower recurrence rate.

In small tumours less than 3 cm, however, RFA may attain comparable oncological outcomes to resection. Following ablation to complete tumour necrosis with a margin over 5 mm, one-year local disease progression rate as low as 3% has been reported^[109,110]. In high-risk patients (elderly, or with significant comorbidities), the considerably lower morbidity associated with ablation could justify its use over resection, provided patients accept the trade-off of potentially inferior long-term results. The ongoing LAVA randomized trial is designed to clarify the optimal treatment strategy in these high-risk individuals^[111].

Borderline resectable disease due to insufficient liver remnant: Patients with

extensive bilobar LM poses a unique challenge. The major limiting factor for a curative resection is the volume and function of the future liver remnant (FLR). Different management strategies and their advantages and disadvantages were listed below.

Portal vein embolization (PVE) is a well-established method for augmentation of small liver remnant, thereby enables extensive curative resection initially deemed high risk of postoperative liver failure. By obliterating portal flow to the liver segments involved by metastases (intended to be resected) and diverting it to the side that will remain, PVE is expected to increase the FLR volume by 43.1% on average. Less than 5% patients had inadequate hypertrophy response precluding them from the planned resection^[112]. In one study, extended right hepatectomy became feasible after PVE in two thirds of patients who initially had inadequate FLR, yielding a survival similar to those who did not need PVE^[113]. There were concerns about post-PVE tumour progression leading to unresectable disease, which could occur in up to 20-30% patients; whether PVE itself accelerates tumour growth remained controversial. Continued administration of chemotherapy after PVE has been shown to reduce tumour progression rate, while minimizing the interval between PVE and resection to 4 wk has also been advocated^[114]. Reassuringly a meta-analysis showed PVE did not adversely affect overall survival or intrahepatic recurrence in patients undergoing major liver resection for CRLM^[115]. Future research goals include identification of individuals at risk of rapid tumour progression and strategies to reduce progression rate without compromising FLR hypertrophy.

Two-stage hepatectomy (TSH) aims to remove all CRLM in two sequential operations for selected patients with advanced bilobar disease, in whom removing all lesions with safe margin is impossible during a single procedure. Pre-operative chemotherapy was frequently employed to select patients with favourable tumour biology. Following the first stage (for tumour clearance of FLR), a 2- to 3-mo interval allows liver tissue to regenerate (often aided by PVE) before a second stage hepatectomy took place to achieve R0 resection. A systematic review reported three quarters of patients successfully underwent second stage hepatectomy and R0 resection rate was 75%; the main reason for non-completion was interval disease progression (88%). Postoperative morbidity rate was 17% and 40% after first and second stage respectively, and overall mortality was less than 5%. A median OS of 37 mo and a 3-year DFS rate of 20% were encouraging, and comparable to other series of lesser-scale CRLM resections^[116].

Both PVE and TSH, although proven effective, have a considerable patient drop-out rate due to tumour progression in the waiting interval. A novel surgical technique was introduced in 2012 to treat advanced bilobar liver tumour in a more rapid fashion.

Associating liver partition and portal vein ligation for staged hepatectomy (ALPPS) comprises liver transection and ligation of portal vein in the first operation, followed by resection of the diseased liver segment 7-14 d later. By redistributing total portal blood flow to FLR and triggering an inflammatory response, ALPPS induces rapid hypertrophy of FLR at an estimated growth rate of 22-35 mL/d (compared with 3-5 mL/d after PVE)^[117]. The benefits of ALPPS include a greater FLR hypertrophy (76% *vs* 37% for PVE) and a higher rate of completion of stage 2 hepatectomy (100% *vs* 77% for PVE)^[118]. A recent randomized controlled trial confirmed ALPPS had superior resection rate compared with TSH in patients with advanced CRLM, 92% *vs* 57%^[119]. ALPPS has also been reported as a salvage treatment for failed PVE^[120]. However, these came at the price of higher morbidity and mortality. The relative risks for overall and major morbidity (*e.g.*, bile leakage and sepsis) were 1.39 and 1.57 compared with TSH^[117]. As per the international ALPPS Registry, the 90-d mortality rate was high at 8.8%, three quarters were due to postoperative liver failure^[121]. Oncological outcomes after ALPPS were also unclear; limited data available came from case series only. For CRLM, recurrence rates ranged from 14.3% (after a median follow-up of 9 mo) to 78.3% (after a median of 22.5 mo)^[122,123]. Patients with "otherwise unresectable" advanced CRLM were identified from the international ALPPS registry, overall survival after ALPPS was not superior to matched controls who received palliative systemic treatment (with median OS of 24.0 mo *vs* 17.6 mo, *P* = 0.88)^[124]. To improve outcomes of this radical surgical strategy, careful patient selection is essential; a consensus on the ideal indications of ALPPS is urgently needed. Recent promising results from high-volume centres suggest that, with surgical advancement, ALPPS can have low perioperative risk (0% mortality and 21% severe complications) and satisfactory survival (3-year OS 50% and 3-year DFS 13%) in experienced hands^[125]. However, given the unclear long-term outcome and questionable safety profile, currently ALPPS should be limited to high-volume institutes in research setting.

Combining ablation with resection allows potential tumour clearance in extensive

bilobar CRLM. This approach could achieve long-term survivals (5-year OS 37%-56%) comparable to that of TSH, with improved perioperative outcomes – less blood loss and shorter length of stay. The short disease-free interval (median DFS around 9 mo), though, suggested a temporary disease control rather than complete cure^[126-128].

ADJUVANT THERAPY (AFTER POTENTIALLY CURATIVE METASTASECTOMY)

Adjuvant systemic therapy aims to reduce recurrence and prolong survival after curative resection, ideally with minimal treatment-related toxicity. In primary CRC, postoperative oxaliplatin-containing chemotherapy has been shown to offer survival benefit for up to 10 years after curative resection for stage III disease^[129].

The role of adjuvant treatment is more controversial for CRLM; according to existing evidence, it only improves DFS but not OS. From pooled analysis of two early randomized trials' results (French FFCD trial and English ENG trial), adjuvant fluorouracil potentially improved OS (median 62.2 mo *vs* 47.3 mo, $P = 0.095$) and PFS (median 27.9 mo *vs* 18.8 mo, $P = 0.058$) compared with surgery alone, but both did not reach statistical significance^[130]. Adjuvant oral uracil-tegafur and leucovorin also only prolonged recurrence-free survival (from 0.70 to 1.45 years) without improving OS, as demonstrated by a Japanese RCT^[131]. Current recommendations for oxaliplatin-containing adjuvant regimen (FOLFOX) were based on extrapolation of result of the EORTC intergroup trial 40983, which showed perioperative FOLFOX conferred a PFS benefit but did not affect OS^[53]. The JCOG0603 trial is now underway to determine if adjuvant mFOLFOX is superior to resection alone^[132]. Meanwhile, adjuvant FOLFIRI did not improve DFS or OS compared with 5FU alone, but was associated with lower treatment tolerance in a randomized study^[133]; therefore, FOLFIRI is not commonly used in the adjuvant setting for CRLM. According to the latest ESMO guidelines, there was no strong evidence supporting the use of adjuvant chemotherapy in patients with good oncological and technical criteria who underwent upfront surgery; on the other hand, patients with unfavourable prognostic criteria or have not received any previous chemotherapy for metastatic disease may benefit from adjuvant therapy (*e.g.*, FOLFOX/ XELOX)^[9].

To date, no evidence supports the combined use of chemotherapeutics and biological targeted agents in the adjuvant setting after resection of CRLM. Addition of bevacizumab to modern chemotherapy or combination of hepatic arterial chemotherapy infusion (HAI) and systemic chemotherapy did not prolong survival, but appeared to increase biliary toxicity^[134,135]. A randomized trial is in progress to assess whether bevacizumab gives additional benefit to adjuvant XELOX^[136]. As the New EPOC trial linked its use in the peri-operative setting to a shorter PFS, cetuximab is generally not recommended in the adjuvant setting after liver metastasectomy^[57].

Taking advantage of CRLM's predilection of hepatic artery neovascularization, infusion of cytotoxics via the hepatic artery can deliver high concentration of therapeutic agents to the tumour while minimizing side-effects. An earlier RCT showed adding floxuridine-HAI to systemic fluorouracil (5-FU) chemotherapy improved PFS but not OS after a median follow-up of 10 years^[137]. In a recent retrospective study, adjuvant HAI offered an OS advantage of approximately 2 years compared to systemic chemotherapy alone, and this benefit was substantiated in patients receiving modern chemotherapy^[138]; the 5- and 10-year OS reached 78% and 61%^[139]. The ongoing PACHA-01 trial compares the outcomes of adding oxaliplatin-HAI *vs* systemic oxaliplatin to adjuvant systemic 5-FU after resection or thermal ablation of at least four CRLM^[140]. More evidence is needed to show whether HAI offers additional benefit to modern doublet or triplet adjuvant chemotherapy. However, the unique expertise required and the need of placing a special port-catheter has limited its use in specialized centres only.

UNRESECTABLE LIVER-ONLY OR LIVER-DOMINANT METASTASES – A MULTIDISCIPLINARY APPROACH

As mentioned, resectability should be determined by a MDT on a per patient basis, taking into consideration of technical and oncological factors. For unresectable CRLM, the former standard of care is palliative systemic chemotherapy. Although modern doublet or triplet chemotherapy (FOLFOX, FOLFIRI, FOLFOXIRI) have considerably improved the median OS to 15-21 mo and these were further extended by targeted agents, long-term survival remained rare^[141,142]. In an attempt to obtain durable local

disease control or even cure, two approaches have been employed – (1) conversion therapy followed by potential curative resection; and (2) use of single or a combination of liver-directed therapies. For systemic treatment regimen and local therapy, clinicians can determine the most appropriate strategy from a ‘toolbox’ of ever-expanding options, according to patient and disease factors, treatment goal and its related morbidity^[9]; again, the decision is best made in a multi-disciplinary setting.

CONVERSION CHEMOTHERAPY

A subset of patients with initially unresectable CRLM (around 15%-30% depending on the definition of unresectability) may be rendered resectable after conversion chemo-therapy. In a systematic review of 10 studies using different downsizing regimens, an objective radiological response was achieved in 64% (range 43%-79%) patients; 22.6% underwent macroscopically curative liver resection (most studies reported a range of 12.5%-45%) and R0 resection rate was 87%. The median OS and DFS after liver metastasectomy were 45 and 14 months respectively^[143].

The optimal regimen for conversion to operable disease remains unclear. Standard doublet chemotherapy FOLFOX or FOLFIRI had conversion rates between 9% to 33%^[144]. Compared with FOLFIRI, intensified triplet chemotherapy FOLFOXIRI improved the secondary R0 resection rate from 12% to 36%, median PFS from 6.9 to 9.8 mo, and median OS from 16.7 to 22.6 mo; albeit at the cost of greater but manageable toxicity *e.g.*, peripheral neuropathy and neutropenia^[145]. Addition of targeted agents is recommended by guidelines, but there is no concrete supporting evidence. In a large RCT, giving bevacizumab together with XELOX/ FOLFOX only moderately improved resectability (from 6.1% to 8.4%) and PFS (from 8 to 9.4 mo), but did not prolong OS^[146]. According to a recent meta-analysis, the combination of bevacizumab and FOLFOXIRI offers more promising results – the R0 surgery conversion rate was 28.1%, and the median OS and PFS were 30.2 and 12.4 mo respectively^[147]. Multiple randomized trials have shown the addition of cetuximab to chemotherapy in *KRAS* wild-type (WT) unresectable disease improved the R0 resection rate by 2-3 folds^[144,148]. An increase in complete resection rate from 11 to 18%, however, did not translate into survival benefit in a meta-analysis^[149]. Panitumumab, another anti-EGFR agent, has also been linked with greater likelihood of curative resection when added to FOLFOX (29% *vs* 17%) in *KRAS*-WT unresectable CRLM^[150].

Adding floxuridine-HAI to best systemic chemotherapy achieved a 47% conversion rate to resectable disease at a median of 6 months; median OS and PFS were 38 and 13 mo^[151]. Oxaliplatin-HAI, meanwhile, was associated with response rates ranging from 24% to 81% in multiple small-scale studies^[152]. In the French multicentric OPTILIV trial, triplet-agent-HAI plus systemic cetuximab gave a 30% secondary resection rate in *KRAS*-WT disease^[153]. Whether HAI strategies offer additional benefit to modern intensive systemic chemotherapy regimens have to be tested by future RCTs.

Of note, patients who required prolonged chemotherapy (> 12 cycles) to achieve resectability had higher perioperative morbidity and inferior oncological outcomes. Conservative strategies rather than radical operation may be more appropriate for this subgroup^[154]. Nonetheless, in general, conversion therapy followed by curative resection should be attempted whenever possible, as survival in this secondary R0 resected group is similar to those who had curative upfront surgery; early recurrence is not uncommon though.

LIVER-DIRECTED THERAPIES IN UNRESECTABLE CRLM

Clinicians nowadays can choose from an ever-growing armamentarium of loco-regional therapies to attain hepatic disease control. Yet, there is a lack of high-quality evidence assessing the benefits of each modality and no large-scale trials compared the different treatment strategies. Most of these liver-directed therapies require specific expertise, and should be recommended only in institutions with extensive experience with the procedure; the choice of treatment modality often depends on the availability of expertise. Here we will present an update of the available evidence regarding popular treatment modalities.

Ablation

Radiofrequency ablation (RFA) produces heat by delivering high-frequency alternating electric current through an electrode. Its widespread application has been limited by the relatively high local recurrence rate (ranged from 5% to 60% across different studies) and associated technical barriers *e.g.*, tissue desiccation (charring)

and heat-sink effect affect energy delivery^[109]. Five-year survival varies between 17% and 51%^[155]. According to an international expert panel position paper, thermal ablation is indicated for limited number (five or fewer) of small-sized (preferably < 3 cm) metastases deemed inoperable because of tumour or patient factors (*e.g.*, multiple comorbidities); well-located tumours up to 5 cm or patients with up to nine tumours could also be considered^[155]. Complete ablation with 10 mm margins in all directions should be attained^[156]. In the landmark EORTC CLOCC study (40004), on top of systemic chemotherapy, RFA offered PFS benefit (median 16.8 mo *vs* 9.9 mo) and a significant prolongation of OS (median 45.6 mo *vs* 40.5 mo) in unresectable CRLM^[157,158].

Producing heat from oscillation of water molecules, microwave ablation (MWA) is less susceptible to charring and heat sink, and could be more efficient in the treatment of large lesions and those near major hepatic vessels. It has been associated with lower local recurrence rate compared to RFA, with similar long-term survival outcomes and safety profiles^[159,160].

Irreversible electroporation (IRE) induces cell death by creating permanent nanopores in cellular membranes while preserving tissue architecture. It can be a good ablative modality for tumours adjacent to major vascular or biliary structures. Early series showed encouraging results with PFS and OS rates of 18 and 62% at 2 years, but further evidence is necessary before advocacy for its more widespread use in clinical practice^[109,161].

Intra-arterial therapies

Intra-arterial therapies (IATs) are used to palliate symptoms or prolong survival in selected patients with unresectable CRLM refractory to chemotherapy. Selective infusion of tumoricidal and/or embolizing agents into hepatic artery branches enhances their delivery to liver tumours, while minimizes their effect on normal liver parenchyma; thereby avoiding significant hepatic and systemic toxicity.

Trans-arterial chemoembolization (TACE) kills cancer cells by means of high concentration of cytotoxic agents and ischemia. For CRLM, it is mainly used as rescue therapy for chemo-refractory diseases, although evidence for that is lacking. Mitomycin C- and cisplatin/doxorubicin-based conventional TACE (cTACE) offered median survivals of 14 and 11 months from the start of salvage therapy^[162,163]. Post-embolization syndrome was reported in two-thirds of patients, but most only have nausea, fever, fatigue and mildly deranged liver function; severe complications *e.g.*, liver abscess, hepatic failure and peptic ulcer were rare^[164].

The newer drug-eluting bead (DEB)-TACE utilizes microspheres loaded with cytotoxics (doxorubicin or irinotecan); the drug is released in a controlled manner and a higher intra-tumoral dose can be delivered, meanwhile reduced peak plasma concentration may improve patient tolerance. In patients who failed previous chemotherapy, the median OS and PFS after DEB-TACE were 25 and 8 mo respectively^[165]. In a small RCT, irinotecan-loaded DEB-TACE alone achieved better OS (median 22 mo *vs* 15 mo), PFS (7 mo *vs* 4 mo) and quality of life than systemic FOLFIRI; this needs to be verified in larger studies though^[166]. Compared with cTACE, fewer patients (30%) experienced drug-related adverse events and most reported only minor symptoms *e.g.*, abdominal pain, vomiting and fever^[164]. Recent studies explored the combination of DEB-TACE with systemic chemotherapy or other treatment modalities. Concurrent capecitabine administration improved disease control but not survival^[167], while adding FOLFOX and bevacizumab increased the conversion rate to resectability^[168]. In non-surgical candidates, RFA combined with TACE attained local tumour control in 92% and OS at 2 years was 88.0%^[169]. With better understanding of the properties of drug-eluting microspheres, results of DEB-TACE will improve and it will become a therapeutic option earlier in the course of disease *e.g.*, neoadjuvant therapy, first-line treatment for unresectable disease.

For selective internal radiation therapy (SIRT), instead of cytotoxics, radiolabeled microspheres (Yttrium-90) were infused into the arterial system, delivering an effective dose of radiation to the tumour without causing intolerable toxicity to normal liver. Similar to TACE, it is typically considered in CRLM not amenable to resection or ablation. Earlier data confirmed its role in chemo-refractory disease – when given together with systemic 5-FU as salvage therapy, SIRT prolonged PFS (from 2.1 to 4.5 mo) in spite of no OS advantage^[170]. However, more recent evidence did not support SIRT as a first-line therapy. Pooled data from 3 randomized trials (FOXFIRE, SIRFLOX and FOXFIRE-Global; including 1103 patients not suitable for curative resection or ablation and have not received any chemotherapy) showed, although associated with a higher objective response rate (72% *vs* 63%), early use of SIRT in combination with FOLFOX did not improve OS, PFS or hepatic resection rate, compared with FOLFOX alone (median OS 22.6 mo *vs* 23.3 mo, median PFS 11.1 *vs* 11.9 mo, hepatic resection rate 17% *vs* 16%). The SIRT group had more grade 3 or

above adverse events (OR 1.42, 74% *vs* 67%). The authors concluded SIRT should not be used in unselected chemotherapy-naïve liver-dominant or liver-only CRLM^[171]. Further studies can help define patient selection and the role of SIRT in the management of CRLM.

In unresectable CRLM, HAI achieved a better tumour response rate but similar survival compared with standard 5-FU chemotherapy^[172]; its role in the era of modern chemotherapy and targeted agents is less well-defined. HAI has been associated with relative high rates of technical failure (hepatic artery dissection and thrombosis 21%, catheter occlusion 5%, pump failure and infection 2% and 3%); together with the special equipment and expertise required, its availability is limited to relatively few centres^[164].

No randomized trial has compared the efficacy and safety of the above three IAT modalities; evidence supporting one over the other is lacking. A recent systematic review tried to settle this issue - the pooled RECIST response rates for cTACE, DEB-TACE and SIRT were 23%, 36% and 23%, while medians survivals from first therapy were 16, 16 and 12 mo respectively^[173]. However, significant heterogeneity in terms of patient characteristics, tumour burden, previous and post-IAT therapies exists between the included studies; and this precluded meaningful comparisons between the three therapies. We need higher-quality evidence to properly answer this question.

Stereotactic body radiation therapy

This technique allows delivery of a conformal high dose radiation to the tumour, while sparing normal liver. It is suitable for patients with adequate hepatic function but unresectable CRLM. Precise selection criteria are not well defined; some included good performance status, not more than 3 CRLMs and the largest tumour size less than 3 cm^[174]. From a systematic review including 18 heterogeneous studies with different RT doses and schedules (most patients had 1-2 oligo-metastases), the pooled 1- and 2-year local control rates were 67% and 59.3%, while one- and two-year OS were 67.2% and 56.5% respectively; mild/moderate and severe liver toxicity occurred in 30.7% and 8.7% patients^[175]. The limited evidence so far showed encouraging results; however, this has to be validated in large prospective trials. Guidelines define stereotactic body radiation therapy as a reasonable treatment option for patients unsuitable for surgery or ablative therapies^[9,10].

Controversy 6: Role of liver transplantation: Traditionally CRLM was regarded as a contraindication to liver transplantation (LT); this concept was challenged by the pilot SECA study^[176]. Twenty-one patients with unresectable liver-only CRC metastases underwent deceased donor liver transplantation after at least 6 weeks of chemotherapy. OS rates of 95%, 68% and 60% at 1, 3 and 5 years were comparable to results of LT for other indications, and significantly better than a similar cohort who received first-line chemotherapy (5-year OS 9%). However, only 35% patients remained recurrence-free at 1 year; most of the recurrences were small slow-growing lung metastases. Compared with the chemotherapy group, the equivalent DFS but markedly superior OS attained by LT can be attributed to the different metastatic patterns - progression of non-resected LM carries a much worse prognosis than the post-LT indolent pulmonary metastases^[177]. Similar OS rates were observed in a small series containing 12 patients - one third remained relapse-free after 4 years, suggesting LT may achieve long-term cure in selected patients with unresectable CRLM^[178].

These results have to be interpreted with caution, though. They were small studies and there were no standardized selection criteria for recruiting these patients; it is questionable whether this survival benefit can be reproduced in other patients. Currently, LT remains experimental until better selection criteria help achieve lower recurrence rate, particularly in the setting of limited liver graft availability. A number of trials are underway to address the potential of LT for unresectable CRLM, including clarification of survival advantage by RCTs, the role of living donor LT, and the safety and efficacy of total hepatectomy after transplantation of left lateral section graft^[179-182].

CONCLUSION

Recent advances in CRLM management have significantly improved outcome on the one hand while complicating the formulation of treatment strategy on the other. Multi-disciplinary involvement from the outset helps define resectability and devise personalized treatment approach. Refined patient selection, with greater emphasis on tumour biology, ensures patients benefit most from the offered interventions.

Surgical resection remains the cornerstone of treatment for curative intent. Liver augmentation strategies and conversion therapy have expanded the definition of resectability and increased the number of patients getting cured. The role of neoadjuvant therapy in operable disease is still controversial, while the use of adjuvant chemotherapy has gained generalized acceptance. Liver-directed therapies are getting more popular, resulting in better local disease control; however, they are currently not recommended as first-line treatment in unresectable CRLM. Liver transplant remains experimental and needs further evidence to validate its use. In the absence of standardized evidence-based protocols, the optimal management of CRLM should be determined by a multi-disciplinary team.

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Hepatic encephalopathy: Lessons from preclinical studies

Luiza Cioglia Dias Lima, Aline Silva Miranda, Rodrigo Novaes Ferreira, Milene Alvarenga Rachid, Ana Cristina Simões e Silva

ORCID number: Luiza Cioglia Dias Lima (0000-0003-1601-0812); Aline Silva Miranda (0000-0003-2811-7924); Rodrigo Novaes Ferreira (0000-0002-5987-8622); Milene Alvarenga Rachid (0000-0002-3142-6552); Ana Cristina Simoes e Silva (0000-0001-9222-3882).

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Luiza Cioglia Dias Lima, Milene Alvarenga Rachid, Departamento de Patologia Geral, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, Minas Gerais 31270-901, Brasil

Aline Silva Miranda, Rodrigo Novaes Ferreira, Departamento de Morfologia, Instituto de Ciências Biológicas, UFMG, Belo Horizonte, Minas Gerais 30130-100, Brasil

Aline Silva Miranda, Ana Cristina Simões e Silva, Laboratório Interdisciplinar de Investigação Médica, Faculdade de Medicina, UFMG, Belo Horizonte, Minas Gerais 30130-100, Brasil

Corresponding author: Ana Cristina Simoes e Silva, MD, PhD, Full Professor, Laboratório Interdisciplinar de Investigação Médica, Faculdade de Medicina, UFMG, Avenida Alfredo Balena, 190, 2o andar, sala 281, Belo Horizonte, Minas Gerais 30130-100, Brazil.

acssilva@hotmail.com

Telephone: +55-31-34098073

Abstract

Hepatic encephalopathy (HE) is a major complication that is closely related to the progression of end-stage liver disease. Metabolic changes in advanced liver failure can promote cognition impairment, attention deficits and motor dysfunction that may result in coma and death. HE can be subdivided according to the type of hepatic injury, namely, type A, which results from acute liver failure, type B, which is associated with a portosystemic shunting without intrinsic liver disease, and type C, which is due to chronic liver disease. Several studies have investigated the pathogenesis of the disease, and most of the mechanisms have been explored using animal models. This article aimed to review the use of preclinical models to investigate HE. The most used animal species are rats and mice. Experimental models of type A HE include surgical procedures and the administration of hepatotoxic medications, whereas models of types B and C HE are generally surgically induced lesions in liver tissue, which evolve to hepatic cirrhosis. Preclinical models have allowed the comprehension of the pathways related to HE.

Key words: Hepatic encephalopathy; Acute liver failure; Preclinical studies; Hepatic cirrhosis; Neuroinflammation; Hyperammonemia

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Core tip: Hepatic encephalopathy (HE) is a major complication closely related to the progression of end-stage liver disease. It can be subdivided according to the type of

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hepatic injury: type A, which results from acute liver failure, type B, which is associated with a portosystemic shunting without intrinsic liver disease, and type C, which is due to chronic liver disease. In this article, we have described the use of preclinical models to investigate HE. We have briefly described the applicability and the characteristics of these experimental models. In conclusion, preclinical models have allowed the comprehension of the pathways related to HE.

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INTRODUCTION

Hepatic encephalopathy (HE) is defined as a neuropsychiatric syndrome that occurs in patients with acute or chronic liver diseases^[1]. Clinically, it is characterized by a spectrum of symptoms, including cognition impairment, altered levels of consciousness that may progress to coma and death^[2]. The risk of mortality has usually been related to higher grades of HE, and the incidence of this disease is increased in patients with frequent infections^[3,4]. Patients with HE are greatly impacted in terms of quality of life and the high costs caused by increased healthcare utilization^[5,6].

The pathophysiology of HE is not completely understood, and several studies have shown biochemical disturbances, with elevation of serum ammonia levels and increased oxidative stress in blood, alterations of neurotransmission systems, development of brain edema, astrocyte swelling and inflammation^[8]. A wide range of animals, including dogs, goats, pigs, rabbits, guinea pigs, rats and mice, have been used for better understanding the mechanisms underlying HE pathogenesis and for the development of novel therapeutic agents for HE, providing a basis for future clinical research. Therefore, the objective of this brief review is to discuss relevant aspects of the HE pathogenesis and the current animal models employed, which may closely resemble the disease in humans.

HEPATIC ENCEPHALOPATHY

HE is a central nervous system (CNS) dysfunction resulting from acute or chronic liver failure that leads to a wide range of neuropsychiatric manifestations^[7]. Clinically, patients with HE may present sleep-wake cycle disturbance, personality changes and cognitive, motor activity and coordination dysfunctions, which ultimately progress to stupor, coma and death. Importantly, HE often affects health-related quality of life, clinical management strategies, liver transplant priority, and patient survival^[8].

According to the American Association for the Study of Liver Disease (AASLD) updated guidelines, HE should be classified as type A, B, or C based on the underlying disease. Type A (acute) results from an acute liver failure (ALF), while type B (bypass) is associated with a portosystemic shunting without intrinsic liver disease and type C (chronic) is the consequence of cirrhosis^[7]. The incidence of ALF is low, affecting approximately 2000 people per year in the United States or Europe^[8,9]. However, the mortality of ALF is high and is mostly attributed to fast progression to HE, leading to cerebral edema, increased intracranial pressure and cerebral herniation^[8,9].

In contrast, chronic liver diseases are highly prevalent, affecting approximately 5.5 million individuals only in the United States. Approximately 80% of patients with cirrhosis will develop a less severe form of HE, known as minimal HE (MHE), which is characterized by mild cognitive impairment, attention deficits, psychomotor slowing and impaired visuomotor and bimanual coordination^[10]. MHE is detected only by employing psychometric or neurophysiological tests and is considered an important predictive factor for the development of HE since 30%-40% of cirrhotic patients progress to this later condition^[10,11]. Apart from its educational and social impact, HE also carries a significant economic burden. For instance, in 2009, in the United States, HE led to approximately 22931 hospitalizations, with an average cost of each stay ranging from 46663 to 63108 USD^[8].

The broad spectrum of HE, especially regarding the type of underlying disease, severity of clinical manifestations, and precipitating factors (e.g., infections, gastrointestinal bleeding and drug toxicities) should be considered to understand the pathophysiological mechanisms of HE as well as to identify potential therapeutic targets^[12].

HEPATIC ENCEPHALOPATHY PATHOPHYSIOLOGY

HE is a complex condition whose cellular and molecular mechanisms remain to be fully elucidated. Over the past decades, some hypotheses have been proposed, including neurotransmitter system dysfunction, impaired energy and lactate metabolism and oxidative stress (for review see^[1,13]). However, the hyperammonemia and the neuroinflammation hypotheses have been the mostly recognized ones and will be briefly revisited in the current review. **Figure 1** shows the main pathophysiological features of the available models of HE.

Hyperammonemia

Currently, increased systemic and brain levels of ammonia are the main factors implicated in HE pathogenesis. Under physiological conditions, the ammonia, resulting from nitrogenous compounds, such as proteins metabolized by gut microflora, is metabolized in the liver *via* the urea cycle, forming urea, which is mainly excreted by the kidneys. ALF, portosystemic shunting or chronic liver disease can impair liver function, leading to increased levels of ammonia in the blood^[14].

Ammonia metabolism in the liver depends on phosphate-activated glutaminase (PAG), which catalyzes the hydrolysis of glutamine to produce glutamate, energy, nucleotide synthesis and ammonia. PAG has two isoforms, the hepatic type (L-PAG), restricted to the liver, and the kidney-type (K-PAG), found in the kidney, brain and enterocyte villi, especially in the small intestine. Interestingly, PAG activity in the intestine has been associated with increased systemic levels of ammonia during liver cirrhosis and seems to play a major role in the pathogenesis of HE^[15,16].

As the levels of ammonia increase systemically, the molecule crosses the blood-brain barrier and starts to be metabolized in the CNS^[14]. The ammonia detoxification in the brain requires its incorporation into glutamine by the action of the enzyme glutamine synthetase, which is present only in astrocytes. The glutamine accumulation in astrocytes as a result of ammonia detoxification results in increased water entry and osmotic forces, ultimately inducing astrocytes to swell and causing cytotoxic edema^[17]. The impact of hyperammonemia on astrocyte function in response to HE remains to be fully elucidated. However, it has been reported that, apart from increasing oxidative stress and osmotic pressure, hyperammonemia may also influence inflammatory and signal transduction pathways^[18,19], gene expression and neurotransmitter release^[20] as well as posttranslational protein modifications^[21].

Although a great deal of attention continues to be focused on ammonia as the main toxin related to HE pathogenesis, there is evidence that patients with elevated levels of systemic and local ammonia may not present HE symptoms; additionally, the ammonia concentration is not always consistent with the severity of HE in cirrhotic patients^[22,23]. Moreover, ammonia-lowering agents, including L-ornithine, L-aspartate and lactulose, have so far been of limited value in preventing HE in ALF and in cirrhosis^[24-26], supporting a role for other factors alone or in association with ammonia in the development of HE. For instance, the effect of the glycerol phenylbutyrate (GPB), approved by the Food and Drug Administration in 2013 for the treatment of urea cycle disorders, was investigated in a randomized, double-blind, placebo-controlled phase II trial with cirrhotic patients who experienced two or more HE events in the last 6 mo. The GPB acts as an ammonia-lowering agent by producing phenylacetyl glutamine, which is excreted in urine. GPB treatment in cirrhotic patients decreased plasma levels of ammonia, the proportion of patients who experienced HE and hospitalizations due to HE. These findings supported the involvement of ammonia in HE pathophysiology and the potential therapeutic role of GPB^[27].

Neuroinflammation

In addition to the ammonia hypothesis, brain inflammation, also known as neuroinflammation, is thought to be a major component in the development of HE. Clinical and experimental evidence of activation of microglia, the brain resident immune cells, in response to ALF and cirrhosis extensively supported the neuroinflammation hypothesis^[28-32]. For instance, increased expression of the major histocompatibility complex class II antigen marker CD11b/c (also termed OX-42), an indicator of microglial activation, was found in an ALF model following liver

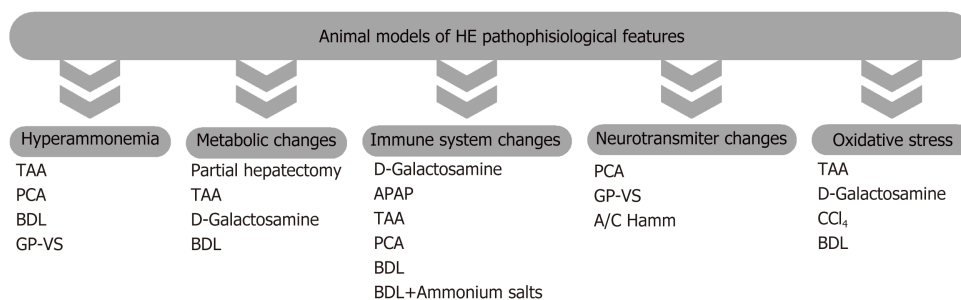


Figure 1 Main pathophysiological feature of the available models of hepatic encephalopathy. APAP: Acetaminophen; A/C Hamm: Acute/chronic hyperammonemia; BDL: Bile duct-ligated; CCl₄: Carbon tetrachloride; GP-VS: Graded portal-vein stenosis; PCA: Portosystemic anastomosis; TAA: Thioacetamide.

ischemia in rats^[30]. Importantly, the administration of minocycline, a potent inhibitor of microglial activation, attenuates the encephalopathy grade and prevents brain edema in the same ALF experimental model^[32]. Corroborating these findings, increased expression levels of microglial activation markers, including human leukocyte antigen DR (CR3/43) and ionized calcium-binding adaptor molecule 1 (Iba-1), were found in the CNSs of patients with ALF associated with viral hepatitis^[29] and in liver cirrhosis^[31], respectively.

Microglial activation has often been associated with increased release of cytokines and chemokines, which are also implicated in the pathogenesis of neurodegenerative and neuropsychiatric diseases^[33,34]. The first evidence of upregulation of inflammatory molecules in HE was obtained in a study of patients with ALF due to acetaminophen overdose. The levels of inflammatory cytokines (TNF, IL-1 β and IL-6) were measured in blood samples from an artery and a reverse jugular catheter. Increased arterial levels of cytokines correlated with intracranial hypertension. Brain cytokine efflux was noted, indicating brain cytokine production in these patients^[35]. Supporting data were also provided by several studies employing pharmacological and nonpharmacological liver failure models in rodents, which also showed increased levels of inflammatory cytokines (TNF, IL-1 β and IL-6) and chemokines, such as CXCL-1, CCL2, CCL3, CCL5 and CX3CL1^[30,32,36-38]. Importantly, anti-inflammatory-based strategies attenuated cognitive decline and motor activity impairment, supporting the involvement of neuroinflammation in HE pathophysiology^[39-42]. It is worth mentioning that ammonia alone is capable of inducing microglial and astrocyte activation, leading to increased expression of inflammatory cytokines, such as IL-1 β and IL-6. This finding suggests that hyperammonemia may trigger neuroinflammation in HE in a synergistic manner. However, a direct link between both mechanisms is still missing^[43].

PRECLINICAL MODELS

Choice of animal species

In 2008, a commission formed by members of ISHEN (International Society of Hepatic Encephalopathy and Nitrogen Metabolism) gathered in Padua, Italy to establish guidelines for HE animal models. Due to the variety of etiologies of hepatic dysfunction and the many factors that may influence the development of neurological symptoms, HE is considered difficult to reproduce faithfully in animals. **Table 1** summarizes the main advantages and disadvantages of the available HE models. Currently, there is no ideal animal model to resemble hepatic failure caused by ethanol, virus or acetaminophen, the most common etiologies in human beings^[44].

The availability of animal models is indispensable for studying the mechanisms of diseases and possible therapies^[45]. Many animal species have been used for the investigation of HE, including dogs, goats, pigs, rabbits, guinea pigs, rats and mice. Large animals are advantageous for neurological examinations and the availability of biological samples, such as blood samples, body fluids and biopsies but are rarely used due to the cost of the animals, the maintenance involved and ethical concerns. The most common species currently used in HE models are rats and mice, mainly due to the accessibility of molecular and anatomical studies and the availability of literature on behavioral, pathological and biochemical methods and findings. Other advantages of these species are the complete characterization of the genome, easy availability of antibodies and molecular probes and low costs for obtaining and maintaining the animals^[44].

Table 1 Advantages and disadvantages of animal models of hepatic encephalopathy

	Model features	Advantages	Disadvantages
Type A Model. Encephalopathy associated with acute liver failure	Type A models have been developed by exclusion (anhepatic models), partial removal of the liver or from the administration of a hepatotoxin	<i>Anhepatic model</i> : The course of HE is relatively rapid. Applied to studies of brain metabolism, neurotransmission abnormalities, gene expression and brain inflammation in ALF. The model responds to hypothermia, ammonia-lowering agents and anti-inflammatory drugs; <i>Hepatotoxic model</i> : Different hepatotoxins could be used to create Type A models. Generally, these models produce hypothermia, hypoglycemia and other systemic complications; <i>Thioacetamide</i> : Model with good repeatability, easy operation, and high similarity to human HE; <i>Acetaminophen</i> : Easy preparation, low price and dose-dependence; <i>D-galactosamine</i> : Good repeatability. Simulates the pathophysiological changes of acute liver failure. Shows manifestations of liver injury that are similar to viral hepatic failure	<i>Anhepatic model</i> : Neither procedure could lead to a potential recovery. The surgical procedure causes great trauma to the animal. Absence of injured or necrotic hepatic cells. The toxic substances and inflammatory mediators present in the injured liver are not perfused into the blood circulation; <i>Partial hepatectomy</i> : The surgery is difficult to control. Severe hypoglycemia leads to death; <i>Hepatotoxic model</i> : Each of these toxins could produce hepatitis with variable pathological nature. Animal-to-animal variations lead to a lack of reproducibility. Some hepatotoxins show extrahepatic toxicity; <i>Acetaminophen</i> : Poor reproducibility. Shows side effects in kidneys and other organs; <i>D-galactosamine</i> : High cost, short survival time and poor stability
Type B Model. Encephalopathy associated with portosystemic bypass without liver disease	Type B models have been developed by portosystemic shunting (portacaval anastomosis, congenital portacaval shunts, graded portal vein stenosis, and biliary duct ligation). Different HE aspects could be assessed using different shunt methods and species (pig, dog, rabbit, rat, and mouse)	<i>Portacaval anastomosis</i> : Can better simulate the clinical mild HE phenotypes in different animal models. (Dog) EEG changes and neurological status correlate with ammonia in the plasma; <i>Congenital portacaval shunts</i> : (Dogs) Naturally develop psychomotor dysfunction, reduced hepatic function, and hyperammonemia and are susceptible to high-protein diets; <i>Graded portal vein stenosis</i> : Easy to perform. The surgery may be reversed. (Rat) Provides a Minimal Hepatic Encephalopathy model. (Rat) Develop loss of activity, altered circadian rhythm, hyperammonemia, and altered ammonia/glutamine in the brain	<i>Portacaval anastomosis</i> : Cause severe coma due to hypersensitivity to ammonia. (Rabbit) Portacaval anastomosis may lead to death of animal in most cases. (Rat) Needs high surgical skills to perform; <i>Congenital portacaval shunts</i> : Access to animals with this congenital alteration
Type C Model. Encephalopathy associated with liver disease	HE associated with cirrhosis and portal hypertension (Type C) is the most common form of HE in patients. At present, there is no appropriate model to study HE that occurs in liver cirrhosis; nevertheless, some models have been developed	<i>Biliary duct ligation</i> : Animals develop liver failure, jaundice, portal hypertension, immune system dysfunction, and bacterial translocation. (Rat) Reproducible model of biliary cirrhosis with the development of hyperammonemia, low-grade encephalopathy, and decreased locomotor activity. (Rat) Bile duct-ligated animals fed with ammonium salts provide a model that reproduces human Alzheimer Type II astrocytosis	No satisfactory Type C animal model induced by alcoholic liver disease or viral hepatitis exists at the present time; <i>Biliary duct ligation</i> : Immune system dysfunction and other diseases may affect the final HE phenotypes. (Rat) Weight loss due to hunger suppression; <i>CCl4</i> : Animal-to-animal variations lead to a lack of reproducibility. Limited neurobehavioral assessment due to the presence of ascites. Possible health damage when a researcher uses this chemical
Models of acute/chronic hyperammonemia	These models are designed to study the effects of hyperammonemia on brain function without liver dysfunction	Inexpensive and simple to perform. The model shows alterations of multiple neurotransmitter systems in the brain. The model shows impairments in learning and memory	Limited to rats and mice. Time-consuming; not suitable for long-term studies. Lacks liver failure

Animal HE models of ALF (type A)

According to the HE guidelines established by ISHEN, an animal model of HE in ALF has to essentially reproduce the clinical picture to facilitate staging of encephalopathy and to show the progression of symptoms, including brain edema and its complications (*i.e.*, intracranial hypertension and brain herniation). The model should also be capable of being reversed, with high concentrations of ammonia and glutamine in the brain, classical hepatic and brain pathology and minimal hazards to personnel from toxins and infectious agents. All animal models of ALF produce

hypothermia and hypoglycemia, making it essential to control the temperature and glucose levels and to provide supportive care to the animals. A brief description of ALF models is presented in Table 2.

Pharmacological models

Several hepatotoxins have been extensively used in the development of animal models of ALF^[46]. The main hepatotoxic substances used to cause ALF in animals include galactosamine, acetaminophen, azoxymethane (AOM) and thioacetamide (TAA)^[44]. Although the administration of carbon tetrachloride (CCL₄) has been primarily described as a type A HE model, the ALF and behavioral changes in this model are not commonly observed. However, the ability of this model to induce liver cirrhosis makes it a valuable tool for studying type C HE^[44]. Thus, in the current review, the CCL₄ model will be discussed as a Type C HE model.

Thioacetamide: Until 1943, TAA was used as fungicide in orange crops. In 1948, it was discovered that chronic administration of the substance led to liver cirrhosis and hepatocellular carcinomas. TAA causes hepatocellular necrosis after biotransformation to an active metabolite via the flavin adenine dinucleotide monooxygenase pathway, resulting in the formation of TAA-S-oxide^[47]. TAA reduces antioxidant activity and enhances lipid peroxidation in the liver, leading to oxidative stress and cellular necrosis^[48]. The most common forms of administration of TAA are oral (in drinking water) and intraperitoneal injection^[49]. TAA has been extensively used to induce ALF in rats and mice, producing encephalopathy, metabolic acidosis, high transaminases, abnormal coagulation and histological centrilobular necrosis. The TAA model of HE shows good reproducibility and well-described hepatic and cerebral changes^[50-52]. Chronic administration can produce hepatic cirrhosis^[49,53,54].

D-Galactosamine: D-Galactosamine (D-Gal) is an amino sugar that is metabolized in the liver, causing consumption of uridine nucleotides and blockade of transcriptional factors in the liver. The hepatic failure resulting from D-Gal administration has been, at least in part, associated with the production of uridine-containing compounds during hepatic biotransformation^[55]. The administration of D-Gal in rats was described by Keppler *et al*^[56] as an acute hepatic failure model that induced encephalopathy and increased aspartate transaminase, prothrombin time, ammonia and intracranial pressure. The same model was described by Blitzer *et al*^[57] in rabbits and by Sielaff *et al*^[58] and Diaz Buxo *et al*^[59] in dogs.

There are differences in species susceptibility. Mice are resistant until high doses are administered, while rats are sensitive to D-Gal-induced hepatic failure^[60]. D-Gal and acetaminophen models cause liver failure, although the development of HE is variable. Both models are difficult to reproduce and have poorly characterized cerebral pathology^[44].

Acetaminophen: Acetaminophen is known as APAP (in the United States) and Paracetamol (in Europe and other areas of the world). Acetaminophen has been widely used as an antipyretic and/or analgesic since 1955, particularly because it is easily accessible in various formulations as an over-the-counter medication. However, acetaminophen is one of the most common causes of ALF, accounting for more than 60% of all cases in the United States^[61].

The metabolism of APAP occurs in the liver. Under normal circumstances, the medication undergoes biotransformation by a combination of glucuronidation and sulfation and is then excreted by the kidneys. When excessive amounts of acetaminophen are administered, these metabolic pathways are saturated, and APAP is metabolized by the P450 cytochrome oxidase enzyme system, leading to the formation of a toxic electrophile, N-acetyl-p-benzoquinoneimine (NAPQI). NAPQI produces cell injury unless conjugated with endogenous glutathione. NAPQI is supposed to disrupt mitochondrial calcium flux, resulting in cell damage by the production of free radical oxygen species, hydroxyl radicals, nitrites and nitrates. The cascade is amplified by the activation of Kupffer cells and the release of cytotoxic mediators (*e.g.*, cytokines and free radicals), leading to apoptosis and cell necrosis^[62-65].

Miller *et al*^[66] reported a model of APAP-induced liver failure in pigs. Animals exhibited metabolic acidosis, encephalopathy, coma, increased transaminase levels and histological evidence of severe centrilobular coagulative necrosis. However, changes produced by APAP administration were variable according to animal species used, as was described by Gazzard *et al*^[67], who showed a variable clinical outcome in greyhound dogs.

Later, Francavilla *et al*^[68] induced ALF by APAP administration for the first time in beagles. The authors compared the routes of administration: intravenous, intramuscular and subcutaneous. APAP toxicity was very variable in different animal models, and the incidence of sudden death due to methemoglobinemia was

Table 2 Brief description of type A animal models of hepatic encephalopathy

Species used		Main findings
Surgical models		
Hepatic devascularization	Rat, rabbit, pig	Increased AST, hypoglycemia, lethargy and coma
Hepatectomy	Rat, pig	Increased AST, TNF, PT, NH ₃ , lactate, hypoglycemia, hepatic necrosis
Pharmacological models		
Galactosamine (IP, IV, SC)	Rat, rabbit, guinea pig	Increased AST, PT, NH ₃ , hepatic necrosis
Acetaminophen (IP, IV, SC, oral)	Rat, dog, pig	Increased AST, NH ₃ , bilirubin, hypoglycemia, metabolic acidosis, centrilobular coagulative necrosis
Thioacetamide (IP, oral)	Rat	Increased AST, PT, NH ₃ , metabolic acidosis, centrilobular necrosis
Azoxymethane (IP, SC)	Mouse	Increased AST, NH ₃ and bilirubin, hepatic necrosis

AST: Aspartate transaminase; TNF: Tumor necrosis factor; PT: Prothrombin time; NH₃: Ammonia; IP: Intraperitoneal; IV: Intravenous; SC: Subcutaneous.

frequently observed. This drug exhibited cardiotoxicity and nephrotoxicity, which was associated with acute lung injury^[69-71].

Azoxymethane: AOM is an active metabolite of the cycad palm nut found on the island of Guam. AOM is a potent hepatotoxin that induces ALF in mice in a dose-dependent manner. Liver toxicity has also been reported in humans, livestock and rats following the ingestion of Guam cycad palm nuts due to AOM toxicity. The AOM model was first described as a model of hepatotoxin-induced liver failure and HE by Matkowskyj *et al*^[72]. This model leads to encephalopathy, cerebral edema, elevated brain ammonia and unbalanced amino acid levels. This model also shows characteristic pathologic aspects^[28].

The AOM model generates microvesicular steatosis, dilation of hepatic sinusoids, and hepatocyte necrosis in addition to elevations in serum transaminases and bile acids, with the largest increases observed when mice progressed toward coma^[73].

Surgical models

The surgical models can be divided into variations of partial and total hepatectomy and partial and complete devascularization of the liver^[46].

Hepatic devascularization: Rappaport was the first to describe a devascularization model, in 1953^[74]. The model is usually produced by a portocaval anastomosis (PCA) with subsequent hepatic artery ligation (HAL). ALF can be reversible by the occlusion of the hepatic artery for only a short period of time. There is no blood lost. The presence of necrotic hepatic tissue is comparable to ALF in humans^[45]. Hypoxic insult results in dysfunction of the mitochondrial respiratory chain, which, in turn, reduces ATP levels due to impaired oxidative phosphorylation, interferes with the intracellular calcium homeostasis and activates enzymes responsible for protein, lipid and DNA damage. After hypoxic injury, the reperfusion process induces more damage in liver tissue^[75-77].

Hepatectomy: Hepatectomy is considered a model of postoperative liver failure (POLF) that can accurately reproduce all neurological and metabolic changes as a consequence of extensive liver resections in humans^[78]. Almost 100% survival and intense regeneration occur in partial hepatectomy that removes approximately 70% of the liver in rats and pigs^[79-81]. In contrast, progressive necrosis follows partial resections of the liver, as reported by Panis *et al*^[82]. Among other disadvantages of partial hepatectomy are the lack of consistency of injury degree and the increased intraoperative blood loss. Additionally, the surgery often hampers the animal's capability to immediately restore drink and food consumption, which may lead to severe hypoglycemia and death^[83].

The altered physiological state is attributed to increased total blood flow through the remnant liver tissue, leading to flow injury and damage to sinusoidal endothelial cells, activation of Kupffer cells and release of inflammatory cytokines^[84]. This model is also associated with increased levels of aspartate transaminase (AST), tumor necrosis factor (TNF) and hypoglycemia^[85].

The "anhepatic" model is made by the total removal of the liver. In clinical practice, total hepatectomy has been performed only in cases of very severe ALF, pending the arrival of a donor liver, which follows the removal of a "toxic" organ^[86,87]. Overall, the anhepatic model seems not to be suitable for evaluating therapies as an artificial liver support for ALF. Moreover, due to the lack of the liver, this model has very poor

reversibility^[46].

Animal models of HE types B and C

An ideal model of HE classified as type B or C should have some essential features, including a precipitant factor and neuropathological findings of HE, such as symptoms of encephalopathy, ranging from MHE to coma. Additionally, increased brain ammonia/glutamine along with low-grade brain edema and Alzheimer Type II astrocytosis at advanced stages of HE might also be observed. These models should exhibit clinical responses to established treatments^[29]. Table 3 displays the animal models of HE associated with portosystemic shunting (type B) and chronic liver disease (type C).

Type B hepatic encephalopathy

As previously mentioned, animal models of type B HE are based on portal-systemic shunting and include the following approaches.

Portacaval anastomosis: PCA is the most common animal model used in the study of chronic HE. The basis of this model is the creation of a portal-systemic shunt (end-to-side PCA) that mimics the situation induced in cirrhosis by collateral circulation^[45].

It is known that the deviation of portal blood decreases the total hepatic blood flow, depriving the liver of oxygen and hepatotrophic factors from the portal vein that are necessary for metabolic processes. These phenomena induce hepatic parenchyma atrophy. Among other factors, the high levels of ammonia, as a consequence of reduced hepatic metabolism, contribute to HE. The severity of neurological manifestations depends on the intensity of liver injury. Animals submitted to PCA have increased brain ammonia/glutamine, altered circadian cycle, hypokinesia and reduced memory and learning ability^[88].

Congenital portacaval shunts: Dogs and cats with congenital portacaval shunts are considered naturally occurring models of type B HE. These animals develop psychomotor dysfunction, abnormal motor signs, altered day-night rhythms, hyperammonemia, and hepatic dysfunction^[89].

Graded portal vein stenosis: Graded portal vein stenosis provides a MHE model in rats. This procedure is easier to perform than the end-to-side portacaval anastomosis. Alterations associated with this model are hypoactivity, altered circadian cycle and increased brain ammonia/glutamine^[44].

Type C hepatic encephalopathy

Animal models of type C HE should lead to decompensated liver cirrhosis. It is worth noting that there is currently no ideal animal model of type C HE^[44]. Some models that resemble human hepatic cirrhosis have been employed, as described below.

CCl₄: CCl₄ was extensively used in the 1970s as a model of acute hepatic failure, but the species variation was significant, and it was difficult to reproduce. It has been used more recently in the study of liver cirrhosis^[90,91]. CCl₄ is metabolized by cytochrome P-450, generating free radicals, causing lipoperoxidation, increased hepatic membrane permeability, tissue fibrosis and hepatic failure. This model does not seem to produce many behavioral changes, except in cases of advanced cirrhosis^[92]. It is used in the study of astrocytic response at the level of RNA synthesis. Inconsistent lesions animal to animal may represent a major disadvantage. Moreover, the presence of ascites may limit neurobehavioral assessments^[44].

Bile duct ligation: The ligation of the common bile duct ligation (BDL) induces a reproducible model of biliary cirrhosis in rats, leading to liver failure, portal hypertension, translocation of bacteria and immune system dysfunction. BDL rats show hyperammonemia and decreased motor activities, but only low-grade encephalopathy^[45,93-95]. It is possible to reproduce the human neuropathology of type C HE, Alzheimer type II astrocytosis, altered brain osmolytes, low-grade brain edema, inflammation and motor activity deficits by feeding BDL rats with ammonium salts. Of note, by giving ammonium salts to BDL rats, it is possible to obtain a model of acute-on-chronic HE^[96].

HE MODELS RESULTING FROM PURE HYPERAMMONEMIA

These models are usually limited to rats and mice and are used to study the effects of hyperammonemia on brain function, in the absence of liver damage or portacaval shunting. They are produced by means of the administration of high-ammonia diets,

Table 3 Brief description of findings obtained with types B and C experimental models of hepatic encephalopathy

Experimental model	Animal species	Biological findings	Clinical Signs
Portacaval anastomosis	Rats, dog, rabbit, pig	Increased brain ammonia/glutamine	Altered circadian cycle, hypokinesia, reduced memory and learning ability
Congenital portacaval shunts	Dogs, cats	Hyperammonemia	Hepatic dysfunction, psychomotor dysfunction, motor signs
Graded portal vein stenosis	Rats	Increased brain ammonia/glutamine	Minimal hepatic encephalopathy, loss of activity, altered circadian cycle
Carbon tetrachloride (CCl ₄)	Rats, mice	Generation of free radicals, lipoperoxidation, tissue fibrosis, increased hepatic membrane permeability	Hepatic failure, motor activity dysfunction
Bile duct ligation	Rats	Bacterial translocation, immune system dysfunction, hyperammonemia	Liver failure, portal hypertension, decreased locomotor activities due to low-grade encephalopathy

parenteral infusion of ammonia or urease treatment. These models are inexpensive and easily reproducible and result in animals with impaired memory and learning skills^[44]. However, the disadvantages of these models include the time involved to produce alterations and their unsuitability for long-term studies^[45].

CONCLUSION

HE is a very severe complication in the context of liver failure. HE can be subdivided according to the type of hepatic injury, namely, type A, which results from ALF, type B, which is associated with a portosystemic shunting without intrinsic liver disease, and type C, which is due to chronic liver disease. The pathophysiology of HE has two major factors, namely, increased ammonia levels and neuroinflammation.

Preclinical models have been very useful in investigating the mechanisms of HE and in evaluating novel therapeutic approaches. The most used animal species are rats and mice. Experimental models of ALF (type A) include surgical procedures (hepatectomy and hepatic devascularization) and the administration of hepatotoxic medications. Surgical models resemble postoperative liver failure in humans, whereas pharmacological models are similar to adverse drug reactions due to hepatotoxicity.

Ideally, models of HE associated with portosystemic shunting (type B) and due to chronic liver disease (type C) must exhibit liver cirrhosis, a precipitant factor, neuropathological and neurochemical alterations. These models are generally surgically induced lesions in liver tissue, which evolve to hepatic cirrhosis.

In conclusion, preclinical models have allowed the comprehension of the pathways related to neurological damage as a consequence of acute and chronic liver injury, resulting in the identification of potential therapeutic targets.

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Case Control Study

Comprehensive analysis of *HFE* gene in hereditary hemochromatosis and in diseases associated with acquired iron overload

Wagner Narciso de Campos, Juliana Doblás Massaro, Eduardo Luiz Rachid Cançado, Cláudia Emília Vieira Wiesel, Aguinaldo Luiz Simões, Andreza Correa Teixeira, Fernanda Fernandes de Souza, Celso Teixeira Mendes-Junior, Ana de Lourdes Candolo Martinelli, Eduardo Antônio Donadi

ORCID number: Wagner Narciso de Campos (0000-0002-4118-2846); Juliana Doblás Massaro (0000-0001-5324-5229); Eduardo Luiz Rachid Cançado (0000-0002-9309-1524); Cláudia Emília Vieira Wiesel (0000-0002-4381-0834); Aguinaldo Luiz Simões (0000-0001-5950-894X); Andreza Correa Teixeira (0000-0003-4878-8215); Fernanda Fernandes de Souza (0000-0002-2369-7686); Celso Teixeira Mendes-Junior (0000-0002-7337-1203); Ana de Lourdes Candolo Martinelli (0000-0002-1713-9039); Eduardo Antônio Donadi (0000-0002-9457-9601).

Author contributions: de Campos WN and Massaro JD contributed equally in the production of the paper; Campos WN performed all experiments and statistical analysis, wrote the final manuscript; Massaro JD wrote the initial project, standardized HLA-*HFE* procedures, performed the statistical analysis, helped on the writing of the final manuscript; Cançado ELR selected and provided patients; Wiesel CEV performed cloning experiments for defining *HFE* alleles; Simões AL provided facilities to perform the *HFE* cloning experiments; Teixeira AC selected and provided patients; Souza FF selected and provided patients; Mendes-Junior CT helped on statistical analyses; Martinelli ALC responsible for the

Wagner Narciso de Campos, Juliana Doblás Massaro, Eduardo Antônio Donadi, Division of Clinical Immunology, Department of Medicine, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto 14048-900, Brazil

Eduardo Luiz Rachid Cançado, Department of Gastroenterology, Clinical Gastroenterology and Clinical Hepatology of Clinical Hospital, University of São Paulo School of Medicine, São Paulo 01329-000, Brazil

Cláudia Emília Vieira Wiesel, Aguinaldo Luiz Simões, Department of Genetics, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto 14048-900, Brazil

Andreza Correa Teixeira, Fernanda Fernandes de Souza, Ana de Lourdes Candolo Martinelli, Division of Gastroenterology, Department of Medicine, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto 14048-900, Brazil

Celso Teixeira Mendes-Junior, Departamento de Química, Laboratório de Pesquisas Forenses e Genômicas, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto 14040-901, Brazil

Corresponding author: Eduardo Antônio Donadi, MD, PhD, Associate Professor, Division of Clinical Immunology, Department of Medicine, Ribeirão Preto Medical School, University of São Paulo, Avenida Bandeirantes 3900, Monte Alegre, Ribeirão Preto 14048-900, Brazil.

eadonadi@fmrp.usp.br

Telephone: +55-16-36022566

Fax: +55-16-36020229

Abstract

BACKGROUND

Patients with hepatitis C virus (HCV) and hepatocellular carcinoma (HCC) may or not develop iron overload (IO), which is associated with worst prognosis, because can cause serious damage to organs. *HFE* gene controls the iron uptake from gut, particularly in patients with hereditary hemochromatosis (HH).

AIM

To identify associations between *HFE* coding region in patients exhibiting hereditary hemochromatosis and in diseases associated with acquired IO.

outpatients followed-up at gastroenterology clinics; Donadi EA mentor of the research, provided financial support, technical facilities and performed final review.

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statement: This study was approved by the local Ethics Research Committee, process number HCRP 4822/2011. The samples are deposited in the Bank of Samples of the Nucleus in Research in Immunogenetics (BAMPI), process number HCFMRP 3530/2007, under the coordination of Eduardo Antônio Donadi, and in the Bank of Samples HCFMRP 3416/2003, under the responsibility of Ana de Lourdes Candolo Martinelli.

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METHODS

We sequenced exons 2 to 5 and boundary introns of *HFE* gene, evaluating all polymorphic sites in patients presenting hereditary (hemochromatosis) or acquired iron overload HCV and HCC) and in healthy controls, using Sanger sequencing. We also determined the ensemble of extended haplotype in healthy control individuals, including several major histocompatibility complex loci, using sequence specific probes. Haplotype reconstruction was performed using the Arlequin and Phase softwares, and linkage disequilibrium (LD) between histocompatibility loci and *HFE* gene was performed using the Haploview software.

RESULTS

The *HFE**003 allele was overrepresented ($f = 71\%$) and *HFE**001 allele was underrepresented ($f = 14\%$) in HH patients compared to all groups. A strong linkage disequilibrium was observed among the *H63D-G*, *IVS2(+4)-C* and *C282Y-G* gene variants, particularly in HH; however, the mutation *IVS2(+4)T>C* was not directly associated with HH susceptibility. The *HFE**001/*HFE**002 genotype conferred susceptibility to HCC in HCV patients exhibiting IO ($P = 0.02$, OR = 14.14). Although *HFE* is telomeric to other histocompatibility genes, the *H63D-G/IVS2(+4)-C* ($P \leq 0.00001/P \leq 0.0057$) combination was in LD with *HLA-B*44* allele group in healthy controls. No LD was observed between *HFE* alleles and other major histocompatibility loci.

CONCLUSION

A differential *HFE* association was observed for HH and for diseases associated with acquired IO (HCV, HCC). Since *HFE* is very distant from other histocompatibility loci, only weak associations were observed with these alleles.

Key words: *HFE* gene; Hepatocellular carcinoma; Hepatitis C; Hemochromatosis hereditary; Alleles; Haplotypes

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Core tip: Patients with hepatitis C virus (HCV) and hepatocellular carcinoma (HCC) may or not develop iron overload (IO), which is associated with worst prognosis. The sequencing of the *HFE* gene permitted to assemble the previously described variation sites (*H63DC>G*-, *S65CA>T* and *C282YG>A*) associated with hereditary hemochromatosis into *HFE* haplotypes, under the standardized HLA nomenclature. A differential association of *HFE* alleles was observed for hereditary and acquired IO (HCV, HCC). In addition to the *HFE* gene, we also typed other major histocompatibility loci (*HLA-A/-B/-C/DRB1/-DQB1*, and *HLA-G 14bp INDEL* and *TNFA-d* microsatellites) in the healthy population to understand how the *HFE* gene variability is associated with these loci.

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INTRODUCTION

The *HFE* gene has seven exons and five introns, which code the α -heavy chain of the molecule. Exon 1 codes the signal peptide, exons 2-4 encode the $\alpha 1$, $\alpha 2$ and $\alpha 3$ domains, exons 5 the transmembrane domains, and the 5' portion of exon 6 the cytoplasmic tail^[1]. Considering that *HFE* gene controls the iron uptake from gut, defects of the encoded molecule have been associated with iron overload (IO), particularly in hemochromatosis hereditary (HH). Major variation sites observed at exons 2 to 4 have been associated with HH, including the *H63DC>G* (exon 2),

S65CA>T (exon 2) and C282YG>A (exon 4) variants^[2]. However, not all HH patients exhibit these mutations^[1].

Besides HH, some acquired liver disorders have been associated with IO and fibrosis, including chronic hepatitis C virus (HCV), cirrhosis and hepatocellular carcinoma (HCC)^[3]. The C282Y-A allele is associated with high iron serum levels, increased hepatic iron content and advanced fibrosis in HCV patients. Increased frequency of the classical HFE mutations has also been reported for HCC patients^[4].

We sequenced exons 2 to 5 and boundary introns in HH patients, HCV patients presenting or not IO, and HCC patients exhibiting or not chronic HCV infection to associate with iron overload. We also evaluated the linkage disequilibrium (LD) between the HFE and HLA-A, HLA-B, HLA-C, HLA-DRB1 and DQB1 genes, as well as HLA-G 14bp INDEL and TNFa-d microsatellites to understand the association between HFE alleles and other major histocompatibility genes.

MATERIALS AND METHODS

This study was approved by the local Ethics Research Committee (Process HCRP-FMRP, USP n° 4822/2011), and informed consent was obtained from all participants.

Subjects

A total of 204 patients followed-up at Gastroenterology Units of University Hospitals of the University of São Paulo (USP) were studied: (1) 14 patients (9 men) aged 32-81 years (55.35 ± 15.16) exhibited HH, defined by high transferrin saturation ($\geq 45\%$) and liver IO in the absence of secondary causes; (2) 130 patients with HCV (93 men) aged 19-69 years (42.60 ± 10.98), exhibiting (71 patients, 57 men) or not IO (59 patients, 36 men) (HCV-IO⁺ and HCV-IO⁻, respectively) in the absence of chronic alcohol ingestion (> 60 g/d). All patients exhibited IgG antibody against recombinant HCV antigens by second-generation ELISA (Abbott, Chicago, IL) for at least 6 mo and positive serum HCV RNA (Roche Diagnostic Systems, Branchburg, NJ). Serum levels of liver enzymes, iron, ferritin, and transferrin saturation were also determined. Liver specimens were scored for necroinflammatory activity, as previously described by Desmet *et al.*^[4]. Iron deposits were assessed and scored on the basis of the amount and cellular/lobular location^[4,5]; and (3) 60 patients (43 men) aged 14-78 years (57 ± 14) exhibiting HCC, of whom 24 (18 men) presented IO and chronic hepatitis C (HCC HCV-IO⁺), and 36 (25 men) presented several underlying disorders including cryptogenic hepatitis, hepatitis B, non-alcoholic steatohepatitis and other comorbidities. Since there is no need for liver biopsy for HCC diagnosis, liver iron was not screened in these patients (HCC-IO⁻). The diagnosis of HCC was performed according to Bruix and Sherman^[6].

Iron overload was defined when iron deposits were detected in liver biopsy using Perl's iron staining^[7,8] and/or when serum transferrin saturation was higher than or equal to 45% with or without elevated ferritin. Patients presenting other types of congenital, virus or autoimmune liver disorders were excluded.

A total of 100 healthy unrelated blood donors (CTL), 80 men, and aged 20-52 years (33.31 ± 8.18) was also studied.

HFE typing

Exons 2 to 5 and boundary introns were evaluated using Sanger sequencing^[9] (Figure 1). HFE nucleotide variations were retrieved from the NCBI (NC_000006.12) and Ensembl (ENSG00000010704) databases. Primer sequences, amplification conditions and allele nomenclature were defined as previously reported^[10]. Sequencing was performed using an ABI 3500 sequencer (Applied Biosystems, Foster City, CA).

Major histocompatibility complex loci typing

HLA-A/-B/-C/-DRB1 and -DQB1 typing was performed using commercial kits (One-Lambda, Canoga Park, CA). HLA-G 14bp INDEL^[11] and TNFa-d microsatellites^[12] were typed as previously described. Haplotype inferences combining major histocompatibility genes were performed only for healthy controls.

Statistical analysis

Allelic and genotype frequencies (f), Hardy Weinberg Equilibrium (HWE), Fisher exact test, and linkage disequilibrium (LD) were performed using the GENEPOP v.4.2 and ARLEQUIN v.3.1 softwares. Image map of the pairwise LD parameters [Log of the Odds (LOD) and Linkage Disequilibrium Coefficient (D')] was generated using the HAPLOVIEW v.3.32 software.

Extended major histocompatibility alleles were reconstructed by means of the EM (ARLEQUIN) and PHASE v.2 algorithms. For all situations, P values ≤ 0.05 were

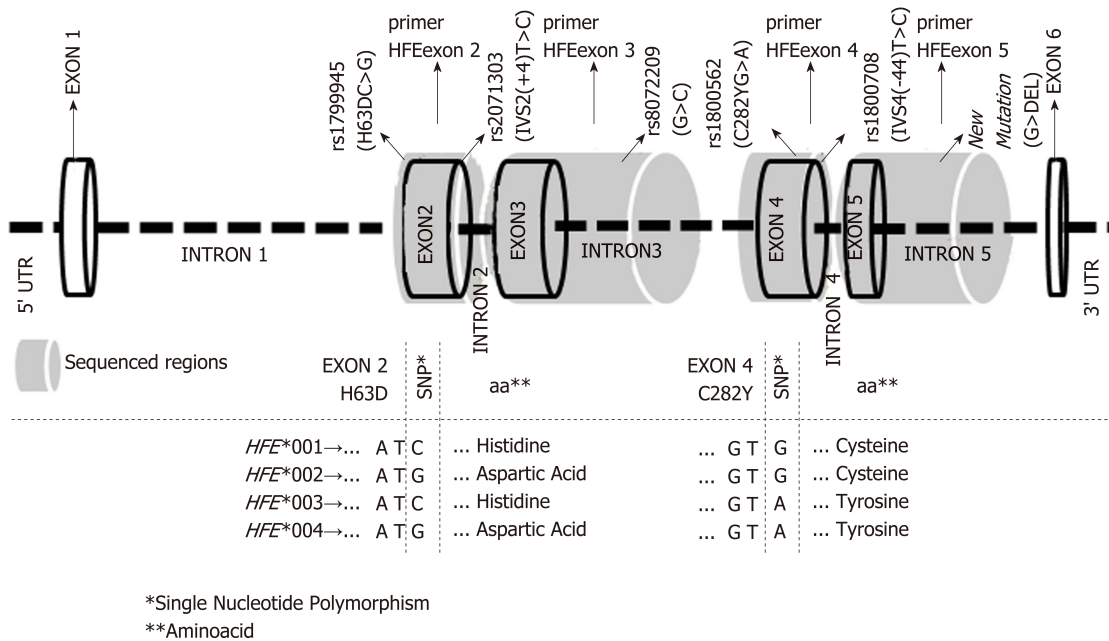


Figure 1 Structure of the *HFE* gene (ID# ENSG0000010704 - <http://www.ensembl.org>) at chromosome region 6p21.3, showing the reference number (rs) of variation sites (NCBI Data base - <http://www.ncbi.nlm.nih.gov/snp>), previously associated with iron disorders. Shaded grey areas indicate the sequenced gene regions and the respective pairs of primers, as previously described^[10]. The combination of these variation sites, translated into the official nomenclature for *HFE* alleles is also shown in the bottom chart; i.e., the combination of the triplet bases and respective encoded residues of the two most important mutations (H63DC>G and C282YG>A) that defined the four major *HFE* allele groups (mutated bases are shown in bold type). *Single nucleotide polymorphism; **Aminoacid.

considered to be significant.

RESULTS

The results regarding *HFE* alleles are presented in two forms: (1) as previously reported in the literature, including the single nucleotide polymorphism (SNP) reference number (rs), the usual SNP names (H63DC>G, C282YG>A, IVS2(+4)T>C and IVS4(-44)T>C) and new variation sites (Table 1); and (2) as the newly described official *HFE* allele nomenclature (Table 2)^[10]. The location of the previously reported variation sites with respect to the nucleotide sequence that defined the new *HFE* nomenclature is illustrated in Figure 1.

HFE alleles and genotypes according to previously described variation sites

All population samples adhered to the HWE, except HCC patients (IO⁺) at the C282YG>A variation site ($P = 0.031$). Overall, patients and healthy controls shared the same most frequent alleles at each SNP, except when HH patients were compared to healthy controls, for whom the C282Y-A ($f = 0.714$) allele was the most frequently observed, significantly associated with susceptibility to HH ($P < 0.001$; OR = 53.06; 95%CI: 18.41-152.90). The C282Y-G allele was protective against HH ($P < 0.001$; OR = 0.01; 95%CI: 0.006-0.05). On the other hand, when the genotype frequencies were compared between HH patients and healthy controls several differences were observed. The IVS2(+4)-TT genotype was associated with susceptibility to HH ($P = 0.04$, OR = 3.91; 95%CI: 1.14-13.34). The C282Y-GG genotype was associated with protection against HH ($P < 0.001$; OR = 0.007; 95%CI: 0.0008-0.065), while the C282Y-AA genotype was associated with susceptibility to HH ($P < 0.001$; OR = 201.00; 95%CI: 10.44-3,871) (Table 1).

The most remarkable LD among these loci included: (1) H63DC>G and IVS2(+4)T>C in almost all groups analyzed separately and in the whole population; (2) IVS2(+4)T>C and IVS4(-44)T>C in most patient samples; and (3) IVS2(+4)T>C and C282YG>A in the HH population (Table S1). The Haploview software was used to analyze and visualize the patterns of linkage disequilibrium observed in these data and confirmed the strong LD between H63DC>G and IVS2(+4)T>C ($D' = 95$) and IVS2(+4)T>C and IVS4(-44)T>C ($D' = 90$), and a less strong linkage between IVS2(+4)T>C and C282YG>A ($D' = 77$) (Figure 2). Therefore, the most relevant SNPs in LD with each other were H63DC>G, IVS2(+4)T>C, IVS4(-44)T>C and C282YG>A.

Table 1 The single nucleotide polymorphism reference number (rs), the usual single nucleotide polymorphism names (H63DC>G, C282YG>A, IVS2(+4)T>C and IVS4(-44)T>C) and new variation sites

SNPs	Allele/genotype	HH	HCV-IO ⁺	HCV-IO ⁻	HCC HCV-IO ⁺	HCC-IO [?]	CTL
H63DC>G (rs1799945) [+3511]	C	0.893	0.859	0.864	0.805	0.921	0.825
	G	0.107	0.141	0.136	0.195	0.079	0.175
	GG	0.000	0.042	0.000	0.024	0.000	0.030
	CG	0.214	0.197	0.271	0.341	0.158	0.290
	CC	0.786	0.761	0.729	0.634	0.842	0.680
	HW <i>P</i> value	1.000	1.000	0.580	1.000	1.000	1.000
IVS2(+4)T>C (rs2071303) [+3668]	T	0.857	0.641	0.669	0.585	0.684	0.610
	C	0.143	0.359	0.331	0.415	0.316	0.390
	TT	0.714 ¹	0.408	0.424	0.390	0.526	0.390
	TC	0.286	0.465	0.492	0.390	0.316	0.440
	CC	0.000	0.127	0.085	0.220	0.158	0.170
	HW <i>P</i> value	0.528	1.000	0.556	0.212	0.295	0.528
G>C (rs807209) [+5197]	G	0.000	0.007	0.017	0.000	0.053	0.035
	C	1.000	0.993	0.983	1.000	0.947	0.965
	GG	0.000	0.000	0.000	0.000	0.000	0.010
	GC	0.000	0.014	0.034	0.000	0.105	0.050
	CC	1.000	0.986	0.966	1.000	0.895	0.940
	HW <i>P</i> value	-	-	1.000	-	1.000	0.103
C282YG>A (rs1800562) [+5473]	G	0.286 ²	0.979	0.983	0.902	1.000	0.955
	A	0.714 ²	0.021	0.017	0.098	0.000	0.045
	GG	0.071 ²	0.958	0.966	0.854	1.000	0.910
	GA	0.429	0.042	0.034	0.098	0.000	0.090
	AA	0.500 ²	0.000	0.000	0.049	0.000	0.000
	HW <i>P</i> value	1.000	1.000	1.000	0.031	-	1.000
IVS4(-44)T>C (rs1800708) [+5635]	T	1.000	0.880	0.907	0.817	0.842	0.925
	C	0.000	0.120	0.093	0.183	0.158	0.075
	CC	0.000	0.014	0.000	0.024	0.000	0.000
	TC	0.000	0.211	0.186	0.317	0.316	0.150
	TT	1.000	0.775	0.814	0.659	0.684	0.850
	HW <i>P</i> value	1.000	1.000	1.000	1.000	1.000	1.000
New mutation (G>Del) at intron 5 [+5811]	G	1.000	1.000	1.000	1.000	1.000	0.995
	Del	0.000	0.000	0.000	0.000	0.000	0.005
	GG	1.000	1.000	1.000	1.000	1.000	0.990
	G Del	0.000	0.000	0.000	0.000	0.000	0.010
	Del Del	0.000	0.000	0.000	0.000	0.000	0.000
	HW <i>P</i> value	-	-	-	-	-	-

Frequency of single nucleotide polymorphism (SNP) alleles and genotypes observed at the *HFE* coding region (ordered from 5' to 3') in patients with hereditary hemochromatosis (HH), hepatitis C exhibiting (HCV-IO⁺) or not (HCV-IO⁻) iron overload, hepatocellular carcinoma and hepatitis C plus iron overload (HCC HCV-IO⁺), hepatocellular carcinoma caused by diverse etiologies other than HCV and without information regarding iron overload (HCC-IO[?]), and in healthy control individuals (CTL). The reference SNP numbers (rs) and the position SNP base [] are also shown for the previously described *HFE* variation sites and were assigned according to NCBI (<http://www.ncbi.nlm.nih.gov>) and Ensembl (<http://www.ensembl.org>) databases. Significant Fisher's exact test *P* values (≤ 0.05) and Hardy-Weinberg equilibrium adherence are shown in table.

¹HH x CTL: TT (*P* = 0.04; OR = 3.91; 95%CI: 1.14-13.34);

²HH x CTL: G (*P* < 0.001; OR = 0.01; 95%CI: 0.006-0.05), A (*P* < 0.001; OR = 53.06; 95%CI: 18.41-152.90), GG (*P* < 0.001; OR = 0.007; 95%CI: 0.0008-0.065) and AA (*P* < 0.001; OR = 201.00; 95%CI: 10.44-3.871). The most frequent allele/genotype different of healthy controls. In italics: alleles and genotypes that presented statistically significant values.

Considering that: (1) H63DC>G and IVS2(+4)T>C were in LD in almost all analyses; (2) H63DC>G and C282YG>A presented LD only in HH patients; and (3) H63DC>G and C282YG>A polymorphic sites were frequently associated with susceptibility to HH in the literature, a third LD approach was performed, analyzing only HH and healthy control individuals to evaluate specifically-linked alleles and the strength of these associations. Accordingly, in both healthy controls and HH populations, a remarkable LD between the H63DC>G and IVS2(+4)T>C was observed ($D' = 1.000$ in

Table 2 HFE coding region allele frequency in individuals exhibiting congenital or acquired iron overload and healthy control population

Allele	SNPs sequences	Population frequencies (f)															
		IO ⁻							IO ⁺							Whole	
		CTL		HCV-IO ⁻		HCV-IO ⁺		HCC HCV-IO ⁺		HH		TOTAL		HCC-IO ²			
		1-2-3-4-5-6 ³															
		n = 100	%	n = 59	%	n = 71	%	n = 24	%	n = 28	%	n = 109	%	n = 36	%	n = 304	%
HFE*001 ⁴	C-T-C-G-T-G	107	0.54	74	0.63	86	0.61	23	0.48	4 ²	0.14	113	0.52	40	0.56	334	0.55
HFE*001:unofficial:02 ⁵	C-C-C-G-T-G	28	0.14	12	0.10	15	0.11	3	0.06	1	0.04	19	0.09	4	0.06	63	0.10
HFE*001:unofficial:03 ⁵	C-T-G-G-T-G	8	0.04	2	0.02	1	0.01	0	0.00	0	0.00	1	0.00	2	0.03	12	0.02
HFE*001:unofficial:04 ⁵	C-T-C-G-C-G	0	0.00	0	0.00	1	0.01	1	0.02	0	0.00	2	0.01	1	0.01	3	< 0.01
HFE*001:unofficial:05 ⁵	C-C-C-G-C-G	13	0.07	11	0.09	16	0.11	7 ¹	0.15	0	0.00	23	0.11	12	0.17	59	0.10
HFE*001:unofficial:06 ⁵	C-C-G-G-T-Del	1	0.01	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	1	< 0.01
HFE*002 ⁴	G-C-C-G-T-G	35	0.17	16	0.13	20	0.14	9 ¹	0.19	3	0.11	32	0.15	8	0.11	92	0.15
HFE*003 ⁴	C-T-C-A-T-G	8	0.04	3	0.02	3	0.02	3	0.06	20 ²	0.71	26	0.12	4	0.06	40	0.06
HFE*004 ⁴	G-C-C-A-T-G	0	0.00	0	0.00	0	0.00	1	0.02	0	0.00	1	0.00	0	0.00	1	< 0.01
Number of alleles		200		118		142		48		28		218		72		608	

Patients with hereditary hemochromatosis (HH), patients with hepatitis C exhibiting (HCV-IO⁺) or not (HCV-IO⁻) iron overload, hepatocellular carcinoma (HCC) plus HCV-IO⁺, HCC caused by diverse etiologies other than HCV and without information regarding iron overload (HCC-IO⁵), and population healthy control individuals (CTL).

¹HCC HCV-IO⁺ x CTL: HFE*001:unofficial:06/HFE*002 ($P = 0.02$; OR = 14.14; 95%CI: 1.40-142.80);

²HH x CTL: HFE*001 ($P < 0.001$; OR = 0.14; 95%CI: 0.04-0.43), HFE*003 ($P < 0.001$; OR = 60.00; 95%CI: 20.31-177.20), HFE*001/HFE*003 ($P = 0.03$; OR = 7.20; 95%CI: 1.40-36.85), HFE*003/HFE*003 ($P < 0.001$; OR = 174.20; 95%CI: 8.92-400.00);

³ Order of base changes for each single nucleotide polymorphism (SNP) observed, encompassing H63DC>G; IVS2(+4)T>C; rs807209G>C; C282YG>A; IVS4(-44)T>C; new deletionG>DEL (5' to 3');

⁴ Alleles recognized by ImMunoGeneTics information system - IMGT;

⁵ Alleles in validation process.

LD of H63D-G and IVS2(+4)-C was detected as well as an absence of the recombinant H63D-G in linkage with the IVS2(+4)-T. Another relevant result was the linkage of both mutant H63D-G and IVS(+4)-C mutants with the C282Y-G ($D' = 1.000$ in both analyses, and $r^2 = 0.3000$ and 0.4267 , respectively) (Table S2).

HFE alleles and genotypes using the HFE nomenclature

The reconstruction of the meiotic phase generated nine alleles, included into four major allele groups (HFE*001 to *004), as standardized by IMGT^[10]. These allele groups encoded four distinct proteins (HFE*001 to *004) on the basis of polymorphic sites along the coding region, encompassing the H63DC>G (exon 2), IVS2(+4)T>C (intron 2), rs807209 (G>C intron 3), C282YG>A (exon 4) IVS4(-44)T>C (intron 4) and the new mutation (G>DEL at intron 5) (Table 2).

The HFE*001:01:01 was the most frequently observed allele in all studied populations (f varying from 48-63%), except in HH patients ($f = 14\%$). In contrast, the HFE*003 allele was underrepresented in all studied populations (f varying from 2%-12%), except in HH patients ($f = 71\%$). Therefore, the HFE*001, containing the H63D-C; IVS2(+4)-T; rs807209-C; C282Y-G; IVS4(-44)-T variation sites (from 5' to 3'), conferred protection against the development of HH ($P < 0.0001$, OR = 0.14) and the HFE*003 allele, containing the H63D-C; IVS2(+4)-T; rs807209-C; C282Y-A; IVS4(-44)-T (from 5' to 3'), conferred a high risk for HH development ($P < 0.0001$, OR = 60.00). The HFE*001/HFE*003 ($P = 0.03$, OR = 7.2) and HFE*003/HFE*003 ($P < 0.001$, OR = 174.20) genotypes, both containing the HFE*003 allele, were also overrepresented in HH patients. On the other hand, the HFE*001/HFE*002 genotype was associated with the development of HCC ($P = 0.02$, OR = 14.14) in patients exhibiting the underlying HCV infection and iron overload (HCC HCV-IO⁺).

Linkage disequilibrium between other major histocompatibility complex genes and HFE

The major histocompatibility complex (MHC) LD analysis was performed using two approaches: (1) considering HFE alleles (Table S3); and (2) considering separately the two HFE SNPs most frequently reported in association with HH (H63DC>G and

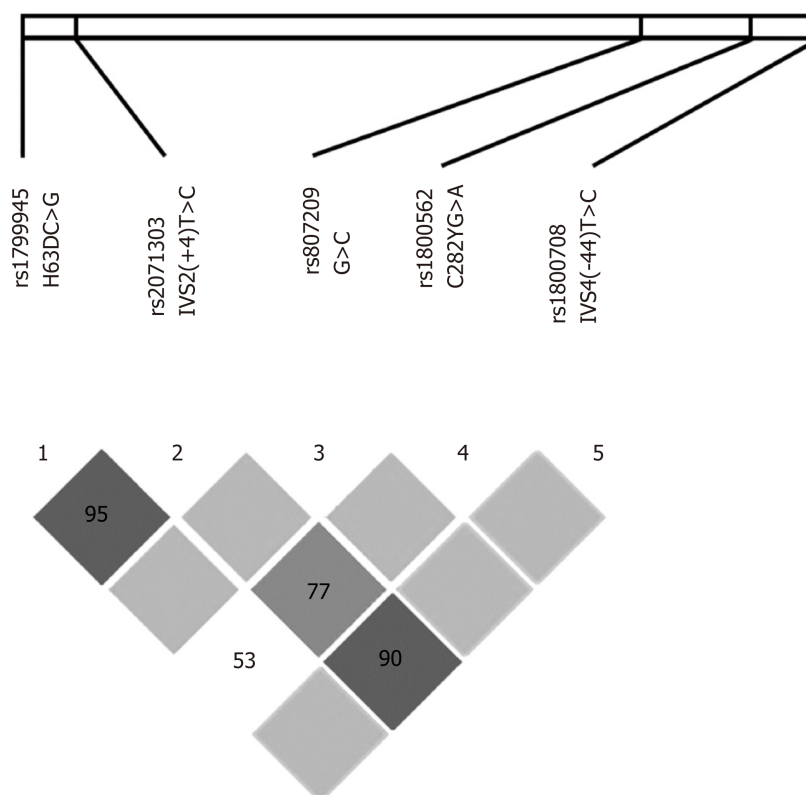


Figure 2 Linkage disequilibrium among single nucleotide polymorphisms observed along the coding region of the *HFE* gene. Areas in dark gray represent strong linkage disequilibrium (LD) ($\text{LOD} \geq 2$ and $D' = 1$), medium gray indicates moderate LD ($\text{LOD} \geq 2$, $D' < 1$), light gray indicates weak LD ($\text{LOD} < 2$, $D' = 1$); and white indicates no LD ($\text{LOD} < 2$, $D' < 1$). Values of D' different from 1.00 are represented as a percentage within the square. LOD: Log of the odds; D' : Linkage disequilibrium coefficient.

two *HFE* SNPs most frequently reported in association with HH (H63DC>G and C282Y>A) (Table 3). Considering the first approach, no LD was observed between *HFE* alleles and MHC alleles, except for H63DC>G and *HLA-B* locus ($P = 0.03$), showing a weak association between H63DC>G and *HLA-B*44* (Table 3). We also observed an absence of LD between the classical C282Y>A SNP and *HLA-A*, *HLA-B*, *HLA-C*, 14bp *HLA-G*, *TNFA-d* microsatellites. Since the variation site IVS2(+4)T>C is located only 157bp downstream from the H63DC>G site and since these loci are in LD, the IVS2(+4)T>C would be a good candidate to be analyzed regarding the disequilibrium between *HFE* and *HLA-B* genes (Table 4). The weak *HLA-B* associations were confirmed.

DISCUSSION

Individual *HFE* gene variation sites

The frequency of the H63D-G allele in healthy controls varies from 7.9% to 17.5% in worldwide populations, exhibiting high frequencies in Netherlands and Iberian Peninsula (around 20%)^[13,14]. The frequency of the C282Y-A allele decreases from North (4%-10%) to South Europe (0%-3%)^[15], and in populations without a high European genetic ancestry, the frequency of this allele is negligible. The frequency of the C282Y-A allele in our healthy control series, as well as in other Southern Brazilian samples^[16-18], is closely similar to South European populations, indicating the European ancestry influenced on the Brazilian gene pool. The mutant S65C-T allele is observed at low frequency (0-1%) in European populations^[19-21], as well as in the Brazilian population^[22,23] (absent in our samples – data not shown).

Although the IVS2(+4)T>C SNP does not change protein sequence, it is in LD with H63DC>G, C282Y>A and IVS2(+4)-T alleles. Considering that IVS2(+4)-T allele is increased in HH population, and considering that this allele is only 157bp distant from the H63D-G allele, this association probably reflects a hitch-hiking effect, and possibly does not present biological significance in the susceptibility to HH. Indeed, de Lucas

Table 3 Linkage disequilibrium between *HLA-B* alleles and *HFE* coding region H63DC>G single nucleotide polymorphism alleles

	Observed frequency		Expected frequency		Standardized value of disequilibrium(<i>D'</i>)		Standardized value of correlation(<i>r</i> ²)	QUI ² value	<i>P</i> value of QUI ²
<i>HLA-B</i>	<i>HFE</i> H63DC>G								
	C	G	C	G	C	G			
07	16	2	14.83	3.17	0.3683	-0.3683	0.0029	0.5728	0.4492
08	12	1	10.71	2.29	0.5626	-0.5626	0.0047	0.9396	0.3324
13	3	1	3.30	0.70	-0.0899	0.0899	0.0008	0.1547	0.6941
14	10	0	8.24	1.76	1.0000	-1.0000	0.0113	2.2471	0.1339
15	17	0	14.01	2.99	1.0000	-1.0000	0.0199	3.9669	0.0464
18	10	0	8.24	1.76	1.0000	-1.0000	0.0113	2.2471	0.1339
27	2	2	3.30	0.70	-0.3933	0.3933	0.0149	2.9586	0.0854
35	17	4	17.31	3.69	-0.0177	0.0177	0.0002	0.0345	0.8526
37	1	2	2.47	0.53	-0.5955	0.5955	0.0254	5.0617	0.0245
38	3	0	2.47	0.53	1.0000	-1.0000	0.0033	0.6500	0.4201
39	4	2	4.94	1.06	-0.1911	0.1911	0.0053	1.0582	0.3036
40	3	0	2.47	0.53	1.0000	-1.0000	0.0033	0.6500	0.4201
41	3	0	2.47	0.53	1.0000	-1.0000	0.0033	0.6500	0.4201
42	1	0	0.82	0.18	1.0000	-1.0000	0.0011	0.2145	0.6433
44	8	11	15.66	3.34	-0.4891	0.4891	0.1183 ¹	23.5443	< 0.00001
45	6	0	4.94	1.06	1.0000	-1.0000	0.0066	1.3203	0.2505
48	2	0	1.65	0.35	1.0000	-1.0000	0.0022	0.4312	0.5114
49	8	0	6.59	1.41	1.0000	-1.0000	0.0089	1.7788	0.1823
50	2	0	1.65	0.35	1.0000	-1.0000	0.0022	0.4312	0.5114
51	9	5	11.54	2.46	-0.2199	0.2199	0.0172	3.4137	0.0647
52	7	0	5.77	1.23	1.0000	-1.0000	0.0078	1.5484	0.2134
53	5	0	4.12	0.88	1.0000	-1.0000	0.0055	1.0946	0.2955
55	2	0	1.65	0.35	1.0000	-1.0000	0.0022	0.4312	0.5114
56	0	1	0.82	0.18	-1.0000	1.0000	0.0237	4.7094	0.0300
57	8	2	8.24	1.76	-0.0293	0.0293	0.0002	0.0423	0.8371
58		2	4.94	1.06	-0.1911	0.1911	0.0053	1.0582	0.3036
67	1	0	0.82	0.18	1.0000	-1.0000	0.0011	0.2145	0.6433

¹The higher value of correlation. Identification of single nucleotide polymorphisms and most frequent allele according to NCBI (<http://www.ncbi.nlm.nih.gov>) and Ensembl (<http://www.ensembl.org>): rs1799945 (H63DC>G). Shaded cells are showing significant LD values.

et al^[24] reported that HH patients presenting homozygosis for the C282Y-A allele did not exhibit the IVS2(+4)-C allele, indicating that the presence of the C282Y-A allele excludes the presence of IVS2(+4)-C allele in the same haplotype. Therefore, the sole analysis of the allelic frequency of the IVS2(+4)T>C SNP is not adequate to evaluate HH susceptibility, since the frequency of the C282Y-A allele is high in HH patients, and consequently, there is a high frequency of IVS2(+4)-T allele in the same sample (Table 1). The C282Y-A allele and the AA genotype have been associated with susceptibility to HH patients^[24,25], including the HH patients of this study and other Brazilian HH populations^[23]. Although the HH cohort is small, the mutated AA genotype appeared in high frequency in patients and was not observed in the healthy control group. The C282Y-G allele and the GG genotype have been associated with protection against HH development in various worldwide populations^[21]. The H63D-G allele and the GG genotype have been associated with HH in European and North American patients^[1,25]. However, these associations were not observed in ours nor in other HH Brazilian samples^[26].

The role of H63DC>G and C282YG>A variation sites in acquired IO disorders is controversial. Apart from HH, no other association involving such polymorphisms was observed in the present study. A previous study evaluating chronic hepatitis C patients reported an association between *HFE* mutations (H63DC>G and C282YG>A) and elevated serum transferrin saturation, but not with liver iron deposits^[5]. On the other hand, some authors have observed an increased prevalence of C282YG>A mutation in hepatitis C patients from North England^[27], Austria^[28], and North

Table 4 Linkage disequilibrium between *HLA-B* alleles and *HFE* coding region IVS2(+4)T>C single nucleotide polymorphism alleles

	Observed frequency		Expected frequency		Standardized value of disequilibrium(<i>D'</i>)		Standardized value of correlation(<i>r</i> ²)	QUI ² value	<i>P</i> value of QUI ²
<i>HLA-B</i>			<i>HFE</i> IVS2(+4)T>C						
	T	C	T	C	T	C			
07	13	5	10.98	7.02	0.2877	-0.2877	0.0052	1.0471	0.3062
08	8	5	7.93	5.07	0.0138	-0.0138	0.0000	0.0017	0.9672
13	4	0	2.44	1.56	1.0000	-1.0000	0.0130	2.6096	0.1062
14	4	6	6.10	3.90	-0.3443	0.3443	0.0098	1.9513	0.1624
15	11	6	10.37	6.63	0.0950	-0.0950	0.0005	0.1073	0.7433
18	8	2	6.10	3.90	0.4872	-0.4872	0.0080	1.5973	0.2063
27	1	3	2.44	1.56	-0.5902	0.5902	0.0111	2.2235	0.1359
35	17	4	12.81	8.19	0.5116	-0.5116	0.0196	3.9264	0.0475
37	0	3	1.83	1.17	-1.0000	1.0000	0.0238	4.7638	0.0291
38	2	1	1.83	1.17	0.1453	-0.1453	0.0002	0.0411	0.8393
39	1	5	3.66	2.34	-0.7268	0.7268	0.0256	5.1103	0.0238
40	3	0	1.83	1.17	1.0000	-1.0000	0.0097	1.9472	0.1629
41	2	1	1.83	1.17	0.1453	-0.1453	0.0002	0.0411	0.8393
42	1	0	0.61	0.39	1.0000	-1.0000	0.0032	0.6426	0.4228
44	6	13	11.59	7.41	-0.4823	0.4823	0.0382 ¹	7.6388	0.0057
45	3	3	3.66	2.34	-0.1803	0.1803	0.0016	0.3146	0.5749
48	2	0	1.22	0.78	1.0000	-1.0000	0.0065	1.2916	0.2558
49	8	0	4.88	3.12	1.0000	-1.0000	0.0266	5.3279	0.0210
50	2	0	1.22	0.78	1.0000	-1.0000	0.0065	1.2916	0.2558
51	10	4	8.54	5.46	0.2674	-0.2674	0.0034	0.6882	0.4068
52	3	4	4.27	2.73	-0.2974	0.2974	0.0050	1.0037	0.3164
53	5	0	3.05	1.95	1.0000	-1.0000	0.0164	3.2787	0.0702
55	0	2	1.22	0.78	-1.0000	1.0000	0.0158	3.1598	0.0755
56	1	0	0.61	0.39	1.0000	-1.0000	0.0032	0.6426	0.4228
57	4	6	6.10	3.90	-0.3443	0.3443	0.0098	1.9513	0.1624
58	1	5	3.66	2.34	-0.7268	0.7268	0.0256	5.1103	0.0238
67	1	0	0.61	0.39	1.0000	-1.0000	0.0032	0.6426	0.4228

¹The higher value of correlation. Identification of single nucleotide polymorphisms and most frequent allele according to NCBI (<http://www.ncbi.nlm.nih.gov>) and Ensembl (<http://www.ensembl.org>): rs2071303 (IVS2(+4)T>C). Shaded cells are showing significant LD values.

America^[29]. These studies have shown an association between *HFE* mutations and higher serum iron indices and liver iron deposits, especially for C282Y homozygotes. In contrast, another study did not show association between *HFE* mutations and liver iron deposits^[30].

The association between the C282YG>A mutation and the HCC risk is also still controversial. HH is a condition characterized by hepatic IO, leading to higher cancer incidence^[31]. However, the role of moderate liver IO and of the carriage of *HFE* mutations on the HCC risk remains unclear. Some studies have shown higher prevalence of the C282YG>A mutation in patients with HCC compared with cirrhotic patients without HCC^[32], whereas other studies found no association between *HFE* and HCC^[33]. Additionally, another study reported an association between liver IO and C282YG>A with a higher risk of HCC in patients with alcoholic but not with HCV-related cirrhosis^[34].

***HFE* alleles and genotypes**

The *HFE**001 allele was underrepresented, while the *HFE**003 was overrepresented in HH patients of this series. These findings corroborate the importance of the C282YG>A SNP on the susceptibility to HH, since only the *HFE**003 allele has an Adenine at this position (C282Y-A), which is the unique difference between both alleles. In addition, the *HFE**001/*HFE**003 and *HFE**003/*HFE**003 genotypes were also significantly associated with high risk for HH development. The homozygosis for

the *HFE**003 allele group, which was not observed in the healthy control population, drastically increased the susceptibility to HH. Indeed, the *HFE**003 allele was present in 13 out of 14 patients and its presence in double doses was observed in 7 out of 14 HH patients.

In relation to acquired diseases exhibiting IO, the *HFE**001/*HFE**002 genotype was overrepresented in HCC patients exhibiting HCV infection and IO. When the *HFE* SNPs were analyzed separately, no significant differences were observed. Noteworthy, these results indicate that these populations are heterogeneous and in some circumstances represented small groups.

Extended MHC haplotypes encompassing the *HFE* SNPs and alleles

HH was initially associated with the *HLA-A3*, *HLA-A14* and *HLA-B14* antigens^[35]. Microsatellite evaluations pointed out a susceptibility locus for HH. This locus was initially named as *HLA-H*^[25], which is the same name of a pseudogene, located close to *HLA-A*, stressing the disequilibrium concept between *HLA-A/B* genes and the HH locus. Later, this HH locus was renamed *HFE* to put an end on this ambiguity^[36]. Considering the great distance between the *HFE* and *HLA-A*, *-B* and *-C* loci, strong LD between these genes are not expected; however, some studies reported LD between H63DC>G and C282YG>A SNPs with *HLA-A* and *HLA-B* alleles. Taking advantage of the fact that our healthy control population was typed for ten additional MHC loci, LD between *HFE* and all these loci was evaluated.

The pairwise test detected no disequilibrium between the *HFE* alleles and other MHC loci (Table S3), which is in agreement with the argument that the *HFE* gene is far from the other loci tested. When LD analyses were performed evaluating the H63DC>G and C282YG>A SNPs, a significant disequilibrium between the H63DC>G and *HLA-B* ($P = 0.03$) was observed, encompassing *HLA-B**15/H63D-C, *HLA-B**37/H63D-G, *HLA-B**44/H63D-G and *HLA-B**56/H63D-G alleles (Table 3), being stronger for *HLA-B**15/H63D-C and *HLA-B**56/H63D-G alleles ($D' = 1$). Since *HLA-B* locus is multiallelic, H63DC>G is biallelic, and H63D-G is rare, it is possible that not all H63DC>G/*HLA-B* haplotypes were represented in our CTL. In addition, the recombination coefficient, which indicates the power of the correlation between alleles, was weak for all these combinations, except for the *HLA-B**44/H63D-G ($r^2 = 0.11$) (Figure 3 and Table 3) which was much stronger than in the other combinations ($r^2 = 0.01-0.02$). Most likely, this *HLA-B**44/H63D-G disequilibrium has a historical origin.

Since the IVS2(+4)T>C SNP exhibited a significant LD with the H63DC>G SNP, as we discussed before, and considering that both SNPs are located at a relatively short distance, we further evaluated the LD between this SNP and *HLA-B*, which showed similar results: *HLA-B**35/IVS2(+4)-T; *HLA-B**37/IVS2(+4)-C; *HLA-B**44/IVS2(+4)-C; *HLA-B**49/IVS2(+4)-T and *HLA-B**58/IVS2(+4)-C. The analyses of LD between *HLA-B* alleles and IVS2(+4)T>C and H63DC>G showed that *HLA-B**37 and *B**44 exhibited weaker correlations in relation to H63DC>G ($r^2 = 0.02$ and 0.03 , respectively) (Table 4). This analysis resulted on the identification of the extended H63D-G/IVS2(+4)-C/*HLA-B**44 haplotype (Figure 3).

Regarding genetic studies in patients with IO, *HLA-B**44 and C282Y-A alleles are reported to be overrepresented in patients with HH^[1] or in patients with acquired diseases associated with IO^[37], however, without reaching significance. Since haplotypes containing *HLA-B**44 are common in Europe, West and North Africa, and in North-American Caucasians^[38], there is a high probability of overrepresentation of the H63D-G/*HLA-B**44 haplotype in these populations. Although the present study revealed that C282Y-A is not a part of this extended haplotype, the mentioned associations suggest an independent role of H63D-G and C282Y-A on HH susceptibility.

In conclusion, this study systematically reports variation sites along the *HFE* gene using *HFE* allelic official nomenclature, previously described by our group. The *HFE**003 was frequently observed in HH patients, whereas the *HFE**001 was frequently observed in healthy controls. The *HFE**001/*HFE**002 genotype was identified as a risk factor for HCC HCV patients exhibiting IO. Even if a strong LD has been observed among the H63D-G, IVS2(+4)-C and C282Y-G alleles, particularly in HH patients, the mutation IVS2(+4)T>C was not directly associated with HH susceptibility. Although the *HFE* gene is distant from other MHC genes, the *HFE* H63D-G/IVS2(+4)-C alleles were in weak LD with the *HLA-B**44 allele.

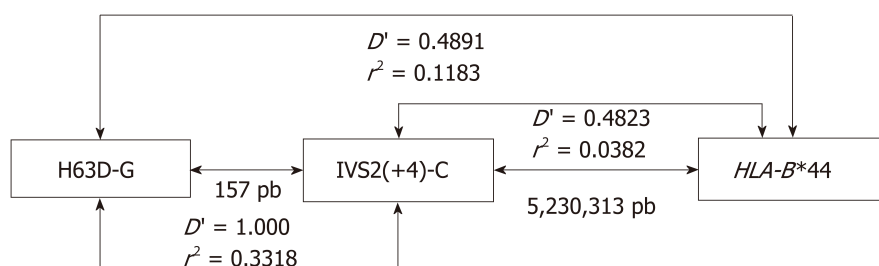


Figure 3 Linkage disequilibrium observed between two relevant *HFE* coding region [H63D>G and IVS2(+4)T>C] single nucleotide polymorphism alleles and *HLA-B* alleles.

ARTICLE HIGHLIGHTS

Research background

HFE gene controls the iron uptake from gut, and defects of the encoded molecule have been associated with iron overload (IO), particularly in hemochromatosis hereditary (HH), which can cause serious damage to the liver. Besides HH, patients with hepatitis C virus (HCV) and hepatocellular carcinoma (HCC) may or not develop IO.

Research motivation

The search for markers associated with IO may be very useful for the early diagnosis of these patients, which is essential for their survival.

Research objectives

The main objectives of this work is to identify associations between *HFE* coding region variable sites in patients exhibiting HH and in diseases associated with acquired IO.

Research methods

We sequenced exons 2 to 5 and boundary introns of the *HFE* gene to evaluate all polymorphic sites in patients presenting HH or acquired IO (HCV and HCC), and in healthy controls, using Sanger sequencing. We also determined the extended haplotype in healthy controls, including other major histocompatibility genes (*HLA-A/-B/-C/-DRB1/-DQB1* alleles, and *HLA-G* 14bp INDEL and *TNFA-d* microsatellites). Haplotype reconstruction was performed using the Arlequin and Phase softwares, and linkage disequilibrium (LD) between histocompatibility loci and *HFE* gene was performed using the Haploview software.

Research results

The *HFE**003 allele was overrepresented ($f = 71\%$) and *HFE**001 allele was underrepresented ($f = 14\%$) in HH patients compared to all groups. A strong LD was observed among the previously reported H63D-G, IVS2(+4)-C and C282Y-G gene variants, particularly in HH; however, the mutation IVS2(+4)T>C was not associated with HH susceptibility. The *HFE**001/*HFE**002 genotype conferred susceptibility to HCC in HCV patients exhibiting IO ($P = 0.02$, OR = 14.14). Although *HFE* is telomeric to other histocompatibility genes, the H63D-G/IVS2(+4)-C ($P \leq 0.00001/P \leq 0.0057$) combination was in LD with *HLA-B**44 allele group in healthy controls.

Research conclusions

This study systematically evaluated variation sites along the *HFE* gene using the HLA official nomenclature, previously described by our group. The *HFE**003 allele that was overrepresented in HH patients encompasses major variation sites previously described in association with HH in several worldwide populations, in contrast with the *HFE**001 allele which does not present HH-associated variation sites and predominates among healthy controls. On the other hand, the *HFE**001/*HFE**002 genotype was identified as a risk factor for HCC and HCV patients exhibiting IO. Although the *HFE* gene is distant from other histocompatibility genes, the *HFE* H63D-G/IVS2(+4)-C alleles were in weak LD with the *HLA-B**44 allele. Thus, a differential *HFE* association was observed for HH and for diseases associated with acquired IO (HCV, HCC).

Research perspectives

Besides the identification of markers associated with IO, which may permit an early detection of patients prone to develop iron deposits, the knowledge of the major gene associated with iron uptake may help on the understanding of the IO pathogenesis.

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Retrospective Cohort Study

Clinical outcomes after major hepatectomy are acceptable in low-volume centers in the Caribbean

Shamir O Cawich, Ravi Maharaj, Vijay Naraynsingh, Neil Pearce, Wesley Francis, Kimon O Bonadie, Dexter A Thomas

ORCID number: Shamir O Cawich (0000-0003-3377-0303); Ravi Maharaj (0000-0002-6583-3164); Vijay Naraynsingh (0000-0002-5445-3385); Neil Pearce (0000-0002-3182-7268); Wesley Francis (0000-0003-3174-1015); Kimon O Bonadie (0000-0001-9387-6121); Dexter A Thomas (0000-0003-3744-744X).

Author contributions: Cawich SO designed the study; Cawich SO, Maharaj R, Naraynsingh V, Pearce NW, Francis W, Bonadie KO and Thomas DA performed the research; Pearce NW, Francis W, Bonadie KO and Thomas DA analyzed the data; Cawich SO, Maharaj R, Naraynsingh V and Pearce NW wrote the paper; and Cawich SO, Maharaj R, Naraynsingh V, Pearce NW, Francis W, Bonadie KO and Thomas DA revised the manuscript for final submission.

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Shamir O Cawich, Ravi Maharaj, Vijay Naraynsingh, Dexter A Thomas, Department of Clinical Surgical Sciences, University of the West Indies, St. Augustine, Trinidad and Tobago 999183, West Indies

Neil Pearce, Department of Surgery, University Hospital Southampton NHS Trust, Southampton, SO16DP, United Kingdom

Wesley Francis, Department Surgery, University of the West Indies, Nassau 999154, Bahamas

Kimon O Bonadie, Department Surgery, Cayman Islands Health Service Authority, Grand Cayman, KY11100, West Indies

Corresponding author: Shamir O Cawich, MBBS, Professor, Department of Clinical Surgical Sciences, University of the West Indies, St. Augustine Campus, St Augustine, Trinidad and Tobago 999183, West Indies. socawich@hotmail.com

Telephone: +1-868-6229909

Abstract

BACKGROUND

Major hepatectomies are routinely performed because they are often the only curative treatment for metastatic liver disease. There has been a trend to concentrate major hepatectomies in referral hospitals that perform these operations at high volumes. These high volume referral centers are usually located in developed countries, but many patients in developing nations are not able to access these centers because of financial limitations, lack of social support and/or travel restrictions. Therefore, local hospitals are often the only options many of these patients have for surgical treatment of metastatic liver disease. This is the situation in many Caribbean countries.

AIM

To determine the clinical outcomes after major liver resections in a low-resource hepatobiliary center in the Caribbean.

METHODS

We prospectively studied all patients who underwent major liver resections over five years. The following data were extracted: patient demographics, diagnoses, ECOG status, operation performed, post-operative morbidity and mortality. Statistical analyses were performed using SPSS ver 16.0

RESULTS

relationships or other scenarios that may represent potential conflicts of interest.

STROBE statement: The authors have read the CONSORT 2010 Statement. The manuscript adheres to the CONSORT 2010 policies, with the exceptions that this is not a randomized trial.

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There were 69 major liver resections performed by two teams at a mean case volume of 13.8 major resections/year. Sixty-nine major hepatic resections were performed for: colorectal liver metastases 40 (58%), non-colorectal metastases 9 (13%), hepatocellular carcinoma 8 (11.6%), ruptured adenomas 4 (5.8%), hilar cholangiocarcinomas 4 (5.8%), hemangiomas 2 (2.9%), trauma 1 (1.5%) and hepatoblastoma 1 (1.5%). Twenty-one patients had at least one complication, for an overall morbidity rate of 30.4%. There were minor complications in 17 (24.6%) patients, major complications in 11 (15.9%) patients and 4 (5.8%) deaths.

CONCLUSION

There are unique geographic, political and financial limitations to healthcare delivery in the Caribbean. Nevertheless, clinical outcomes are acceptable in the established, low-volume hepatobiliary centers in the Eastern Caribbean.

Key words: Liver; Surgery; Resection; Caribbean; Volume; Outcomes

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Core tip: Although there has been a global trend to concentrate major liver resections in tertiary referral centers, it is not practical in the Caribbean region. However, the hepatobiliary centers in the Caribbean do not meet the criteria to be defined as high-volume centers. This study prospectively evaluated outcomes after 69 consecutive major liver resections in a Caribbean center that only performed 13.8 resections per year. With a major morbidity rate of 15.9% and mortality rate of 5.8%, we have shown that the clinical outcomes after major liver resections are acceptable in the established, low-volume hepatobiliary centers in the Eastern Caribbean.

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INTRODUCTION

Major hepatectomies are routinely performed because they are often the only curative treatment for metastatic liver disease^[1]. They are accepted to be safe procedures when performed by trained hepatobiliary teams in specialized, high-volume centers^[1-3].

There has been a trend to concentrate major hepatectomies in referral hospitals that perform these operations at high volumes^[3,4,5]. These high volume referral centers are usually located in developed countries, but many patients in developing nations are not able to access these centers because of financial limitations, lack of social support and/or travel restrictions. Therefore, local hospitals are often the only options many of these patients have for surgical treatment of metastatic liver disease. This is the situation in many Caribbean countries.

While there are hepatobiliary units in the Caribbean, none meet the criteria to be defined as high-volume^[3,4,5,6,7]. Additionally, hepatobiliary units in the Caribbean operate in challenging, resource-poor environments. In this study, we sought to determine whether the clinical outcomes were acceptable when major hepatectomies were performed in a low-volume, resource-poor hepatobiliary unit in the Eastern Caribbean.

MATERIALS AND METHODS

In 2011, an attempt was made to achieve service centralization in the Caribbean with the establishment of three hepatobiliary units in the Bahamas, Jamaica and Trinidad and Tobago. They were intended to serve as regional referral centers for patients requiring major hepatectomies across the English-speaking Caribbean^[8]. This was supported by the Americas Hepatopancreatobiliary Association (AHPBA), culminating with the formation of a Caribbean Chapter of the AHPBA in 2015.

The hepatobiliary unit in Trinidad and Tobago is the largest referral unit in the English-speaking Caribbean^[8-9]. This unit is comprised of two hepatobiliary teams each headed by fellowship-trained hepatobiliary surgeons. All cases are discussed in a multidisciplinary team meeting where decisions are made for treatment of patients with hepatobiliary diseases.

Ethics

The local institutional review board granted permission to collect and examine data from all patients who underwent major hepatectomies in this setting.

Study population

We prospectively recorded data from all patients who underwent major hepatectomies with the hepatobiliary unit in Trinidad and Tobago over a five-year period from January 1, 2012 to December 30, 2016. We used the standardized definition of major hepatectomies as defined by Reddy *et al*^[10]: resection of four or more liver segments.

Data analysis

The following data were recorded for all patients who underwent major hepatectomies during the study period: patient demographics, diagnoses, ECOG status, operation performed, operative details, therapeutic outcomes, post-operative morbidity and mortality. Complications were classified according to the modified Clavien-Dindo system^[11]. Statistical analyses were performed using SPSS ver 16.0.

RESULTS

There were 69 major hepatectomies performed over the five-year study period. Therefore, the mean annual case volume was 13.8 major hepatectomies per annum. When examined chronologically, there was a steady increase in the number of hepatectomies performed each year, except in the year 2016 (Figure 1). During this time, the nation experienced an economic recession.

Indications for operation

All major hepatectomies were performed by one of two trained hepatobiliary surgeons for the following indications: colorectal liver metastases 40 (58%), non-colorectal metastases 9 (13%), hepatocellular carcinoma 8 (11.6%), ruptured adenomas 4 (5.8%), hilar cholangiocarcinomas 4 (5.8%), hemangiomas 2 (2.9%), trauma 1 (1.5%) and hepatoblastoma 1 (1.5%).

Patient demographics

The patients in this series consisted of 40 men and 29 women, with a mean age of 63 years (Range 34-80; SD +/- 10.3; Median 65). Sixty-four (93%) patients had at least one co-morbidity. Overall, there were 40 (58%) patients with ASA scores \geq III, as detailed in Table 1, and 39 (56.5%) patients with ECOG scores \geq 2, as detailed in Table 2.

After pre-operative multidisciplinary review, we anticipated that the hepatectomy procedure would be technically complex in 26 (37.7%) patients for: emergency hepatectomy for ruptured tumours or trauma (6), multiple intra-hepatic hepatico-jejunostomies for hilar cholangiocarcinomas (4), IVC resection and reconstruction (4), borderline future liver remnants (4), synchronous colorectal operations (3), synchronous gastric resections (2), prior open hepatectomy scheduled for repeat laparoscopic resections (2) and synchronous nephrectomy (1).

Operative details

Fourteen (20.3%) hepatectomies were attempted using the laparoscopic approach, with 3 (21.4%) conversions for unclear anatomy (1), bleeding (1) and repair of IVC injury (1). The remaining 55 (79.7%) operations were planned using an open approach. No patients in this series underwent veno-venous bypass during major hepatectomies. The hanging maneuver with anterior parenchymal transection technique was used to complete hepatectomy in 18 (26.1%) patients and the conventional technique was used in the remaining 51 (73.9%) cases.

Clinical outcomes

Excluding patients who had synchronous resections performed, the mean operating time for a major hepatectomy alone was 380 min (Range 260-600; SD +/- 75.8; Median 350). The operations in these patients were accompanied by a mean blood loss of 1405 mL (Range 600-4000; SD +/- 729; Median 1200) and mean transfusion requirements of 1.8 units of packed cells (Range 0-5; SD +/- 1.43; Median 2).

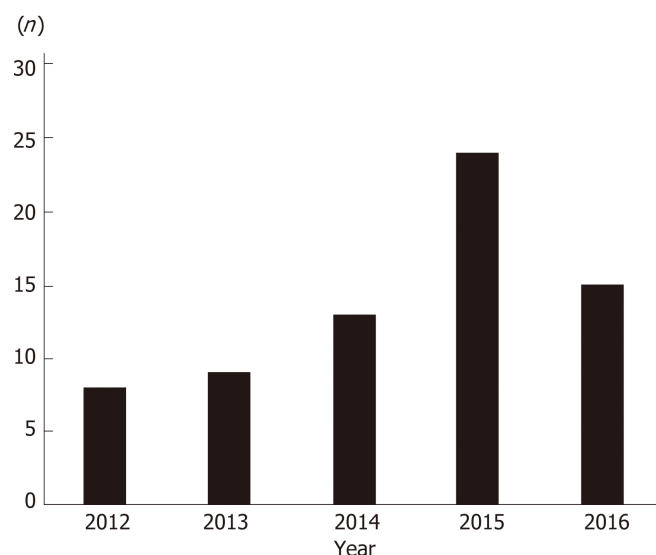


Figure 1 Chronologic relationship of major liver resections performed.

When we evaluated the subset of 26 patients in whom technically complex operations were anticipated, the mean operating time was 461.5 min (Range 300-650; SD \pm 95.6; Median 455), mean estimated blood loss was 2009 mL (Range 800-3500; SD \pm 667.4; Median 2000) and the mean transfusion requirement was 3.2 units of packed cells (Range 1-5; SD \pm 1.05; Median 3).

In the 43 cases where technical difficulty was not anticipated pre-operatively, the mean operating time was 367 min (Range 260-600; SD \pm 69.4; Median 350), mean EBL of 1236.7 mL (Range 600-4000; SD \pm 679.5; Median 1000) and mean transfusion requirements of 1.37 units (Range 0-4; SD \pm 1.3; Median 1).

In this setting, we maintained a policy of mandatory ICU admission after major hepatectomy because institutional limitations generally did not allow the expected level of supportive care outside of the ICU setting. Therefore, all patients were admitted to the ICU post-hepatectomy, with a mean ICU stay of 5.3 d (Range 1-40; SD \pm 7.37; Median 3). Fifteen (21.7%) patients required a prolonged ICU stay beyond 72 h for invasive treatment, ventilator and/or inotropic support. Overall, the mean duration of hospitalization after major hepatectomy was 16 d (Range 9-103; SD \pm 13.35; Median 12).

Morbidity / mortality analysis

There were 58 patients with no complications or minor morbidity. These patients had a mean ICU stay of 3.2 d (Range 1-8; SD \pm 1.55; Median 3) and mean hospital stay of 13.2 d (Range 9-35; SD \pm 6.85; Median 10). In comparison, the 11 patients with major morbidity had a mean ICU stay of 16.3 d (Range 5-40; SD \pm 14.1; median 6) and mean overall hospital stay of 31.1 d (Range 13-103; SD \pm 25.4; Median 23).

Twenty-one patients experienced at least one complication in this series. Minor complications were recorded in 17 (24.6%) patients and major complications in 11 (15.9%) patients. The individual complications are outlined in Table 3.

There were 4 (5.8%) reported deaths within 30 d of operation in this series. These included: (1) a 69 year-old man who underwent an abdomino-perineal resection and synchronous major hepatectomy. He developed intra-abdominal sepsis after a leak from a bladder injury; (2) an 80-year old man who underwent an extended right hepatectomy for colorectal liver metastases. He developed a significant bile leak, with resultant collections and eventually succumbed to intra-abdominal sepsis; (3) a 69-year old woman who had extended right hepatectomy for hepatocellular carcinoma and developed post-hepatectomy liver failure despite a 40% functional liver remnant; and (4) a 79-year old man who had an extended right hepatectomy for hilar cholangiocarcinoma. He developed a small bowel anastomotic leak and eventually succumbed to intra-abdominal sepsis.

DISCUSSION

At the turn of the 21st century, we witnessed the era of service centralization where

Table 1 ASA scores for patients undergoing major liver resections in a low volume Caribbean center

Score	ASA descriptor	n (%)
I	Completely healthy	5 (7.3)
II	Mild systemic disease	24 (34.8)
III	Severe systemic disease that is not incapacitating	31 (44.9)
IV	Incapacitating disease that is a threat to life	9 (13)
V	Moribund and not expected to survive > 24 h	0

surgical treatment for complex diseases was concentrated in specific centers in order to support sub-specialty teams performing these operations at high volumes^[7,12,13]. This trend was supported by accumulating data to suggest that there were better peri-operative outcomes in high-volume referral hospitals^[3,4,5,6,14,15,16,17].

Specifically for major hepatectomies, the data demonstrated that high-volume centers achieved significant reductions in overall morbidity^[3,5,6,14,16], 30-day mortality^[3,5,14,15,16,17], readmission rates^[16], cost^[14] and the duration of hospital stay^[6,14,16]. Lu *et al*^[14] also reported that high-volume centers achieved longer 5-year survival rates. These data seem to lend strong support to the principle of centralization.

However, a closer look at the existing data revealed that there is no standardized definition of “high volumes”, with researchers applying ad-hoc definitions that range from as low as 10 as cases per annum^[16] to as high as 150 cases per annum^[18]. Most papers in the literature quote numbers in excess of 20 cases per annum^[3,19,20,21,22,23]. Using these definitions, the hepatobiliary unit in Trinidad and Tobago does not qualify as high volume, with a mean case volume of 13.8 major hepatectomies per year.

Furthermore, the high volume centers are often tertiary referral hospitals that serve large catchment populations and attract significant funding. They are usually located in major cities within developed countries. Unfortunately, many patients in less-developed Caribbean countries cannot access care in these high volume centers because of travel restrictions, financial limitations, lack of health insurance coverage and/or a paucity of social support structures. Even within the United States, Eppsteiner *et al*^[17] noted that there was socio-economic inequity for access to care at high volume centers.

We observed that most current reports, even those supporting centralization, documented that the majority of major hepatectomies are being performed in low-volume centers - even in developed countries in the 21st century. Fong *et al*^[3] reported in 2005 that only 1% of the hospitals that offered major hepatectomies in the United States of America actually qualified as high-volume centers. In fact, Fong *et al*^[3] reported that the 1272 low-volume centers in the United States performed an average of 1 major hepatectomy per annum - substantially below the “high-volume mark”.

Similar findings were reported by other researchers: Choti *et al*^[5] reported that only 2.7% (1) of the 37 facilities performing major liver resections in the state of Maryland qualified as high-volume. The low-volume facilities performed an average of 1.5 cases annually^[5]. Similarly, Glasgow *et al*^[6] reported that only 3% of 138 hospitals performing liver resections in the state of California qualified as high volume. The low-volume hospitals performed ≤ 3 hepatectomies per annum^[6].

It seems that there is still not universal buy in to the concept of centralization for major hepatectomies. One reason for this may be the lack of practicality. This is especially true in developing countries and it can be appreciated by examining the health care environment in the Anglophone Caribbean. Narayansingh *et al*^[24] outlined the unique challenges to healthcare delivery in this setting: (1) many countries are island states that are geographically separated by the Caribbean Sea; (2) there are political barriers since each country is independent and separately governed; (3) each island has distinctly different cultures; and (4) many surgeons, even those with subspecialty training, are required to perform a wide repertoire of general surgical procedures at low volumes. In addition, most of these countries have underfunded health care systems^[8], leadership deficiencies^[25,26], cultural resistance to multidisciplinary collaboration^[8] and limited access to specialists and subspecialists^[9,24]. These factors were all obstacles to service centralization in the Anglophone Caribbean.

Despite the obstacles, surgical leaders recognized the need and established a hepatobiliary unit in Trinidad and Tobago to serve patients in the Eastern Caribbean^[8]. There has been some success in this regard, as measured by the

Table 2 Performance scores for patients undergoing major liver resections in a low volume Caribbean center

Grade	ECOG Performance status	n (%)
0	Fully active, able to carry out all activities without restriction	10 (14.5)
1	Restricted in physically strenuous activity, but ambulatory and able to carry out light work	20 (29)
2	Ambulatory and capable of self care, but unable to carry out work activities. Up and about > 50% of waking hours	33 (47.8)
3	Capable of limited self care and confined to bed or chair for more than 50% of waking hours	5 (7.3)
4	Completely disabled and cannot carry on self care. Confined to bed or chair	1 (1.5)
5	Dead	0

consistent increase in the annual number of major liver resections performed by this unit (Figure 1). The reduction in case volumes in the year 2016 correlated with the country experiencing a recession. This led to a lack of consumables in Government-funded hospitals and it highlights our point that these demanding operations require significant resources. Coupled with the fact that these hospitals face unique challenges (scarcity of specialized equipment, blood products, ICU space and operating lists), one can realize that the environment is not always conducive to observing best practice recommendations.

Nevertheless, the clinical outcomes in this established low-volume hepatobiliary unit were acceptable. The perioperative mortality rate was 5.8% in our setting. Although reported 30-d mortality in high-volume centers ranged widely from 1.5%^[5] to 9.4%^[6], most high volume centers reported 30-d mortality between 4% and 6.5%^[3,5,16,19]. Our results compared favourably to these high-volume centers. In contrast, the reported 30-day mortality in low volume centers range from 5%-22.7%^[3,5,6,17,22].

Major complications occurred in 15.9% of our patients. This was comparable to reports in the existing medical literature, where major hepatectomies in high-volume centers resulted in major morbidity in 13.2%^[22] to 27%^[27] of cases. Similarly, minor morbidity (24.6%) rates were comparable to existing reports from high-volume centers, ranging from 9.3%^[18] to 26.9%^[22].

Potential critics may suggest that therapeutic outcomes may appear reasonable because of “case selection”, where high-risk patients are referred onward to high-volume referral centers. Obviously, it could have skewed the results toward improved outcomes if only low-risk patients were selected for major hepatectomies in our setting. However, more than half of the major hepatectomies at our facility were performed in high-risk patients, with ASA scores \geq III (58%), ECOG scores \geq 2 (57%) and at least one co-morbidity (93%). Moreover, after pre-operative MDT assessment a further 38% of the major hepatectomies performed in this setting were technically difficult operations.

We do acknowledge that high volume referral centers treat more patients, including high-risk cases with multiple co-morbidities and complicated surgical histories. However, in our setting in the Caribbean, we did not have the luxury of “case selection” because the patients treated at our facilities had no other options for care, for reasons already discussed. We believe, therefore, that referral practices/case selection could not account for the clinical outcomes in this setting. Furthermore, this was a resource-poor environment with limited support services and numerous institutional limitations. These results demonstrated that, despite multiple challenges, the outcomes are not solely dependent on numbers.

We agree with Gasper *et al*^[28] that modern hospitals are complex adaptive systems whose outputs are determined by interactions between internal agents. We also agree with Hashimoto *et al*^[29] that annual volume only contributes a partial assessment and that there is also a substantial contribution by surgeon training and experience. In this regard, we attribute our outcomes to the unit staff (1) having appropriate training; (2) developing an intimate knowledge of the health care system in which they work; (3) fostering a spirit of collective teamwork; (4) maintaining due diligence in care administration; (5) continued audit; and (6) knowledge of population-based data^[30,31,32].

In conclusion, Caribbean hospitals do not, and possibly never will, qualify as high-volume centers due to unique geographic, political and financial limitations to healthcare delivery in the region. Nevertheless, there can be good short-term outcomes when major hepatectomies are performed in low-volume hepatobiliary units in the Eastern Caribbean, despite a high proportion of high-risk patients requiring technically complex operations. This demonstrates that case volume is not the only determinant of good outcomes after major hepatectomy. To achieve good

Table 3 Complications after major liver resections in patients undergoing major liver resections in a low volume Caribbean center

Morbidity	Description	n	Percent
Overall	Number of patients with any complication	21/69	30.4%
Minor	Clavien-Dindo I or II	17/69	24.6%
	Pneumonia	2	2.9%
	Deep Vein Thrombosis	2	2.9%
	Surgical site infections	2	2.9%
	Bile leaks	4	5.8%
	ISGLS Grade-B post-hepatic liver failure	7	10.1%
Major	Clavien-Dindo III or IV	11/69	15.9%
	Anastomotic dehiscence	1	1.5%
	Intra-abdominal collection	4	5.8%
	Right hepatic artery injury	1	1.5%
	Strangulated internal hernia	1	1.5%
	ISGLS Grade-C post-hepatic liver failure	4	5.8%
Mortality	30-d mortality: All causes (1) 69 yr-old man: Sepsis after bladder leak after abdomino-perineal resection for synchronous colorectal liver metastases (2) 80 yr-old man: bile leak after extended right hepatectomy for colorectal liver metastases (3) 69 yr-old woman: Post-Hepatic Liver Failure after extended right hepatectomy for hepatocellular carcinoma (4) 79 yr-old man: Anastomotic leak after extended right hepatectomy for hilar cholangiocarcinoma	4/69	5.8%

outcomes, there is also the need for teamwork, appropriately trained staff, due diligence in care administration, continued audit and knowledge of population-based data.

ARTICLE HIGHLIGHTS

Research background

In the past two decades, there was a trend to concentrate major hepatectomies in specific centers in order to support sub-specialty teams performing these operations at high volumes. This trend was supported by accumulating data to suggest that there were better peri-operative outcomes in high-volume referral hospitals. However, this is not practical in the Caribbean and other resource-poor countries.

Research motivation

Clinicians in the Caribbean do not have the luxury of “case selection” because most patients treated at our facilities have no other options for care. Therefore, these patients must receive treatment at low-volume, resource-poor centers with limited support services and numerous institutional limitations. The motivation for our research was to determine if the clinical outcomes are acceptable despite the numerous limitations.

Research objectives

To determine the clinical outcomes after major hepatectomies in a low-volume, resource-poor center in the Caribbean.

Research methods

We prospectively studied post-operative morbidity and mortality in all patients undergoing major hepatectomies in a low-volume Caribbean hepatobiliary center over a five-year study period. Statistical analyses were performed using SPSS ver 16.0.

Research results

There were 69 major hepatectomies performed over the study period (mean case volume of 13.8 major resections/year). More than half of the major hepatectomies were performed in high-risk

patients, with ASA scores \geq III (58%), ECOG scores \geq 2 (57%) or at least one co-morbidity (93%). A further 38% of the major hepatectomies performed in this setting were technically difficult operations. Twenty-one patients experienced at least 1 complication, for an overall morbidity rate of 30.4%. There were minor complications in 17 (24.6%) patients, major complications in 11 (15.9%) patients and 4 (5.8%) deaths.

Research conclusions

Although Caribbean hospitals do not qualify as high-volume centers, there can be good short-term outcomes after major hepatectomies are performed in established hepatobiliary units. This demonstrates that case volume is not the only determinant of good outcomes after major hepatectomy.

Research perspectives

To achieve good outcomes, there is the need for teamwork, appropriately trained staff, due diligence in care administration, continued audit and knowledge of population-based data. Case volume is not the only determinant of good outcomes after major hepatectomy.

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Retrospective Study

Central line-associated bloodstream infection among children with biliary atresia listed for liver transplantation

Nicole D Triggs, Stacey Beer, Sonam Mokha, Kat Hosek, Danielle Guffey, Charles G Minard, Flor M Munoz, Ryan W Himes

ORCID number: Nicole D Triggs (0000-0003-0587-8026); Stacey Beer (0000-0001-7244-2033); Sonam Mokha (0000-0002-2633-8638); Kat Hosek (0000-0002-0667-3826); Danielle Guffey (0000-0003-3721-614X); Charles G Minard (0000-0003-4631-6943); Flor M Munoz (0000-0002-0457-7689); Ryan W Himes (0000-0002-0116-2613).

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Nicole D Triggs, Stacey Beer, Ryan W Himes, Department of Pediatrics, Section of Gastroenterology, Hepatology, and Nutrition, Baylor College of Medicine, Houston, TX 77030, United States

Sonam Mokha, College of Arts and Sciences, Washington University in St. Louis, St. Louis, MO 63130, United States

Kat Hosek, Outcomes and Impact Service, Texas Children's Hospital, Houston, TX 77030, United States

Danielle Guffey, Charles G Minard, Dan L. Duncan Institute for Clinical and Translational Research, Baylor College of Medicine, Houston, TX 77030, United States

Flor M Munoz, Department of Pediatrics, Section of Infectious Disease, Baylor College of Medicine, Houston, TX 77030, United States

Corresponding author: Ryan W Himes, MD, Assistant Professor, Department of Pediatrics, Section of Gastroenterology, Hepatology, and Nutrition, Baylor College of Medicine, 6701 Fannin St, MWT 1010, Houston, TX 77030, United States. rwHimes@texaschildrens.org

Telephone: +1-832-8221050

Fax: +1-832-8253633

Abstract

BACKGROUND

Pre-transplant nutrition is a key driver of outcomes following liver transplantation in children. Patients with biliary atresia (BA) may have difficulty achieving satisfactory weight gain with enteral nutrition alone, and parenteral nutrition (PN) may be indicated. While PN has been shown to improve anthropometric parameters of children with BA listed for liver transplantation, less is known about the risks, particularly infectious, associated with this therapy among this specific group of patients.

AIM

To describe the incidence, microbiology, and risk factors of central line-associated bloodstream infection (CLABSI) among children with BA listed for liver transplantation.

METHODS

Retrospective review of children aged ≤ 2 -years of age with BA who were listed for primary liver transplantation at Texas Children's Hospital from 2008 through

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2015 ($n = 96$). Patients with a central line for administration of PN ($n = 63$) were identified and details of each CLABSI event were abstracted. We compared the group of patients who experienced CLABSI to the group who did not, to determine whether demographic, clinical, or laboratory factors correlated with development of CLABSI.

RESULTS

Nineteen of 63 patients (30%, 95%CI: 19, 43) experienced 29 episodes of CLABSI during 4800 line days (6.04 CLABSI per 1000 line days). CLABSI was predominantly associated with Gram-negative organisms (14/29 episodes, 48%) including *Klebsiella* spp., *Enterobacter* spp., and *Escherichia coli*. The sole polymicrobial infection grew *Enterobacter cloacae* and *Klebsiella pneumoniae*. Gram-positive organisms (all *Staphylococcus* spp.) and fungus (all *Candida* spp.) comprised 9/29 (31%) and 6/29 (21%) episodes, respectively. No demographic, clinical, or laboratory factors were significantly associated with an increased risk for the first CLABSI event in Cox proportional hazards regression analysis

CONCLUSION

There is substantial risk for CLABSI among children with BA listed for liver transplantation. No clinical, demographic, or laboratory factor we tested emerged as an independent predictor of CLABSI. While our data did not show an impact of CLABSI on the short-term clinical outcome, it would seem prudent to implement CLABSI reduction strategies in this population to the extent that each CLABSI event represents potentially preventable hospitalization, unnecessary healthcare dollar expenditures, and may exact an opportunity cost, in terms of missed allograft offers.

Key words: Parenteral nutrition; Central line-associated bloodstream infection; Pediatric; Microbiology; Central venous catheter

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Core tip: Rates of central line-associated bloodstream infection (CLABSI) are high among children with biliary atresia listed for liver transplantation. While most CLABSI represented enteric flora, *Candida* was isolated in 21% of events, suggesting that it may be appropriate to consider the use of antifungals in this population when empiric therapy doesn't lead to expected clinical improvement. Since no factors we tested appeared to predict CLABSI, we propose that prevention efforts should be focused on universal and meticulous application of known CLABSI-reducing strategies, such as line insertion bundles.

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INTRODUCTION

Biliary atresia (BA) is a progressive obliterative cholangiopathy which presents in the first months of life and it is the most common indication for liver transplantation in children. While a patient's pre-operative nutritional status is an important driver of transplant outcomes^[1-3], malnutrition is prevalent among patients with BA^[3-5]. Common nutritional rehabilitation strategies include provision of calorically dense enteral formulas, enrichment in medium chain triglycerides, and use of nasogastric feeding tubes. In spite of these efforts, many children with BA will fail to achieve the desired catch-up growth, and will ultimately be prescribed parenteral nutrition (PN). Although PN has been shown to improve the nutritional status of children with BA on the liver transplant waitlist^[5,6], PN delivered through a central venous catheter (CVC) introduces a new set of risks to the patient, including mechanical, infectious,

and metabolic complications. We sought to characterize infectious complications of PN, specifically central line-associated bloodstream infection (CLABSI) among children with BA on the liver transplant waitlist. The aim of our investigation was to describe the incidence and microbiology of CLABSI among this cohort of patients and to elucidate potential risk factors which might be leveraged for prevention efforts.

MATERIALS AND METHODS

We identified children aged ≤ 2 -years of age with BA who were listed for primary liver transplantation at Texas Children's Hospital from 2008 through 2015. Utilization of a CVC for administration of PN was ascertained through pharmacy records, radiology reports, and clinical documentation. Demographic, anthropometric, laboratory, and clinical data were collected retrospectively through review of electronic medical records. The decision to use PN and its specific prescription was at the clinical discretion of the treating hepatologist, working together with a registered dietitian. Weight-for-length z-scores at the time of listing were calculated according to the World Health Organization standards using an online calculator available at <https://peditools.org/>.

Central line days accrued from the time of line insertion until line removal, liver transplantation, or removal of the patient from the transplant waitlist for a reason other than transplantation (*i.e.*, death, clinical deterioration, or clinical improvement), whichever came first.

Our operational definition of CLABSI is based on the Center for Disease Control and Prevention/National Healthcare Safety Network (CDC/NHSN) definition: laboratory-confirmed bloodstream infections, not secondary to infection at another body site, among patients with a CVC^[7]. In contrast to the CDC/NHSN definition, however, which is designed to capture healthcare facility acquired infections, we regarded all CLABSI events as relevant, even if the place of origin was in the community. To that end, central line days accrued, and CLABSI events were recorded, for children while admitted in a healthcare facility and while at home.

According to our clinical practice, febrile patients with a CVC and no other localizing source were admitted to hospital. Broad-spectrum antibiotics were initiated, guided by sensitivities of prior blood cultures, when applicable. Antibiotics were tailored to the specific isolate when sensitivities were available. Daily blood cultures were obtained until two consecutive cultures were sterile. CVCs were removed if sterility was not achieved, or sooner, if clinical circumstances warranted. Seven to 14 d of parenteral antibiotic therapy was completed, starting from the first sterile day.

Data are presented as frequency with percent, means with standard deviations, or medians with 25th and 75th centiles. Chi-squared, Wilcoxon rank sum, or *t*-tests were used to compare groups, as appropriate. Kaplan-Meier survival analysis and Cox proportional hazards regression were used to analyze the time-to-the-first CLABSI event and Cox proportional hazards regression was used to evaluate risk factors for CLABSI. This study was approved by the Baylor College of Medicine institutional review board.

RESULTS

Ninety-six patients with BA, ≤ 2 -years of age, were listed for liver transplantation between 2008 and 2015 at our center. Sixty-three (66%) patients had a CVC placed for administration of PN. Nineteen of 63 patients (30%, 95%CI: 19, 43) experienced 29 episodes of CLABSI during 4800 line days (6.04 CLABSI per 1000 line days) (Figure 1).

Baseline demographic and clinical data

Clinical and demographic data at the time of listing for liver transplantation are shown in Table 1. In univariate analyses, there was a higher proportion of patients with public or no insurance in the CLABSI group, compared to the no CLABSI group (84.2% *vs* 56.8%, $P = 0.036$). There were uniform, but non-significant trends, towards greater growth retardation in the CLABSI group, as mean length-for-age z-scores, weight-for-age z-scores, and weight-for-length z-scores were lower. Among all 63 patients with a CVC for administration of PN, the median age at the time of line placement was 6.6 mo (IQR 5.4, 9) and the median number of line days were 58 (IQR 30, 96), with a range of 4-255 d (data not shown).

Characteristics of CLABSI events

Among patients who developed CLABSI, the median time to first event was 28 d (IQR

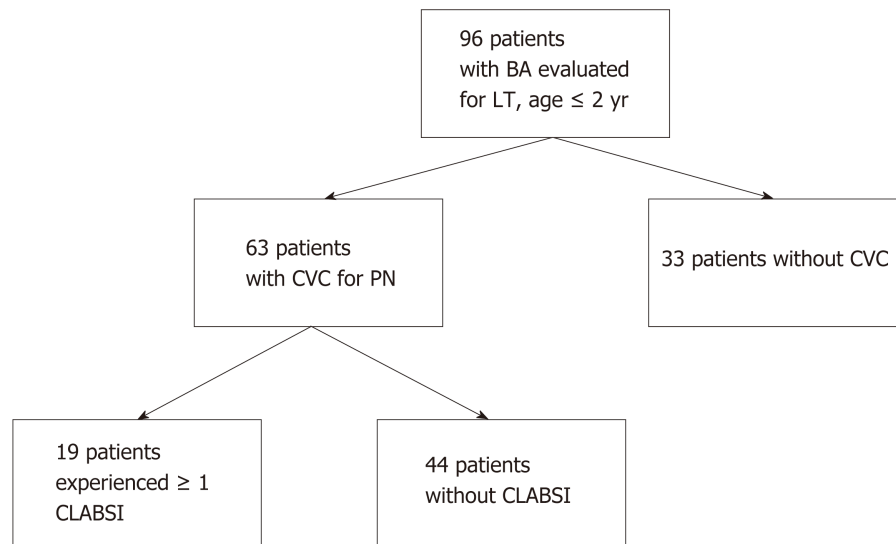


Figure 1 Patient flowchart. BA: Biliary atresia; LT: Liver transplant; CVC: Central venous catheter; PN: Parenteral nutrition; CLABSI: Central line-associated bloodstream infection.

12, 53). The earliest CLABSI occurred on line day 3 and the latest event on line day 80. Kaplan-Meier analysis revealed that 75% of patients remained free of CLABSI after 43 line days (95%CI: 21, 62, **Figure 2**). No demographic, clinical, or laboratory factors were significantly associated with an increased risk for the first CLABSI event in Cox proportional hazards regression analysis (**Table 2**).

Fourteen of 19 (74%) affected patients experienced a single episode of CLABSI. There were 3 patients with two episodes, and 1 patient experienced four and five episodes each. Only the patient with five episodes had the same organism isolated on more than one occasion, in this case *Klebsiella pneumoniae*. Overall, CLABSI was predominantly associated with Gram-negative organisms (14/29 episodes, 48%) including *Klebsiella* spp., *Enterobacter* spp., and *Escherichia coli*. The sole polymicrobial infection grew *Enterobacter cloacae* and *Klebsiella pneumoniae*. Gram-positive organisms (all *Staphylococcus* spp.) and fungus (all *Candida* spp.) comprised 9/29 (31%) and 6/29 (21%) episodes, respectively (**Figure 3**).

Clinical outcomes

While the short-term clinical outcomes did not significantly differ between the two groups (**Table 1**), there were trends towards a higher rate of transplantation and a lower rate of clinical deterioration in the CLABSI group. The single waitlist death in the CLABSI group was not directly attributable to the CLABSI event.

DISCUSSION

Nutritional rehabilitation is a cornerstone in the management of pediatric liver transplant candidates. Those ≤ 2-years of age with BA comprise a large and fairly homogenous group of patients whose nutritional deficits have been well-documented^[2,4,8]. In spite of calorically dense enteral formulas and modular supplements, often delivered via nasogastric tubes, it is not uncommon for patients to still not achieve satisfactory growth. On the other hand, two studies have now demonstrated that PN improves the nutritional status (*i.e.*, mid-arm circumference and triceps skinfold thickness) of malnourished patients with BA on the transplant waitlist^[5,6], underscoring the important role this therapy plays in the nutritional optimization of liver transplant candidates.

In our large pediatric liver transplant program, about 2/3 of liver transplant candidates, ≤ 2-years of age, with BA received PN. This is higher than the 41% reported by Wendel *et al*^[6], and to the 53% reported by Sullivan *et al*^[5], though inclusion criteria were not identical. On a per patient basis, we observed that 30% of patients with a CVC for PN met rigorous criteria for CLABSI, similar to the 33% reported by Wendel *et al*^[6]. While Sullivan *et al*^[5] found 52% of their cohort with a CVC for PN had a positive blood culture, it is not known how many of these represented CLABSI, as opposed to secondary bacteremia or contaminants, making direct comparison impossible. Our CLABSI rate, 6.04/1000 line days, is greater than that of

Table 1 Clinical and demographic data at time of liver transplant listing and short-term clinical outcome of patients with and without central line-associated bloodstream infection

	No CLABSI	CLABSI	P value
<i>n</i> (%)	44 (70)	19 (30)	
Male, <i>n</i> (%)	11 (25)	5 (26)	0.91
Race, <i>n</i> (%)			
White	38 (86)	15 (79)	0.71
Black	5 (11)	3 (16)	
Asian	1 (2)	1 (5)	
Ethnicity, <i>n</i> (%)			
Hispanic	25 (57%)	9 (47%)	0.49
Primary language non-English, <i>n</i> (%)	12 (27)	3 (16)	0.32
Insurance, <i>n</i> (%)			0.036
Public/none	25 (57)	16 (84)	
Private	19 (43)	3 (16)	
Kasai portoenterostomy timing, <i>n</i> (%)			0.99
≤ 100 d	28 (64)	12 (63)	
> 100 d	2 (5)	1 (5)	
Not performed	14 (32)	6 (32)	
Age at listing, years, mean (SD)	0.59 (0.27)	0.61 (0.31)	0.89
Any home parenteral nutrition, <i>n</i> (%)	16 (36)	9 (47)	0.41
Anthropometrics			
Length, cm, mean (SD)	64.5 (3.8)	64.3 (3.6)	0.82
Weight, kg, mean (SD)	6.9 (1.3)	6.6 (1.1)	0.38
Length z-score, mean (SD)	-1.26 (1.47)	-1.57 (1.24)	0.43
Weight z-score, mean (SD)	-0.96 (1.26)	-1.44 (0.98)	0.15
WFL z-score, mean (SD)	-0.21 (1.13)	-0.58 (0.91)	0.21
Laboratory			
ALT, U/L, median (25 th , 75 th)	133 (90, 204)	128 (90, 233)	0.98
GGT, U/L, median (25 th , 75 th)	257 (106, 504)	364 (205, 800)	0.14
Albumin, g/dL, mean (SD)	3.1 (0.46)	3 (0.68)	0.88
Conjugated bilirubin, mg/dL, median (25 th , 75 th)	4.2 (0.95, 8.2)	3.7 (2, 5.7)	0.73
Total bilirubin, mg/dL, median (25 th , 75 th)	10.7 (3.5, 16.2)	11.3 (6.2, 12.7)	0.90
White blood cells, × 10 ³ /μL, median (25 th , 75 th)	10.2 (8.3, 15.6)	10.6 (7.4, 14.4)	0.88
Hemoglobin, g/dL, mean (SD)	9.9 (1.6)	10.2 (1.3)	0.56
Platelets, × 10 ³ /μL, median (25 th , 75 th)	123 (95, 217)	160 (102, 230)	0.41
INR, mean (SD)	1.6 (0.6)	2.3 (4.1)	0.28
BUN, mg/dL, mean (SD)	9.5 (5)	9.9 (4.6)	0.73
Prealbumin, mg/dL, mean (SD)	9.5 (3.6)	9.5 (4.3)	0.95
Clinical outcome			0.29
Transplanted	35 (80)	18 (95)	
Died on the waitlist or removed for too ill	6 (14)	1 (5)	
Removed from waitlist for too well	3 (7)	0	

CLABSI: Central line-associated bloodstream infection; SD: Standard deviation; WFL: Weight-for-length; ALT: Alanine aminotransferase; GGT: Gamma glutamyl transferase; INR: International normalized ratio; BUN: Blood urea nitrogen.

Wendel *et al*^[6] (3.8/1000 line days), though it is not clear whether the same definition of CLABSI was used in their study. While these methodological differences may be barriers to generalizing CLABSI rates from center-to-center, and population-to-population, collectively our data calls attention to a facet of the care of transplant candidates which may be underappreciated. We would suggest that calculation and internal benchmarking of an individual program's CLABSI rate may be a good balancing measure on initiatives to improve the nutritional status of pediatric transplant candidates.

Table 2 Demographic, clinical, and laboratory factors assessed as potential risk factors for central line-associated bloodstream infection

	HR	95%CI	P value
Male	0.934	0.335-2.6	0.89
Race			
White	Reference		
Black	1.156	0.334-4	0.819
Asian	1.089	0.143-8.298	0.934
Ethnicity			
Hispanic	0.642	0.259	0.339
Primary Language non-English	0.531	0.154-1.832	0.317
Insurance			
Private	Reference		
Public/None	2.435	0.705-8.406	0.159
Kasai portoenterostomy timing			
≤ 100 d	0.852	0.319-2.275	0.749
> 100 d	1.33	0.159-11.126	0.792
Not performed	Reference		
Age at listing	1.883	0.379-9.36	0.439
Any home parenteral nutrition	0.811	0.323-2.035	0.656
Anthropometrics			
Length at listing	1.069	0.937-1.219	0.323
Weight at listing	1.036	0.67-1.672	0.872
Length z-score at listing	0.942	0.674-1.317	0.728
Weight z-score at listing	0.836	0.55-1.271	0.403
WFL z-score at listing	0.858	0.579-1.271	0.445
Laboratory			
ALT at listing	0.999	0.998-1.002	0.861
GGT at listing	1	0.999-1.001	0.176
Albumin at listing	0.853	0.314-2.314	0.775
Conjugated bilirubin at listing	0.991	0.91-1.079	0.842
Total bilirubin at listing	0.997	0.946-1.051	0.927
White blood cells at listing	1.016	0.93-1.109	0.724
Hemoglobin at listing	1.106	0.816-1.5	0.516
Platelets at listing	1.001	0.998-1.005	0.458
INR at listing	1.084	0.9593-1.225	0.196
BUN at listing	1.007	0.918-1.104	0.889
Prealbumin at listing	0.966	0.842-1.109	0.628

HR: Hazard ratio; CI: Confidence interval; WFL: Weight-for-length; ALT: Alanine aminotransferase; GGT: Gamma glutamyl transferase; INR: International normalized ratio; BUN: Blood urea nitrogen.

In our univariate analyses, children with public/no insurance were overrepresented in the CLABSI group. While our data do not offer potential explanations for this finding, it is congruent with an evolving body of literature which documents disparities in access to, or outcomes of, pediatric liver transplantation^[9-11]. In agreement with Sullivan *et al*^[5], we did not find that CLABSI impacted short-term clinical outcomes, namely whether the patient was removed from the waitlist for transplantation, deterioration, or improvement. The single waitlist death in our CLABSI cohort was unrelated, however, Sullivan *et al*^[5] reported 3 deaths among the 25 patients with BA who received PN, one of which was due to fungal sepsis, highlighting that this threat is tangible. Due to the relatively small number of individuals in each group, these findings should be interpreted with caution, however.

The microbiology of our CLABSI was predominantly Gram-negative, enteric organisms. Among children with BA, secondary bacteremia from ascending cholangitis is also a diagnostic consideration. However, care was taken in

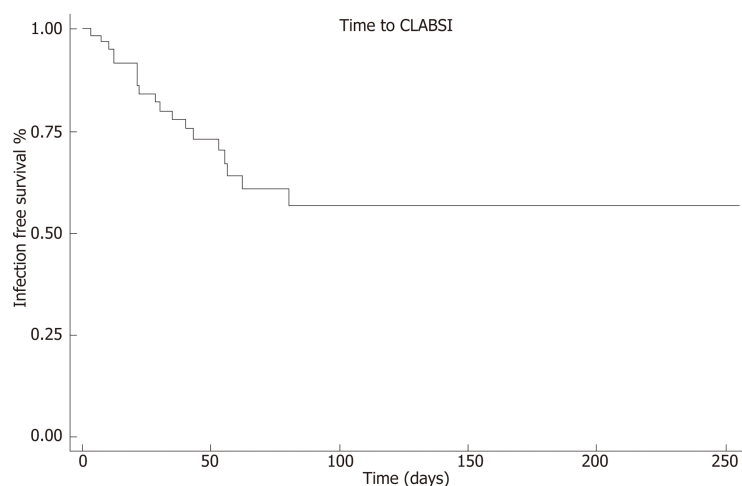


Figure 2 Time to central line-associated bloodstream infection survival analysis. Kaplan-Meier curve of time to CLABSI indicates that 75% of patients remained free of CLABSI on line day 43. CLABSI: Central line-associated bloodstream infection.

adjudicating CLABSI to exclude positive blood cultures when a concurrent diagnosis of cholangitis was made. Given the difficulties in diagnosing cholangitis in young children though, the possibility remains that some cholangitis episodes were not recognized, which may have led to an overestimation of CLABSI. *Candida* comprised about 20% of our CLABSI events, therefore it may also be prudent to consider a fungal etiology for CLABSI among young transplant candidates with BA receiving PN.

As expected, time with a CVC is related to incident CLABSI; according to our survival analysis, 75% of our patients remained free of CLABSI on line day 43. While interventions to reduce total line days may reduce CLABSI, this strategy is difficult to implement in the transplant candidate. Uncertainty with regard to timing of transplantation and the lack of data to support specific clinical or laboratory thresholds for nutritional intervention breeds an environment in which PN is prescribed from the time enteral nutrition is deemed to be insufficient until transplantation. A better understanding of anthropometric or nutritional thresholds associated with specific clinical outcomes may help to more precisely utilize PN. In the meantime, strategies for CLABSI reduction which have been studied in other patient populations should be explored in transplant candidates as well. CVC insertion bundles and parental line care training^[12], ethanol locks^[13], and taurolidine locks^[14] have been suggested for CLABSI reduction in children with other conditions receiving PN.

Strengths of our data include the comparatively large size of the cohort and the application of a CLABSI definition which captures both the events occurring within a healthcare environment and in the community. A principal limitation of our data is its retrospective nature; this precluded standardized data collection and led to non-uniform utilization criteria and prescriptions for PN. And while our sample size is large for study of a very specific sub-group of pediatric patients, in absolute terms, its size limits statistical power. Post-hoc power calculations suggest that the sample sizes observed in this study has about 67% power to detect a hazards ratio of 2.0 between two groups, assuming 30% of patients in one group have CLABSI and 9% in the second group, and $\alpha = 0.05$ (two-sided). It is likely that only a concerted multi-center effort, like Studies in Pediatric Liver Transplantation, would be able to overcome the power problem, however information on CVC use is not currently collected in this registry.

In conclusion, our series calls attention to the substantial risk for CLABSI among children with BA listed for liver transplantation. No clinical, demographic, or laboratory factor we tested emerged as an independent predictor of CLABSI, but time with a CVC was directly related to incident CLABSI. While our data did not show an impact of CLABSI on the short-term clinical outcome, it would seem prudent to implement CLABSI reduction strategies in this population to the extent that each CLABSI event represents potentially preventable hospitalization, unnecessary healthcare dollar expenditures, and may exact an opportunity cost, in terms of missed allograft offers.

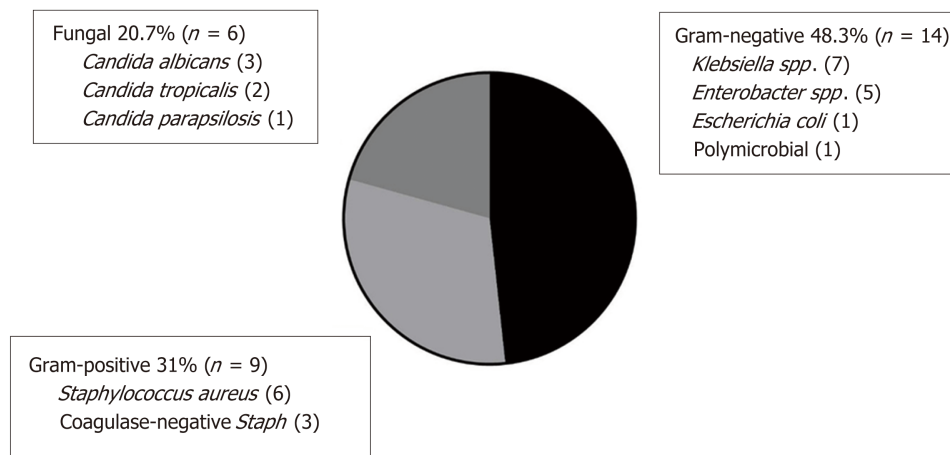


Figure 3 Microbiology of central line-associated bloodstream infection events.

ARTICLE HIGHLIGHTS

Research background

Children with biliary atresia (BA) undergoing liver transplantation benefit from pre-operative optimization of their nutritional status. When feeding enterally is insufficient to rehabilitate these patients, parenteral nutrition (PN) may be a useful adjunct. While this modality has been shown to improve the growth of children with BA listed for liver transplantation, it is also associated with distinct risks, chief among them the risk of infection associated with an indwelling central venous catheter.

Research motivation

Our group was motivated to pursue this project so that the field might have a better understanding of the infectious risks of PN given to children with BA on the liver transplant waitlist, and thus make informed decisions regarding risk and benefit to the patient.

Research objectives

The objective of our study was to describe the incidence, microbiology, and risk factors of central line-associated bloodstream infection (CLABSI) among children with BA listed for liver transplantation.

Research methods

Retrospective, single-center review.

Research results

Nineteen of 63 patients (30%) experienced 29 episodes of CLABSI during 4800 line days (6.04 CLABSI per 1000 line days). CLABSI were predominantly associated with Gram-negative organisms (14/29 episodes, 48%) including *Klebsiella spp.*, *Enterobacter spp.*, and *Escherichia coli*. The sole polymicrobial infection grew *Enterobacter cloacae* and *Klebsiella pneumoniae*. Gram-positive organisms (all *Staphylococcus spp.*) and fungus (all *Candida spp.*) comprised 9/29 (31%) and 6/29 (21%) episodes, respectively. There were no demographic, laboratory, or clinical features associated with CLABSI risk in our model.

Research conclusions

CLABSI events are not rare among children with BA, receiving PN, while listed for liver transplantation. In spite of the frequency of events, CLABSI were not associated with mortality, or removal from the transplant waitlist due to becoming too ill to transplant. Since none of the factors tested in our model were associated with CLABSI risk, we propose meticulous application of known CLABSI-reducing strategies, such as line insertion bundles.

Research perspectives

Owing to the relatively small volume of pediatric liver transplants performed, even at the largest centers, future efforts should consider leveraging existing databases, such as Studies in Pediatric Liver Transplantation, to address these questions.

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Parallel transjugular intrahepatic portosystemic shunt with Viatorr® stents for primary TIPS insufficiency: Case series and review of literature

Driss Raissi, Qian Yu, Michael Nisiewicz, Steven Krohmer

ORCID number: Driss Raissi (0000-0002-6751-2997); Qian Yu (0000-0001-9774-9226); Michael Nisiewicz (0000-0001-8439-4024); Steven Krohmer (0000-0003-4338-0640).

Author contributions: Raissi D designed the literature review, drafted and revised the manuscript and selected images; Yu Q performed research, drafted manuscript and created the table; Nisiewicz M performed research and drafted manuscript; Krohmer S reviewed and guided the manuscript.

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Driss Raissi, Qian Yu, Michael Nisiewicz, Steven Krohmer, Department of Radiology, University of Kentucky, Lexington, KY 40536, United States

Corresponding author: Driss Raissi, MD, Assistant Professor of Radiology, Medicine and Obstetrics & Gynecology. Department of Radiology, University of Kentucky, 800 Rose Street, Lexington, KY 40536, United States. driss.raissi@uky.edu

Telephone: +1-859-3231004

Fax: +1-859-2574884

Abstract

BACKGROUND

Transjugular intrahepatic portosystemic shunts (TIPS) can alleviate complications of portal hypertension such as ascites and variceal bleeding by decreasing the portosystemic gradient. In limited clinical situations, parallel TIPS may be only solution to alleviate either variceal bleeding or ascites secondary to portal hypertension when the primary TIPS fails to do so. Data specifically addressing the use of this partially polytetrafluoroethylene covered nitinol stent (Viatorr®) is largely lacking despite Viatorr® being the current gold standard for modern TIPS placement.

CASE SUMMARY

All three patients had portal hypertension and already had a primary Viatorr® TIPS placed previously. All patients have undergone failed endoscopy to manage acute variceal bleeding before referral for a parallel stent (PS). PS were placed in patients presenting with recurrent variceal bleeding despite existence of a widely patent primary TIPS. Primary stent patency was verified with either Doppler ultrasound or intra-procedural TIPS stent venography. Doppler ultrasound follow-up imaging demonstrated complete patency of both primary and parallel TIPS. All three patients did well on clinical follow-up of up to six months and no major complications were recorded. A review of existing literature on the role of PS in the management of portal hypertension complications is discussed. There are three case reports of use of primary and PS Viatorr® stents placement, only one of which is in a patient with gastrointestinal variceal bleeding despite a patent primary Viatorr® TIPS.

CONCLUSION

Viatorr® PS placement in the management of variceal hemorrhage is feasible with promising short term patency and clinical follow-up data.

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Core tip: To our knowledge, we are first to report a three-case series with 6-month follow-up data using Viatorr® for both primary transjugular intrahepatic portosystemic shunt (TIPS) and parallel TIPS placement for the management of recurrent upper gastrointestinal variceal hemorrhage. Although, parallel TIPS placement has been previously reported, it was largely using bare metal stents and/or fully covered stents. Data regarding the use of the partially polytetrafluoroethylene-covered nitinol stent (Viatorr®) in parallel TIPS placement is largely lacking despite this device being the current gold standard for TIPS placement.

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INTRODUCTION

Gastroesophageal varices and ascites are common complications of portal hypertension and cirrhosis. It is estimated that the mortality during the first episode of acute upper gastrointestinal variceal bleeding in cirrhotic patients is between 15%-30%^[1-3], whereas the mortality rates of ascites in those patients increases from 15% to 44% between one-year and five-year follow-ups^[4,5]. Transjugular intrahepatic portosystemic shunt (TIPS) can alleviate these two complications by decreasing the portosystemic gradient. This is achieved by decompressing the portal venous system directly via a stent supported shunt into the systemic circulation^[6-9]. A previous study suggested an association between the early use of TIPS in cirrhotic patients with variceal bleeding with a reduction in mortality^[10]. Additionally, according to a meta-analysis of four randomized trials, cirrhotic patients with recurring ascites that have received TIPS demonstrated a higher transplant-free survival compared to those receiving paracentesis at 6, 12, 24, and 36 months: 75.1% *vs* 65.3%, 63.1% *vs* 52.5%, 49.0% *vs* 35.2%, and 38.1% *vs* 28.7% ($P = 0.035$)^[11]. Yet, one complication of TIPS was shunt dysfunction, which could be manifested by the persistence or recurrence of ascites, variceal bleeding after the initial procedure, unsuccessful reduction of portal venous pressure, elevated portosystemic gradient, and decreased mid-shunt velocity on Doppler imaging during surveillance^[12-16]. Once shunt dysfunction has been confirmed, multiple approaches may be adopted: shunt revision with angioplasty, repeat stenting, deployment of endoprosthesis, and/or catheter directed thrombolysis^[16,17]. Rarely, a second parallel stent (PS) can be placed to overcome primary TIPS insufficiency and alleviate upper gastrointestinal variceal bleeding or ascites. Here, we report a case series of PS placements in three patients with portal hypertension secondary to alcoholic cirrhosis, alpha-1 antitrypsin deficiency (AATD), and non-alcoholic steatohepatitis (NASH) respectively.

CASE PRESENTATION

Case 1

Chief complaints: Vomiting blood and lightheadedness.

History of present illness: A 46-year-old female with alcoholic cirrhosis and esophageal varices presented to the emergency department (ED) with recent 2 episodes of hematochezia. She endorsed nausea and lightheadedness for the past few days, denying hematemesis or coffee-ground emesis. Four months ago, she presented with GI bleeding from esophageal varices and failed repeated esophageal bandings. TIPS and coronary vein varices coil-embolization were performed at that time: Portosystemic gradient (PSG) was reduced from 17 mmHg to 10 mmHg; direct portal

pressure decreased from 27 mmHg to 23 mmHg. She was discharged 3 d later. During one-month follow-up after the procedure, she was clinically asymptomatic with adequate flow-velocities in her TIPS (Table 1).

History of past illness: Chronic pancreatitis;; non-bleeding grade 1 gastric varices status post coronary vein coil-embolization; hypertension; endometriosis.

Personal and family history: No current tobacco, alcohol, or substance use. Family history was non-contributory.

Physical examination upon admission: Mildly jaundiced; abdomen was soft and non-distended; clear bilateral lung sounds, intact neurological exams with appropriate mood and affect.

Laboratory examinations: Hemoglobin: 4.7 g/dL and platelets: 25000/mL consistent with anemia secondary to acute blood loss; abnormal liver function tests (Table 1).

Imaging examinations: Esophagogastroduodenoscopy (EGD) showed blood-filled stomach and type-1 isolated gastric varices. Contrast enhanced computed tomography of the abdomen showed a blood-filled stomach and large gastric wall varices. Four months ago, a 10 mm × 8 cm Viatorr® (GORE, Flagstaff AR, United States) stent was placed from right hepatic vein to the left portal vein with a 4-cm bare metal stent extension for optimal outflow positioning into the inferior vena cava (Figure 1A-C). Doppler ultrasound (US) demonstrated a patent stent with adequate velocities (Table 1).

Multidisciplinary expert consult

Kinjal Dave, MD, Assistant Professor of Medicine, Division of Gastroenterology; Jen Rosenau, MD, Assistant Professor of Medicine, Division of Hepatology.

Diagnosis

Primary TIPS insufficiency with recurrent variceal bleeding.

Treatment

Parallel TIPS placement: A TIPS revision procedure demonstrated a widely patent stent and a portosystemic gradient of 9 mmHg. A parallel 10 mm × 8 cm Viatorr® TIPS was inserted to manage continued bleeding, reducing PSG to 4 mmHg. The direct portal pressure remained constant (25 mmHg) before and after the procedure. Patient later developed urinary sepsis and was successfully treated with broad spectrum antibiotics.

Follow-up

Six months later, patient returned to ED reporting progressive abdominal pain and swelling. Both TIPS stents were found to be patent by US (Table 1). No ascites was seen on US.

Case 2

Chief complaints: Persistent engorged esophageal varices on surveillance EGD.

History of present illness: A 56-year-old male with cirrhosis secondary to AATD status post TIPS was referred to interventional radiology (IR) in June 2017 because of persistent large esophageal varices on EGD. He was clinically asymptomatic at presentation. Previously, he was admitted in April 2017 with abdominal distension, hematemesis and acute blood loss anemia. At that time, US showed large volume ascites and an occluded TIPS stent. In addition to paracentesis and blood transfusion, sharp recanalization of the shunt was performed reducing PSG from 15 mmHg to 10 mmHg. He was discharged 3 d after the May 2017 admission.

History of past illness: Cirrhosis with prior esophageal variceal bleeding status post TIPS in 2009; esophageal banding; hypertension; inguinal hernia; umbilical hernia.

Personal and family history: No tobacco, alcohol, or substance use. Family history was positive for AATD affecting his mother's lungs.

Physical examination upon admission: Jaundiced; abdomen was soft and non-distended; clear bilateral lung sounds, intact neurological exams with appropriate mood and affect.

Laboratory examinations: Abnormal liver function tests (Table 1).

Imaging examinations: Given concern for shunt occlusion, we obviated the TIPS US

Table 1 Laboratory values, Child-Pugh class, model for end-stage liver disease score and medications given for hepatic encephalopathy of patient 1, 2, and 3 before the transjugular intrahepatic portosystemic shunts placement, before the parallel placement, and 6-mo follow-up

Status	Patient 1	Patient 2	Patient 3
Before TIPS	Child-Pugh B; MELD 11; bilirubin 1.1, INR 1.5, PTT 18.6, albumin 2.6; DV post TIPS: 153 (TIPS), 33 cm/s (MPV). Lactulose 10 g/d	Child-Pugh B; MELD 11; bilirubin 0.7, INR 1.3, PTT 16.6, and albumin 3.5. Rifaximin 550 mg × 2/d, Lactulose 20 g × 3/d	Child-Pugh C; MELD 12; bilirubin 1.6, INR 1.4, PTT 17.1, and albumin 2.5; DV immediately post-TIPS: 96 (TIPS), 89.5 cm/s (MPV). Rifaximin 550 mg × 2/d, Lactulose 30 g/45 mL × 3/d
Before PS	Child-Pugh B; MELD 15; bilirubin 4.6, INR 1.3, PTT 17.0, and albumin 2.5; DV: 155 (TIPS), 18 cm/s (MPV). Rifaximin 550 mg × 2/d Lactulose 10 g/d	Child-Pugh B; MELD 10; bilirubin 0.7, INR 1.4, PTT 17.1, and Albumin 2.1; DV 2 wk post-PS: 147 (TIPS), 138 (PS), and 16 cm/s (MPV). Rifaximin 550 mg × 2/d, Lactulose 20 g × 3/d	Child-Pugh C; MELD 13; bilirubin 0.7, INR 1.4, PTT 17.1, and Albumin 2.5; DV: 90.9 (TIPS) 37 cm/s (MPV). Rifaximin 550 mg × 2/d, Lactulose (20 g) 30 mL × 3/d, Piperacillin 4.5 g/6 h
6 mo post-PS	Child-Pugh B; MELD 21; bilirubin 4.5, INR 1.3, PTT 16.5, and albumin 3.5; DV: 78 (TIPS), 144 (PS), 39.3 cm/s (MPV). Rifaximin 550 mg × 2/d Lactulose 10 g/d	Child-Pugh B; MELD 21; bilirubin 2.5, INR 1.5, PTT 18.5, and albumin 2.8; DV: 85.8 (TIPS), 109 (PS), and 83 cm/s (MPV). Rifaximin 550 mg × 2/d, Lactulose 20 g × 3/d	Child-Pugh C; MELD 14; bilirubin 2.1, INR 1.3, PTT 16.7, and albumin 2.0; DV: 72 (TIPS), 92 (PS), and 68 (MPV) cm/s. Rifaximin 550 mg × 2/d, Lactulose (20 g) 30 mL × 3/d

Doppler velocities of the transjugular intrahepatic portosystemic shunts, parallel stent, and main portal vein were also measured. Laboratory values include total bilirubin, international normalized ratio, partial thromboplastin time, and albumin. TIPS: Transjugular intrahepatic portosystemic shunts; PS: Parallel stent; MELD: Model for end-stage liver disease; DV: Doppler velocities; MPV: Main portal vein; INR: International normalized ratio; PTT: Partial thromboplastin time.

and proceeded immediately to TIPS revision procedure. The first Viatorr® TIPS stent was inserted directly from the middle hepatic vein. Contrast venography demonstrated patency of the primary TIPS stent. The PSG was 13 mmHg despite 12-mm balloon angioplasty.

Diagnosis

Primary TIPS insufficiency with recurrent variceal bleeding.

Treatment

Parallel TIPS placement: A 10 mm × 10 cm PS Viatorr® TIPS was inserted from right hepatic vein to the confluence of right portal and left portal veins and coronary vein varices were coil embolized (Figure 2A-C). PS reduced PSG from 13 mmHg to 7 mmHg and direct portal pressure from 34 mmHg to 21 mmHg.

Follow-up

Doppler US revealed patency in both stents during 2-wk and 6-mo follow-ups (Table 1). In December 2017, patient presented to ED with worsening bilateral lower extremity edema. Whereas Doppler showed an average velocity of 121 cm/s for the new shunt and 95 cm/s for portal vein, there was markedly decrease flow in the old shunt with a peak velocity of 10 cm/s. IR was consulted to evaluate suspected shunt dysfunction. However, the measured portosystemic gradient of 7 mmHg was deemed adequate, and contrast flow through both stents was excellent. Considering the increased model for end-stage liver disease (MELD) score (Table 1), the patient was evaluated for liver transplant in February 2018 and at that time, his follow-up demonstrated patency of both shunts with the following velocities of 55 cm/s, 60 cm/s, and 66 cm/s for the old shunt, new shunt, and portal vein respectively.

Case 3

Chief complaints: Hematemesis.

History of present illness: A 54-year-old female with esophageal varices secondary to NASH cirrhosis presented with hematemesis. Initial EGD showed type 2 gastroesophageal varices (GOV2) and endoscopic banding was performed. However, the patient continued having hematemesis and was referred to IR. A 10 mm × 8 cm Viatorr® TIPS was inserted from right hepatic vein to the right portal vein. The procedure decreased PSG from 20 mmHg to 12 mmHg. Doppler US confirmed TIPS patency (Table 1). Five days following the procedure, patient began to experience significant hematemesis.

History of past illness: Type 2 diabetes mellitus, hypertension, gastroesophageal reflux disease, chronic obstructive pulmonary disease, umbilical hernia,

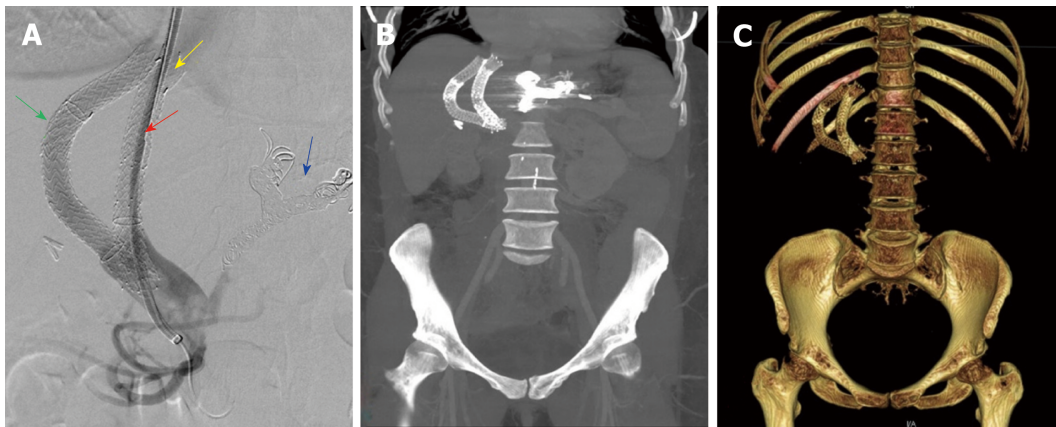


Figure 1 Imaging examinations of patient 1. A: Digital subtracted portal angiography showing successful placement of the parallel stent (red arrow) with caval extension (yellow arrow). Embolization coils can be seen the coronary vein branches (blue arrow). Primary stent (green arrow) is seen alongside the second transjugular intrahepatic portosystemic shunt stent. B: Maximum intensity projection with 30-mm slab of post procedural computed tomography (CT) abdomen showing primary and parallel stents in tandem. C: 3D reconstruction of a post procedural CT abdomen showing primary and parallel stents in tandem.

hysterectomy.

Personal and family history: No tobacco, alcohol, or substance use. Family history was non-contributory.

Physical examination upon admission: Sclera icteric; abdomen was soft and non-distended; tachypnea, clear bilateral lung sounds, intact neurological exams with appropriate mood and affect.

Laboratory examinations: Hemoglobin 10.95 g/dL, white blood cells 21900/mm³, platelets 84000/mL; abnormal Liver function tests (Table 1).

Imaging examinations: EGD showed recurrent bleeding from GOV2 with no evidence of isolated gastric varices. Doppler US revealed TIPS patency (Table 1).

Multidisciplinary expert consult

Sara Pasha, MD, Assistant Professor of Medicine, Department of Internal Medicine-Pulmonary Division; Alla Grigorian, MD, PhD, Assistant Professor of Medicine, Medical Director of Liver Transplant; Terrence Barrett, MD, Professor of Medicine and Microbiology/Immunology, Chief, Division of Digestive Diseases and Nutrition.

Diagnosis

Primary TIPS insufficiency with recurrent variceal bleeding.

Treatment

Parallel TIPS placement: Due to recurrent variceal bleed and decompensated cirrhosis, a second 10 mm × 8 cm Viatorr® TIPS was inserted from right hepatic vein to the left portal vein (Figure 3A-C). Procedure was a technical success, decreasing PSG from 9 to 4 mmHg. Direct portal pressure decreased from 40 mmHg to 35 mmHg. Following the procedure, patient developed hyponatremia which was managed with intravenous fluids. Patient was discharged home seven days after.

Follow-up

Six-month US follow-up was unremarkable. Doppler demonstrated patency of both shunts with adequate flow (Table 1).

DISCUSSION

TIPS failure can either be due to stent occlusion or stenosis, which has become significantly less frequent with the widespread use of covered stents^[14,18,19]. This complication can be diagnosed with Doppler imaging and confirmed during TIPS revision procedure^[20-24]. However, in the presence of a well-functioning primary stent, the recurrence or persistence of ascites and variceal bleeding can be a therapeutic challenge. We present a short case series to illustrate three patients with portal hypertension who underwent a second TIPS stent placement using Viatorr® stents. This is commonly known as parallel TIPS (PS) or double barrel TIPS. As early as

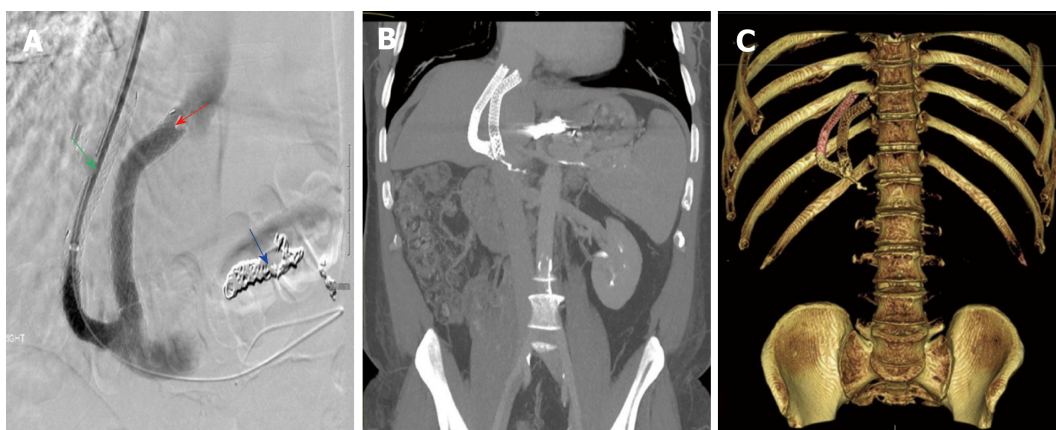


Figure 2 Treatment for patient 2. A: Digital subtracted portal angiography showing successful placement of the parallel stent (red arrow). Embolization coils can be seen the coronary vein branches (blue arrow). Primary stent (green arrow) is seen alongside the second transjugular intrahepatic portosystemic shunts stent. B: Maximum intensity projection with 30-mm slab of a post procedural computed tomography (CT) abdomen showing primary and parallel stents in tandem. C: 3D reconstruction of post procedural CT abdomen showing primary and parallel stents in tandem.

1990s, the effectiveness of PS in treating shunt insufficiency had been demonstrated along with other options such as re-stenting and balloon angioplasty^[25]. In our practice, PS is pursued in symptomatic patients in whom the primary TIPS shunt insufficiency or is thrombosed and recanalization techniques have failed.

In our case series, the primary TIPS were insufficient in alleviating portal hypertension symptoms despite their patency being verified during follow-ups and/or before PS placement. Furthermore, the PSGs prior to PS were below 10 mmHg in cases 1 and 3. No laboratory values were predictive of shunt insufficiency or the need for a PS. A study by Haskal *et al*^[13] reported a cohort of 10 out of 93 patients that underwent PS placement. This study alluded to insufficiency of the primary TIPS stent in decreasing the PSG as a predictor for the need of PS placement; the mean post procedural PSG in after the first TIPS was 10.2 ± 3.7 mmHg. In patients who received a PS, the mean post procedural gradient was 19.1 ± 3.8 mmHg after placement of the first TIPS and 12.5 ± 3.5 mmHg after placing a PS^[13]. As the previous gradients indicate, the initial TIPS were suboptimal to begin with even at maximum diameter of 10 mm expansion.

A similar trend was observed in another retrospective study, in which 40 out of 338 TIPS patients underwent parallel Wallstent® placement^[26]. In this study by Helmy *et al*^[26], PS patients exhibited lower portal pressure gradient drop compared to single TIPS only patients with means of (10.4 ± 5.4 mmHg *vs* 12.4 ± 7.1 mmHg) after the first TIPS procedure. Whether the patients from these two studies would have benefited from primary shunts with larger diameters to begin with and therefore avoid subsequent PS should be further investigated. In our report, single TIPS were not sufficient for symptomatic relief despite achieving maximum stent expansion, and in cases 1 and 3 despite optimal PSGs. This is similar to Helmy *et al*^[26] patient cohort requiring PS placement despite achieving a mean portal pressure gradient of 7.9 ± 4.8 mmHg after the initial shunt placement. However, as previously mentioned, his study used bare metal stent with its inherent lower patency rate overtime.

Case 2 was secondary to an occluded primary TIPS, though it provided an impressive symptom-free period of 8 years. The successful recanalization of this occluded shunt failed to provide symptomatic relief requiring us to place a PS to further decrease the PSG from 13 mmHg to 7 mmHg. Also, the patient's MELD score increased from 10 to 21 over the following 6 mo (Table 1); on one hand, patients with AATD tend to have rapidly progressive cirrhosis with extensive inflammation and fibrosis far beyond other causes for cirrhosis^[27,28]. On the other hand, excessive shunting of portal venous blood away from liver parenchyma after PS placement may have contributed to worsening liver function. This way addressed in Helmy *et al*^[18] study and they advocated for the use of smaller PS to decrease the risk of hepatic encephalopathy and mortality after PS placement.

In addition to the studies mentioned above, literature on PS is rather limited. More recently, one group reported 18 out of 132 TIPS patients underwent PS placement after primary TIPS stent dysfunction, indicating that shunt patency of 1-year post-PS was higher in the patients who received Fluency® endoprosthesis than those who received Wallgraft® (87.5% *vs* 70.0% , $P = 0.358$)^[29]. Another study described the application of PS in 10 cases of cirrhotic patients due to hepatitis B infection^[30]. Although covered stents were placed for both primary TIPS stent and PS,

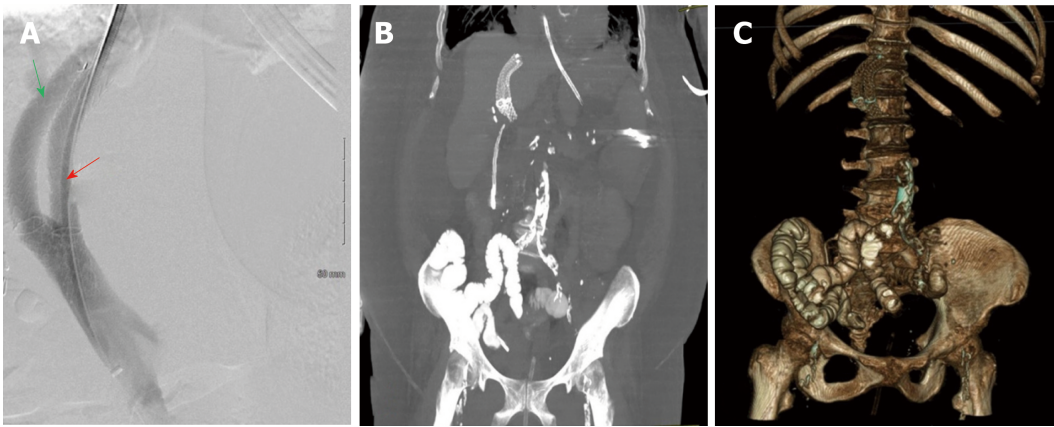


Figure 3 Treatment for patient 3. A: Digital subtracted portal angiography showing successful placement of the parallel stent (red arrow). Primary stent (green arrow) is perfectly parallel along the second transjugular intrahepatic portosystemic shunts stent. B: Maximum intensity projection with 30-mm slab of a post procedural computed tomography (CT) abdomen showing primary and parallel stents in tandem. C: 3D reconstruction of post procedural CT abdomen showing primary and parallel stents in tandem.

unfortunately, the authors did not specify the exact type and brand of stents used. Like our cases, most primary TIPS dysfunctions were manifested by symptoms such as ascites and recurrent variceal bleeding rather than surveillance US findings. In addition to the PSG decrease after PS placement in both studies (25.5 ± 7.3 mmHg to 10.9 ± 2.3 mmHg, and 35.60 ± 2.72 mmHg to 15.30 ± 3.27 mmHg, respectively), the most common post procedural symptom was hepatic encephalopathy, requiring only medical management with Lactulose and Rifaximin, which were also prescribed for all our patients (Table 1). With average follow-ups of 16.7 ± 10.8 mo and 14.0 ± 1.13 mo, these two studies reported adverse outcomes including worsening liver function, recurrent variceal bleed and death. In comparison, our case series of PS achieved patency at 6-mo follow-up. However, only patient 1 and 3 from our report remained symptom free post-PS for those 6 mo, while patient 2 developed progressive worsening of hepatic function, likely a combination of ATTD progression of the disease and excessive portal blood shunting away from liver parenchyma. In terms of portal hypertensive symptoms, PS successfully prevented recurrent variceal bleeding and ascites in all 3 cases for at least 6-mo follow-up.

A pre-procedural review of abdominal cross-sectional imaging and of the initial TIPS procedure can assist in optimal planning of PS placement. For the patient discussed in case 2, the initial TIPS was placed from the middle hepatic vein to the right main portal vein. This left us ample space for placement of the second TIPS from the right hepatic vein to the left portal vein. As for cases 1 and 3, the first TIPS was placed from the right hepatic vein to the right portal vein. In each case, the origin of the right hepatic vein was peripheral enough into the liver to allow for a new access of the right hepatic vein for a subsequent PS placement into the left portal vein. Overall, placement of a second TIPS stent is not technically challenging after careful review of the hepatic venous anatomy and of the target portal venous branch. The left portal vein can be accessed from the hepatic vein aided by a more acute angle of the TIPS needle to allow a more anterior reach and can be easily targeted from either the right or the middle hepatic veins. We typically avoid placing a PS within the same portal vein of the primary stent to avoid a crowded space that would limit stent expansion. Shunt reduction maybe performed if needed to address medically uncontrollable hepatic encephalopathy, however, this was not observed in our series.

Despite the widespread use of Viatorr® stents in TIPS placement for over 20 years, there are only two reports of use of primary and PS Viatorr® stents placement with limited follow-up data. The first of which is a single case report describing the use of Viatorr® stent for both primary TIPS and PS in an alcoholic cirrhotic patient that initially presented with recurrent variceal bleeding after being lost to follow-up for 3 years^[31]. Similar to cases 1 and 3, his PSG was as low as 10 mmHg prior to PS placement and was then reduced to 5 mmHg post-PS. Unfortunately; the authors did not provide clinical or imaging follow-up data. Another case report of two cases described the successful placement of primary and parallel Viatorr® stents in recurrent hydrothorax and ascites respectively. In both instances the PSG at the time was suboptimal at ≥ 12 mmHg prior to PS placement, but with no radiological evidence of primary stent dysfunction. The only follow-up data provided was clinical resolution of hydrothorax and ascites at two and three months respectively. No follow-up Doppler US data was provided on these shunts^[23].

In our case series, all patients underwent Viatorr® PS placement for recurrent upper gastrointestinal variceal bleeding despite adequate PSG in two out of three cases. Also, we were fortunate to have follow-up data showing technical and clinical success of up to 6 mo including Doppler US velocities of primary and PS shunts.

To our knowledge, we are the first to report a three-case series with 6-mo follow-up data, using Viatorr® for both primary TIPS and PS placement for the management of recurrent upper gastrointestinal variceal hemorrhage. Patients after TIPS placement should be monitored routinely using Doppler US of the stents to avoid TIPS stent occlusion. Previous studies recommend US follow-up every 3-12 mo after TIPS procedure^[18]. Within our institution, shunt patency evaluations are performed at 3 mo, 6 mo, 1 year and yearly thereafter. A mean shunt velocity < 90 cm/s or > 200 cm/s, or a value lower than 30 cm/s in the main portal vein should raise the suspicion of shunt dysfunction^[18]. TIPS catheter venography and PSG are performed in patients with suspicious US findings and/or in those with persistent portal hypertensive symptoms despite US evidence of a well-functioning primary TIPS. We pursue a PS in patients with either a non-salvageable primary TIPS or with symptom persistence despite a well-functioning primary TIPS.

CONCLUSION

In conclusion, the addition of a second Viatorr® TIPS stent to a preexisting primary TIPS stent in patients with recalcitrant upper gastrointestinal variceal hemorrhage after a single Viatorr® stent placement proves insufficient in the control of variceal bleeding despite maintaining adequate patency, is feasible and has promising short term follow-up data. These findings are supported by Haskal *et al*^[13] retrospective data on the role of parallel TIPS stents for primary TIPS insufficiency. And while Haskal's study used bare metal stents, Bureau *et al*^[19] randomized controlled trial from 2004 supported the superiority and safety of several covered stents over bare metal stents when placed as a primary TIPS. Hence, considering the added design specific advantages of Viatorr® stents when placed as the primary TIPS, their therapeutic value in treating recurrent variceal bleeding after primary shunt insufficiency should be investigated further with a prospective data that addresses this unique cohort of patients in need of parallel TIPS stents for adequate control of their portal hypertension.

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Necrolytic acral erythema in a human immunodeficiency virus/hepatitis C virus coinfecting patient: A case report

Katerina G Oikonomou, Dost Sarpel, Alexandra Abrams-Downey, Adnan Mubasher, Douglas T Dieterich

ORCID number: Katerina G Oikonomou (0000-0002-2142-1387); Dost Sarpel (0000-0002-1826-8684); Alexandra Abrams-Downey (0000-0001-8181-7327); Adnan Mubasher (0000-0003-0934-7396); Douglas T Dieterich (0000-0001-7786-8594).

Author contributions: Oikonomou KG manuscript preparation, clinical data collection and literature search and review; Sarpel D clinical data and literature review, critical review of the manuscript, clinical images preparation; Abrams-Downey A clinical data and literature review, critical review of the manuscript, clinical images preparation; Mubasher A pathology slides review and histologic description of the biopsy samples, pathology images/photomicroscopy preparation; Dieterich DT critical review of manuscript, clinical data and literature review.

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Katerina G Oikonomou, Dost Sarpel, Alexandra Abrams-Downey, Division of Infectious Diseases, Icahn School of Medicine at Mount Sinai, Mount Sinai St Luke's-West, New York, NY 10025, United States

Adnan Mubasher, Department of Pathology, Icahn School of Medicine at Mount Sinai, Mount Sinai St Luke's-West, New York, NY 10025, United States

Douglas T Dieterich, Division of Liver Diseases, Icahn School of Medicine at Mount Sinai, New York, NY 10029, United States

Corresponding author: Katerina G Oikonomou, MD, PhD, Academic Fellow, Division of Infectious Diseases, Icahn School of Medicine at Mount Sinai, Mount Sinai St Luke's-West, 1111 Amsterdam Avenue, S and R 13, New York, NY 10025, United States.

katerina.oikonomou@mountsinai.org

Telephone: +1-212-5232525

Fax: +1-212-5233931

Abstract

BACKGROUND

Necrolytic acral erythema (NAE) is a rare dermatological disorder, which is associated with hepatitis C virus (HCV) infection or zinc deficiency. It is characterized by erythematous or violaceous lesions occurring primarily in the lower extremities. The treatment includes systemic steroids and oral zinc supplementation. We report a case of NAE in a 66-year-old human immunodeficiency virus (HIV)/HCV co-infected woman with NAE. NAE is rarely reported in co-infected patients and the exact mechanisms of pathogenesis are still unclear.

CASE SUMMARY

A 66-year-old HIV/HCV co-infected female patient presented with painless, non-pruritic rash of extremities for one week and underwent extensive work-up for possible rheumatologic disorders including vasculitis and cryoglobulinemia. Punch skin biopsies of right and left thigh revealed thickened parakeratotic stratum corneum most consistent with NAE. Patient was started on prednisone and zinc supplementation with resolution of the lesions and improvement of rash.

CONCLUSION

Clinicians should maintain high clinical suspicion for early recognition of NAE in patients with rash and HCV.

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Core tip: Necrolytic acral erythema (NAE) is a rare dermatological entity associated with hepatitis C virus (HCV) and zinc deficiency. Aim of the case report is to describe the occurrence of NAE in a human immunodeficiency virus/HCV coinfecting patient, elucidate the clinical characteristics, pathophysiologic mechanisms and increase clinician awareness about diagnosis and management.

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INTRODUCTION

Necrolytic acral erythema (NAE) is a rare dermatological entity. While the disease is frequently associated with hepatitis C virus (HCV) infection or zinc deficiency^[1-4], the pathogenesis is poorly understood. NAE is characterized by erythematous lesions, violaceous papules, bullae and superficial skin erosions occurring primarily in the lower extremities and dorsal feet. Associated symptoms include pruritus, pain, burning and dysesthesia. NAE is an infrequent extrahepatic manifestation of hepatitis C with a much less frequent overall prevalence of 1.7% compared to cryoglobulinemia, porphyria cutanea tarda, lichen planus^[5,6]. Additionally, NAE has been reported in patients with zinc deficiency and less frequently in association with vaccination against hepatitis B^[2,7]. NAE should be differentiated from psoriasis and eczematous dermatitis, lichen simplex chronicus, hypertrophic lichen planus, acrokeratoelastoidosis, and acrokeratosis paraneoplastica. Zinc supplementation and treatment of underlying hepatitis C have been related to favorable response. We describe a case of a patient with human immunodeficiency virus (HIV) and hepatitis C co-infections diagnosed with NAE.

CASE PRESENTATION

Chief complaints and history of past illness

A 66-year-old woman, with past medical history of well-controlled HIV infection on antiretroviral (ARV) therapy with azatanavir/ritonavir and abacavir/lamivudine and untreated chronic hepatitis C (Genotype 1b) with cirrhosis, who presented with chief complaint of painless, non-pruritic rash for one week. The rash began as diffuse, patchy erythematous lesions of bilateral lower extremities, starting at her feet but progressing up her legs to her thighs. She noted associated edema, but denied fevers, chills, joint pain, oral lesions or ulcers and weakness or numbness in her extremities. She was not sexually active and denied any allergies. Patient was recently discharged from the hospital after a COPD exacerbation. She was discharged on a brief oral prednisone taper, which she completed prior to presentation, but was still taking when the rash developed. On admission patient was afebrile and hemodynamically stable.

Physical examination upon admission

Her physical exam revealed dusky erythematous patches of non-blanching palpable petechiae and purpura on bilateral calves and thighs as well as on her right forearm. She also had vesiculobullous lesions on bilateral lower extremities with several scattered erosions, without lesions on palms or soles and no oral or genital lesions (Figure 1). Nikolsky sign was negative. Patient underwent extensive work-up for possible rheumatologic disorders including vasculitis and cryoglobulinemia.

Laboratory examinations



Figure 1 Typical appearance of necrolytic acral erythema involving the right upper and right and left lower extremities.

Laboratory findings are shown in [Table 1](#). Dermatology consulted during her hospital stay and performed punch skin biopsies of right and left thigh. Pathology reported thickened parakeratotic stratum corneum most consistent with NAE ([Figure 2](#)).

FINAL DIAGNOSIS

NAE in an HIV/HCV co-infected patient.

TREATMENT

Patient was started on prednisone 20 mg daily along with zinc supplementation given her low serum zinc levels. She had resolution of her vesiculobullous lesions and improvement of erythema. Unfortunately, no clinical images were obtained after her clinical improvement.

OUTCOME AND FOLLOW-UP

Patient was discharged to follow-up with her infectious diseases provider for initiation of hepatitis C treatment. She was initiated on sofosbuvir/veltapasvir and her ARV was transitioned to bictegravir/emtricitabine/tenofovir alafenamide to avoid any drug drug interactions.

DISCUSSION

Necrolytic erythemas include NAE, necrolytic migratory erythema, acrodermatitis enteropathica, and various dermopathies due to nutrient deficiencies^[6]. NAE was first

Table 1 Basic laboratory findings

Parameters	Reference range
White blood cell count - 15.3	4.5-11 K/uL
Hematocrit - 44.5	34%-47%
Hemoglobin - 13.6	11.7-15 g/dL
Platelet count - 376000	150-450 K/uL
Blood urea nitrogen - 42	7-20 mg/dL
Creatinine - 1.28	0.5-1.1 mg/dL
AST - 17	< 36 U/L
ALT - 23	< 46 U/L
Total bilirubin - 2.0	0.1-1.2 mg/dL
Direct bilirubin - 1.0	< 0.9 mg/dL
gGT - 145	0-60 IU/L
Total protein - 6.4	6-8.3 g/dL
Albumin - 2.6	3.5-5.0 g/dL
Erythrocyte Sedimentation rate - 68	(0-24 mm/h)
C-reactive protein - 78.32	< 5.1 mg/L
INR - 1.0	0.9-1.1
C3 - 156	90-180 mg/dL
cANCA - negative	Negative
pANCA - negative	Negative
Rheumatoid factor - < 15	0-15 IU/mL
Anti-SSA - negative	Negative
Anti-SSB - negative	Negative
Anti-CCP - negative	Negative
RPR - non-reactive	Non-reactive
Cryoglobulins - negative	Negative
Antinuclear antibodies - negative	Negative
AntidsDNA - negative	Negative
HIV RNA - undetectable	< 20 copies/mL
CD4 - 564/25%	Cells/mL
HCV-RNA - 346755 genotype 1B	< 15 IU/mL

HCV: Hepatitis C virus; HIV: Human immunodeficiency virus; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; INR: International normalized ratio; cANCA: Cytoplasmic anti-neutrophil cytoplasmic antibody; pANCA: Perinuclear anti-neutrophil cytoplasmic antibody; CCP: Cyclic citrullinated peptides; RPR: Rapid plasma regain.

described by El-Ghandour *et al*^[4] in a cohort of Egyptian patients. NAE is observed most commonly in women and with age of onset around 40 years^[2]. In early stages, skin changes consist of erythematous papules and plaques with early skin erosion. During the second stage, there is increasing thickness of the papules and lichenification followed by hyperpigmentation often associated with necrosis of superficial epidermis. In the late stage hyperpigmentation becomes more prominent. The most common location of lesions is the back of the feet and toes, and also in lower extremities along the surface of the Achilles tendon, the malleoli, legs and knees. Histological characteristics include acanthosis, spongiosis in early stages of the disease process along with psoriasiform hyperplasia in the later stages. In advanced disease, parakeratosis and possible necrosis of keratinocytes can be seen. These histopathological findings are non-specific and high clinical suspicion is required for early diagnosis^[9].

The pathogenesis of NAE is unknown and several mechanisms have been proposed. Potential etiologies include the metabolic changes associated with liver dysfunction and diabetes^[10-12]. Hypoalbuminemia, hypoaminoacidemia and hyperglucagonemia are all associated with inducing inflammatory responses^[13].

Other proposed mechanisms include mineral deficiencies, primarily zinc. Moneib *et al*^[10] reported that serum levels of zinc are low in patients with NAE. Zinc deficiency causes a reduction in serum transport proteins, such as retinol-binding protein and prealbumin, which impair delivery of the vitamin A, major factor for epidermal

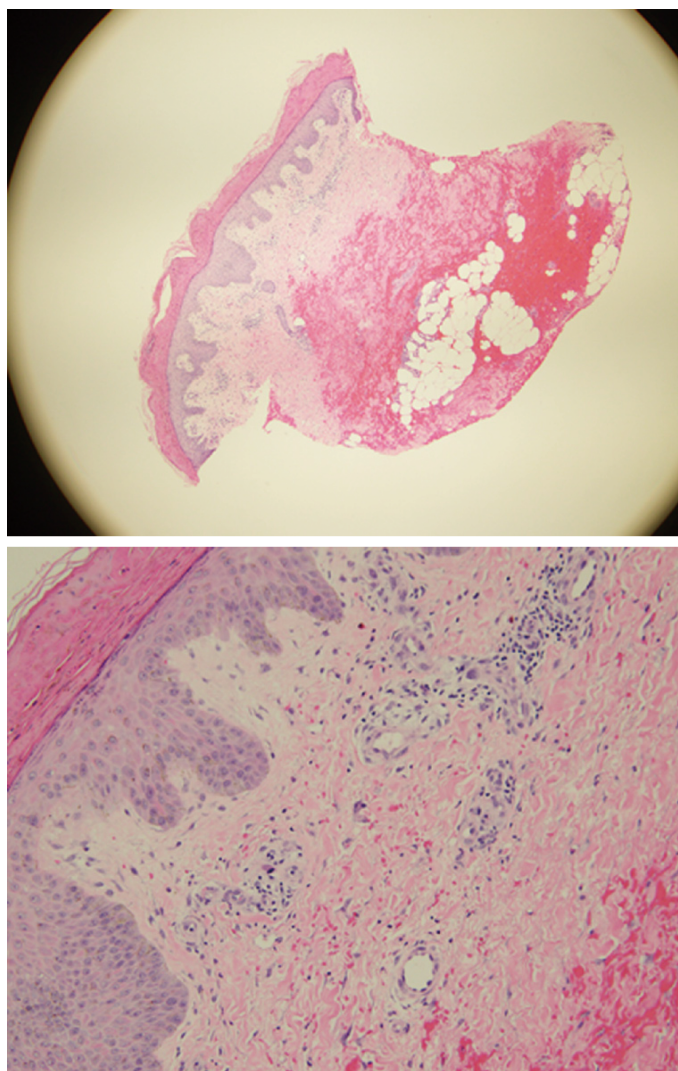


Figure 2 Low and high power of skin histopathology demonstrating bullous/hemorrhagic cellulitis with thick parakeratosis, impetiginization of the dermis and rare scattered neutrophilic infiltrates with dermal hemorrhage.

proliferation and differentiation^[9]. The significance of zinc deficiencies in NAE is further limited by the role of blood measurements for the detection of zinc. Determination levels of zinc levels in future patients with NAE is indicated^[11,12].

Additional association has been reported in the setting of hepatitis C. Hepatitis C viral load and genotype may be related to the etiopathogenesis of NAE^[13,14]. Although no clear correlation with genotype has been reported in the literature, most article reports describe patients with genotypes 1 and 4^[5]. Moreover, in setting of HCV, it seems that there is correlation between severity of lesions and liver damage. While the exact role of zinc deficiency in NAE is unknown and controversial^[12].

The treatment of NAE is challenging due to the lack of available data. There are no prospective randomized control trials regarding optimal treatment and most available information is provided from retrospective case series. Regarding treatment of NAE lesions after zinc supplementation, the current literature data are inconsistent. Oral zinc supplementation showed a variable response rate. Zinc with topical tacrolimus, vitamin B1, and vitamin B6 subcutaneous interferon alpha was also reported with variable rates of response and inconsistent benefits^[8].

Similarly, there are controversial data about topical or systemic corticosteroids, and zinc supplementation ranging from no response to complete resolution^[1]. A trial of brief systemic steroids and oral zinc supplementation and close monitoring for clinical resolution is usually warranted in patients with clinical manifestations of NAE regardless of the serum zinc levels.

In HCV-associated NAE complete or partial resolution has been demonstrated previously with interferon alpha-2b and/or ribavirin, and also with combinations of interferon α -2b and zinc^[8]. Interferon free direct acting antiviral regimens have also

been shown to be effective and should be offered to chronic HCV patients with the goal of sustained viral response.

It is interesting to note that zinc dysregulation and metabolic alterations can also occur as a result of hepatitis C and HIV infections^[2], but to our knowledge case reports of NAE in HCV/HIV co-infected patients are rare. Najarian *et al*^[12] reported a case of NAE in a woman with well controlled HIV and untreated HCV. Patient presented with well-demarcated, painful, pruritic plaques with a distinctive erythematous rim and a distinctive sandal-like pattern of bilateral lower extremities. She was found to have low zinc levels and was treated successfully with oral zinc supplementation. One of the proposed mechanisms for the pathogenesis of NAE in co-infected patients is the increased zinc loss with urine that can be observed with both HCV and HIV. In our patient, urine zinc levels were not routinely checked, and zinc levels after treatment with zinc supplementation were not available.

It is well known that patients with HIV/HCV co-infection have accelerated fibrosis progression due to multiple mechanisms and perhaps this may play a role in NAE. In addition these patients have higher levels of pro-inflammatory cytokines such as TGF-beta and IFN gamma along with higher levels of lipopolysaccharides which all can enhance inflammatory response. Perhaps this also plays a role in NAE in HIV/HCV co-infected patients.

In terms of appearance of lesions and distribution, there is no significant difference in HCV/HIV co-infected versus mono-infected patients with HCV, or in seronegative patients with isolated zinc deficiency. Skin biopsy can be a powerful tool, but given characteristics of skin lesions, NAE can also be a clinical diagnosis. Table 2 describes the reported cases of NAE, the serologic profiles of patients, and the methods of diagnosis and treatment.

The early diagnosis of NAE is crucial, regardless of the underlying disorder, and early and effective treatment can improve patients quality of life and limit secondary infections through skin lesions. The interpretation of skin histopathology should be performed by experienced pathologists, in order to avoid misinterpretation of the results. Kapoor *et al*^[2] reported a case of NAE in a 44-year-old man with history of HCV, who had findings consistent with eczema or psoriasis on skin biopsy. Patient received multiple courses of treatment with immunosuppressants without significant improvement, was hospitalized multiple times with episodes of cellulitis and suicidal ideation, and after nine years he was treated successfully with oral zinc supplementation with improvement of lesions, pain and functional status^[4].

CONCLUSION

NAE as a rare skin disorder often represents clinical manifestation of underlying and frequently undiagnosed hepatitis C. The etiology is likely multifactorial, as demonstrated in our patient who had both untreated hepatitis C cirrhosis as well as documented zinc deficiency. This case highlights the importance of clinical recognition of NAE and early skin biopsy to confirm the diagnosis. Additionally, this case provides further cause for the expedient treatment of HCV, particularly in HIV/HCV co-infected patients. High clinical suspicion, physician awareness and early diagnosis play a pivotal role in appropriate management and optimal clinical outcomes.

Table 2 Literature review – cases of necrolytic acral erythema

	HCV	HIV	Zinc deficiency	Diagnosis	Treatment
Srisuwanwattana <i>et al</i> ^[1]	Yes	NA	No	Skin biopsy	Zinc supplementation and topical steroids
Kapoor <i>et al</i> ^[2]	Yes	NA	Yes	Clinical diagnosis	Oral zinc supplementation
Jakubovic <i>et al</i> ^[3]	No	NA	Yes	Skin biopsy	Nutritional Supplementation
Abdallah <i>et al</i> ^[5]	Yes	NA	No	Skin biopsy	Zinc supplementation
Pernet <i>et al</i> ^[7]	No	NA	No	Skin biopsy	Resolved spontaneously
Tabibian <i>et al</i> ^[8] Case 1	Yes	NA	Yes	Skin biopsy	Zinc supplementation
Tabibian <i>et al</i> ^[8] Case 2	Yes	NA	Yes	Skin biopsy	Zinc supplementation
Das <i>et al</i> ^[9]	No	NA	NA	Skin biopsy	Zinc supplementation
Najarian <i>et al</i> ^[12]	Yes	Yes	Yes	Skin biopsy	Zinc supplementation
Shumez <i>et al</i> ^[15]	Yes	NA	Yes	Skin biopsy	Zinc supplementation
Shaikh <i>et al</i> ^[16]	Yes	NA	No	Skin biopsy	Ledipasvir/sofosbuvir
Wu <i>et al</i> ^[17]	No	NA	NA	Skin biopsy	Systemic steroids
Rahman <i>et al</i> ^[18]	Yes	NA	NA	Skin biopsy	Systemic steroids and zinc supplementation
Botelho <i>et al</i> ^[19]	Yes	NA	Yes	Skin biopsy	Zinc supplementation
Panta <i>et al</i> ^[20]	No	NA	Low normal levels	Skin biopsy	Oral zinc supplementation and topical steroids
Pandit <i>et al</i> ^[21] Case 1	No	NA	Yes	Skin biopsy	Oral zinc supplementation
Pandit <i>et al</i> ^[21] Case 2	No	NA	Yes	Clinical diagnosis	Oral zinc supplementation

NA: Not available; HCV: Hepatitis C virus; HIV: Human immunodeficiency virus.

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Acute portal vein thrombosis after liver transplant presenting with subtle ultrasound abnormalities: A case report and literature review

Thomas Couri, Carla Harmath, Talia Baker, Anjana Pillai

ORCID number: Thomas Couri (0000-0002-1610-8978); Talia Baker (0000-0002-0561-8497); Anjana Pillai (0000-0001-6783-2109).

Author contributions: Couri T and Pillai A designed the report; Couri T and Harmath C collected the patient's data; Harmath C, Baker T, and Pillai A provided critical revision of the report; Couri T and Pillai A wrote the report.

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Thomas Couri, Department of Internal Medicine, University of Chicago, Chicago, IL 60637, United States

Carla Harmath, Department of Radiology, University of Chicago, Chicago, IL 60637, United States

Talia Baker, Department of Surgery, Section of Transplant Surgery, University of Chicago, Chicago, IL 60637, United States

Anjana Pillai, Department of Internal Medicine, Section of Gastroenterology, Hepatology, and Nutrition, University of Chicago, Chicago, IL 60637, United States

Corresponding author: Thomas Couri, MD, Department of Internal Medicine, University of Chicago, 5841 South Maryland Avenue, Chicago, IL 60637, United States.

thomas.couri@uchospitals.edu

Telephone: +1-773-7021000

Fax: +1-773-7022230

Abstract

BACKGROUND

Portal vein thrombosis (PVT) after liver transplantation (LT) is an uncommon complication with potential for significant morbidity and mortality that transplant providers should be cognizant of. Recognizing subtle changes in post-operative ultrasounds that could herald but do not definitively diagnose PVT is paramount.

CASE SUMMARY

A 30-year-old female with a history of alcohol-related cirrhosis presented with painless jaundice and received a deceased donor orthotopic liver transplant. On the first two days post-operatively, her liver Doppler ultrasounds showed a patent portal vein, increased hepatic arterial diastolic flows, and reduced hepatic arterial resistive indices. She was asymptomatic with improving labs. On post-operative day three, her resistive indices declined further, and computed tomography of the abdomen revealed a large extra-hepatic PVT. The patient then underwent emergent percutaneous venography with tissue plasminogen activator administration, angioplasty, and stent placement. Aspirin was started to prevent stent thrombosis. Follow-up ultrasounds showed a patent portal vein and improved hepatic arterial resistive indices. Her graft function improved to normal by discharge. Although decreased hepatic artery resistive indices and increased diastolic flows on ultrasound are often associated with hepatic arterial stenosis post-LT, PVT can also cause these findings.

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CONCLUSION

Reduced hepatic arterial resistive indices on ultrasound can signify PVT post-LT, and thrombolysis, angioplasty, and stent placement are efficacious treatments.

Key words: Portal vein thrombosis; Portal vein stent; Liver transplant; Case report

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Core tip: Acute portal vein thrombosis (PVT) after liver transplant is uncommon but can cause significant morbidity and mortality. PVT can present with subtle ultrasound abnormalities in the hepatic artery, such as decreased resistive indices and increased diastolic flows, in the absence of frank thrombosis in the portal vein. Long term portal vein patency has been seen with percutaneous thrombolysis, angioplasty, and stent placement as treatment.

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INTRODUCTION

Acute portal vein thrombosis (PVT) after liver transplantation (LT) is an uncommon yet consequential complication with potential for significant morbidity and mortality. The incidence of PVT after LT varies, however most case series report occurrences in 1%-3% of patients^[1-4]. Similarly, presentations of this condition vary, with case reports documenting abnormalities in liver function tests to signs of portal hypertension as initial signs of PVT^[1,3,4]. While the diagnosis can often be readily made with ultrasound, recognizing subtle changes in routine post-operative ultrasounds that herald but do not definitively diagnose portal venous abnormalities is paramount. Furthermore, no rigorous studies have determined the most optimal treatment for PVT post-LT. We report a case of acute PVT post-LT in a patient who developed subtle vascular changes on serial ultrasounds. The patient required percutaneous thrombolysis, angioplasty, and ultimately stenting of the portal vein, a rarely reported treatment for acute PVT after LT.

CASE PRESENTATION

A 30-year-old woman presented to an outside hospital with painless jaundice. She had no other complaints. Her past medical history was notable for alcohol use disorder. She had no prior surgeries, was not on any medications, had no allergies, and denied cigarette or illegal drug use. She had no family history of liver disease. She was then transferred to our institution for consideration for LT.

At our institution, her vital signs were notable for fever, tachycardia, and hypotension. On physical exam, she appeared diffusely jaundiced and confused. She was oriented to person but not to time or place, and she was noted to have asterixis. Her abdomen was non-tender but distended, and a fluid wave was present. She had scleral icterus, numerous spider angiomas on her chest, and 3+ bilateral lower extremity edema. Her initial laboratories are found in [Table 1](#). She had a MELD-Na score of 44 and a Maddrey's discriminant function of 105.5. In addition, she had a negative work-up for acute viral hepatitis, Wilson's disease, and autoimmune hepatitis. She was cytomegalovirus (CMV) IgG positive but IgM negative. A liver MRI with and without contrast was notable for a cirrhotic appearing liver without evidence of PVT. She was ultimately diagnosed with alcoholic hepatitis with underlying alcohol cirrhosis.

She was evaluated and subsequently listed for LT at our center. On the third day of admission, she received a CMV donor positive deceased donor orthotopic liver transplant. The operation was described as uneventful with no technical complications.

Table 1 Presenting laboratories

Laboratory	Result
White blood cell count (cells/mm ³)	54.5
Hemoglobin (g/dL)	7.0
Platelet count (cells/mm ³)	76
Creatinine (mg/dL)	2.3
Blood urea nitrogen (mg/dL)	52
Sodium (mmol/L)	143
Potassium (mmol/L)	4.8
Protein (g/dL)	5.0
Albumin (g/dL)	3.2
Total bilirubin (mg/dL)	30.1
Aspartate aminotransferase (U/L)	215
Alanine aminotransferase (U/L)	37
Alkaline phosphatase (U/L)	178
International normalized ratio	2.7

On post-operative day (POD) #1 her clinical condition improved with successful weaning of her vasopressor and ventilator support. Liver doppler ultrasound showed a patent portal vein in the hilar region, with hepatopetal flow, but low velocity at 12 cm/s (normal 16-40 cm/s)^[5]. The hepatic arteries were patent, with high diastolic flows and resultant low resistive indices in the right and left hepatic arteries (0.35-0.44, normal 0.55-0.7), shown in [Figure 1](#)^[5]. Her post-operative laboratories are listed in [Table 2](#).

A follow-up POD #3 liver doppler ultrasound demonstrated a patent main portal vein with appropriate flow direction. The flow velocity was lower than expected at 14 cm/s. The left portal vein demonstrated reversal of flow (hepatofugal) which was new. Hepatic arterial waveforms exhibited increased diastolic flows, and the resistive indices declined further, to 0.38-0.40.

Given the persistency of the abnormal hepatic arterial wave forms and new reversal of flow in the left portal vein, further evaluation of the transplant vasculature was performed with computed tomography (CT) of her abdomen and pelvis with and without contrast. The CT showed unremarkable arterial anatomy and non-opacification of a long extra-hepatic segment of the portal vein, shown in [Figure 2](#), indicating acute PVT.

The patient then underwent emergent percutaneous venography. [Figure 3](#) shows her pre-intervention venography, notable for PVT. Eight milligrams of tissue plasminogen activator (tPA) were injected at the thrombus site, angioplasty was performed with a 12 mm x 40 mm balloon, and an uncovered 14 mm x 40 mm stent was deployed in the main portal vein, which successfully eradicated the PVT. In addition, coils were deployed into the inferior mesenteric vein to prevent hepatofugal shunting of blood flow ([Figure 4](#)). The patient was also started on aspirin 81 mg once daily to prevent stent thrombosis.

This patient's follow-up ultrasound showed a patent portal vein with visualization of hilar and retropancreatic segments and normalization of the flow and direction of the left portal vein and velocities. The hepatic resistive indices improved significantly. Her graft function improved to normal by time of discharge ([Table 2](#)).

MULTIDISCIPLINARY EXPERT CONSULTATION

Interventional radiology, transplant surgery, and hepatology.

FINAL DIAGNOSIS

Acute PVT.

TREATMENT

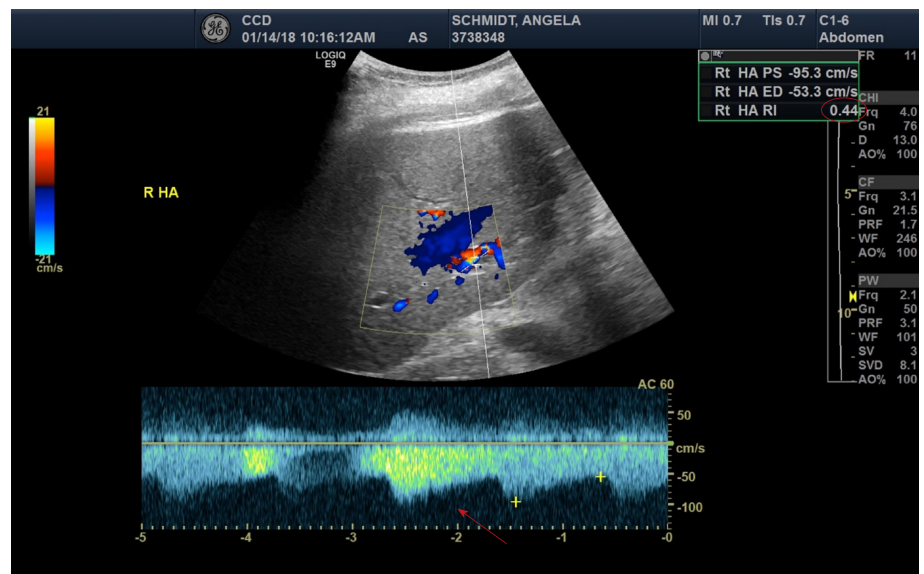


Figure 1 Liver ultrasound with doppler demonstrating increased diastolic flows (red arrow) and reduced resistive indices (noted to be 0.44 in the upper right corner, red oval) of the right posterior hepatic artery.

Tissue plasminogen activator administration, angioplasty, portal vein stent placement, and aspirin.

OUTCOME AND FOLLOW-UP

Successful eradication of PVT and normal graft function with improved hepatic resistive indices at discharge.

DISCUSSION

Reports of acute PVT post-LT typically state an incidence rate of 1%-3%, however one case series of adult and pediatric patients documented an incidence of 5.7%^[2-4]. While PVT in this scenario can present with hepatic enzyme abnormalities, relying on laboratory derangements to screen for PVT in the acute post-operative period is unreliable given that hepatic enzyme laboratories are often abnormal during this time. PVT post-LT can also present with sequelae of portal hypertension, including ascites, variceal formation, and hemorrhage^[1]. Graft failure and re-transplantation, although rare, have been reported in cases of acute PVT post-LT^[1,2].

Prevalence rates vary for PVT in other populations, with one study reporting a prevalence rate of 1.0% in the general population at time of autopsy, and other studies documenting PVT in 1.0%-26.0% of patients with cirrhosis^[6-10]. Risk factors for PVT include hypercoagulable states (such as malignancy or genetic defects), LT, increased portal vein resistance, and decreased portal vein flow^[11-17]. PVT can be classified several different ways, including according to the degree of portal vein occlusion, whether it is acute or chronic, its anatomic extent, and whether it is associated with cavernoma formation^[18-20].

Because of the potential ramifications of vascular complications post-LT (including both PVT and hepatic artery stenosis or thrombosis), routine serial ultrasounds in the immediate post-operative period are often employed after LT based on surgical practice. The high diastolic flows and low resistive indices seen in the patient's hepatic arteries were the first clues that portal venous dysfunction may have been present. PVT is a known etiology of decreased hepatic artery resistive index (RI)^[21]. Resistive index is the difference between the peak systolic and end diastolic velocities divided by the peak systolic velocity, and low RI is defined as < 0.55^[5]. The mechanism of reduced RI in PVT is explained by the disparate dual blood supply to the liver. If thrombosis occurs within the portal vein, the hepatic artery must compensate and supply more blood to the liver; in order to increase perfusion through the hepatic artery, resistance must decrease, which is accomplished by increasing hepatic arterial diastolic flow^[5]. The sensitivity and specificity for these findings in PVT are sub-optimal and vary, ranging from 73%-83% and 60%-73%,

Table 2 Relevant laboratories during the patient's hospital course

Labs	1 d before LT	POD0	POD1	POD2	POD3	POD4	POD10	POD17
AST (U/L)	142	163	264	124	74	70	21	16
ALT (U/L)	33	32	192	162	125	115	30	17
Total Bilirubin (mg/dL)	28.1	24.6	6.2	4.8	5.0	4.6	2.0	0.9
INR	2.6	2.8	1.7	1.6	1.3	1.3	1.1	1.2
AP (U/L)	133	125	58	84	116	179	147	128

LT: Liver transplant; POD: Post-operative day; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; INR: International normalized ratio; AP: Alkaline phosphatase.

respectively^[22]. While the hepatic arterial abnormalities seen on this patient's ultrasound can often indicate hepatic artery stenosis, noticeable arterial narrowing was not visualized on the patient's ultrasounds or CT scan. The sonographic findings were initially presumed to be due to her recent post-operative status and possible arterial anastomotic edema given her clinical improvement and lack of stenoses and/or thromboses seen in the hepatic arteries or portal veins on ultrasound.

Further workup during her first two PODs, such as an angiogram or CT scan, was not performed due to her continued clinical improvement, including improvement of her liver synthetic function as noted in the INR trend on Table 2. CT scan without an angiogram component was ultimately performed because of the newly discovered hepatofugal flow of the left hepatic vein on POD #3 which was concerning for PVT.

While no inherited hypercoagulability testing was done for this patient as her PVT was presumed to be secondary to the hypercoagulable state of surgery and because LT is a known risk factor for PVT, case reports exist documenting PVT in the setting of acute CMV infection in immunocompromised and immunocompetent patients, likely due to local inflammation and the development of anti-phospholipid antibodies^[23-27]. Although pre-LT testing confirmed that the patient was not actively infected with CMV and had immunity, the donor was CMV IgG positive. Transplant providers should be aware of the association between PVT and CMV, particularly in CMV donor positive/recipient negative patients and in the setting of immunosuppression.

The sensitivity and specificity for ultrasound diagnosis of PVT are better than the aforementioned secondary hepatic artery findings, with estimates of 80%-100% for both parameters^[18]. However, the Doppler exams for this patient were falsely negative for PVT, showing main portal vein patency in the hilar region. This may have been because the entire portal vein is not always visualized on the ultrasound, due to artifact from bowel gas and/or lack of adequate window on immediate postoperative days. To the best of our knowledge, this is the first case report post-LT that details these ultrasound findings as the presenting signs of acute PVT.

Anticoagulation is generally recommended for patients diagnosed with acute PVT even though there are no randomized controlled trials assessing this intervention. Early anticoagulation has been shown to lead to higher recanalization rates and prevent intestinal infarction compared to no anticoagulation, however it is unknown if these data apply to post-LT patients^[28]. Of note, no rigorous studies have assessed the efficacy of thrombolysis, angioplasty, and stent placement in patients with PVT, although rare case reports exist documenting these treatments after liver transplant with long term portal vein patency^[1,20,29,30].

CONCLUSION

Acute PVT is a rare and serious complication of LT. While ultrasound has a sensitivity and specificity between 80%-100% for diagnosing PVT, false negative results can occur; subtle findings on ultrasound, however, such as high diastolic flows and low resistive indices, can indicate that PVT may be present. A low threshold to perform more accurate diagnostic imaging should be employed if these ultrasound abnormalities are seen. Thrombolysis, angioplasty, and portal venous stent placement, although rare, have been successfully implemented as treatment for PVT post-LT. The optimal follow-up imaging regimen and anti-platelet or anticoagulation regimen is unknown and warrants further investigation.



Figure 2 Contrast enhanced venous phase computed tomography abdomen coronal image with red arrow and bracket showing non-opacification of the portal vein, indicating thrombosis.

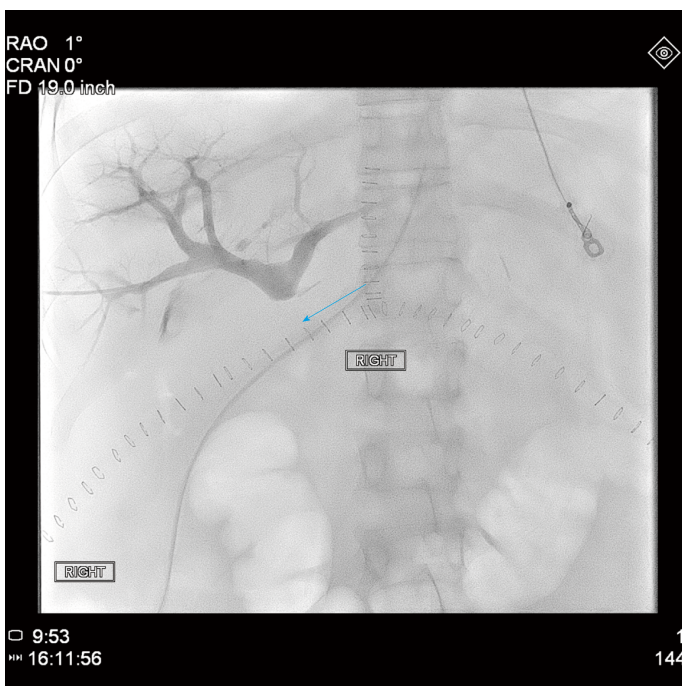


Figure 3 Pre-intervention venography, with the blue arrow denoting the beginning of the patient's portal vein thrombosis.

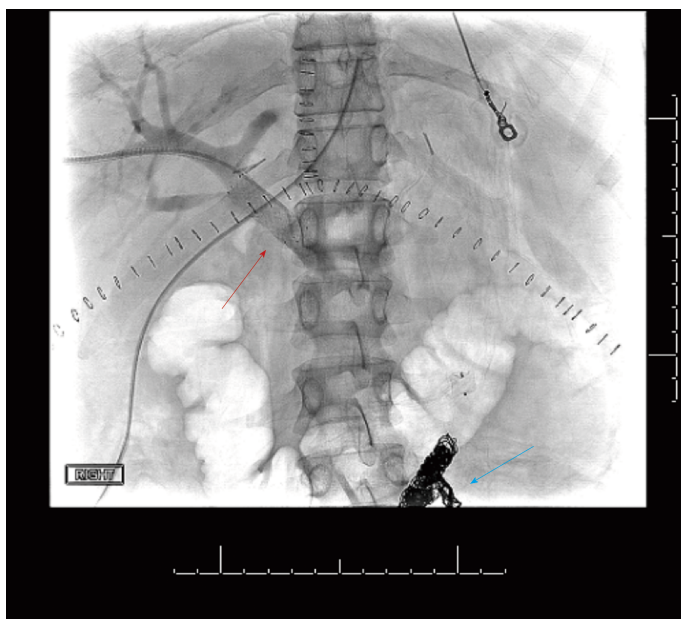


Figure 4 Post-intervention venography, with the red arrow showing recanalization of the portal vein and the uncovered stent, and the blue arrow indicating the coils placed in the inferior mesenteric vein.

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Two-stage liver transplant for ruptured hepatic adenoma: A case report

Marc Salhanick, Malcolm P MacConmara, Mark R Pedersen, Lafaine Grant, Christine S Hwang, Justin R Parekh

ORCID number: Marc Salhanick (0000-0003-0763-6346); Malcolm P MacConmara (0000-0001-7683-6945); Mark R Pedersen (0000-0002-4782-925X); Lafaine Grant (0000-0002-0837-5689); Christine S Hwang (0000-0003-4262-122X); Justin R Parekh (0000-0002-1725-6155).

Author contributions: Salhanick M and Pedersen MR wrote the paper; MacConmara MP, Hwang CS, Parekh JR and Grant L provided critical revision.

Informed consent statement: Given the nature of the patient's condition at the time of hepatectomy and liver transplant, consent was obtained from the patient's legal surrogate decision maker (her parents).

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Marc Salhanick, Division of Vascular Surgery, Department of Surgery, the University of Texas Southwestern Medical Center, Dallas, TX 75390, United States

Malcolm P MacConmara, Christine S Hwang, Justin R Parekh, Division of Surgical Transplantation, Department of Surgery, the University of Texas Southwestern Medical Center, Dallas, TX 75390, United States

Mark R Pedersen, Lafaine Grant, Division of Digestive and Liver Diseases, Department of Internal Medicine, the University of Texas Southwestern Medical Center, Dallas, TX 75390, United States

Corresponding author: Malcolm P MacConmara, MD, Attending Doctor, Division of Surgical Transplantation, Department of Surgery, the University of Texas Southwestern Medical Center, 6201 Harry Hines Blvd, Dallas, TX 75390, United States.

malcolm.maconmara@utsouthwestern.edu

Telephone: +1-214-6458300

Fax: +1-214-6456771

Abstract

BACKGROUND

Only one case of liver transplantation for hepatic adenoma has previously been reported for patients with rupture and uncontrolled hemorrhage. We present the case of a massive ruptured hepatic adenoma with persistent hemorrhagic shock and toxic liver syndrome which resulted in a two-stage liver transplantation. This is the first case of a two-stage liver transplantation performed for a ruptured hepatic adenoma.

CASE SUMMARY

A 23 years old African American female with a history of pre-diabetes and oral contraceptive presented to an outside facility complaining of right-sided chest pain and emesis for one day. She was found to be in hemorrhagic shock due to a massive ruptured hepatic adenoma. She underwent repeated embolizations with interventional radiology with ongoing hemorrhage and the development of renal failure, hepatic failure, and hemodynamic instability, known as toxic liver syndrome. In the setting of uncontrolled hemorrhage and toxic liver syndrome, a hepatectomy with porto-caval anastomosis was performed with liver transplantation 15 h later. She tolerated the anhepatic stage well, and has done well over one year later.

CONCLUSION

When toxic liver syndrome is recognized, liver transplantation with or without

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hepatectomy should be considered before the patient becomes unstable.

Key words: Hepatic adenoma; Toxic liver syndrome; Two-stage liver transplantation; Hepatectomy; Ruptured adenoma; Case report

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Core tip: This case describes a rare and dramatic complication of a hepatic adenoma that resulted in both massive hemorrhage and toxic liver syndrome which could only be treated with hepatectomy. Recognition of toxic liver syndrome is essential when dealing with patients who suffer massive liver necrosis in attempts to control bleeding. Early consideration should be given to liver transplantation with or without hepatectomy before the patient becomes too unstable to proceed.

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INTRODUCTION

Hepatic adenomas are an uncommon solid tumor of the liver with an estimated incidence of 3-4 per 100000 women^[1]. Hemorrhagic rupture occurs in 10%-31% of patients with hepatic adenomas, with treatment options including embolization and surgical resection with good outcomes^[2,3].

One case of liver transplantation has previously been reported for patients with rupture and uncontrolled hemorrhage from a hepatic adenoma^[4]. We present the case of a massive ruptured hepatic adenoma with persistent hemorrhagic shock and toxic liver syndrome which resulted in a two-stage liver transplantation. This is the first case of a two-stage liver transplantation performed for a ruptured hepatic adenoma.

CASE PRESENTATION

Chief complaints

A 23 year old African American female with a history of pre-diabetes and oral contraceptive use since age 11, presented to an outside facility complaining of right-upper-quadrant pain, generalized weakness, and emesis for one day. She had been in her usual state of health until that morning when she experiences the acute onset of stabbing right-upper-quadrant pain that radiated to her chest. She quickly felt nauseous and had several episodes of non-bloody emesis.

History of past illness

She had a past medical history significant for pre-diabetes and oral contraceptive use, but otherwise had no other medical problems and took no other medications.

Personal and family history

She had no history of alcohol, tobacco, or drug abuse, and no family history of liver disease.

Physical examination upon admission

Her initial physical exam was remarkable for pallor. She was afebrile with an initial blood pressure of 96/52 mmHg and a heart rate of 126 beats per minute. Her abdominal exam was notable for right-upper-quadrant tenderness and fullness. Her cardiopulmonary exam was normal except for tachycardia. Initial labs revealed a hemoglobin 8.7 gm/dL, platelets $396 \times 10^9/L$, lactic acid 5.6 mmol/L, alanine aminotransferase (ALT) 100 IU/L, aspartate aminotransferase (AST) 166 IU/L, total bilirubin 0.3 mg/dL, creatinine 1.30 mg/dL, blood urea nitrogen 13 mg/dL, and bicarbonate 18 mEq/L. A urinary pregnancy test was negative.

Imaging examinations

Initial imaging would include a CT of the abdomen and pelvis with contrast demonstrated a large 22 cm x 15 cm heterogenous, hypoattenuating mass encompassing nearly the entire liver. The mass demonstrated hypervascularity along the border and hyperattenuating areas, suggesting a large hemorrhagic liver mass with active hemorrhage. There was no rupture of the liver and no perihepatic, subcapsular hematoma (Figure 1). She was given preliminary diagnosis of hemorrhagic shock due to this hemorrhagic liver mass and was transferred to a second hospital for interventional radiology.

On arrival, her hemoglobin was 9.6 gm/dL after transfusion of 6 units of packed red blood cells. She underwent gel foam embolization of the right hepatic artery. However, throughout the evening she required ongoing blood transfusions. A repeat CT scan demonstrated enlargement of the intrahepatic hematoma with new intraperitoneal fluid, retroperitoneal fluid, and bilateral pleural effusion concerning for ongoing hemorrhage. She was then taken back for a mesenteric angiogram with embolization of the middle hepatic artery and repeat embolization of the right hepatic artery. During this period, her ALT increased to 1023 IU/L, AST 2287 IU/L, bilirubin 3.2 mg/dL, alkaline phosphatase 506 IU/L, and lactic acid 8.3 mmol/L. She had ongoing hemodynamic instability and anuric kidney injury (creatinine 1.73 mg/dL). She was then transferred to our facility.

FINAL DIAGNOSIS

Here, an angiogram demonstrated active extravasation from the liver lesion (Figure 2). Repeat embolization of the entire right hepatic artery was performed. Despite these interventions and additional resuscitation, she had progressive acidosis, increasing pressor requirement, and worsening of her bilirubin, INR, and lactate (Table 1). With this rapidly deteriorating hepatic function with hemodynamic instability and renal failure, she was diagnosed with toxic liver syndrome.

TREATMENT

She was then taken to the operating room where the liver was found to have a rupture extending across the entire right lobe into segment 4 anteriorly, as well as a separate rupture posteriorly. Both fractures were at least 4 cm deep and more than 10 cm long with active rupture into the abdomen (Figure 3). Her right lobe and most of the left had been almost entirely replaced by coagulated blood inside of the adenoma. There was significant compressive effect of the enlarged liver on the portal vein and hepatic artery. Only a small lateral portion of segments II and III was uninvolved.

Given the size of the mass with compressive effect on adjacent vasculature, ongoing bleeding during the operation, and the ischemic injury to the remaining liver, it was decided that total hepatectomy followed by transplant would be her best chance at survival. Resection was not thought to be viable as only a small remnant of uninvolved liver remained, and this small portion was felt to already be heavily injured by preceding ischemia. The liver was then dissected off the cava. The hepatic vein stumps were oversewn and a porto-caval shunt created. Final pathology demonstrated a liver size of 34.5 cm x 22.5 cm x 8.5 cm with a red-tan hepatic adenoma measuring 30 cm x 22.5 cm x 8.5 cm. Coagulative necrosis was noted throughout tumor with intravascular foreign material consistent with embolization. The remnant liver tissue demonstrated massive necrosis with only a few remaining periportal hepatocytes.

After hepatectomy, her hemodynamics stabilized and her urine output increased. She underwent urgent liver transplant evaluation and was listed as status 1, anhepatic. She was maintained intubated on continuous venovenous hemodialysis with target sodium 145-150 mEq/L, a fresh frozen plasma drip, a 50% dextrose solution drip, empiric antibiotics, frequent calcium checks, and elevation of the head of bed. She required minimal sedation during this period with an intermittent low dose fentanyl drip. She was anhepatic for a total of 15 h before going back to the operating room for an orthotopic liver transplantation. A standard piggyback transplant was performed and a supra-celiac aortic conduit was created given the celiac dissection that had been noted earlier.

OUTCOME AND FOLLOW-UP

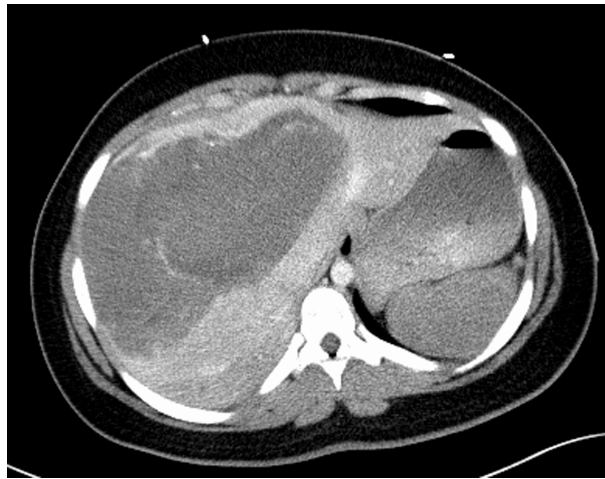


Figure 1 CT scan prior to interventions. A CT of the abdomen and pelvis with contrast demonstrated a large 22 cm × 15 cm heterogenous, hypoattenuating mass encompassing nearly the entire liver. The mass demonstrated hypervascularity along the border and hyperattenuating areas, suggesting a large hemorrhagic liver mass with active hemorrhage.

She tolerated the procedure well, and had one take-back surgery due to elevated liver function tests with findings of increased resistive indices on ultrasound. Intraoperatively, the vessels were found to be intact with some compression from abdominal wall edema which did not require any intervention other than additional volume removal. Her recovery was otherwise unremarkable. She was extubated on post-transplant day 4. She was discharged from the hospital on post-transplant day 8.

DISCUSSION

When adenomas rupture, they are managed with resuscitation to achieve hemodynamic stability and nonsurgical modalities such as embolization to control bleeding^[5,6]. When conservative measure fail, partial hepatectomy or packing of the liver may be used to control the hemorrhage^[3,7]. In rare cases, liver transplantation may be considered^[4]. To date, 67 patients have been transplanted with hepatic adenoma as the primary diagnosis according to the Organ Procurement and Transplantation Network (exact indications are not specified, but presumably due to size, malignant transformation, multifocality, or hemorrhage)^[8]. Only one case of liver transplant for a hemorrhagic hepatic adenoma has been reported in the literature^[4].

We present the case of a massive ruptured hepatic adenoma that would ultimately require a hepatectomy prior to liver transplant to manage. While the patient suffered a significant hemorrhage with rupture of her liver, the degree of hepatic and physiologic dysfunction she experienced was out of proportion solely to the degree of hemorrhage that she experienced. It is unusual for otherwise healthy patients to have such marked liver dysfunction, even in the setting of prolonged hypotension^[9]. However, with the mass effect of a ruptured liver on the perihepatic vasculature, almost complete replacement of the hepatic parenchyma by adenoma and hematoma, and further damage from hemorrhagic shock and three embolization procedures, her liver parenchyma started to necrose and resulted in toxic liver syndrome.

Ringe *et al*^[10] first coined the term toxic liver syndrome to describe patients with a non-functioning liver associated with hemodynamic instability and renal failure. The condition, though rare, is important to recognize. A recent case series from Kaltenborn *et al*^[11] found that the cause for mortality in similar patients is not hemorrhage, which can usually be halted with packing or ligation of the porta, rather the liver necrosis and subsequent toxic liver syndrome. Cessation of hemorrhage would not have rescued this patient. In this case, the segment of remaining liver was too small to be viable, and the ongoing egress of necrotic byproducts from any retained liver would have continued to propagate her unstable state. Total hepatectomy was necessary to control hemorrhage and to relieve the physiologic sequelae of toxic liver syndrome. As a result, post-hepatectomy her heart rate normalized, her urine output tripled, and her vasoactive medications were stopped.

The use of a hepatectomy with a portocaval shunt prior to liver transplant is sometimes referred to as a two-stage liver transplant and can temporize patient awaiting an organ to transplant. Two-stage liver transplantation was first reported in

Table 1 Peri-transplant hemodynamics and labs

	On arrival at OSH	Mid-hospitalization	Prior to transfer, at OSH	Prior to hepatectomy	1 h post hepatectomy	6 h post hepatectomy	Prior to transplant	1 h after transplant	6 h after transplant
Norepinephrine (mcg/kg per minute)	0	0	0	0.04	0	0	0	0	0
Lactate (mmol/L)	4.9	6.4	8.3	9.5	9.7	5.2	5.1	5	4.8
Continuous venovenous hemodialysis	No	No	No	Adjuncts	Initiated	-100 mL/h	-150 mL/h	-200 mL/h	-130 mL/h
Urine Output (mL/h)	65	9	35	Anuric	400	75	22	75	43
Potassium (meq/L)	4.2	5.4	6.9	4.5	4	3.8	4	5	4.5
Creatinine (mg/dL)	1.22	1.07	1.73	1.53	1.45	0.87	1.03	1.00	0.84
INR	1.2	1.4	1.9	NA	1.4	1.3	1.6	1.3	1.3
pH	NA	7.28	7.2	7.24	7.48	7.45	7.37	7.46	7.45
P _{CO2} (torr)	NA	26	34	35	31	34	37.5	34	37
Total Bilirubin (mg/dL)	NA	2.1	2.1	3.3	NA	NA	NA	3.9	3.7
AST (IU/mL)	NA	784	2178	3882	NA	NA	NA	2440	1985
ALT (IU/mL)	NA	366	894	1464	NA	NA	NA	1138	938

Rate indicates rate of fluid removal (sum of all fluid administered – fluid removed on continuous venovenous hemodialysis). OSH: Outside hospital; INR: International normalized ratio; P_{CO2}: Partial pressure of carbon dioxide; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; NA: Not available.

1988 and is used as a last resort for patients who are unstable due to exsanguinating hemorrhage (from trauma or an irreparable laceration due to a ruptured hepatic adenoma) or overwhelming inflammation (such as from primary graft non-function or acute liver failure)^[12-14]. While early mortality for the two-stage liver transplant was as high as 60%-76% within the first year^[10,15,16], the mortality in the last decade has been reported to be as low as 24% in some series^[12-14].

Our patient tolerated the hepatectomy remarkably well. She had rapid hemodynamic stabilization and improvement in her urine output. During this stage, other consequences of the anhepatic state were carefully monitored. Hypoglycemia, due to a lack of hepatic gluconeogenesis, was controlled with a 50% dextrose drip. Hypocalcemia, a consequence of multiple citrate-containing blood transfusion combined with the inability to metabolize citrate, was carefully monitored and corrected^[15]. Volume status and acid-base balance was managed with continuous veno-venous hemodialysis. Increased intracranial pressure was avoided by elevation of the head of the bed and maintenance of mild hyponatremia. She was transplanted 15 hours later, and was discharged within approximately one week of liver transplantation. At the time of submission, the patient continues to do well over a year from transplant and has not had to be re-hospitalized.

This case describes a rare and dramatic complication of a hepatic adenoma that resulted in both massive hemorrhage and liver dysfunction which could only be treated with hepatectomy. Recognition of toxic liver syndrome is essential when dealing with patients who suffer massive liver necrosis in attempts to control bleeding. Though embolization to control bleeding is an important first step, ultimately these patients will not be definitively managed by embolization procedures. Early consideration should be given to liver transplantation with or without hepatectomy before the patient becomes too unstable to proceed.

CONCLUSION

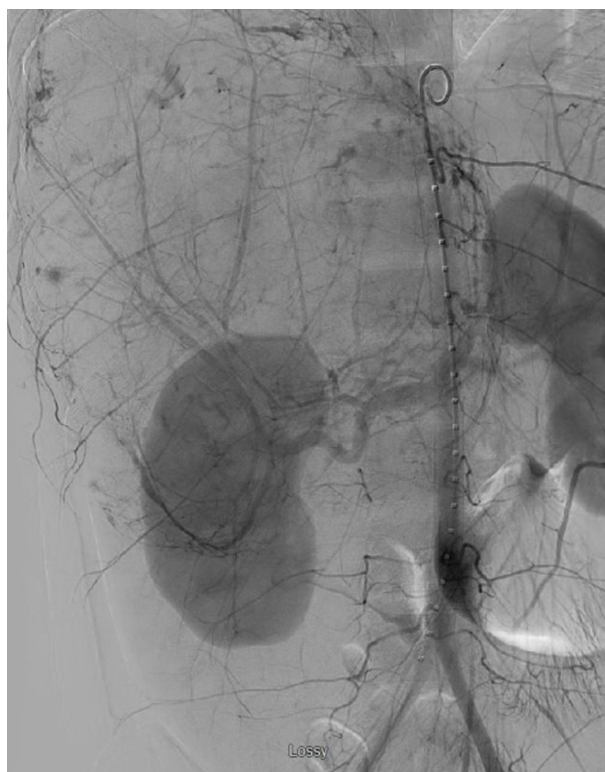


Figure 2 Mesenteric angiogram prior to transplant. Mesenteric angiogram demonstrating a large right hepatic lobe with multiple areas of abnormal contrast accumulation indicative of ongoing hemorrhage. Gelfoam embolization of the right hepatic artery was performed.

Toxic liver syndrome describes patients with a non-functioning liver associated with hemodynamic instability and renal failure. Mortality in these patients are not from the hemorrhage itself, rather the liver necrosis and subsequent toxic liver syndrome. A two-stage liver transplantation, or the use of a hepatectomy with a portocaval shunt prior to liver transplant, should be considered in patients with toxic liver syndrome. Anhepatic patients require careful management of hypoglycemia, hypocalcemia, volume status, acid-base balance, and intracranial pressure, among other parameters. Further research is needed to determine the optimal management of anheptic patients and ways to identify the point when hepatectomy would be most useful in patients developing toxic liver syndrome.

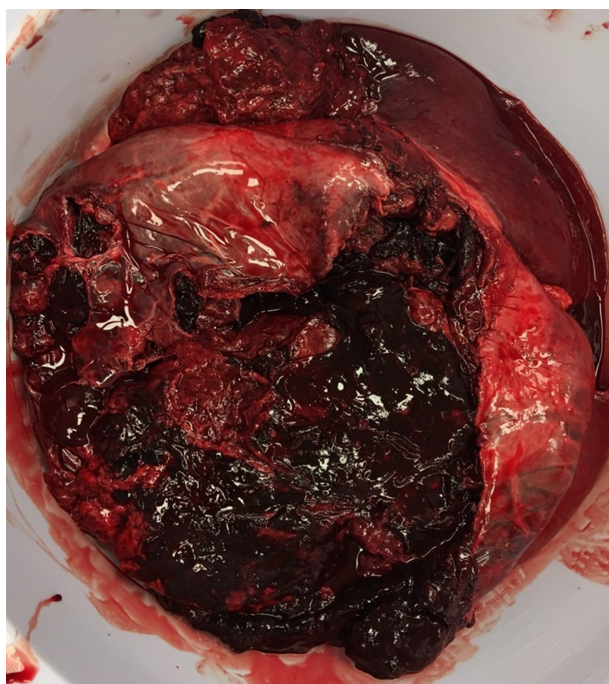


Figure 3 Explanted liver. Explanted liver, measuring 34.5 cm × 22.5 cm × 8.5 cm, with a large surface disruption with adenomatous tissue and significant adherent clot.

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