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MINIREVIEWS

Pathologic and molecular features of hepatocellular carcinoma: An update

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Abstract

Morphological diversity and several new distinct pathologic subtypes of hepatocellular carcinoma (HCC) are now well-recognized. Recent advances in tumor genomics and transcriptomics have identified several recurrent somatic/genetic alterations that are closely related with histomorphological subtypes and have therefore, greatly improved our understanding of HCC pathogenesis. Pathologic subtyping allows for a diagnosis which is clinically helpful and can have important implication in patient prognostication as some of these subtypes are extremely aggressive with vascular invasion, early recurrence, and worst outcomes. Several targeted treatments are now being considered in HCC, and the reporting of subtypes may be quite useful for personalized therapeutic purpose. This manuscript reviews the recently identified histomorphological subtypes and molecular alterations in HCC.

Key Words: Pathology; Hepatocellular carcinoma subtypes; Macrotrabecular massive; Steatohepatitic; Fibrolamellar; Molecular alterations

Core Tip: We summarize several new distinct histologic subtypes of hepatocellular carcinoma (HCC) and recurrent molecular alterations in HCC. Major histologic subtypes like macrotrabecular massive, fibrolamellar HCC, steatohepatitic HCC, scirrhous HCC, lymphoepithelioma-like HCC, and combined hepatocellaular-cholangiocarcinoma are discussed in detail. Rare and provisional histological variants are also discussed.

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INTRODUCTION

The incidence of hepatocellular carcinoma (HCC) has been increasing steadily over the past two decades and currently ranks as the fifth most common cancer in men and seventh in women[1,2]. HCC is now the fourth-most common cause of cancer-related deaths and the most frequent primary liver neoplasia, causing more than 80%-85% of liver cancer cases globally[3]. Major risk factors associated with HCC are chronic infection with hepatitis B virus and hepatitis C virus, chronic alcohol consumption, and non-alcoholic fatty liver disease associated with metabolic syndrome, diabetes and obesity. Prognosis of patients with HCC remains poor with 5-year survival rate of 18%, as the majority of these tumors are detected at a clinically advanced stage[3]. Hepatocarcinogenesis is a multistep process of malignant transformation of hepatocytes through the sequential accumulation of multiple genomic and epigenomic alterations. HCC is a histologically and genetically diverse cancer[4]. Indeed, several new pathologic subtypes of HCC have been reported recently and new underlying genetic alterations have been described. HCC histological growth patterns are closely related to molecular alterations and oncogenic pathways.

PATHOLOGY OF PRECANCEROUS LESIONS AND CONVENTIONAL HCC

It is now well-established that HCC evolves from precancerous lesions (dysplastic foci/dysplastic nodules). By consensus, the sequence of hepatocarcinogenesis includes low-grade dysplastic nodule (LGDN), high-grade dysplastic nodule (HGDN), early HCC, and small progressed HCC[5,6] (Figure 1). This classification is also supported by molecular studies on increasing accumulation of clonal molecular alterations[7]. Dysplastic foci (< 1 mm in size) are identified incidentally in chronic liver disease (CLD) and are microscopic lesions composed of dysplastic hepatocytes (Figure 2A). The nature of dysplasia is similar to that observed in dysplastic nodules: Large cell change, small cell change, or focal iron free area. Large cell change is characterized by cellular enlargement with enlarged pleomorphic nuclei, abundant cytoplasm, and frequent multinucleation of hepatocytes. The nuclear-cytoplasmic ratio is preserved in large cell change. Small cell change is characterized by decreased cell volume, increased nuclear-cytoplasmic ratio, cytoplasmic basophilia, mild nuclear pleomorphism, and hyperchromasia. Iron-free foci in patients with marked hepatic iron overload show immunohistochemical evidence of proliferative activity and are associated with a high incidence of HCC. Dysplastic nodules are usually identified in livers with cirrhosis but are also occasionally found along with CLD without cirrhosis. These are around 5-15 mm in diameter and can be single or multiple lesions. A LGDN is a distinctly nodular lesion displaying a monotonous cell population with a mild increase in cellular density, a clear trabecular arrangement, and no architectural atypia in comparison to the neighbouring cirrhotic liver. HGDNs are characterized by hepatocyte proliferation with atypical cytological and/or architectural features that are not sufficient for a diagnosis of HCC. HGDN show higher cellular density and frequently demonstrates small cell change.

Macroscopically, lesions with foci of malignant transformation may demonstrate variable features like vaguely nodular, expansile nodular, multinodular, multicentric, cirrhotomimetic, nodular with perinodular extension, and infiltrative types (Figure 2B-D). Small HCCs, ≤ 2 cm, are divided into two groups. Early HCC are vaguely nodular with indistinct margins and usually show higher cellular density than the surrounding cirrhotic tissue with increased nuclear to cytoplasmic ratio, irregular trabeculae, pseudoacini formation, and unpaired arterioles (Figure 3A). Stromal invasion is one of the most important characteristics to differentiate early HCC from HGDN, but is however difficult to identify. Progressed HCC are distinctly nodular with distinguishable margins, frequently capsulated, and show infiltrative or expansile growth pattern. Morphologically, conventional HCC show 4 major architectural growth patterns: Trabecular, solid, pseudoglandular/acinar, and macrotrabecular

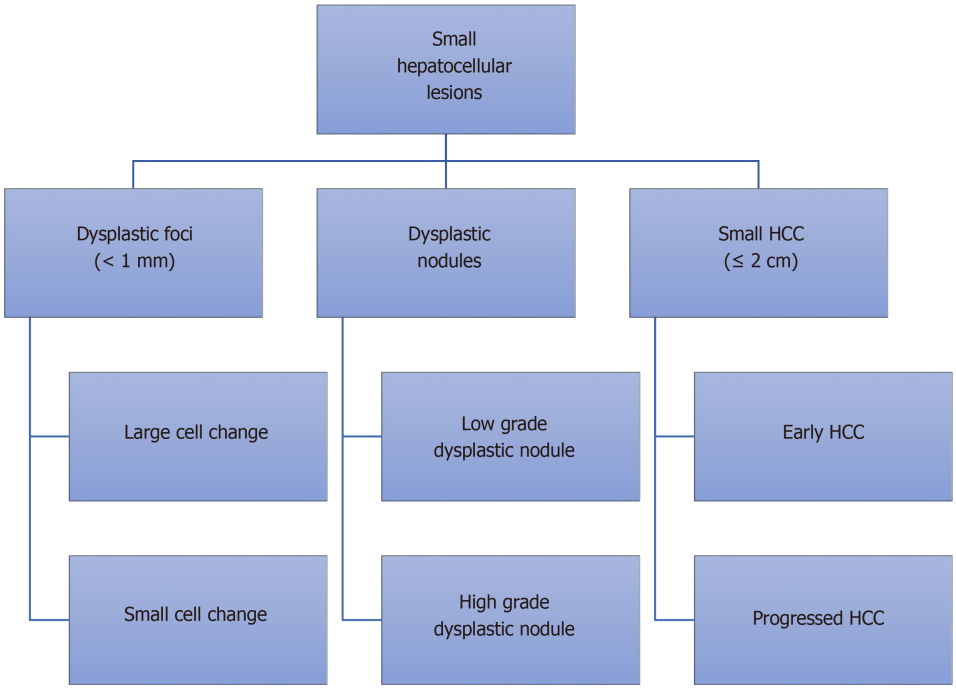

Figure 1 International consensus group for hepatocellular neoplasia classification of small hepatocellular lesions. HCC: Hepatocellular carcinoma.

(Figure 3B-D) and several cytological features (clear cell, steatosis, pleomorphism, multinucleation, foamy cells, oncocytic cells, spindle cells), with frequent co-existence of several features (Figure 4)[8,9]. Various intra-hepatocytic inclusions may be seen like hyaline globules (Figure 5A), Mallory-Denk bodies, bile, and pale bodies. Two histological grading systems for HCC are available. The WHO three-tiered grading system is based on a combination of cytological features and differentiation, and further grades the tumor into well, moderately, and poorly differentiated types[10]. Primary hepatic undifferentiated carcinoma is not included in the WHO grading system as it shows no evidence of either hepatic or biliary differentiation. It is the system most commonly used by pathologists[10,11]. Edmondson and Steiner grading system divides HCC into four grades based on histological differentiation with grade 1 being very well differentiated[12]. A correlation between the histological grade and patient prognosis has been reported[13]. Poorly differentiated HCC are associated with higher recurrence after surgery[14].

ANCILLARY STUDIES FOR THE PATHOLOGIC DIAGNOSIS OF HCC

Differentiation of HCC from other malignancies can be difficult; immunostaining can be helpful to differentiate between these lesions. Arginase-1 is a binuclear manganese metalloenzyme and is the most sensitive and specific marker of hepatocytic differentiation[15]. It shows diffused nuclear and cytoplasmic staining. Carcinoma with hepatoid differentiation and rare cases of adenocarcinoma (including colorectal, pancreatic, breast, and prostatic primaries), cholangiocarcinoma, and may however, show focal or weak Arginase-1 positivity[16]. Hepatocyte paraffin 1 (Hep-Par 1) is a monoclonal antibody that reacts with the urea cycle enzyme carbamoyl phosphate synthetase 1 of liver mitochondria. It shows diffuse granular cytoplasmic staining in normal and neoplastic hepatocytes[17]. Hep-Par 1 is unfortunately frequently negative for poorly differentiated HCCs. Few cholangiocarcinoma and metastatic adenocarcinoma may show Hep-Par 1 immunopositivity. Glypican 3 is excellent marker for neoplastic hepatocytes with cytoplasmic, membranous, or golgi-zone pattern of immunopositivity[18]. Other immunostains like polyclonal carcinoembryonic antigen (pCEA), CD10 and villin shows a distinct canalicular immunostaining pattern in HCC[16]. Alpha-fetoprotein (AFP) immunohistochemistry is not very useful in diagnosis of HCC as it has low sensitivity and is often only focally positive. Albumin RNA *in situ* hybridization has been shown to be a highly sensitive maker for hepatocellular differentiation[19]. Its specificity is however suboptimal and it can be

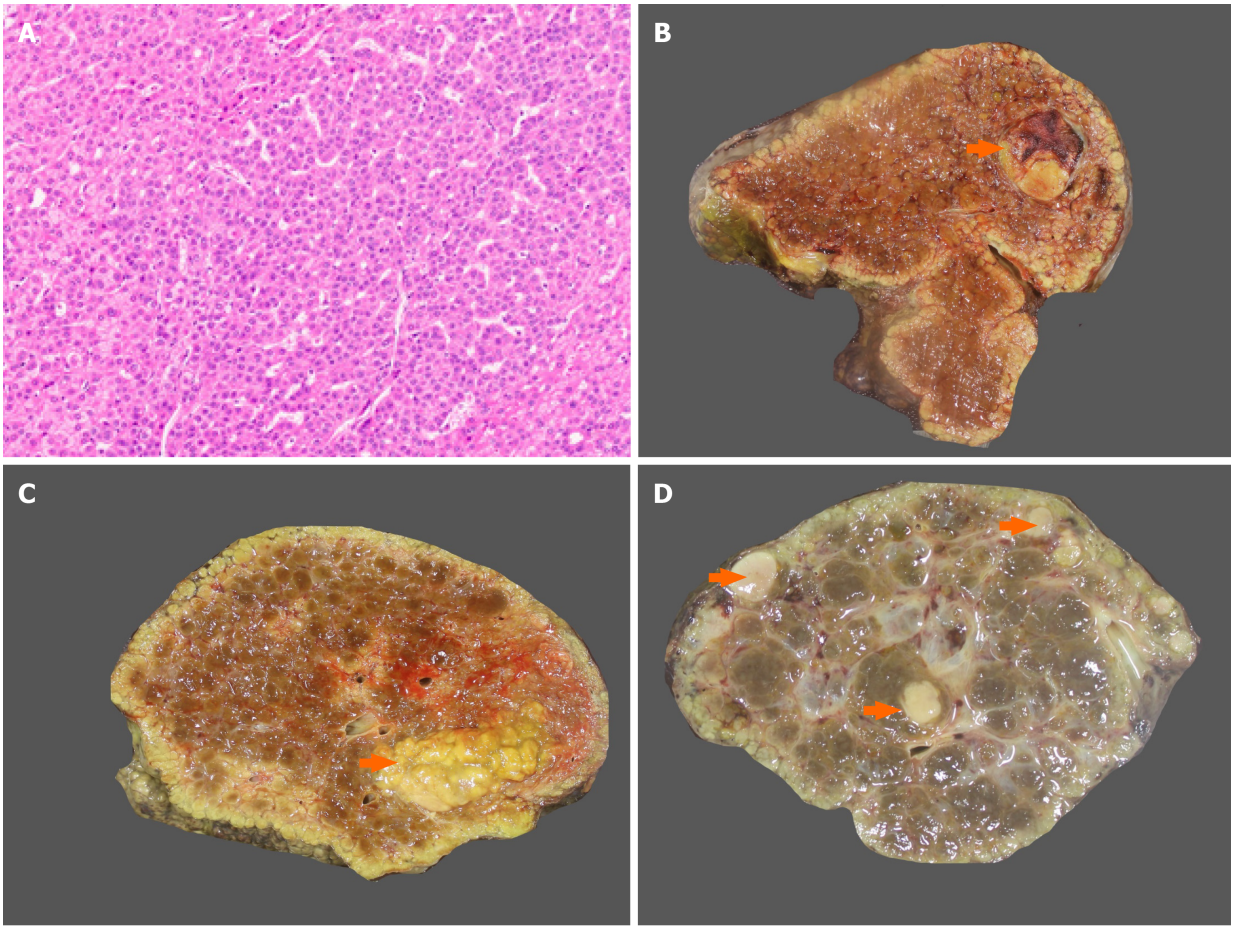

Figure 2 Dysplasia and gross morphology of hepatocellular carcinoma. A: Dysplastic foci with small cell change (hematoxylin and eosin); B: Nodular hepatocellular carcinoma (HCC) in a cirrhotic liver (arrow); C: Multinodular HCC in a cirrhotic liver (arrow); D: Multicentric HCC (arrow).

positive in tumors demonstrating hepatocytic differentiation, such as hepatoid carcinomas of various sites, intrahepatic cholangiocarcinoma (iCCA), gall bladder adenocarcinoma, and yolk sac tumour[20]. Well-differentiated HCCs may also be difficult to distinguish from dysplastic nodules. Loss of reticulin, stromal invasion, and neoarteriolization are particularly useful in these cases. The combination of 3 immunomarkers-glypican 3, glutamine synthetase (GS), and heat shock protein 70-can be used to differentiate early HCC from HGDN[12].

DISTINCT PATHOLOGICAL SUBTYPES WITH MOLECULAR FEATURES

Table 1 summarizes distinct pathological subtypes and their molecular features.

MACROTRABECULAR MASSIVE HCC

The Macrotrabecular-Massive HCC (MTM-HCC) subtype represents a novel histomorphological subtype of HCC. It represents 10%–20% of all cases of HCC. Histologically, it is defined by a macrotrabecular (> 6 cells thick) architectural pattern involving > 50% of the entire tumour, regardless of the associated cytological features (Figure 5B)[4]. Most trabeculae in MTM-HCC are ≥ 10 cells thick[10]. On trucut biopsy analysis, MTM-HCC case is classified if at least 1 focus of macrotrabecular pattern is observed, and the percentage of the macrotrabecular pattern is not taken into account. Pathologists robustly identify MTM-HCC with good inter-observer agreements. MTM-HCC is also characterized by an association with tumor protein 53 (TP53) mutations and fibroblast growth factor 19 (FGF19) amplifications[21]. Being an aggressive form of HCC, it is associated with poor prognostic factors, such as higher Barcelona Clinic Liver Cancer (BCLC) stage B or C, higher AFP levels (> 100 ng/dL), larger tumor size,

Table 1 Hepatocellular carcinoma distinct subtypes with pathological and molecular features

Distinct subtypes	Pathological features	Molecular features
Macrotrabecular massive	Macrotrabeculae > 50% of the tumor, staellite nodules, vascular invasion	TP53 mutations and FGF19 amplifications
Steatohepatitic	Steatohepatitis in the tumor	IL6/JAK/STAT pathway activation
Scirrhous	Dense fibrosis in > 50% of the tumor	Activation of (TGF-β) pathway, with overexpression of VIM, SNAIL (SNAI1), SMAD4 and TWIST
Fibrolamellar	Large polygonal tumor cells with abundant eosinophilic granular cytoplasm and dense bands of intratumoral fibrosis	Recurrent chimeric *DNAJB1-PRKACA* gene fusion
Lymphoepithelioma-like	Neoplastic epithelial cells with a prominent lymphoid infiltrate	Marked focal amplification of chromosome 11q13.3
Progenitor	Immunohistochemical expression of biliary marker CK19 in > 5% of tumor cells	TP53 mutations
Combined hepatocellular-cholangiocarcinoma	Unequivocal presence of both hepatocytic and cholangiocytic differentiation	TP53, TERT, IDH mutations

DNAJB1-PRKACA: DnaJ heat shock protein family member B1 (DNAJB1) and protein kinase 3'-5'-cyclic adenosine monophosphate-activated catalytic subunit alpha; IL6: Interleukin-6; JAK: Janus kinas; STAT: Signal transducer and activator of transcription; FGF19: Fibroblast growth factor 19; TERT: Telomerase reverse transcriptase; IDH: Isocitrate dehydrogenase; SNAIL (SNAI1): SNAIL family transcriptional repressor 1; SMAD4: SMAD family member 4; TWIST: *Twist*-related protein; TGF-β: Transforming growth factor beta.

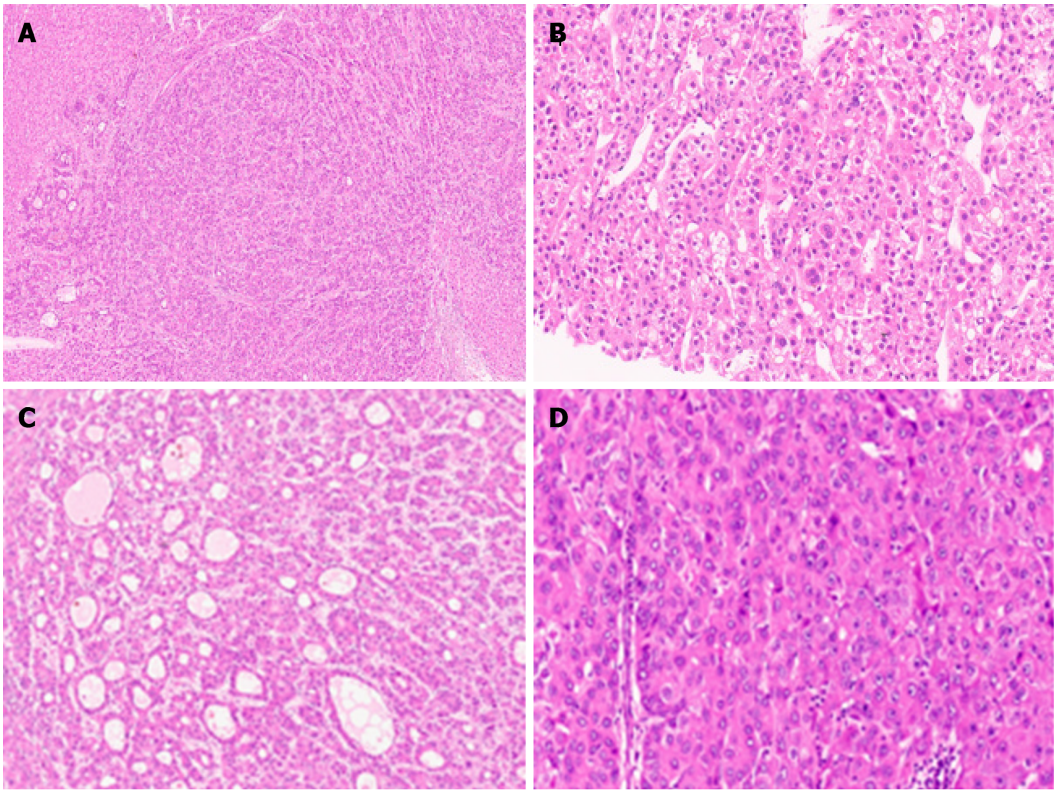

Figure 3 Well differentiated hepatocellular carcinoma. A: Early hepatocellular carcinoma (HCC) with pseudoacinar pattern [hematoxylin and eosin (H&E)]; B: Well differentiated HCC with thin trabeculae (H&E); C: Well differentiated HCC with pseudoacini (H&E); D: HCC with solid sheet growth pattern (H&E).

frequent satellite nodules, substantial necrosis, and macro or microvascular invasion; hence, there is a higher risk of early tumor recurrence and poor disease-free and overall survival rate (Figures 5C and D)[22]. These findings have been further validated by several groups. The other characteristics are its association with viral hepatitis B infection and profound activation of angiogenesis[23]. Presence of the satellite nodule on the multiphase liver magnetic resonance imaging (MRI) has been described as independent factor associated with both early and overall tumor recurrence[24]. Rhee

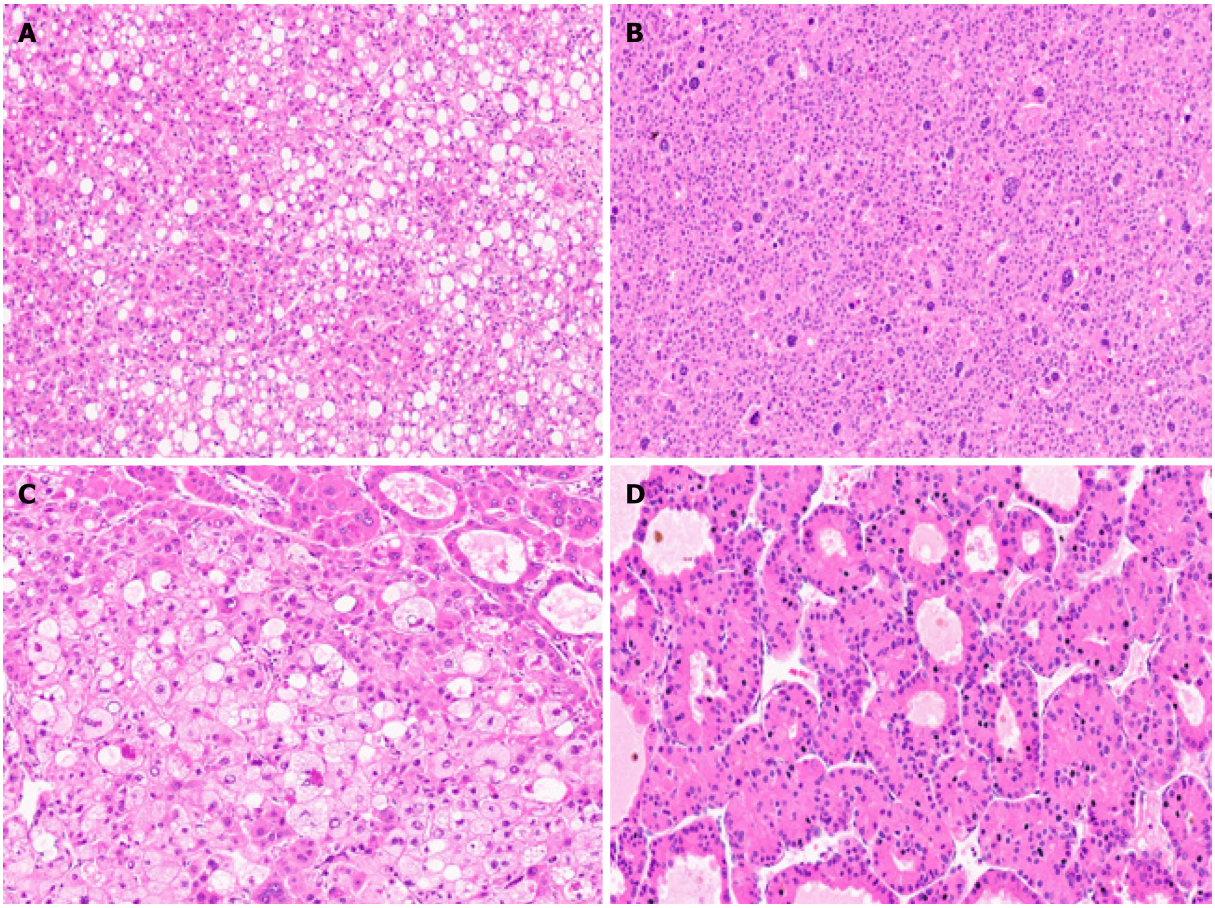

Figure 4 Hepatocellular carcinoma cytological features. A: Hepatocellular carcinoma (HCC) with fatty change [hematoxylin and eosin (H&E)]; B: Marked pleomorphism in an HCC (H&E); C: Foamy cell cytoplasm in an HCC (H&E); D: HCC with oncocytic cells (H&E).

et al[25] reported imaging findings of MTM-HCC by gadoxetic acid-enhanced MRI. With gadoxetic acid-enhanced MRI findings, including arterial phase hypovascular component, they were able to stratify the probability of MTM-HCC and obtain prognostic information[25]. The gene expression profile associated with the MTM-HCC subtype is characterized by the activation of neoangiogenesis, with overexpression of angiopoietin 2 and Vascular Endothelial Growth Factor A (VEGFA). Angiopoietin 2 is responsible for the destabilization of established vasculature and subsequent neoangiogenesis, and also disturbs interactions between endothelial and periendothelial cells, which results in an increased receptiveness to VEGFA[4]. These tumors have high expression of neoangiogenesis-related genes, which led to the discovery of Endothelial-Specific Molecule 1 as a reliable immunostaining marker[26]. Immune assessment of MTM-HCC using expression of the programmed death ligand 1 (PD-L1) and Chemokine-like factor MARVEL transmembrane domain containing 6 (CMTM6) protein coded immune-checkpoint inhibitors showed higher tumoral PD-L1 expression, higher density of inflammatory cells, and higher CMTM6 expression. Therefore, combined expression of PD-L1 and CMTM6 were associated with shorter overall and disease-free survival[27].

STEATOHEPATITIC HCC

Steatohepatitic HCC (SH-HCC) first described in 2010 by Salomao *et al*[28], and is a distinct histological subtype strongly associated with underlying steatosis and/or steatohepatitis and metabolic syndrome[28]. SH-HCC demonstrates morphological features similar to steatohepatitis with macrovesicular steatosis, hepatocellular ballooning with cytoplasmic clarification, Mallory-Denk bodies, pericellular fibrosis, and patchy inflammation (Figure 6A)[29]. The steatohepatitis should be a dominant part of the tumor morphology, and at least 50% of the tumor should show this pattern. Fibrosis can be best demonstrated on histochemical stain like Masson trichrome. The

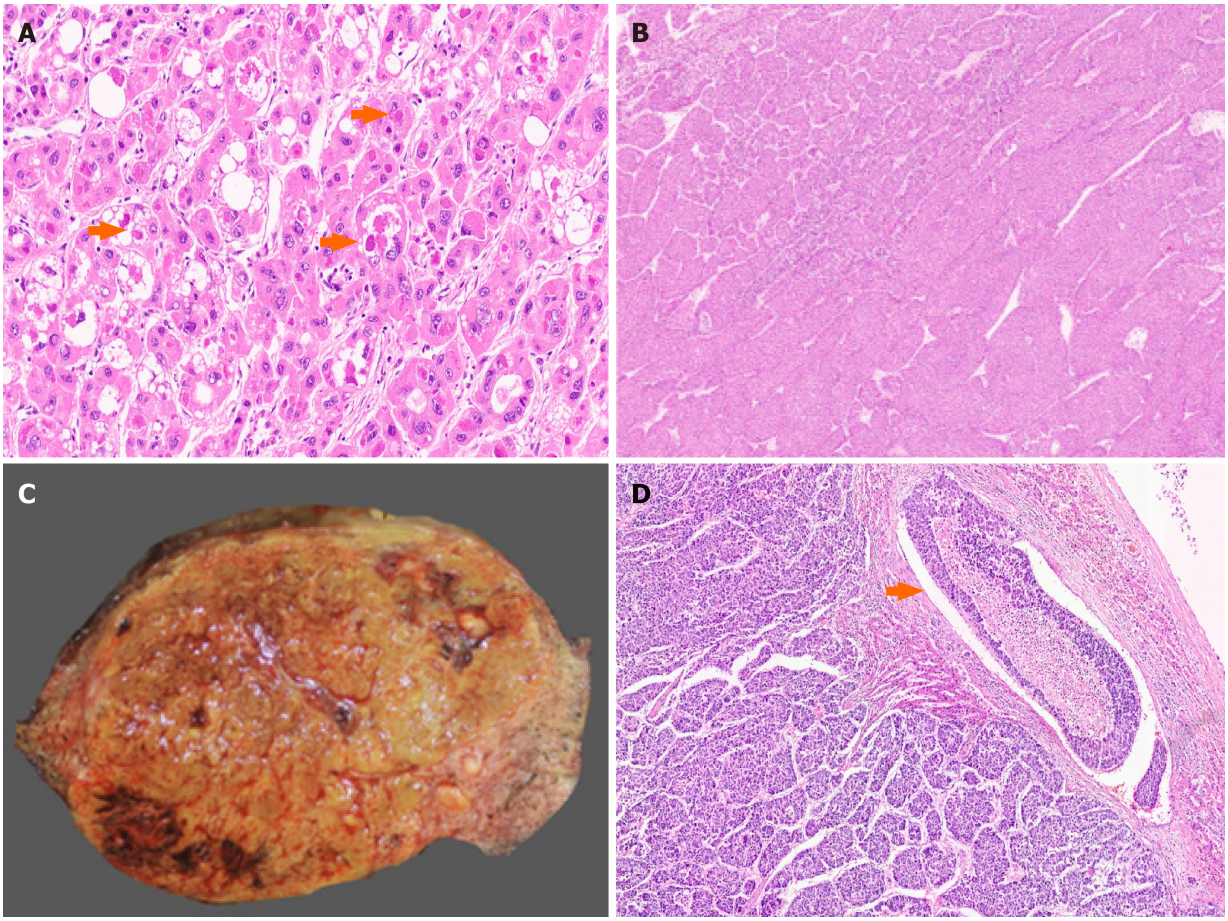

Figure 5 Conventional and macrotrabecular massive hepatocellular carcinoma. A: Hyaline globules in a conventional hepatocellular carcinoma (HCC) [arrow, hematoxylin and eosin (H&E)]; B: Macrotrabecular massive HCC (H&E); C: Large macrotrabecular massive HCC with satellite nodule; D: Macrotrabecular massive HCC with vascular invasion (arrow, H&E).

immunophenotyping of SH-HCC is similar to conventional HCC; however, it shows increased immunostaining with markers of inflammation like C-reactive protein due to interleukin (IL)-6/Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathway activation[21]. SH-HCC are well-differentiated to moderately differentiated tumors and are associated G4 transcriptomics subclass. In a recent transcriptomic analysis by Van Treeck *et al*[30] SH-HCC demonstrated a distinctive differential gene expression profile with upregulation of the sonic hedgehog signal transduction pathway based on GLI1 family zinc finger 1 (GLI1) overexpression. *GLI1* gene encodes a protein that functions as a transcription factor protein and plays a role in the regulation of stem cell proliferation. There was reduced expression of carnitine palmitoyltransferase 2 (CPT2) transcripts. CPT2 is a mitochondrial enzyme with an essential role in fatty acid β-oxidation and carnitine metabolism. In a mouse model of obesity-driven and non-alcoholic steatohepatitis-driven HCC, metabolic reprogramming mediated by the downregulation of CPT2 enables protection of neoplastic hepatocytes from lipotoxicity[31]. Therefore; reduced level of CPT2 is believed to facilitate survival of malignancy in obesity-associated HCC. Lee *et al*[32] recently suggested that alteration of the tumor stroma might play an important role in SH-HCC development, and as compared to classical HCC, cancer-associated fibroblasts in SH-HCC and non-tumoral stellate cells were characterized by increased expression of IL-6, a key governor of the JAK/STAT pathway[32]. SH-HCC appears to have similar overall and disease-free survival, development of metastasis, or local recurrence compared with conventional HCC[29].

SCIRRHOUS HCC

Scirrhous HCC represent approximately 5% of all cases[33]. Radiologic findings are atypical and often show arterial phase peripheral enhancement and venous phase

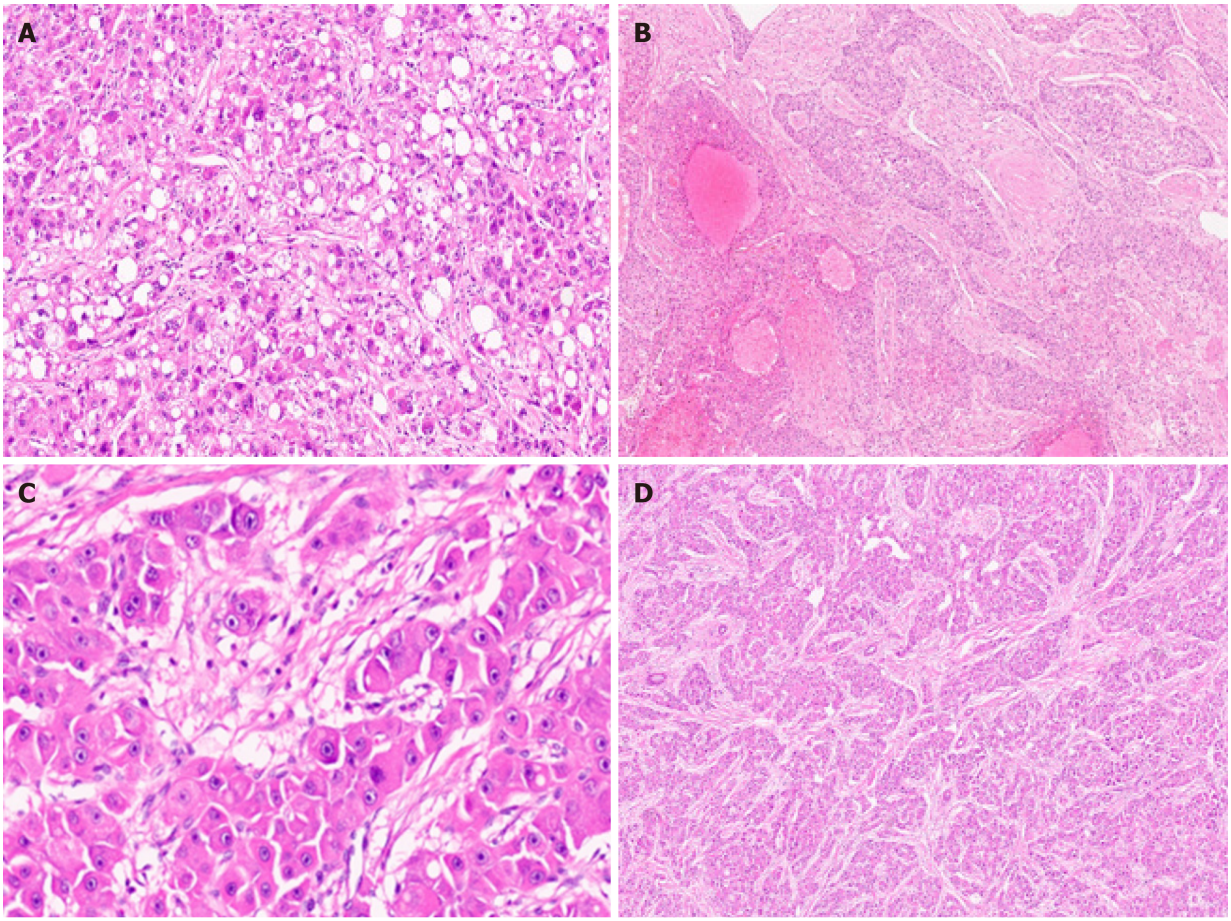

Figure 6 Hepatocellular carcinoma subtypes. A: Hepatocellular carcinoma (HCC) with steatohepatitic pattern [hematoxylin and eosin (H&E)]; B: Sclerotic HCC (H&E); C: Fibrolamellar HCC with large cells and prominent nucleoli (H&E); D: Fibrolamellar HCC with lamellar fibrosis (H&E).

persistent enhancement[34]. Scirrhous HCC is characterized by tumor cell clusters surrounded by abundant fibrous stroma which should constitute at least 50% of the tumor (Figure 6B)[11]. The presence of marked intratumoural fibrosis may lead to a faulty impression of intrahepatic CCA on radiology and macroscopic examination. Scirrhous HCC are mostly well to moderately differentiated HCC. Steatosis, clear cell change, pale bodies, and hyaline bodies have also been reported. Immunohistochemically, there is lack of positive staining for primary hepatocellular stains like HepPar-1 and pCEA in more than 60% of scirrhous HCC, with arginase and glypican 3 positivity in around 80% of cases[35]. Immunostains used for adenocarcinoma, like cytokeratin (CK) 7, CK19, and epithelial cell adhesion molecule, are positive in more than 60% of cases and can lead to erroneous diagnosis of adenocarcinoma[36]. Scirrhous HCC may resemble fibrolamellar HCC histologically, and molecular testing for DNAJ heat shock protein family member B1 (DNAJB1) and protein kinase 3'-5'-cyclic adenosine monophosphate (cAMP)-activated catalytic subunit alpha (PRKACA) fusion can be performed in histologically difficult cases[37]. There is no significant difference in prognosis in Scirrhous HCC compared with conventional HCC[38]. Expression of various cholangiocarcinoma-like and stem-cell-like genomic traits, including CK7 (KRT7), CK19 (KRT19), THY1, and CD133/Prominin-1, have been reported in scirrhous-HCC, and it has therefore been suggested that scirrhous HCC harbour intermediate molecular features, between HCC and cholangiocarcinoma[4,39]. Scirrhous HCC genomic profile also shows activation of transforming growth factor beta pathway/epithelial-to-mesenchymal transition related genes, with overexpression of Vimentin, SNAIL family transcriptional repressor 1, SMAD family member 4, and Twist-related protein[21].

FIBROLAMELLAR HCC

Fibrolamellar HCC (FL-HCC) is a rare and unique histologic subtype of liver cancer

with a predilection for adolescent and young adults (male:female, 1:1) without underlying liver disease, a characteristic morphological pattern with large neoplastic cells, distinct immunostaining, and recurrent genomic abnormalities typically involving PRKACA[40]. FL-HCC comprises approximately only 1% of primary liver cancer[41]. FL-HCC commonly presents as an abdominal mass with enlargement of liver, pain in abdomen, and features of biliary obstruction secondary to external compression by the mass lesion[42]. Rarely FL-HCC can present with paraneoplastic manifestations. These tumors are mostly solitary, large, and well circumscribed grossly with a yellow tan colored cut surface, and areas of central scarring are identified in almost 70% of cases[43,44]. Importantly, FL-HCC are much more likely to invade regional lymph nodes. Histologically, the tumor cells are large, polygonal with abundant eosinophilic granular cytoplasm (because of numerous mitochondria), centrally located nuclei with vesicular chromatin, and prominent nucleoli (Figure 6C). Focal bi-or multi-nucleation are also reported. Dense bands of intratumoural fibrosis arranged in lamellar (parallel arrangement) pattern separates the trabeculae and clusters of tumor cells (Figure 6D). FL-HCC also show presence of pale or hyaline bodies; however, these are not specific and may be observed in conventional HCC. Immunophenotyping shows neoplastic cells are positive of CD68 and CK-7 (biliary lineage) apart from markers of hepatic differentiation (Arginase 1, Hep-Par 1 and albumin mRNA as detected by *in situ* hybridization). Honeyman *et al*[37] first reported a specific 400-kilobase deletion on chromosome 19 in FL-HCC leading to recurrent chimeric *DNAJB1-PRKACA* gene fusion, genetic footprint of FL-HCC. DNAJB1 encodes a member of heat shock protein 40 which is involved in protein folding within cells, while PRKACA codes for the cAMP-dependent protein kinase catalytic subunit alpha; the molecular alteration results in upregulation of PRKACA activity by a promoter switch mechanism[45,46]. Both fluorescence *in situ* hybridization (FISH) or reverse transcription polymerase chain reaction are available now to detect DNAJB1-PRKACA fusion for confirming the diagnosis of FL-HCC. Recently, the genetic alteration (*DNAJB1-PRKACA* gene fusion) has also been identified in a set of oncocytic pancreaticobiliary neoplasm; however, DNAJB1-PRKACA fusion is still the most accurate test when the diagnosis of FL-HCC is doubtful[47,48]. FL-HCC has a unique gene expression profile, with Erb-b2 receptor tyrosine kinase (ERBB) 2 overexpression and glycolysis upregulation leading to compensatory mitochondrial hyperplasia, and various neuroendocrine genes, including Proprotein Convertase Subtilisin/Kexin Type 1, Neurotensin, Delta/Notch Like EGF Repeat Containing and Calcitonin Related Polypeptide Alpha[49].

LYMPHOEPITHELIOMA-LIKE HCC

Lymphoepithelioma-like HCC (LEL-HCC) also known as lymphocyte-rich-HCC is an uncommon variant of HCC and comprises < 1% of primary liver cancer[11]. LEL-HCC are associated with lower rates of recurrence after surgery and has an overall favorable survival rate when compared with conventional HCC[50]. LEL-HCC morphologically resembles lymphoepithelioma-like carcinomas, a poorly differentiated epithelial tumor first described in nasopharynx, characterized by a prominent immune stroma/microenvironment[4]. Subsequently it has been diagnosed in various organs such as stomach, colon, salivary glands, lungs, thymus, uterus, and ovaries[51]. These liver tumors are composed of poorly or undifferentiated neoplastic epithelial cells with a prominent lymphoid infiltrate[52]. A study of 11 cases of LEL-HCC by Wada *et al*[53] proposed quantitative criteria > 100 tumor infiltrating lymphocytes in 10 high power microscopic filed to define significant lymphocytic infiltration[53]. WHO defines LEL-HCC subtype as the condition in which lymphocytes outnumber pleomorphic neoplastic cells in most microscopic fields, but no clear cutoffs for lymphocyte number has been provided[10]. In contrast to LEL cholangiocarcinoma, which are frequently associated with EBV infection and are well described in literature, LEL-HCC are not associated with EBV infection and are not well characterized in literature[52,54,55]. Grossly, these are well circumscribed tumors with variable encapsulation. Histologically, the tumors are composed of atypical cells with syncytial cytoplasm and nuclei with prominent nucleoli and infiltrated by abundant lymphocytes (Figure 7A). Tumor cells show positivity for markers like Hep-Par 1 and Glypican 3 indicating hepatocellular origin. Immunohistochemical profile of the infiltrating immune cells shows a predominance of cytotoxic CD8+ lymphocytes[52]. Rare molecular studies are available on LEL-HCC. A recent study by Chan *et al*[56] showed marked focal amplification of chromosome 11q13.3 in LEL-HCC. Calderaro *et al*[57] showed high level

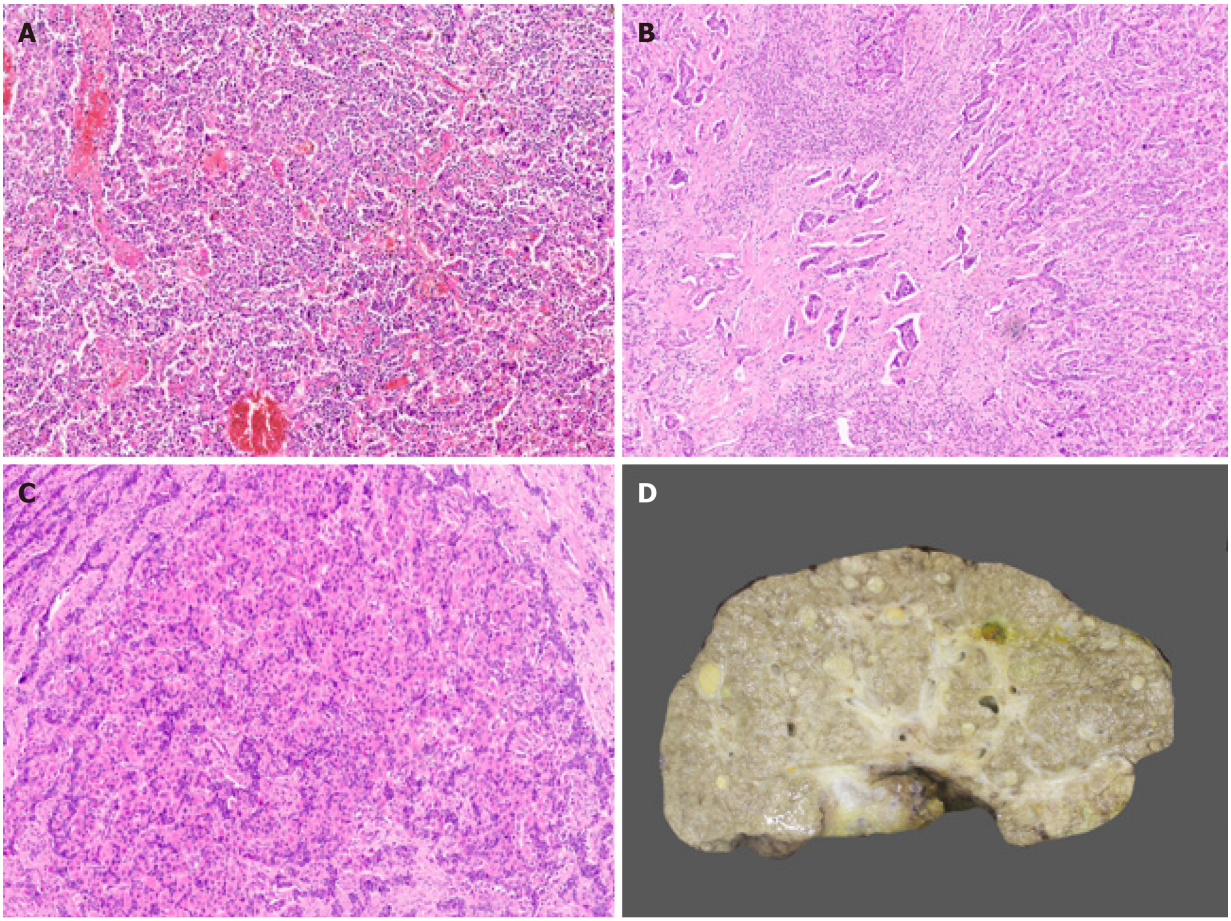

Figure 7 Hepatocellular carcinoma subtypes. A: Lymphoepithelioma like hepatocellular carcinoma (HCC) [hematoxylin and eosin H&E)]; B: Combined hepatocellular-cholangiocarcinoma (cHCC-CCA) with hepatocytic and cholangiocytic component (H&E); C: cHCC-CCA with stem/progenitor cell features (H&E); D: Cirrhotomimetic HCC with numerous tumor nodules.

of PD-L1 and programmed cell death 1 expression in intratumoural inflammatory cells in LEL-HCC. These findings indicate LEL-HCC might be sensitive to drugs targeting immune checkpoint inhibitors. No association of LEL-HCC with a transcriptomic subclass has been identified. Immune class of HCC reported by Sia *et al*[58] characterized by markers of an adaptive T-cell response or exhausted immune response was also not associated with increased number of somatic mutations[58].

PROGENITOR HCC

The progenitor subtype of HCC is defined by the immunohistochemical expression of biliary marker CK19, in more than 5% of neoplastic cells[59,60]. Dedifferentiation of malignant hepatocytes or malignant transformation of hepatic progenitor/stem cells may give rise to this histological subtype[4]. There is growing evidence that progenitor cells, activated during acute and CLD, can directly give rise to HCC. This phenotype is associated with mutation in TP53 and particular genomic subclasses (GI-G3, S2) of HCC[21]. CK19 expression is also reported in HCC after transarterial chemoembolization[61].

COMBINED HEPATOCELLULAR-CHOLANGIOCARCINOMA

Combined hepatocellular-cholangiocarcinoma (cHCC-CCA) is a rare primary liver cancer. Diagnosis of cHCC-CCA is challenging because of its pathological heterogeneity, unique molecular alterations, poorly defined radiological features, and non-specific clinical features. The WHO 2010 Classification defined a classical type of cHCC-CCA (tumor containing unequivocal, intimately mixed elements of both HCC

and intrahepatic CCA), and 3 subtypes of cHCC-CCA with stem/progenitor cell features: Typical, intermediate cell, and cholangiocellular[62]. The WHO consensus classification published in 2019 removed the 3 different stem/progenitor cell subtypes and defined cHCC-CCA as a primary liver carcinoma with unequivocal presence of both hepatocytic and cholangiocytic differentiation (Figure 7B) within the same tumor[63]. This change was implemented because "stem/progenitor cells" identified as small cells with scant cytoplasm, a high nuclear/cytoplasmic ratio, and hyperchromatic nuclei may potentially be seen in all forms of cHCC-CCA; cholangiocellular carcinoma is not always associated with hepatocellular component and subtyping has no prognostic or clinical relevance.

The hepatocellular and cholangiocarcinoma components in cHCC-CCA may be intimately mixed or lie in separate regions of a tumor. Collision of HCC and iCCA arising separately in the same liver should not be included under cHCC-CCA. The diagnosis of cHCC-CCA should be based on hematoxylin and eosin staining only and immunophenotyping can be performed to confirm histologic components. However, IHC alone should not define the diagnosis of cHCC-CCA[64]. Stem/progenitor cell features (Figure 7C) can be mentioned in the comment section of the histology report. Intermediate cell carcinoma is a unique form of cHCC-CCA comprising of monomorphic tumor cells, smaller than hepatocytes but larger than stem/progenitor cells, and has features intermediate between hepatocytes and cholangiocytes. These malignant cells are arranged in strands or trabeculae in an abundant fibrous stroma. Molecular studies of cHCC-CCA are limited and the earlier reported literature suggested that these tumors have a distinct mutational profile with isocitrate dehydrogenase (IDH) mutations usually observed in intrahepatic CCA[4,65]. However this remains debated as a recent study performed by Joseph *et al*[66] demonstrated that the genetics of cHCC-CCA classical type, are distinct from intrahepatic CCA but similar to conventional HCC with alteration in telomerase reverse transcriptase (TERT), p53, and cell cycle genes[66]. Few studies have also reported enrichment in stem/progenitor-like signatures, supporting the concept of a stem/progenitor cell origin of cHCC-CCA[67]. cHCC-CCA has a dismal prognosis, worse than that of either HCC or iCCA, and currently, there are no accepted international management guidelines for cHCC-CCA.

RARE AND PROVISIONAL PATHOLOGICAL SUBTYPES OF HCC

These pathological subtypes are rare and provisional because limited published literature is available.

FIBRONODULAR HCC

Fibronodular HCC (FN-HCC) is a recently described candidate variant[68]. FN-HCC histology is characterized by extensive fibrosis dividing a single tumor into multiple well circumscribed distinct nodules with no significant intranodular fibrosis between single or clusters of neoplastic cells[54]. These tumors show well to moderate differentiation with trabecular or solid growth pattern. Scattered pseudoacini are also described. FN-HCC are reported to be more likely to arise in liver with lower fibrosis stage and lower advanced BCLC stage. They have lower rates of tumor progression. Imaging analysis of FN-HCCs revealed higher rates of non-peripheral washout and a new distinct pattern of enhancement which is characterized by the presence of multiple rounded nodules within a lesion embedded in fibrotic-appearing parenchyma, called as 'popcorn' appearance of the lesion[68].

CHROMOPHOBE HCC WITH ABRUPT ANAPLASIA

This histological subtype is characterized by a unique set of morphological features: smooth chromophobic cytoplasm which can be either slightly eosinophilc or basophilic, abrupt focal nuclear anaplasia (small tumor cell clusters with marked nuclear anaplasia in a background of tumor cells with bland round nuclei and inconspicuous nucleoli), and scattered microscopic pseudocysts[9,69]. This subtype is associated with distinct molecular features with respect to telomere maintenance resulting in alternative lengthening of telomeres (ALT), which can be detected by

telomere FISH. ALT is a telomerase-independent mechanism of telomere maintenance and is found in > 90% of chromophobe HCC with abrupt anaplasia and < 10% of unselected HCCs. Wood *et al*[69] also investigated somatic mutations of alpha-thalassemia/mental retardation, X-linked, Histone H3, and Death Domain Associated Protein identified in various ALT positive tumors reported at other sites in two cases of chromophobe HCC with abrupt anaplasia; however, no mutations were identified[69-71].

GRANULOCYTE COLONY-STIMULATING FACTOR PRODUCING HCC/NEUTROPHIL-RICH HCC

This rare subtype is characterized by production of granulocyte colony-stimulating factor (G-CSF), leading to diffuse infiltrates by neutrophils[72-74]. There is no clear histological definition for this variant. Morphologically, these tumors are poorly differentiated HCC, usually with areas of sarcomatous differentiation and numerous neutrophils. These generally occur in older individuals, grow rapidly, have a high probability of distant metastases, and the overall prognosis seems to be poor as compared with conventional HCC. The mechanism of the production of G-CSF in HCC remains unclear; a close relationship between G-CSF production in malignant cells and their dedifferentiation has been reported[74].

LIPID-RICH HCC

Lipid-rich HCCs have a foamy cytoplasm resulting from lipid accumulation, with numerous very tiny droplets of fat[75-77]. These can be associated with few larger fat droplets. The differential includes lipid-rich variants of metastatic carcinoma. Immunostaining with Hep-Par 1 and Arginase is helpful in doubtful cases.

CIRRHOTOMIMETIC OR DIFFUSE CIRRHOSIS LIKE-HCC

Cirrhotomimetic (CM) or diffuse cirrhosis like-HCC is a rare variant of liver cancer characterized by small cirrhosis-like tumor nodules that are intimately admixed within the cirrhotic liver parenchyma[78-81]. This tumor pattern is often diagnosed incidentally on the native liver explanted at the time of transplantation or autopsy liver specimen, as most of the times, it is clinically and radiologically undetectable (Figure 7D). These tumors are well to moderately differentiated and majority of patients show no significant elevation in serum AFP values[79]. Pseudoacinar architectural growth pattern with bile production and numerous Mallory-Denk bodies have been demonstrated in these tumors. Few studies have investigated tumor nodules in CM-HCC and suggested that these are synchronous multiclonal HCCs[82,83]. One recent study evaluated the liver explants post transcatheter arterial chemoembolization in CM-HCC and non-CM-HCC and reported lower rates of complete pathologic necrosis and poorer overall survival in CM-HCC after liver transplantation as compared with non-CM-HCCs[84].

CLEAR CELL HCC

Clear cell HCC is an uncommon histological variant of HCC. WHO defines this tumor as the condition when > 80% of the neoplastic cells show clear cell morphology[10]. Glycogen accumulation leads to clearing of the cytoplasm; admixed minor steatosis is also acceptable. These are well to moderately differentiated tumors with similar or better prognosis than conventional HCC[85-87]. There is, however, no distinct definition of this subtype and clear cells may be observed in other subtypes.

HEPATIC CARCINOSARCOMA

Hepatic carcinosarcomas are composed of both malignant epithelial component and

mesenchymal components[9]. These neoplasms are extremely rare. The carcinomatous component is moderately to poorly differentiated HCC. The sarcomatous component shows morphologic or immunohistochemical evidence of mesenchymal differentiation, such as leiomyosarcoma, rhabdomyosarcoma, chondrosarcoma, fibrosarcoma, or rarely osteosarcoma. There is scant data on molecular alterations[88,89]. One earlier study revealed mutation in TP53, Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha and FGFR3 genes[88]. One recent study using targeted next-generation sequencing with a panel of 329 cancer-related genes identified TP53, Neurofibrin 1/2 mutations, and VEGFA amplification in both carcinomatous and sarcomatous components[89]. Amplifications involving MET and platelet-derived growth factor receptor A were identified only in the sarcomatous components, whereas mutation affecting ERBB4 and amplifications of Cyclin D1 and FGF 3/4/19 were present only in the carcinomatous components.

MYXOID HCC

This rare morphological subtype of HCC shows well to moderately differentiated neoplastic cells with a trabecular growth pattern, separated by abundant extracellular myxoid/mucin material[9,90]. The neoplastic cells stain strongly with HepPar1 and Arginase-1, and are negative for biliary marker CK19. These tumors typically show loss of liver fatty acid binding protein and also immunostaining with strong and diffuse positivity for GS.

CONCLUSION

Pathology of HCC has evolved significantly in the last two decades. We are now well versed with various dysplastic liver lesions and multiple distinct pathologic subtypes of HCC. There is also remarkable improvement in our understanding of HCC pathogenesis as tumor genome sequencing has identified recurrent molecular alterations and oncogenic pathways and how this correlates with various morphological findings. Identification of genetic alterations also gives us an opportunity to develop targeted therapies that can prevent recurrence and improve patient survival.

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MINIREVIEWS

Infantile giant cell hepatitis with autoimmune hemolytic anemia

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Abstract

Giant cell hepatitis (GCH) is characterized by large and multinucleated (syncytial) hepatocytes in the context of liver inflammation. Infantile GCH is typically associated with autoimmune hemolytic anemia in the absence of any other systemic or organ-specific autoimmune comorbidity. The etiology is unknown; concomitant viral infections (as potential trigger factors) have been identified in a few patients. The pathogenesis reportedly relies upon immune-mediated/autoimmune mechanisms. This condition should be considered in any infant developing Coombs-positive anemia; indeed, anemia usually precedes the development of hepatitis. The clinical course is usually aggressive without the appropriate immunosuppressive therapy, which may include steroids, conventional immunosuppressors (*e.g.*, azathioprine and cyclophosphamide as first-line treatments), intravenous immunoglobulin, and biologics (rituximab). Improvements in medical management (including the availability of rituximab) have significantly reduced the mortality of this condition in the last decade.

Key Words: Giant cell hepatitis; Autoimmune hemolytic anemia; Rituximab; Infantile hepatitis; Jaundice; Hyperbilirubinemia

Core Tip: This review discusses the main characteristics of giant cell hepatitis associated with autoimmune hemolytic anemia including etiology, pathogenesis, pathophysiology, clinical aspects, prognosis, and therapy. All of the available case reports and case series have been considered to provide an overall picture of this disease and its general clinical management.

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INTRODUCTION

Giant cell hepatitis (GCH) refers to a histopathological picture of liver inflammation characterized by large and multinucleated (syncytial) hepatocytes; ≥4-5 nuclei can be seen in the affected cells of the liver parenchyma, along with other features of hepatitis such as lobular fibrotic rearrangements, Kupffer cell hypertrophy, and spotty necrosis[1,2].

In adults, GCH is rare; indeed, it is mainly observed and described in children, mostly in the first years of life. The giant cell transformation of hepatocytes is considered as an altered/dysfunctional regenerative response of hepatocytes in the context of different underlying liver diseases, such as chronic autoimmune hepatitis (AIH), and/or exposure to various noxious agents including drugs and viral infections[2-4].

The histopathological finding of partial or diffuse giant cell transformation of hepatocytes is more frequent in infantile, and in particular, neonatal hepatitis. Indeed, GCH is considered in the differential diagnosis of neonatal cholestasis, where biliary atresia and idiopathic/GCH account for 70% to 80% of all cases; the diagnostic work-up usually includes liver biopsy to achieve a complete and final diagnosis[1,5,6]. GCH is also associated with congenital atresia, and thus, both conditions may coexist. However, neonatal GCH has been described in patients with pathological non-obstructive neonatal jaundice (*e.g.*, blood group incompatibility, hereditary spherocytosis), congenital syphilis, perinatal hemochromatosis, viral infections (*e.g.*, cytomegalovirus, rubella) and metabolic diseases[7,8]. Torbenson *et al*[1] analyzed the etiology of GCH in 62 newborns: 49% of cases were idiopathic, whereas the remaining patients were variably affected with hypopituitarism (15%), biliary atresia (8%), Alagille syndrome (6%), progressive familial intrahepatic cholestasis or other bile salts defects (6%), neonatal hemochromatosis (5%), viral infections (4%), and other diseases (8%, *i.e.* cystic fibrosis, alpha-1-antitrypsin deficiency, severe combined immuno-deficiency, AIH)[1].

Infantile GCH is rarely described in patients with post-neonatal hepatitis, and interestingly, is typically associated with autoimmune hemolytic anemia (AHA); this condition is mostly diagnosed in children aged 1 mo to 2 years[9]. Such a pathological association is unusual in post-infantile (childhood and adult) GCH. Indeed, Coombs-positive anemia is found in < 10%-15% cases[10]. Infantile GCH + AHA, as a specific disease pattern, was first recognized in 1981 by Bernard *et al*[11], who described 4 children developing chronic AHA combined with liver disease, which was histologically characterized by severe hepatitis with "diffuse giant cell transformation and extensive fibrosis"[11].

ETIOLOGY AND PATHOGENESIS

The etiology of GCH + AHA is unknown and specific and/or clear trigger factors have not been identified. Indeed, no individual etiological clues have been identified in most patients, except for some cases in whom viral infections (*e.g.*, paramyxoviruses, varicella-zoster virus, cytomegalovirus) have been reported[4,12-14].

The pathogenesis of GCH + AHA reportedly relies on immune mediated/autoimmune mechanisms, even though this was not included in the classification of pediatric autoimmune liver diseases, according to a recent European Society for Paediatric Gastroenterology Hepatology and Nutrition hepatology committee position statement, which considered three liver disorders: AIH, autoimmune sclerosing cholangitis, and *de novo* AIH after liver transplant[15]. However, several clinical and pathological findings suggest the involvement of immunological mechanisms in infantile GCH, in addition to the AHA comorbidity by itself. Indeed, Nastasio *et al*[16] summarized these aspects, including the response to immunosuppressive therapies, the evidence of complement-mediated (C3a- and C5a-driven) hepatocyte injury and liver inflammation, and the sporadic association with autoimmune diseases other than

AHA[16]. Importantly, the typical histological features of GCH + AHA differ from those described in the aforementioned "classical AIH," and in fact, autoimmune liver disease-related autoantibodies are absent. However, a "strong immune/autoimmune component" characterizes the pathogenesis of GCH + AHA[17].

Interestingly, Whitington *et al*[18] emphasized that the histopathology of GCH + AHA is similar to that of Gestational Alloimmune Liver Disease (GALD), which accounts for most cases of neonatal hemochromatosis, characterized by a prominent liver giant cell transformation as well[18,19]. The authors showed that, unlike AIH patients, children with GCH + AHA had diffuse and intense C5b-9 complex deposition in the liver, suggesting that the giant cell transformation in these patients was the result of complement-mediated hepatocyte injury, similar to GALD fetuses and newborns, in whom immunoglobulin G-induced complement-mediated hepatocyte injury has been demonstrated[20,21].

These observations support the fact that GCH + AHA is an autoimmune disease in which giant cell transformation is an "unspecific" reactive response to antibody- and complement-mediated hepatocyte injury. Both hepatitis and Coombs-positive anemia may be consequences of a common systemic B cell immune dysregulation leading to autoantibody production.

PATHOPHYSIOLOGY AND CLINICAL ASPECTS

In general, GCH + AHA should be suspected in any child aged 1 mo to 2 years, who presents with severe liver disease and anemia. The median age of the onset is about 8 mo, and thus, most cases manifest before 1 year of age[22]. Both males and females can be affected, without a clear gender preponderance; in the largest cases series published by Maggiore *et al*[9], there were 9 male and 7 female patients[9]. If all other case reports and small case series are considered (Table 1)[9,11-14,18,22-41], among the 51 reported patients with infantile GCH + AHA, 25 were female and 19 were male (no gender specification was available for 7 patients).

In detail, GCH should be considered in any infant developing Coombs-positive AHA, especially if jaundice is direct, namely characterized by a component of conjugated bilirubin > 20% of total bilirubin. Indeed, AHA and, in general, all hemolytic anemia cases usually show jaundice deriving from the accumulation of indirect bilirubin, because its excessive production (due to the increased heme catabolism) cannot be readily cleared from the bloodstream and metabolized by the liver[42]. In summary, whereas isolated AHA (which may also show mild-moderate increase of liver enzymes) is characterized by indirect jaundice, GCH is accompanied by clear signs of cholestasis, and thus direct jaundice, in addition to the fact that the increase in liver enzymes is usually very pronounced.

Indeed, the increase in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) is usually at least 15-20 times higher than the upper normal limit of the respective age-related reference range[9], but cases with milder liver enzyme elevation (< 5-10 times the upper normal limit) have been described, especially in the initial phases of hemolytic disease[31,37,39]. In this regard, the development of anemia usually precedes the onset of liver disease by a variable period of time, ranging from 1 mo to > 1 year. Therefore, the diagnosis of GCH + AHA often follows a previous diagnosis of isolated AHA[31].

Moreover, the increase in gamma-glutamyl transferase (GGT) is not usually very pronounced and is often not greater than 2-3 times the age-related upper normal limit[9,38]. Such a GGT increase, especially when associated with mild to moderate AST/ALT abnormalities, could be consistent with several common infectious illnesses (*e.g.*, cytomegalovirus, Epstein-Barr virus, mycoplasma pneumoniae)[43-45], which may also trigger immune-mediated hemolytic diseases, and thus, should be appropriately excluded through diagnostic work-up[46,47].

Therefore, the measurement of ALT, AST, and GGT is recommended in all young children diagnosed with AHA at the onset and during follow-up of the disease. If liver enzymes are highly and/or persistently elevated without any clear (infectious) explanation, these children should undergo liver biopsy to assess the liver histopathological features, and in detail, whether GCH is present[9]. In addition to a histopathological picture inconsistent with AIH, these patients are serologically negative for significant titers of anti-mitochondrial, anti-smooth muscle, anti-liver kidney microsomal autoantibodies, and anti-nuclear antibodies[31].

Table 1 Overview of the demographic features and outcome in patients with infantile giant cell hepatitis and autoimmune hemolytic anemia

Ref.	Clinical cases, n	Age in mo	Gender	Outcome	Rituximab	Follow-up in mo	Cause of death
Before 2011 (case reports/series providing individual data on infantile GCH + AHA)							
Bernard *et al*[11], France, 1981	1	10	F	Fatal	N	-	Liver failure
	2	9	M	Fatal	N	-	Liver failure
	3	24	M	Fatal	N	-	Liver failure
	4	6.5	M	Alive	N	N/A	-
Imgrueth *et al*[23], Switzerland, 1986	5	5	F	Alive	N	9	-
	6	8	M	Alive	N	24	-
Brichard *et al*[24], Belgium, 1991	7	7	F	Fatal	N	-	Encephalopathy
Weinstein *et al*[25], United States, 1993	8	5	F	Alive	N	30	-
Perez-Atayde *et al*[26], United States, 1994	9	23	F	Fatal	N	-	Sepsis
	10	9	M	Alive	N	8	-
Choulot *et al*[27], France, 1996	11	15	M	Alive	N	144	-
Melendez *et al*[28], United Kingdom, 1997	12	8	M	Fatal	N	-	Liver and renal failure
Hartman *et al*[29], Israel, 2001	13	6	M	Fatal	N	-	Liver failure
Gorelik *et al*[30], United States, 2004	14	4	F	Alive	Y	36	-
Kashyap *et al*[31], India, 2006	15	4	F	Alive	N	2	-
Vajro *et al*[12], Italy, 2006	16	10	F	Alive	N	36	-
Miloh *et al*[32], United States, 2007	17	2	M	Alive	Y	24	-
Rovelli *et al*[33], Italy, 2007	18	14	M	Alive	Y	48	-
Baran *et al*[13], Turkey, 2010	19	3	F	Fatal	Y	-	Sepsis and renal failure
Ünal *et al*[14], Turkey, 2010	20	2	F	Fatal	Y	-	Sepsis
	21	6	M	Fatal	N	-	N/A
	22	11	M	Alive	Y	18	-
Maggiore *et al*[9](largest case series providing aggregate data on infantile GCH + AHA)							
Maggiore *et al*[9], Italy, 2011	16 cases	2.5-17	M (*n* = 9); F (*n* = 7)	Alive (*n* = 12); Fatal (*n* = 4)	Y (*n* = 2); N (*n* = 10)	2-28 yr	OLT (*n* = 1); Sepsis (*n* = 3)

After 2011 (case reports/series providing individual data on infantile GCH + AHA)							
Raj *et al*[22], United States, 2011	1	6	F	Alive	N	30	-
Lega *et al*[34], Italy, 2013	2	8	M	Alive	N	6	-
Bouguila *et al*[35], Tunisia, 2013	3	9	N/A	Fatal	N	-	Sepsis
Whitington *et al*[18], Canada & United States, 2014	4	22	F	Alive	Y	48	-
	5	14	F	Alive	Y	48	-
	6	6	F	Alive	N	48	-
	7	4	F	Fatal	N	-	N/A
	8	6	M	Alive	Y	36	-
Bakula *et al*[36], Poland, 2014	9	7	N/A	Alive	Y	30	-
	10	8	N/A	Alive	Y	26	-
	11	2	N/A	Alive	Y	5	-
	12	12	N/A	Alive	Y	76	-
	13	7	N/A	Fatal	N	-	Hemophagocytosis (after HSCT)
Paganelli *et al*[37], Italy, 2014	14	3	F	Alive	Y	N/A[1]	-
	15	14	F	Alive	Y	N/A[1]	-
	16	12	F	Alive	Y	N/A[1]	-
	17	16	M	Alive	Y	N/A[1]	-
Marsalli *et al*[38], Italy, 2016	18	5	F	Alive	N	N/A[1]	-
	19	8	M	Alive	N	N/A[1]	-
	20	10	F	Alive	N	N/A[1]	-
	21	10	F	Alive	N	N/A[1]	-
	22	6	F	Alive	Y	N/A[1]	-
	23	7	F	Alive	N	N/A[1]	-
	24	8	M	Alive	N	N/A[1]	-
Cho *et al*[39], South Korea, 2016	25	2	N/A	Alive	N	36	-
Matarazzo *et al*[40], Italy, 2020	26	5	F	Alive	Y	141	-

	27	9	F	Alive	Y	91	-
	28	8	M	Alive	Y	76	-
Kim *et al*[41], South Korea, 2020	29	7	M	Alive	Y	19	-

[1]The authors did not provide the follow-up length for individual patients; however, they provided general information on follow-up in their respective case series (Paganelli *et al*[37]: "At last follow-up visit, all patients were alive with their native liver 2 to 16 year after disease presentation"; Marsalli *et al*[38]: "Follow-up (median 17.4 mo, range 7-24 mo)." F: Female; HSCT: Hematopoietic stem cell transplantation; M: Male; N/A: Not available; N: No; OLT: Orthotopic liver transplantation; Y: Yes.

PROGNOSIS AND THERAPY

The clinical course of GCH + AHA is usually aggressive. According to the analysis of 22 cases reported to 2006, the mortality rate was about 45%. The Italian-French multicentric analysis including 16 pediatric patients (evaluated over a 28-year period and published by Maggiore *et al*[9]) reported a lower mortality rate (25%), probably due to a better therapeutic (*i.e.* immunosuppressive) approach over time. Indeed, if the cases reported after 2011 are specifically considered, only 3 of 29 patients died, which corresponds to an overall mortality rate as low as 10% (Table 1)[9,11-14,18,22-41]. The therapeutic regimens described in these case reports and small case series were widely heterogeneous. Such a discussion goes beyond the scope of this review, but it is worth mentioning that the biological therapy with rituximab was part of the treatment of many more patients after 2011 (rituximab used in 16 of 29 cases) compared to the previous period (rituximab used in 8 of 48 cases), which may have contributed to the reduced mortality in the cases described in the last decade. Indeed, despite an initial response to immunosuppressive therapy, relapses occur in many cases, and liver disease/failure is the main pathological component accounting for a negative prognosis. The hematological component is usually better controlled with immunosuppressive therapy. In fact, persistent and clinically relevant hemolysis has been described in a few patients, who required splenectomy and/or plasmapheresis to control a severe and resistant hematological condition[27,29].

In general, liver disease can be controlled in half of patients with initial immunosuppressive therapy, which may be withdrawn in very few patients. The remaining patients develop more severe disease, which is only partially responsive (or not responsive at all) to immunosuppressive therapy; in some of these cases, the clinical course is rapid and fatal, because of the liver failure by itself and/or its complications, such as severe seizures disorder/encephalopathy and/or concomitant infections[12,29]. Indeed, these children may also develop hemophagocytosis leading to a clinical picture of macrophage activation syndrome, as first described by Hartman *et al*[29].

In those clinical cases with the most severe prognosis, orthotopic liver transplantation (OLT) was also considered. However, the prognosis remained poor. In 1997, Melendez *et al*[28] revised 4 cases undergoing this procedure and 3 of them ultimately

died. Importantly, all of these patients showed recurrence of GCH in the transplanted liver within the first few months[28]. A positive transplantation outcome without relapse was described by Kerkar *et al*[48] in a patient developing progressive hepatic encephalopathy. However, despite the association with Coombs-positive anemia, this patient may have not been a case of infantile GCH + AHA, since he had positive anti-liver kidney microsome antibodies, and only partial/patchy giant cell transformation was observed in the liver. Moreover, anemia was associated with thrombocytopenia, suggesting the possibility of type 2 AIH or systemic autoimmune dysfunction leading to several organ immune-mediated disorders, as further supported by the appearance of bullous pemphigoid after liver transplant[48]. Due to constant disease relapses after OLT, such a therapeutic approach has been basically abandoned in the clinical setting of GCH + AHA[12].

Without rapid and appropriate immunosuppressive treatment, the liver function rapidly deteriorates in these patients with infantile GCH + AHA, leading to a progressive and fatal course, as already mentioned. The early institution of an aggressive steroids therapy usually has beneficial effects on both liver function and autoimmune hemolytic anemia. Combination therapy with steroids and azathioprine/cyclophosphamide is often the first-line therapy, which is able to significantly reduce mortality in the early phases of disease activity[31]. However, due to the frequent steroid-resistant cases and/or relapses after immunosuppression step-down/withdrawal, several and additional immunosuppressive agents have been variably used (based upon all the available case reports and series), including cyclosporine, tacrolimus, 6-mercaptopurine, mycophenolate and vincristine[12].

In addition to these immunosuppressive drugs, some immunomodulatory therapies have also been used[23,26,29]. In this regard, the first experiences included the use of intravenous immunoglobulins (IVIGs), which were administered according to variable therapeutic schemes, as reviewed by Lega *et al*[34]. Actually, these authors used a high-dose regimen (2 g/kg) that was repeated on a monthly basis for more than 6 mo, in association with immunosuppressive therapy[34]. Marsalli *et al*[38] focused their study on IVIG use and concluded that this treatment can help to significantly and rapidly reduce the activity of the liver disease, in combination with prednisone and other immunosuppressive therapies[38]. Some authors also reported the use of plasmapheresis[23,29,30].

However, as mentioned above, the most important advances in infantile GCH + AHA derived from the use of rituximab. In 2004, Gorelik *et al*[30] reported its use to treat the hematological component, but Miloh *et al*[32] first reported a GCH + AHA infant affected with severe liver disease resistant to steroids, azathioprine, sirolimus, and IVIG, who significantly improved after the therapy with rituximab[30,32]. Eventually, several authors reported the successful use of rituximab. For instance, Bakula *et al*[36] reported 4 GCH + AHA infants, who achieved complete remission with rituximab after the failure of the first-line therapy with steroids and azathioprine. Therefore, these authors and others proposed rituximab as the treatment of choice for the early stages of the disease[36,40]. Indeed, unresponsiveness to rituximab is suggested to be more likely when its use is delayed[13]. Additional experiences confirmed the safety and effectiveness of rituximab, even in association with other immunosuppressive agents (*e.g.*, cyclosporine). Moreover, the early treatment could reduce the use of steroids and, thus, prevent several side effects[37]. In some patients, rituximab induced a complete and long-lasting remission and allowed the discontinuation of all immunosuppressive drugs[40]. To conclude, Rovelli *et al*[33] reported a positive result by using alemtuzumab, which is a humanized monoclonal antibody directed against CD52 (cluster of differentiation 52, a glycoprotein expressed on circulating T and B lymphocytes and natural-killer cells). Even though long-term remission of the liver disease was reported in this case of GCH + AHA, to date, this is the only experience with alemtuzumab.

CONCLUSION

Infantile GCH is a clinical condition that should be considered in any infant developing Coombs-positive anemia, in the presence of significant abnormalities of liver function tests and direct hyperbilirubinemia. Indeed, anemia usually precedes the development of hepatitis. This clinical condition requires timely and appropriate immunosuppressive therapy, which may include steroids, conventional immunosuppressors, intravenous immunoglobulin, and biologics (rituximab). Improvements in the medical management (including the availability of rituximab)

have significantly reduced the mortality of this condition in the last decade.

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MINIREVIEWS

Long-term albumin infusion in decompensated cirrhosis: A review of current literature

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Abstract

Decompensated cirrhosis is characterized by chronic inflammation and severe portal hypertension leading to systemic circulatory dysfunction. Albumin infusion has been widely used in decompensated cirrhosis in patients with spontaneous bacterial peritonitis, large-volume paracentesis and hepatorenal syndrome. Emerging data suggest long-term albumin infusion has both oncotic and non-oncotic properties which may improve the clinical outcomes in decompensated cirrhosis patients. We review the current literature on both the established and potential role of albumin, and specifically address the controversies of long-term albumin infusion in decompensated cirrhosis patients.

Key Words: Albumin; Cirrhosis; Hepatic encephalopathy; Hepatorenal syndrome; Acute-on-chronic liver failure; Spontaneous bacterial peritonitis; Large-volume paracentesis

Core Tip: Decompensated cirrhosis is characterized by chronic inflammation and severe portal hypertension leading to systemic circulatory dysfunction. Albumin infusion has been widely used in decompensated cirrhosis in patients with spontaneous bacterial peritonitis, large-volume paracentesis and hepatorenal syndrome. Emerging data suggest long-term albumin infusion has both oncotic and non-oncotic properties which may improve the clinical outcomes in decompensated cirrhosis patients.

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INTRODUCTION

Long-term albumin infusion in decompensated cirrhosis: A critical review of current literature

Concept of compensated and decompensated cirrhosis: Cirrhosis represents the common pathway of all chronic liver disease resulting in over a million deaths every year[1]. The natural history of liver cirrhosis includes an asymptomatic compensated stage and a decompensated cirrhosis stage with clinically overt complications as ascites, jaundice, variceal bleeding and hepatic encephalopathy (HE)[2]. The median survival reduces significantly from 12 to 2 years as patients progress from the compensated to decompensated cirrhosis at an annual rate of 5%-7%[2,3].

Decompensated cirrhosis is characterized by chronic inflammation and severe portal hypertension leading to systemic circulatory dysfunction[4]. As a corrective response to portal hypertension, excessive nitric oxide secretion results in both splanchnic and arterial vasodilatation, which thus impairs organ perfusion[5,6]. To ensure adequate organ perfusion, the arterial pressure is maintained by increased activity of the renin-aldosterone-angiotensin system[7]. The understanding of circulatory dysfunction in patients with decompensated cirrhosis has led to the use of albumin and vasoconstrictors to improve circulatory dysfunction and prevent kidney injury[8]. Such an approach is paramount because the presence of acute kidney injury (AKI) is associated with significantly longer hospitalization stay and higher mortality in patients with decompensated cirrhosis[9].

Human albumin has widely been used in decompensated cirrhosis patients for varying indications. While the established indications of albumin infusion as endorsed by the current societal guidance include spontaneous bacterial peritonitis (SBP), large-volume paracentesis (LVP) and hepatorenal syndrome (HRS)[10], albumin infusion is often used beyond these indications in the daily clinical practice. Although some of the recently published studies have reported the beneficial effect of regular long-term albumin infusion in patients with decompensated cirrhosis[11-13], regular long term albumin infusion is not completely innocuous. Not only is albumin more expensive than crystalloids as volume expander, serious adverse events as pulmonary oedema and even death have also been reported[14].

With this background, we aim to critically review the current literature on both the established and potential role of albumin, and specifically addressed the controversies of long-term albumin infusion in decompensated cirrhosis patients.

WHAT IS ALBUMIN?

Albumin is the main circulating protein in healthy adults. Structurally it is a small (66500 Dalton), negatively-charged protein that consists of 2 sub-domains[15,16]. Albumin is exclusively synthesized within the liver. It is up-regulated by hormones (insulin, cortisol and growth hormone)[17-19] and down-regulated by inflammatory mediators (tumor necrosis factor and interleukin-6)[20]. Once produced, up to 40% of albumin is released into the bloodstream. The half-life of albumin ranges from 12 to 19 d[21]. The degradation of albumin occurs mostly within the liver, kidney and muscle[22].

Function of albumin

Albumin has both oncotic and non-oncotic properties[15,23]. The potent oncotic property of albumin is primarily derived from the direct oncotic effect from high plasma concentration, which accounts for about two-thirds of its osmotic effect. The Gibbs-Donnan effect, where the negatively-charged albumin molecule also attracts positively charged molecules such as sodium within the bloodstream, is responsible for the remaining one-third of the osmotic effect of albumin[23].

Albumin transports hydrophobic molecules (such as bilirubin, bile acid, long-chain fatty acids) to hepatocytes for detoxification and elimination[24]. Recent evidence suggests that the effect of albumin goes beyond the oncotic functions and transport, but also include immunomodulatory and antioxidant functions as well. Albumin is shown to attenuate prostaglandin E2 mediated immune-dysfunction in patients with

decompensated cirrhosis[25]. It also exerts immunomodulatory effect by down-regulating the expression of tumor necrosis factor-alpha and pro-inflammatory nuclear factor-kappa B[26]. Another property attributed to albumin is that it also functions as an antioxidant to scavenge reactive oxygen and nitrogen species in our body[27,28].

Albumin in decompensated cirrhosis

Hypoalbuminemia is a known predictor of poor survival in decompensated cirrhosis and serves well as a constituent of Child-Turcotte-Pugh score. What is less well appreciated is the fact that abnormalities with serum albumin in decompensated cirrhosis patients are both quantitative and qualitative[29]. The quantitative reduction of serum albumin concentration is a result of dilution from sodium and water retention, reduced synthesis from hepatocytes as well as increased trans-capillary leak, particularly amongst patients with refractory ascites[30,31]. The quality of albumin is further compromised in decompensated cirrhosis due to a higher proportion of oxidized albumin[32]. The oxidized albumin differs from native albumin because it has a lower binding capacity, impaired antioxidant properties and a shortened half-life[31]. Oxidized albumin not only correlates with the severity and complication of cirrhosis but also with short and long-term mortality[29,32]. This understanding on both the quantitative and qualitative alterations of albumin has resulted in the concept of "effective albumin concentration" in decompensated cirrhosis, which takes into account both the amount of albumin and its structural integrity[33].

ESTABLISHED INDICATION OF ALBUMIN IN DECOMPENSATED CIRRHOSIS

SBP

SBP is defined based on the presence of > 250 polymorphonuclear cells/mm^3 or positive ascitic fluid cultures, in the absence of an intraabdominal source of infection or malignancy[34]. Renal impairment is reported in up to 33% patients following SBP and is associated with inpatient mortality, despite resolution of infection[34,35]. In the first randomized trial which investigated the role of intravenous albumin infusion in SBP, Sort *et al*[36] demonstrated that albumin infusion and cefotaxime significantly reduced the risk of renal impairment (33% *vs* 10%), inpatient mortality (29% *vs* 10%) and 3-month mortality (41% *vs* 22%)[36]. The benefits of albumin especially in patients at high risk of developing renal impairment (baseline serum bilirubin ≥ 4 mg/dL or creatinine ≥ 1 mg/dL) were subsequently confirmed in a meta-analysis of randomized trials[37].

Is albumin mandatory in SBP patients with low risk of renal impairment, particularly those who did not fulfil the above criteria? A meta-analysis reported a low pooled incidence of renal impairment and death (2.8% and 3.8%, respectively) among the patients with low risk of renal impairment[37]. The number needed-to-treat to prevent one case of renal impairment and death is 45 and 27, respectively. Given the limited data in low-risk SBP patients, further prospective randomized trials are required to confirm the benefit of albumin infusion in SBP patients with low risk of renal impairment.

Post-paracentesis circulatory dysfunction

Paracentesis-induced circulatory dysfunction (PICD) is a known complication of LVP in patients with decompensated cirrhosis. The reported incidence varies widely between 17.1% to as high as 72.7%, depending on whether albumin infusion was given during LVP[38]. PICD classically has been defined as at-least 50% or more rise in serum renin levels up to 6 d following a large volume paracentesis[39]. PICD can lead to arterial hypotension and the resultant renal impairment has been associated with readmissions and mortality[39].

Several studies have evaluated the role of albumin infusion in large volume paracentesis. Albumin infusion (given at 6-8 g/L of ascitic fluid drained) has shown to prevent PICD in paracentesis beyond 5 L[39,40]. In a meta-analysis of randomized trials, albumin infusion is associated with a lower risk of PICD (OR = 0.39, 95%CI: 0.27-0.55) and mortality (OR = 0.64, 95%CI: 0.41-0.98) following paracentesis[38]. Specifically, all the included trials removed beyond 5 L of ascitic fluid; the majority of the studies administered 6-8 g of albumin 20% *per* L of ascitic fluid removed. With this understanding, the current guidelines recommending albumin replacement in

paracentesis beyond 5 L to prevent PICD[38].

HRS

HRS is the functional renal failure secondary to intrarenal vasoconstriction in patients with decompensated cirrhosis or acute liver failure[10]. Emerging data suggest HRS to be driven by both renal hypoperfusion from systemic circulatory dysfunction as well as increased circulating pro-inflammatory cytokines[41].

Currently, most of the evidence for albumin infusion in HRS is derived from HRS type 1 (also known as HRS-AKI). In a prospective, non-randomized study to investigate the role of albumin infusion, with and without terlipressin, in patients with HRS-AKI, Ortega *et al*[42] demonstrated that albumin infusion significantly improves HRS-AKI in addition to terlipressin alone (albumin: 77% *vs* 25%)[42]. Ever since then, albumin has become an integral part of HRS treatment with vasoactive drugs such as terlipressin, noradrenaline or octreotide[42-53]. Most studies administer 20-40 g of albumin *per* day and titrate according to fluid status to avoid fluid overload. Combination of albumin and terlipressin reverse HRS-AKI in up to 56% of patients in randomized clinical trials[43-45]. However, treatment-related adverse events leading to treatment discontinuation still occur in up to 43% of patients during the clinical trials. These complications (namely acute coronary syndromes and peripheral vascular ischemia) are mostly caused by intense systemic vasoconstriction attributable to terlipressin and can be partially mitigated by continuous terlipressin infusion (complication rates of 35% *vs* 62%), without compromising the treatment efficacy[46].

Even though albumin and terlipressin infusion achieves reversal of HRS-AKI in up to 60% of patients, it may not eventually result in reduced mortality. Several notable studies have evaluated the mortality benefit of albumin and vasoconstrictor in HRS-AKI with conflicting results[43-45,47-53]. Based on two of the recent meta-analyses, there is no conclusive survival benefit of albumin and vasoconstrictor infusion in HRS-AKI when compared to placebo[54,55].

Type-2 HRS is different from type-1 as it has a more subtle course and lower short-term mortality. Albumin and terlipressin infusion has also been shown to improve renal function in HRS type 2. However, the recurrence rate of HRS type 2 after treatment discontinuation is high and there is no clear benefit on mortality of these patients[56-58].

THE ROLE OF ALBUMIN IN DECOMPENSTAED CIRRHOSIS: BEYOND GUIDANCE

Non-SBP infection

As the circulating human albumin is less than optimal both quantitatively and qualitatively in decompensated cirrhosis. Theoretically, the benefit of albumin infusion may be expanded to non-SBP infection, especially those with renal impairment. It is also widely accepted that while renal impairment is often reversible in patients with decompensated cirrhosis with non-SBP infection, the 3-mo mortality is significantly higher compared to patients without renal impairment (55% *vs* 13%)[59]. Some notable literatures have tried to answer this quandary with help of randomised clinical trials (RCT). In a single-center RCT, Guevara *et al*[60] randomized 110 patients with non-SBP infections to receive standard antibiotics with or without albumin[60]. The dose of albumin administered was similar to SBP (1.5 g/kg on day 1 and 1 g/kg on day 3) regimen. Despite a reduction in serum creatinine, renin and aldosterone (which indicates an improvement in renal and circulatory function), the 3-mo survival rates were similar between the two groups[60]. In another RCT, Thévenot *et al*[61] randomized 191 patients with decompensated cirrhosis (Child-Pugh score > 8) with sepsis to receive albumin in addition to antibiotic. The rate of renal failure and mortality at three months were similar in both groups (albumin: 14.3% *vs* 13.5%, and, albumin: 70.2% *vs* 78.3%, respectively)[61]. However, 8.3% of patients developed pulmonary oedema following albumin infusion, and two patients died as a result of pulmonary oedema. These findings were confirmed in a recent meta-analysis of randomized trials, which showed that albumin infusion did not reduce the risk of renal impairment or death in non-SBP infection[14]. As albumin infusion did not improve renal function or survival, yet may result in adverse events such as pulmonary oedema or even death, the current guideline does not recommend albumin infusion for patients with non-SBP infection[10].

HE

HE is a neuropsychiatric manifestation associated with poor prognosis in decompensated cirrhosis resulting from the complex interplay between effective circulatory volume, ammonia, systemic inflammation and portosystemic shunting. As albumin is known to improve systemic circulatory dysfunction and oxidative stress-mediated tissue injury, there has been growing interest in using albumin to treat or prevent HE.

The preventive role of albumin infusion was investigated in a single center cohort study by Riggio *et al*[62] The author enrolled 23 patients following Transjugular intrahepatic portal-systemic shunt (TIPSS) to receive albumin infusion for three weeks. The risk of developing new HE was similar to a historical cohort which did not receive albumin infusion[62], suggesting that infusion of albumin may not have any role in preventing TIPSS or systemic shunting-related HE.

The role of albumin for the treatment of HE was first studied in 15 alcoholic cirrhosis patients with diuretic-induced HE. Patients were randomized to receive albumin or colloid infusion titrated accordingly to the central venous pressure[63]. Despite having a similar reduction in serum ammonia in both groups, the albumin group has a greater improvement in HE grade. Similar beneficial effects were observed in a prospective, open-labelled randomized study, Sharma *et al*[64] enrolled 120 patients with overt HE (graded based on the West Haven criteria) to receive either lactulose, with and without albumin[64]. Albumin was administered at 1.5 g/kg/d until the resolution of HE or day 10 of admission. Albumin group was more likely to achieve complete resolution of HE (albumin: 75% *vs* 53%), shorted hospitalization stays (albumin: 6.4 d *vs* 8.6 d) and lower mortality (albumin: 18% *vs* 32%). Furthermore, the albumin group had a greater decline in the serum tumor necrosis factor alfa, interleukin-6 and endotoxin level when compared to lactulose alone. However, this beneficial effect of albumin is not consistently demonstrated across studies. In a multicenter, double-blind, randomized controlled study, 56 patients with HE were randomized to receive albumin infusion (1.5 g/kg on day 1 and 1 g/kg on day 3) *vs* 0.9% saline[65]. This study remarkably did not find any significant difference in HE resolution by day 4, even though albumin infusion was associated with better transplant-free survival in patients with HE [hazard ratio (HR) 0.27, 95%CI: 0.11-0.74]. The current societal guidelines do not endorse the use of long-term albumin infusion for either the treatment or prevention of HE in patients with decompensated cirrhosis[10].

Acute-on-chronic liver failure

Acute-on-chronic liver failure (ACLF) is a distinct clinical entity characterized by systemic inflammation associated with multiorgan failure and high short-term mortality among decompensated cirrhosis patients[66]. As systemic inflammation is the hallmark of ACLF, the pleiotropic properties of albumin to rapidly expand the intravascular volume and ameliorate systemic inflammation makes albumin a promising treatment option in ACLF. Although clinical studies in past investigating the role of extracorporeal devices[67,68] provide the proof of concept that albumin infusion could play an effective role in the management of patients with ACLF, only a few studies have been carried out to specifically investigate the effect of albumin infusion in patients with ACLF.

In a recent multicenter randomized study (INFECIR-2 trial), Fernández *et al*[69] randomized 108 patients with decompensated cirrhosis and non-SBP infection resulting in ACLF to receive albumin or placebo in addition to antibiotic[69]. More patients in the albumin group experienced resolution of ACLF (82.3% *vs* 33.3%), even though the overall mortality were similar to patients receiving antibiotics alone[69]. Though promising, more robust data is required to support the use of albumin in ACLF.

LONG-TERM ALBUMIN IN DECOMPENSATED CIRRHOSIS

There have been growing interests in long-term albumin use among decompensated cirrhosis patients. We summarize all the relevant studies describing the use of long-term albumin in decompensated cirrhosis in Table 1. Wilkinson and Sherlock[70] first studied the role of long-term albumin infusion in the 1960s. They randomized 16 patients with diuretic refractory ascites to receive albumin infusion *vs* standard medical therapy (SMT)[70]. Albumin infusion was titrated based on serum oncotic pressure and maintained up to 19 mo. Apart from improving general "well-being",

Table 1 Characteristics of studies on long-term albumin infusion in decompensated cirrhosis patients

No	Ref.	Country	Study design	Follow-up duration[1]	Study population	Exclusion criteria	Duration of albumin infusion (d)	Sample size	Child-Pugh Score (A/B/C)	MELD score (albumin *vs* SMT)[1]	Intervention	Control
1	Wilkinson and Sherlock[70], 1962	England	Single centre, non-randomized	22 mo	Cirrhosis patients with ascites despite 6 wk of dietary and diuretic therapy	HCC	616	16	NA	NA	Albumin 25-100 g until serum colloid oncotic pressure 38-40 cm of water	SMT
2	Gentilini *et al*[71], 1999	Italy	Single centre, randomised controlled trial	3 yr	Adult cirrhosis patients with ascites after 1 wk of bed rest and low sodium diet	Renal or cardiac failure, HCC or other malignancies, HE (grades 2-4), infections, gastrointestinal bleeding or DIVC	1095	126	0/67/59	NA	Albumin 12.5 g/d	SMT
3	Romanelli *et al*[72], 2006	Italy	Single centre, randomised controlled trial	84 mo (2-120)	Adult cirrhosis patients with ascites	Active alcohol abuse; previous ascites (grades 2 and 3) or HE; cardiac, respiratory or renal impairment; diabetes; refractory ascites; HCC or other malignancies; gastrointestinal bleeding; infections or DIVC	1440	100	0/46/54	NA	Albumin 25 g weekly in the first year, 25 g every two weeks thereafter	SMT
4	Caraceni *et al*[11], 2018	Italy	Multicentre, randomised controlled trial	18 mo	Adult cirrhosis patients with medically controlled uncomplicated ascites	Refractory ascites, recent decompensation, TIPS, HCC, liver transplantation, ongoing alcohol abuse, extrahepatic organ failure and albumin use for the treatment of ascites within one month	540	431	64/282/85	12 (10–15), 13 (10–16)	Albumin 40 g twice weekly for 2 wk, and 40 g weekly up to 18 mo	SMT
5	Sola-Vera *et al*[40], 2003	Spain	Multicentre, randomised controlled trial	1 yr	Cirrhotic patients with ascites on the liver transplantation waiting list	Arterial hypertension; treatment with psychotropic drugs or antibiotic; TIPS; cardiac or respiratory failure; previous or currently listed for liver transplant; HIV or HCV infection, contraindications to midodrine	365	196	NIL	16 ± 6.2, 17 ± 6.0,	Midodrine 15-30 mg/d and Albumin 40 g/15 d for 1 yr	SMT
6	Di Pascoli *et al*[13], 2019	Italy	Non-randomised, prospective study	Mean 408 +/- 394 d	Adult cirrhosis patients with refractory ascites	HCC beyond Milan criteria or severe extrahepatic diseases	720	70	CTP 9.3 ± 1.7; 9.5 ± 1.6	15.2 ± 5.4, 14.9 ± 5	Human albumin 20 grams twice *per* week	SMT, LVP when indicated
7	China *et al*[75], 2018	United Kingdom	Multicentre randomised controlled trial	6 mo	Adult cirrhosis patients hospitalised with acute decompensation and hypoalbuminemia (serum albumin < 30 g/L)	Advanced HCC; heart failure	14	828	NA	NA	Albumin 20-80 g/d until serum albumin ≥ 35 g/L	SMT

[1]Presented in mean (± SD) or median (interquartile range).

SMT: Standard medical therapy, HCC: Hepatocellular carcinoma; HE: Hepatic encephalopathy; DIVC: Disseminated intravascular coagulopathy; TIPS: Transjugular intrahepatic portosystemic shunt; LVP: Large-volume paracentesis; NA: Not available; NIL: Nanoimprint lithography; HIV: Human immunodeficiency virus; HCV: Hepatitis C virus; CTP: Cytoplasmic transduction peptide.

long-term albumin infusion did not improve overall survival or reduce the need for diuretics.

In another single center randomized study, Gentilini *et al*[71] enrolled 126 patients with refractory ascites to receive either albumin infusion or SMT[71]. Patients received weekly albumin infusion of 25 g in the first year, followed by 25 g every two weeks up to 3 years. Long-term albumin infusion reduced ascites recurrence and ascites-related readmission without improving the overall survival. Subsequently, the same group performed a follow-up study 7 years later in 2006, evaluating the long-term outcomes of long-term albumin infusion with an extension of the follow-up period to a median of 84 mo[72]. They recruited 100 patients with new-onset, clinically significant ascites and randomized them to receive either albumin or SMT. The effect of long-term albumin in ascites management was again demonstrated, with less ascites recurrence (39% *vs* 85%) in the albumin group. More importantly, long-term albumin infusion improved 5-year transplant-free survival (albumin: 62% *vs* 26%) for the first time, even though the sample size was relatively small.

The ANSWER study (the human Albumin for the TreatmeNt of aScites in patients With hEpatic cirRhosis) enrolled 431 patients of decompensated cirrhosis with medically controlled ascites and compared the clinical outcomes in patients receiving long term albumin infusion *vs* SMT[11]. In this study, long term albumin infusion (40 g twice weekly for two weeks, followed by 40 g weekly) in addition to SMT was associated with significantly lower mortality (HR 0.62, 95%CI: 0.40-0.95). The ascites control were better in albumin group with a lower risk for paracentesis (HR 0.48, 95%CI: 0.35-0.54) and refractory ascites (HR 0.43, 95%CI: 0.29-0.62). Also, long-term albumin infusion was associated with a lower risk of both SBP and non-SBP related bacterial infection, grade III and IV HE, HRS, renal dysfunction and hyponatremia. Long-term albumin infusion was well-tolerated. Finally, long-term albumin infusion was also shown to be cost-effective, primarily by a reduction in hospital admission, risk of paracentesis and HRS.

In another prospective but non-randomized study, Di Pascoli *et al*[13] enrolled 70 patients with cirrhosis and refractory ascites to receive either long-term albumin infusion *vs* SMT[13], with the primary endpoint of 24-mo survival. Subjects in the albumin group received 20 g of albumin twice weekly. The study demonstrated a significant improvement in 24-mo survival in the albumin group when compared to the SMT (58% *vs* 35% in SMT) over a mean follow up of 408 d. Furthermore, the albumin group had a lower risk of emergency hospitalizations from SBP, non-SBP infection and HE. While the liver transplantation rate was similar in both groups (11% *vs* 8% in SMT), it should be highlighted that none of the patients with refractory ascites received Transjugular intrahepatic portosystemic shunt (TIPS). More data is required to evaluate the comparative efficacy of long-term albumin and TIPS for refractory ascites.

The MACHT trial (midodrine and albumin for cirrhotic patients in the waiting list for liver transplantation) however offered a contrasting view on the survival benefit of long-term albumin in decompensated cirrhosis patients[12]. In this multicenter, randomized, double-blind, placebo-controlled trial, 196 patients on the transplant waiting list were enrolled to receive either SMT or albumin infusion (40 g every 15 d for one year) plus midodrine with cirrhosis-related complications being the primary end-point. In contrast to the ANSWER trial, the cirrhosis-related complications, ascites control and overall survival were similar between albumin and SMT group. However, 3 important features of the MACHT trial must be considered and the results interpreted in accordingly. First, a relatively higher proportion of patients in both groups received transplantation, (68% in albumin *vs* 55% in SMT group). Second, the duration of albumin therapy was relatively short (median duration of 80 d). Thirdly, the dose of albumin therapy used was also lower than that used in all the other studies. A dosage of 40 g every 15 d was used, as compared to higher dosages in all the other trials. The failure of albumin therapy group to show a better outcome may potentially be attributed to these three factors.

IS LONG-TERM ALBUMIN READY FOR PRIME TIME?

The ANSWER study has provided valuable insights on using long-term albumin infusion as a pathophysiological approach to prevent cirrhosis related complications and death in stable cirrhosis patients with medically-controlled ascites. Nevertheless, it is worth noting that the ANSWER study excluded more advanced-cirrhosis patient with refractory ascites and recent decompensation (variceal bleeding, bacterial

infection). In patients with refractory ascites, the comparative efficacy between long-term albumin infusions *vs* TIPS, which is a one-off procedure with good efficacy, remains unanswered. Besides, only 3.2% (14/431) of patients with hepatitis C related cirrhosis received direct-acting antiviral therapy in the ANSWER study. As the treatment with direct-acting viral therapy is expected to improve the clinical outcomes in these patients[73,74], whether this specific subset of decompensated patients would benefit from long-term albumin infusion following sustained virological response remains unanswered.

The most recent published data, although in abstract form, evaluating the benefits of albumin infusion comes from the ATTIRE (Albumin to prevent infection in chronic liver failure) study which included patients with cirrhosis hospitalized for acute decompensation and hypoalbuminemia (serum albumin < 30 g/L)[75]. In this multicenter randomized trial which enrolled 778 patients to receive albumin infusion *vs* SMT, the primary endpoint was having a new infection, renal dysfunction or mortality from day 3 to 15 of treatment. The results of this study show that the risk of renal dysfunction and death were similar between albumin and SMT group and thus albumin infusion may not be beneficial in these patients. The PRECOISA (Effect of long-term administration of albumin in subjects with decompensated cirrhosis and ascites) study which aims to investigate the impact of long-term albumin on the 1-year mortality and ACLF, is currently ongoing (NCT03451282). The results of PRECOISA will hopefully provide robust evidence for the use of long-term albumin infusion in decompensated cirrhosis patients.

CONCLUSION

Decompensated cirrhosis is characterized by systemic circulatory dysfunction from portal hypertension and systemic inflammation. In decompensated cirrhosis, albumin dysfunction both in terms of quantity and quality. The established therapeutic role of albumin infusion in decompensated cirrhosis includes SBP, HRS and in patients undergoing LVP. Although long-term albumin seemed promising to prevent ascites-related complications in decompensated cirrhosis, the existing studies were heterogeneous in terms of their study population, follow-up duration, and the dose of albumin infusion, thus making the interpretation on the survival benefit particularly challenging. The positive results of long-term albumin infusion will likely increase the global demand for intravenous albumin, particularly among decompensated cirrhosis patients. Meanwhile, the cell-free concentrated ascites reinfusion therapy (CART) may be a novel alternative to intravenous albumin infusion in patients with ascites[76], however more data is required to evaluate the efficacy and safety of CART, particularly among cirrhosis patients with refectory ascites.

Future upcoming studies evaluating the role of long-term albumin infusion to ameliorate systemic inflammation and cirrhosis-related complications are expected in the next few years. Till then, the use of albumin beyond the established indication should be individualized. Future studies should focus on refining the dosages, schedule of long-term albumin infusion and on the specific population groups which would benefit the most.

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ORIGINAL ARTICLE

Clinical and Translational Research

Bile acid indices as biomarkers for liver diseases I: Diagnostic markers

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Abstract

BACKGROUND

Hepatobiliary diseases result in the accumulation of toxic bile acids (BA) in the liver, blood, and other tissues which may contribute to an unfavorable prognosis.

AIM

To discover and validate diagnostic biomarkers of cholestatic liver diseases based on the urinary BA profile.

METHODS

We analyzed urine samples by liquid chromatography-tandem mass spectrometry and compared the urinary BA profile between 300 patients with hepatobiliary diseases *vs* 103 healthy controls by statistical analysis. The BA profile was characterized using BA indices, which quantifies the composition, metabolism, hydrophilicity, and toxicity of the BA profile. BA indices have much lower inter- and intra-individual variability compared to absolute concentrations of BA. In addition, BA indices demonstrate high area under the receiver operating

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Clinical trial registration statement: This study is registered at ClinicalTrials.gov. The registration identification number is NCT01200082.

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characteristic curves, and changes of BA indices are associated with the risk of having a liver disease, which demonstrates their use as diagnostic biomarkers for cholestatic liver diseases.

RESULTS

Total and individual BA concentrations were higher in all patients. The percentage of secondary BA (lithocholic acid and deoxycholic acid) was significantly lower, while the percentage of primary BA (chenodeoxycholic acid, cholic acid, and hyocholic acid) was markedly higher in patients compared to controls. In addition, the percentage of taurine-amidation was higher in patients than controls. The increase in the non-12α-OH BA was more profound than 12α-OH BA (cholic acid and deoxycholic acid) causing a decrease in the 12α-OH/ non-12α-OH ratio in patients. This trend was stronger in patients with more advanced liver diseases as reflected by the model for end-stage liver disease score and the presence of hepatic decompensation. The percentage of sulfation was also higher in patients with more severe forms of liver diseases.

CONCLUSION

BA indices have much lower inter- and intra-individual variability compared to absolute BA concentrations and changes of BA indices are associated with the risk of developing liver diseases.

Key Words: Hepatobiliary diseases; Bile acids; Bile acid indices; Diagnosis; Biomarker; Liver diseases

Core Tip: We have developed the concept of "bile acids (BA) indices" based on the detailed quantitative analysis of the urinary BA profile in patients with cholestatic liver diseases. We demonstrated the use of BA indices as diagnostic biomarkers for cholestatic liver diseases. BA indices had much lower inter- and intra-individual variability compared to absolute concentrations of the total and individual BA. In addition, BA indices demonstrated high area under the receiver operating characteristic curves, and changes of BA indices were associated with the risk of having a liver disease as determined by the logistic regression analysis.

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INTRODUCTION

Bile acids (BA) have many physiological functions such as cholesterol absorption and elimination, fat absorption, and maintenance of healthy microbiome[1,2]. BA are also signaling molecules/hormones, which are involved in the regulation of their own homeostasis, thyroid hormone signaling, glucose and lipid metabolism, energy expenditure, and cellular immunity[2-5]. Conversely, certain BA are also cytotoxic at high concentrations and have deleterious effects on hepatocytes and cholangiocytes, which play a major role in liver injury during various liver diseases[5-8].

Cholestatic liver diseases are associated with a reduction in bile flow due to impairment of bile flow or defects in bile production[9]. This causes accumulation of BA in the liver, which spills out into the systemic circulation, extrahepatic tissues, and eventually into urine. Numerous clinical and preclinical studies have shown up to a 100-fold increase in BA concentrations in the blood and urine during various liver diseases[8,10-13]. Elevated BA concentrations were shown to correlate with the progression of damages to the liver and bile duct in cholestatic rats, rabbits, and in humans[14-18].

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Biomarkers currently used in the clinic for the diagnosis and prognosis of liver diseases are primarily serum liver enzymes such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) as well as bilirubin[19,20]. However, they are not specific to the liver or bile duct injuries, may increase in non-hepatobiliary diseases, and require severe cell injury at advanced disease stages before their blood levels increase[19,20]. BA were extensively investigated for decades as biomarkers for numerous hepatobiliary diseases[13,21-23]. However, these efforts never translated into the clinic, with the few exception of limited use in the diagnosis of intrahepatic cholestasis of pregnancy and biliary atresia in infants. This could be attributed to the marked differences in the physiological and pathological properties of the different individual BA. For example, detailed profiling of the more toxic and relevant individual BA rather than total BA concentration may better correlate with the liver condition during hepatobiliary diseases[10,12,24]. Also, the extreme inter-and intra-individual variability of total and individual BA concentrations due to many factors such as food ingestion and diurnal variation, makes it challenging to determine the normal baseline ranges[25,26].

We have developed the concept of "BA indices", which are ratios calculated from the absolute concentrations of individual BA and their metabolites (Table 1). These ratios provide comprehensive quantification of the composition, metabolism, hydrophilicity, formation of secondary BA, and toxicity of the BA profile[9,26]. BA indices have much lower variability than the absolute BA concentrations used to calculate them. Indeed, we have demonstrated that BA indices offered numerous advantages over absolute total and individual BA concentrations including low inter- and intra-individual variability and were resistant to covariate influences such as age, gender, body mass index (BMI), food consumption, and moderate alcohol consumption[9,26].

We have expanded on our previous pilot study, where we have recruited 300 patients with liver diseases and 103 control subjects over a period of 7 years. This study includes a series of two papers. In this article, we have shown the utility of BA indices as diagnosing markers for liver diseases by compared the urinary BA profile between healthy controls and patients and between patients with different severity levels of liver disease. In the 2nd article, we have built a survival model, the Bile Acid Score (BAS), to predict the prognosis of liver diseases using significant BA indices identified in this article.

MATERIALS AND METHODS

Study participants

For controls, 103 healthy subjects (32 male and 71 female) without liver diseases between the ages of 19 and 65 years were recruited by the Clinical Research Center at the University of Nebraska Medical Center (UNMC) (Omaha, NE, United States). The registry URL was (https://www.clinicaltrials.gov/ct2/show/NCT01200082?term=alnouti&draw=2&rank=1). The clinical trial number was NCT01200082. Inclusion criteria for the healthy controls included normal liver functions, as verified by ALT < 50 U/L, AST < 56 U/L, gamma-glutamyl transferase < 78 U/L, absence of diabetes, and no- or moderate alcohol drinking[27] The study was approved by Institutional Review Board at UNMC and written informed consents were provided for all participating subjects. Thirty milliliters urine samples were collected from controls at fasting conditions in the first visit, and 1, 2, and 4 wk thereafter.

Patients diagnosed with one or multi-hepatobiliary conditions due to chronic hepatitis C (n = 71) , hepatitis B (n = 15), alcoholic liver disease/alcoholic cirrhosis (n = 117), primary biliary cholangitis (PBC) (n = 12), primary sclerosing cholangitis (n = 17), autoimmune hepatitis (n = 27), alpha-1-antitrypsin deficiency (n = 6), nonalcoholic fatty liver disease/nonalcoholic steatohepatitis (n = 56), carcinoma (n = 26), cryptogenic cirrhosis (n = 11), polycystic liver disease (n = 5), elevated liver function test (LFT) (n = 22), and unknown etiology (n = 5), were enrolled in the hepatology clinic in UNMC. A total of 300 patients (157 male and 143 female) between the ages of 19 years and 83 years were recruited. Thirty milliliters of urine samples were collected on their first and follow-up visits to the hepatology clinic. All urine samples were stored in -80 °C until analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Patients were divided into three disease-severity groups based on their model for end-stage liver disease (MELD) score: low-MELD (6-15 score), medium-MELD (16-25), and high-MELD (26-40). High MELD group was not included while performing the statistical analysis, because there were only four subjects in that group.

Table 1 List of bile acid indices

Composition	Hepatic metabolism	Hydrophilicity	CYP8B1 activity	Intestinal contribution
Concentration of individual BA	Total sulfated	Total mono-OH	Total 12α-OH	Total primary
% of individual BA	Total G-amidated	Total di-OH	Total non-12α-OH	Total secondary
	Total T-amidated	Total tri-OH	12α-OH/non-12α-OH	Primary/secondary
	% Sulfation	% Mono-OH	CA/CDCA	% Primary
	% Amidation	% Di-OH	% 12α-OH	% Secondary
	% G-amidation	% Tri-OH	% Non-12α-OH	
	% T-amidation			

BA: Bile acids; G: Glycine; T: Taurine; CDCA: Chenodeoxycholic acid; CA: Cholic acid.

In addition, patients were also categorized according to hepatic decompensation (presence or history of encephalopathy, bleeding varices, ascites, or jaundice)[28].

Non-BA parameters

AST, ALT, albumin, and serum creatinine were measured using the Beckman Coulter reagents (Beckman Coulter, Inc, Brea, California). Protime and international normalized ratio (INR) were measured using STANeoplastine "CI PLUS 10" reagent kit (Diagnostica Stago Inc, Parsippany, New Jersey). Total bilirubin in serum was analyzed using QuantiChromTM Bilirubin assay kit (BioAssay Systems, Hayward, CA, United States). AST/ALT ratio and AST/platelet ratio index (APRI) were calculated.

BA quantification by LC-MS/MS

Urine samples were extracted using solid phase extraction as described previously[9,26,29,30]. BA concentrations were quantified by LC-MS/MS, as we described previously[31].

Calculation of BA indices

In addition to the absolute concentration of individual and total BA, the BA profile in urine was characterized using "BA indices" (Table 1), and as we have described previously[9,26,30,31]. BA indices describe the composition, hydrophilicity, formation of 12α-OH BA by CYP8B1, metabolism, and formation of secondary BA by intestinal bacteria. The composition indices were calculated as the ratio of the concentration of individual BA in all of their forms (sulfated, unsulfated amidated, and unamidated) to the total concentration of BA. The percentages of mono-OH BA: [lithocholic acid (LCA)], di-OH BA: [ursodeoxycholic acid (UDCA), murideoxycholic acid (MDCA), chenodeoxycholic acid (CDCA), hyodeoxycholic acid (HDCA), and deoxycholic acid (DCA)], and tri-OH BA: [cholic acid (CA), muricholic acid (MCA), and hyocholic acid (HCA)] were calculated as the ratio of the concentration of the sum of the respective BA in all their forms to the total concentration of BA.

The 12α-OH BA are formed by CYP8B1 in the liver and include DCA, CA, nor-DCA, and 3-dehydroCA. Therefore, CYP8B1 activity can be measured by the ratio of 12α-OH BA to the remaining of all other BA (non-12α-OH BA). Another marker for CYP8B1 is the ratio of CA to CDCA because CA is formed by the 12α hydroxylation of CDCA. In the same way, the ratio of 12α-OH (DCA, CA, nor-DCA, and 3-dehydroCA in all of their forms) to non-12α-OH (CDCA, HDCA, LCA, UDCA, MDCA, HCA, MCA, 12-oxo-CDCA, 6-oxo-LCA, 7-oxo-LCA, 12-oxo-LCA, isoLCA, isoDCA in all of their forms) was calculated.

BA are metabolized primarily by sulfation, glycine (G), and taurine (T) amidation in the liver. The percentage of individual BA sulfation was calculated as a ratio of the concentration of sulfated BA, in both the amidated and unamidated forms, to the total individual BA concentration in all their forms (amidated, unamidated, sulfated, and unsulfated). In both the sulfated and unsulfated forms, the percentage of individual BA amidation have been calculated as the ratio of the concentration of amidated BA, to the total concentration of individual BA in all of their forms (amidated, unamidated, sulfated, and unsulfated). Additionally, percentages of amidation were divided into the percentages of BA existing as G or as T amidates.

The ratio of primary (CA, CDCA, MCA and HCA in all of their forms) to secondary BA (DCA, LCA, UDCA, HDCA, MDCA, Nor-DCA, 12-oxo-CDCA, 3-dehydroCA, 6-oxo-LCA, 7-oxo-LCA, 12-oxo-LCA, isoLCA, and isoDCA in all of their forms) was calculated.

Statistical analysis

Independent sample-*t*-test and Mann-Whitney test were used to study the demographic differences between controls and patients because the sample size was > 30[32]. Independent sample-*t*-test was used for continuous variables and Mann-Whitney test was used for categorical variables. The demographic variables were (age, BMI, gender, and race). Subjects were divided into four age groups (19-29, 30-41, 42-53, 54-83 years), and the variable age was studied as both a continuous and a categorical variable. Subjects were also divided into three BMI groups (normal: BMI < 25, overweight: BMI 25-29.9, and obese: BMI ≥ 30) and the effect of BMI was studied as both a continuous and a categorical variable. Also, subjects were divided into five race groups (White, Black, Asian, Hispanic, others), and the variable race was studied as a categorical variable.

Urine samples were collected from controls and patients on their first visit and follow-up visits. Mixed effects models were used to compare patients *vs* controls and the demographic variables were included as covariates. Statistically significant covariates were returned to the mixed effects models as interaction terms with the primary group, *i.e.*, patients *vs* control.

BA indices were compared between controls, low-MELD (patients), and medium-MELD (patients) groups using mixed effects models followed by pairwise comparisons using Bonferroni's adjustment if the *P* value was < 0.05. BA indices were compared between compensated and decompensated patients using mixed effects models. Mixed effects models were also used to determine the association between non-BA parameters including (AST, ALT, bilirubin, MELD score, AST/ALT, creatinine, INR, APRI, protime, and albumin) and BA indices. Receiver operating characteristic curve (ROC) analyses were used to determine cut-off values of BA as markers for the diagnosis of liver diseases with optimum sensitivity and specificity. The areas under the ROC curve (AUC) values were compared between urinary BA profiles and non-BA parameters. The mixed effects models were used to compare BA indices with AUC > 0.7 between controls and the patients with specific disease subtypes described in the "Study Participants" section (same patients can belong to different disease groups). Polycystic liver disease and unknown etiology subtypes were not included in the comparison between the disease subtypes because they had < six subjects.

Univariate logistic regression analysis was used to determine the association between BA concentrations and indices and the likelihood of developing a liver disease. From logistic regression analysis, the odds ratios (ORs) were calculated for a 10% and 20% change from the mean value of BA indices in the healthy controls.

P value of 0.05 was considered significant for all the statistical tests described above. All statistical analysis was performed using the Statistical Product and Service Solutions (SPSS) software, version 25 (IBM corporation, Armonk, NY, United States).

RESULTS

Demographics

Table 2 shows a summary of the demographics of both patients and controls participants. We enrolled 103 controls (32 males and 71 females) and 300 patients (157 males and 143 females), who were treated for cholestatic liver diseases in UNMC, over the period from November of 2011 to December of 2018. To compare the demographics between the two groups, age and BMI covariates were compared as both continuous and categorical variables using *t*-test, and Mann-Whitney test, respectively. While gender and race were compared as categorical variables using Mann-Whitney test. Age, gender, and BMI were significantly different between control and patients (*P* value < 0.05), while race was not different. Therefore, the statistically significant demographic variables (age, BMI, and gender) were included as covariates in the mixed effects models to compare BA indices between patients and controls.

Table 2 Demographics

	Controls	Patients
n	103	300
Gender[1]		
Male, female	32, 71	157, 143
Age (yr)[1]		
mean ± SE	44.3 ± 0.64	52.1± 0.54
19-29	17	11
30-41	28	40
42-53	30	92
54-83	28	157
BMI[1]		
mean ± SE	27.5 ± 0.28	30.9 ± 0.32
Normal BMI < 25	30	69
Overweight BMI = 25-29.9	45	104
Obese BMI ≥ 30	28	127
Race		
White	88	247
Black	7	14
Asian	7	13
Hispanic	1	8
Others	0	18

[1]Significant difference between controls and patients ($P < 0.05$).
BMI: Body mass index.

Differences in BA between patients vs controls are not due to differences in demographics

Because some of the covariates (age, BMI, and gender) were significantly different between the two groups (Table 2), we reran the univariate mixed effect analysis with these covariates (multivariate analysis). First, association between these covariates and BA indices was identified, and then the covariates with significant association with BA indices were incorporated in the multivariate mixed effect analyses as interaction terms with the group (patients and controls). We did not find any difference in the association between covariates and BA indices between the two groups except for the % primary and % secondary BA with gender (Supplementary Table 1).

BA profiles in controls vs patients

Table 3 shows the absolute concentrations of major urinary BA in controls and patients. Table 4 compares representative absolute BA concentrations and indices between controls and patients. Supplementary Table 2 shows the full list of BA concentrations and indices. Total BA was 5.9-fold higher in patients compared with controls. All individual BA concentrations were also higher in patients, except MDCA, but to different extents. The highest increase was in UDCA (11.9-fold), while the lowest increase was for DCA and HDCA (1.6-fold). The percentage of UDCA, CDCA, MCA, CA, and HCA were higher (1.2-1.6-fold), while the percentage of LCA, DCA, HDCA, and MDCA were lower (0.5-0.8-fold) in patients *vs* controls.

Unamidated, G-amidated, and T-amidated BA which were 3.3-, 5.9-, and 9.4-fold higher in patients than controls. Therefore, the overall % amidation and % G-amidation did not change or slightly decreased in patients, whereas % T-amidation increased from 8.0% in controls to 10.8% in patients. Similarly, the concentrations of both sulfated and unsulfated were approximately 6-fold higher in patient; so that the

Table 3 Absolute concentrations of major bile acids in controls and patients

BA	Unamidated	G-BA	T-BA	Total
	mean ± SE, μmol/L			
Controls				
Unsulfated BA				
LCA	0.000 ± 0.00	0.000 ± 0.00	0.000 ± 0.00	0.001 ± 0.00
UDCA	0.004 ± 0.00	0.033 ± 0.00	0.002 ± 0.00	0.038 ± 0.00
CDCA	0.003 ± 0.00	0.008 ± 0.00	0.002 ± 0.00	0.013 ± 0.00
DCA	0.022 ± 0.00	0.011 ± 0.00	0.002 ± 0.00	0.035 ± 0.00
HDCA	0.01 ± 0.00	0.00 ± 0.00	ND	0.007 ± 0.00
MDCA	0.060 ± 0.01	ND	ND	0.058 ± 0.01
CA	0.179 ± 0.03	0.067 ± 0.00	0.009 ± 0.00	0.255 ± 0.03
MCA	0.028 ± 0.00	0.287 ± 0.02	0.041 ± 0.00	0.356 ± 0.02
HCA	0.008 ± 0.00	0.016 ± 0.00	0.001 ± 0.00	0.026 ± 0.00
Other BA[1]	0.160 ± 0.01	-	-	0.160 ± 0.01
Total unsulfated	0.464 ± 0.04	0.422 ± 0.02	0.057 ± 0.00	0.943 ± 0.05
Sulfated BA				
LCA	0.010 ± 0.00	0.780 ± 0.04	0.220 ± 0.01	1.010 ± 0.05
UDCA	0.450 ± 0.02	1.040 ± 0.05	0.030 ± 0.00	1.520 ± 0.07
CDCA	0.070 ± 0.01	2.380 ± 0.13	0.060 ± 0.00	2.510 ± 0.13
DCA	0.010 ± 0.00	2.900 ± 0.14	0.220 ± 0.02	3.130 ± 0.16
CA	0.004 ± 0.00	0.056 ± 0.01	0.126 ± 0.01	0.190 ± 0.01
Total sulfated	0.535 ± 0.03	7.170 ± 0.28	0.650 ± 0.03	8.350 ± 0.31
Overall total	1.000 ± 0.05	7.590 ± 0.29	0.710 ± 0.03	9.300 ± 0.33
Patients				
Unsulfated BA				
LCA	0.004 ± 0.00	0.001 ± 0.00	0.0001 ± 0.00	0.005 ± 0.00
UDCA	0.079 ± 0.03	0.410 ± 0.17	0.012 ± 0.00	0.500 ± 0.21
CDCA	0.020 ± 0.00	0.090 ± 0.01	0.100 ± 0.02	0.210 ± 0.03
DCA	0.040 ± 0.00	0.040 ± 0.00	0.010 ± 0.00	0.090 ± 0.01
HDCA	0.010 ± 0.00	0.00 ± 0.00	ND	0.010 ± 0.00
MDCA	0.050 ± 0.01	ND	ND	0.050 ± 0.01
CA	0.240 ± 0.03	0.550 ± 0.07	0.320 ± 0.08	1.120 ± 0.14
MCA	0.120 ± 0.02	1.940 ± 0.29	0.730 ± 0.09	2.790 ± 0.34
HCA	0.010 ± 0.00	0.170 ± 0.02	0.090 ± 0.02	0.270 ± 0.04
Other BA[1]	0.860 ± 0.13	-	-	0.860 ± 0.13
Total	0.460 ± 0.04	0.42 ± 0.02	0.06 ± 0.00	5.910 ± 0.57
Sulfated BA				
LCA	0.030 ± 0.01	2.230 ± 0.20	0.650 ± 0.06	2.910 ± 0.24
UDCA	1.560 ± 0.23	15.30 ± 2.68	1.230 ± 0.27	18.10 ± 3.08
CDCA	0.190 ± 0.03	18.70 ± 1.79	1.910 ± 0.38	20.80 ± 2.07
DCA	0.040 ± 0.01	4.280 ± 0.54	0.520 ± 0.07	4.840 ± 0.58
CA	0.080 ± 0.01	0.910 ± 0.13	1.030 ± 0.21	2.010 ± 0.31

Total	1.900 ± 0.24	41.40 ± 4.12	5.340 ± 0.74	48.70 ± 4.77
Overall total	3.330 ± 0.33	44.60 ± 4.46	6.610 ± 0.85	54.60 ± 5.20

[1]Other bile acids: Nor-deoxycholic acid, 12-oxo-chenodeoxycholic acid, 3-dehydrocholic acid, 6-oxo-lithocholic acid, 7-oxo-lithocholic acid, 12-oxo-lithocholic acid, isolithocholic acid, and isodeoxycholic acid.
ND: Not detected; -: Not quantified; BA: Bile acids; G: Glycine; T: Taurine; CDCA: Chenodeoxycholic acid; CA: Cholic acid; LCA: Lithocholic acid; UDCA: Ursodeoxycholic acid; DCA: Deoxycholic acid; HDCA: Hyodeoxycholic acid; MDCA: Murideoxycholic acid; MCA: Muricholic acid; HCA: Hyocholic acid.

% sulfation of BA was unchanged.

The absolute concentrations of mono-, di-, and tri-OH BA were also higher in patients compared with controls, but the % mono-OH decreased (0.8-fold), di-OH remained unchanged, and % tri-OH increased (1.4-fold) due to increasing % CA (1.2-fold), % MCA (1.6-fold), and % HCA (1.5-fold).

Total 12α-OH and non-12α-OH BA were 2.3-fold and 8.2-fold higher in patients, so that the ratio of 12α-OH/ non-12α-OH and the % 12α-OH decreased (approximately 0.5-fold), while % non-12α-OH BA increased (1.2-fold).

Total primary and secondary BA were 8.1-fold and 4.6-fold higher in patients, so that the ratio of primary/secondary BA was 3.6-fold higher. Therefore, % primary BA was 1.4-fold higher, while % secondary BA was 0.80-fold lower in patients *vs* controls.

BA profile in low vs medium-MELD patients

Table 5 compares representative urinary BA concentrations and indices between low- and medium-MELD patients. Total BA concentrations was twice and individual BA concentrations were (1.15-fold to 3.9-fold) higher in medium *vs* low-MELD patients (Table 5).

Unamidated BA concentration was lower, while G-amidated and T-amidated BA were higher in the medium-MELD patients. Therefore, % T-amidation was 1.5-fold higher, while there was minimal difference in the % amidation and % G-amidation between medium and low-MELD patients. Similarly, the concentrations of both sulfated and unsulfated were 1.3- and 2-fold higher in medium *vs* low-MELD. On the other hand, the % sulfation of BA was only 1.07-fold higher, but it was statistically significant.

The absolute concentrations of mono-, di-, and tri-OH BA were also (1.8-2-fold) higher in medium-MELD patients, but the % mono-OH decreased (0.86-fold); while % di- and % tri-OH remained unchanged.

Total 12α-OH and non-12α-OH BA were both higher in medium *vs* low-MELD patients, but to different extents so that % non-12α-OH BA remained unchanged, while % 12α-OH decreased and the ratio of 12α-OH/ non-12α-OH was approximately 0.7-fold lower.

Total primary BA were 3.4-fold higher, while total secondary BA were slightly (0.9-fold) lower in medium-MELD patients, so that the ratio of primary/secondary BA was 2.3-fold higher. Similarly, % primary BA was 1.4-fold higher, while % secondary BA was 0.6-fold lower in medium- MELD patients.

BA profile in compensated vs decompensated patients

Table 6 compares representative urinary BA concentrations and indices between decompensated and compensated patients. In general, the same trend in the higher *vs* lower MELD patients comparison was observed in the decompensated *vs* compensated patients. Total BA was 1.3-fold higher, all individual BA were higher, but to variable extents. The percentage of CDCA, HDCA, CA, and HCA were higher (1.3-2.1-fold), while the percentage of LCA, UDCA, DCA, and MDCA were lower (0.3-0.7-fold) in decompensated *vs* compensated patients.

The % T-amidation was 1.3-fold higher in decompensated vs. compensated patients, while there was no difference in the % amidation, % G-amidation, or % sulfation. The % mono-OH decreased (0.73-fold), % di-OH remained unchanged, and % tri-OH slightly increased (1.13-fold) due to increasing % CA and % HCA. The ratio of 12α-OH/ non-12α-OH lower, the % 12α-OH, and CA/CDCA ratio decreased (0.7-0.8-fold), while % non-12α-OH BA remained unchanged.

Total primary BA were two-fold higher, while total secondary BA were 0.8-fold lower, so that the ratio of primary/secondary BA was 2.6-fold higher in decompensated patients. Therefore, % primary BA was 1.5-fold higher, while % secondary BA was 0.56-fold lower in decompensated patients.

Table 4 Representative bile acids concentrations and indices in controls *vs* patients

BA (μmol/L) or BA indices	Controls		Patients		Patients *vs* controls	
	mean	SE	mean	SE	Ratio	*P* value
Total BA	9.30	0.33	54.6	5.20	5.87	0.000
Total LCA	1.01	0.05	2.92	0.24	2.88	0.000
Total UDCA	1.56	0.07	18.6	3.23	11.9	0.001
Total CDCA	2.52	0.13	21.0	2.09	8.35	0.000
Total DCA	3.16	0.16	4.92	0.58	1.56	0.072
Total HDCA	0.01	0.00	0.01	0.00	1.57	0.051
Total MDCA	0.06	0.01	0.05	0.01	0.90	0.992
Total CA	0.44	0.03	3.13	0.44	7.09	0.003
Total MCA	0.36	0.02	2.79	0.34	7.83	0.000
Total HCA	0.03	0.00	0.27	0.04	10.6	0.001
Other BA[1]	0.16	0.01	0.86	0.13	5.54	NA
% LCA	11.5	0.38	9.20	0.39	0.79	0.002
% UDCA	17.7	0.49	21.3	0.88	1.21	0.138
% CDCA	27.1	0.65	36.3	0.94	1.34	0.000
% DCA	31.1	0.68	14.6	0.53	0.47	0.000
% HDCA	0.07	0.01	0.04	0.00	0.54	0.052
% MDCA	0.64	0.04	0.36	0.05	0.56	0.135
% CA	5.25	0.27	6.27	0.25	1.19	0.064
% MCA	4.03	0.16	6.39	0.34	1.58	0.003
% HCA	0.30	0.02	0.45	0.04	1.52	0.018
Total Unamidated	1.00	0.05	3.33	0.33	3.34	0.000
Total G-amidated	7.59	0.29	44.6	4.46	5.88	0.000
Total T-amidated	0.71	0.03	6.61	0.85	9.37	0.001
% Amidation	87.7	0.47	86.9	0.65	0.99	0.053
% G-amidation	79.7	0.49	76.0	0.71	0.95	0.000
% T-amidation	7.98	0.26	10.8	0.46	1.35	0.005
Total Unsulfated	0.94	0.05	5.91	0.57	6.26	0.000
Total Sulfated	8.35	0.31	48.7	4.77	5.83	0.000
% Sulfation	88.5	0.46	82.9	0.60	0.94	0.000
Total Mono-OH	1.01	0.05	2.92	0.24	2.88	0.000
Total Di-OH	7.30	0.29	44.6	4.58	6.11	0.000
Total Tri-OH	0.82	0.04	6.19	0.65	7.52	0.000
% Mono-OH	11.5	0.38	9.16	0.39	0.79	0.002
% Di-OH	76.6	0.50	72.7	0.65	0.95	0.001
% Tri-OH	9.58	0.33	13.1	0.43	1.37	0.000
Total 12α-OH	3.62	0.17	8.35	0.83	2.30	0.001
Total non-12α-OH	5.67	0.20	46.2	4.68	8.15	0.000
12α-OH/non12α-OH	0.65	0.02	0.33	0.01	0.51	0.000
CA/CDCA	0.24	0.01	0.24	0.02	1.00	0.625
% 12α-OH	36.7	0.62	22.1	0.54	0.60	0.000

% non-12α-OH	63.3	0.62	77.9	0.54	1.23	0.000
Total Primary	3.34	0.15	27.2	2.59	8.15	0.000
Total Secondary	5.95	0.23	27.4	3.52	4.59	0.000
Primary/ Secondary	0.69	0.03	2.52	0.22	3.63	0.000
% Primary	36.7	0.70	49.4	1.06	1.35	0.000
% Secondary	63.3	0.70	50.6	1.06	0.80	0.000

[1]Other bile acids: Nor-deoxycholic acid, 12-oxo-chenodeoxycholic acid, 3-dehydrocholic acid, 6-oxo-lithocholic acid, 7-oxo-lithocholic acid, 12-oxo-lithocholic acid, isolithocholic acid, and isodeoxycholic acid.

NA: Not available; BA: Bile acids; G: Glycine; T: Taurine; CDCA: Chenodeoxycholic acid; CA: Cholic acid; LCA: Lithocholic acid; UDCA: Ursodeoxycholic acid; DCA: Deoxycholic acid; HDCA: Hyodeoxycholic acid; MDCA: Murideoxycholic acid; MCA: Muricholic acid; HCA: Hyocholic acid.

ROC curve analysis

Supplementary Table 3 lists the AUC for BA concentrations and indices. Supplementary Table 4 shows the full list of BA concentrations and indices. Total BA, CDCA, CA, % DCA, % HDCA, % MDCA, total G-Amidated, total unsulfated, total sulfated, total di-OH, total tri-OH, total non-12α-OH, 12α-OH/non12α-OH, % 12α-OH, % non-12α-OH, total primary, primary/secondary, % primary, and % secondary produced AUC > 0.7. Figure 1 shows ROC curves of BA indices with AUC > 0.7. Potential cut-off values selected based on the optimum specificity and sensitivity for BA indices with AUC > 0.7 are listed in Supplementary Table 5.

Risk analysis: Logistic regression analysis

Table 7 shows the results of logistic regression analyzes for BA indices with ROC (AUC) > 0.7. Logistic regression analysis detects whether there is a risk of liver disease associated with changes in BA indices. The risk of liver disease increased with changing levels of all BA indices ($P < 0.05$) except (% HDCA and % MDCA). Additionally, the OR from the logistic regression analysis quantifies the magnitude of the risk of developing liver diseases per unit (10% and 20% of the normal value) changes in BA indices. For example, for every 20% increase in the % non-12α-OH BA, the likelihood of having a liver disease increases 2.72-folds (OR: 2.72; $P < 0.05$). In contrast for every 20% increase in the % 12α-OH BA, the likelihood of having a liver disease decreases 0.56-folds (OR: 0.56; $P < 0.05$).

BA profile in different liver disease subtypes

Table 8 compare BA indices with ROC-AUC > 0.7 between controls *vs* patients with specific liver disease subtype. Mixed effects models were used to compare disease subtypes individually *vs* controls. The goal was to identify BA indices that can serve as diagnostic biomarkers for specific liver disease subtypes.

We have found that most BA indices were significantly different between controls *vs* all individual liver disease subtypes. Total BA, total CDCA, total CA, total G-amidated, total unsulfated, total sulfated, total di-OH, total tri-OH, Total non-12α-OH, % non-12α-OH and total primary were higher (1.1- to 39.5-fold) in every liver disease group compared with controls. % Primary and primary/secondary were higher (1.1-fold to 9.27-fold) in all liver disease group compared with controls except in PBC. % DCA, % HDCA, % 12α-OH, and 12α-OH/non-12α-OH were lower (0.07-fold to 0.85-fold) in every liver disease group compared with controls. % MDCA and % secondary was lower in all liver disease group compared with controls except in elevated LFT and PBC, respectively.

Non-BA parameters

In addition to BA indices, we have also examined other biomarkers currently used in the clinic to evaluate liver functions. These non-BA parameters include AST, ALT, AST/ALT, bilirubin, albumin, INR, protime, creatinine, APRI, and MELD. Table 9 compares the non-BA parameters in controls and patients using mixed effects models. All the non-BA parameters were higher in patients compared to controls except albumin and protime, which were lower in patients. Within the patient population, all non-BA parameters were higher in medium compared to low- MELD patients except albumin, and ALT. The same results also applied to decompensated *vs* compensated patients.

Table 5 Representative bile acids concentrations and indices in medium- *vs* low- model for end-stage liver disease patients

BA (μmol/L) or BA indices	Low-MELD		Medium-MELD		Medium- *vs* low-MELD	
	mean	SE	mean	SE	Ratio	*P* value
Total BA	59.2	7.94	116	24.8	1.96	1.000
Total LCA	3.40	0.35	6.01	1.72	1.77	0.175
Total UDCA	24.4	5.34	18.6	6.30	0.76	0.172
Total CDCA	18.3	2.31	71.4	16.3	3.90	0.000
Total DCA	5.30	0.96	6.08	1.47	1.15	1.000
Total HDCA	0.01	0.00	0.01	0.00	0.61	1.000
Total MDCA	0.05	0.01	0.06	0.01	1.28	1.000
Total CA	2.80	0.48	10.6	4.45	3.79	0.000
Total MCA	3.58	0.57	2.15	0.46	0.60	0.210
Total HCA	0.25	0.04	0.86	0.36	3.48	0.002
% LCA	9.31	0.53	7.97	1.47	0.86	1.000
% UDCA	23.1	1.29	14.3	2.52	0.62	1.000
% CDCA	34.7	1.21	55.6	3.17	1.60	0.000
% DCA	13.8	0.65	7.18	1.33	0.52	0.005
% HDCA	0.04	0.01	0.01	0.00	0.32	0.661
% MDCA	0.29	0.04	0.13	0.03	0.43	1.000
% CA	5.75	0.30	8.70	1.25	1.51	0.145
% MCA	7.15	0.48	3.70	0.91	0.52	0.000
% HCA	0.46	0.07	0.75	0.15	1.61	0.148
Total Unamidated	4.24	0.55	2.87	0.72	0.68	0.062
Total G-amidated	48.4	6.89	92.8	19.7	1.92	1.000
Total T-amidated	6.58	1.04	20.7	7.30	3.15	0.040
% Amidation	86.7	0.87	94.4	1.28	1.09	0.005
% G-amidation	75.5	0.96	77.2	2.73	1.02	1.000
% T-amidation	11.2	0.64	17.1	2.15	1.53	0.002
Total unsulfated	6.99	0.93	9.04	2.42	1.29	1.000
Total sulfated	52.3	7.21	107	23.2	2.05	1.000
% Sulfation	82.4	0.81	88.3	1.34	1.07	0.009
Total mono-OH	3.40	0.35	6.01	1.72	1.77	0.175
Total di-OH	48.1	7.01	96.2	20.9	2.00	1.000
Total tri-OH	6.63	0.90	13.6	4.90	2.06	0.301
% Mono-OH	9.31	0.53	7.97	1.47	0.86	1.000
% Di-OH	72.0	0.90	77.2	2.14	1.07	0.058
% Tri-OH	13.4	0.59	13.1	1.40	0.98	0.274
Total 12α-OH	8.55	1.23	16.8	4.86	1.96	0.053
Total non-12α-OH	50.7	7.21	99.6	21.5	1.96	1.000
12α-OH/non12α-OH	0.30	0.01	0.20	0.02	0.68	0.135
CA/CDCA	0.21	0.01	0.17	0.03	0.81	1.000
% 12α-OH	21.0	0.69	16.1	1.44	0.77	0.008
% non-12α-OH	79.0	0.69	83.9	1.44	1.06	0.008

Total primary	25.0	3.08	85.1	19.5	3.41	0.000
Total secondary	34.3	5.78	31.3	8.05	0.91	0.316
Primary/secondary	2.19	0.24	5.02	1.16	2.29	1.000
% Primary	48.1	1.40	68.7	3.10	1.43	0.014
% Secondary	51.9	1.40	31.3	3.10	0.60	0.014

BA: Bile acids; MELD: Model for end-stage liver disease; G: Glycine; T: Taurine; CDCA: Chenodeoxycholic acid; CA: Cholic acid; LCA: Lithocholic acid; UDCA: Ursodeoxycholic acid; DCA: Deoxycholic acid; HDCA: Hyodeoxycholic acid; MDCA: Murideoxycholic acid; MCA: Muricholic acid; HCA: Hyocholic acid.

The AUC for non-BA parameters was > 0.7 for all of them except creatinine, protime, and AST/ALT ratio. Also, per logistic regression analysis, the risk of being diagnosed with a liver disease increased to various extents with changing levels of all non-BA parameters ($P < 0.05$) except creatinine and AST/ALT. For example, for every 20% increase in the albumin and protime, the likelihood of having a liver disease decreases 0.28-fold and 0.85-fold, respectively. In contrast for every 20% increase in the other non-BA parameters, the likelihood of having a liver disease increases 1.13-fold to 3-fold (Supplementary Table 6).

In addition, we have found that most non-BA parameters were significantly different between controls *vs*. all individual liver disease subtypes (Supplementary Table 7). Creatinine, INR, AST, ALT, bilirubin, AST/ALT, and MELD were higher in most liver disease group compared with controls. In contrast, albumin and protime were lower in most liver disease group compared with controls.

Association between non-BA parameters and BA indices

Supplementary Table 8 shows the association between non-BA parameters and BA indices using mixed effects models. We have found that all non-BA parameters were significantly associated with most BA concentrations/indices, except creatinine ($P > 0.05$).

DISCUSSION

To ensure that the difference in the BA profiles between patients and controls are not due to the differences in the demographics we showed that: (1) Most of BA were not associated with demographic covariates, and (2) The ones that were associated had the same extent of association in the patient and control groups (Supplementary Table 1).

Patients were categorized based on the severity of the liver disease using MELD[33-37] and the compensation status[28]. Accordingly, we have compared the BA profiles between entire patient *vs* control populations as well as among the patients with different levels of disease severity. Most BA (except MDCA) were higher, but to different extents, in patients *vs* controls (Table 4) and in the more-severe patient groups, *i.e.*, medium *vs* low-MELD (Table 5) as well as decompensated *vs* compensated (Table 6). In particular, the percentages of the primary BA (CDCA, CA, and HCA) were higher, while the percentage of the secondary BA (DCA) was lower. The % primary BA was 1.4-fold higher, while % secondary BA was 0.8-fold lower and the ratio of primary/secondary BA was 3.6-fold higher in patients *vs* controls (Table 4). The same trend was also observed in the patients with more severe form of the disease, where the % of primary BA also increased with the severity of the liver disease (medium-MELD > low-MELD > controls) and (decompensated > compensated > controls), whereas % secondary BA decreased with the severity of the disease. (Tables 5 and 6).

Cholestatic diseases are associated with impaired bile flow to the intestine, which translates into reduced transformation of primary into secondary BA by intestinal bacteria[9,25,38-40]. Therefore, while all BA concentrations were higher in patients due to the impairment of bile flow, the proportion of secondary BA (formed in the intestine) decreased with the severity of the cholestatic disease, which may reflect the extent of bile flow impairment.

The conjugation of BA with G and T decreases their pKa, increases their ionization and solubility, enhances their urinary elimination, and decreases their toxicity[30,41-44]. However, T-amidated BA are generally less cytotoxic and more ionized than G-

Table 6 Representative bile acids concentrations and indices in compensated *vs* decompensated patients

BA (μmol/L) or BA indices	Compensated		Decompensated		Decompensated *vs* compensated	
	mean	SE	mean	SE	Ratio	*P* value
Total BA	66.6	10.8	86.9	14.9	1.31	0.160
Total LCA	3.73	0.54	4.26	0.70	1.14	0.547
Total UDCA	27.0	6.49	21.0	9.82	0.78	0.687
Total CDCA	20.4	3.42	45.0	6.28	2.20	0.001
Total DCA	6.85	1.76	4.93	0.73	0.72	0.394
Total HDCA	0.01	0.00	0.02	0.01	1.61	0.430
Total MDCA	0.06	0.01	0.05	0.01	0.86	0.619
Total CA	2.62	0.40	6.28	1.51	2.39	0.024
Total MCA	4.48	0.93	4.07	0.83	0.91	0.864
Total HCA	0.20	0.04	0.64	0.14	3.23	0.002
% LCA	9.00	0.64	6.61	0.64	0.73	0.020
% UDCA	24.9	1.97	12.0	1.32	0.48	0.007
% CDCA	33.2	1.62	54.74	2.05	1.65	0.000
% DCA	14.3	0.98	9.17	1.00	0.64	0.000
% HDCA	0.02	0.01	0.03	0.01	1.42	0.532
% MDCA	0.34	0.14	0.11	0.01	0.33	0.264
% CA	6.07	0.54	7.58	0.48	1.25	0.262
% MCA	7.26	0.72	7.21	0.82	0.99	0.542
% HCA	0.35	0.05	0.74	0.08	2.09	0.005
Total unamidated	4.35	0.69	3.88	1.04	0.89	0.876
Total G-amidated	56.2	9.81	70.5	12.5	1.25	0.240
Total T-amidated	5.97	0.79	12.6	2.58	2.11	0.010
% Amidation	87.9	1.15	93.6	0.75	1.06	0.003
% G-amidation	76.5	1.30	78.8	1.23	1.03	0.161
% T-amidation	11.5	0.93	14.8	1.02	1.29	0.161
Total unsulfated	7.84	1.19	9.53	1.85	1.22	0.310
Total sulfated	58.7	9.97	77.4	13.4	1.32	0.156
% Sulfation	82.7	1.12	85.2	0.99	1.03	0.054
Total mono-OH	3.73	0.54	4.26	0.70	1.14	0.547
Total di-OH	54.4	9.55	70.9	13.1	1.31	0.174
Total tri-OH	7.30	1.25	11.0	1.96	1.51	0.085
% Mono-OH	9.00	0.64	6.61	0.64	0.73	0.020
% Di-OH	72.7	1.14	76.0	1.31	1.05	0.016
% Tri-OH	13.7	0.92	15.5	0.95	1.13	0.674
Total 12α-OH	10.1	2.08	11.44	1.75	1.14	0.554
Total non-12α-OH	56.5	9.36	75.51	14.0	1.34	0.137
12α-OH/non12α-OH	0.33	0.02	0.24	0.02	0.71	0.002
CA/CDCA	0.21	0.02	0.17	0.01	0.79	0.043
% 12α-OH	22.0	1.07	17.3	0.99	0.79	0.001
% non-12α-OH	78.0	1.07	82.7	0.99	1.06	0.001

Total primary	27.7	4.46	56.0	7.59	2.02	0.001
Total secondary	38.8	7.43	31.0	10.3	0.80	0.874
Primary/secondary	2.27	0.44	5.98	0.69	2.64	0.001
% Primary	46.9	2.05	70.3	1.88	1.50	0.000
% Secondary	53.1	2.05	29.7	1.88	0.56	0.000

BA: Bile acids; G: Glycine; T: Taurine; CDCA: Chenodeoxycholic acid; CA: Cholic acid; LCA: Lithocholic acid; UDCA: Ursodeoxycholic acid; DCA: Deoxycholic acid; HDCA: Hyodeoxycholic acid; MDCA: Murideoxycholic acid; MCA: Muricholic acid; HCA: Hyocholic acid.

Table 7 Univariate logistic regression analysis of bile acids concentrations and indices[1]

BA (μmol/L) or BA indices	*B* value (regression coefficient)	*P* value	Exp(B)-odds ratio		
			1-unit change	10% change	20% change
Total BA	0.080	0.000	1.08	1.08	1.16
Total CDCA	0.226	0.000	1.25	1.06	1.12
Total CA	1.181	0.000	3.26	1.05	1.11
% DCA	-0.080	0.000	0.92	0.78	0.61
% HDCA	-1.898	0.069	0.15	0.99	0.97
% MDCA	-0.174	0.162	0.84	0.99	0.98
Total G-amidated	0.084	0.000	1.09	1.07	1.14
Total unsulfated	0.784	0.000	2.19	1.08	1.16
Total sulfated	0.080	0.000	1.08	1.07	1.14
Total di-OH	0.094	0.000	1.10	1.07	1.15
Total tri-OH	0.731	0.000	2.08	1.06	1.13
Total non-12α-OH	0.146	0.000	1.16	1.09	1.18
12α-OH/non12α-OH	-2.349	0.000	0.10	0.86	0.74
% 12α-OH	-0.079	0.000	0.92	0.75	0.56
% non-12α-OH	0.079	0.000	1.08	1.65	2.72
Total primary	0.190	0.000	1.21	1.07	1.14
Primary/secondary	0.834	0.000	2.30	1.06	1.12
% Primary	0.033	0.000	1.03	1.13	1.27
% Secondary	-0.033	0.000	0.97	0.81	0.66

[1]Bile acids with receiver operating characteristic (ROC)-areas under the ROC curve > 0.7 were included in this table.
BA: Bile acids; G: Glycine; CDCA: Chenodeoxycholic acid; CA: Cholic acid; DCA: Deoxycholic acid; HDCA: Hyodeoxycholic acid; MDCA: Murideoxycholic acid.

amidated BA[43,45,46]. Even though unamidated as well as T-and G-amidated BAs were higher in patients, the increase in T-amidated BA was the most profound. Therefore, % T-amidation increased, while % G-amidation decreased in patients *vs* controls (Table 4) as well as in medium-MELD *vs* low-MELD (Table 5) and decompensated *vs* compensated patients (Table 6). The preferential accumulation of T-amidated BA can be interpreted as an adaptive compensating response to protect the liver from BA toxicity by increasing elimination of the more toxic G-amidated and unamidated compared to the less toxic T-amidated BA[9,26,47]. In addition, T-amidated BA has the highest affinity as substrates for the canalicular transporter, Bile Salt Export Pump (BSEP) (T-amidated > G-amidated > unamidated BA)[48-50]. Therefore, an impairment of the BA transport by BSEP, as documented in some cholestatic diseases[51-53], is expected to preferential accumulation T-amidated BA.

Table 8 Bile acids concentrations and indices in controls and patients with specific liver disease subtype[1]

BA (μmol/L) or BA indices	Controls		Hepatitis C		Hepatitis B		Laennec cirrhosis		Primary biliary cholangitis		Primary sclerosing cholangitis		Autoimmune Hepatitis		α-1 antitrypsin deficiency		NASH		Carcinoma		Cryptogenic cirrhosis		Elevated LFT	
	n = 103		*n* = 71		*n* = 15		*n* = 117		*n* = 12		*n* = 17		*n* = 27		*n* = 6		*n* = 56		*n* = 26		*n* = 11		*n* = 22	
	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
Total BA	9.30	0.33	53.3[a]	9.96	13.7[a]	5.23	62.0[a]	9.44	237[a]	69.8	124[a]	27.4	71.9[a]	15.2	25.4[a]	6.79	29.8[a]	4.31	90.9[a]	26.7	56.0[a]	16.3	106[a]	69.3
Total CDCA	2.52	0.13	27.0[a]	4.89	6.76[a]	4.16	30.0[a]	4.25	28.6[a]	9.99	39.4[a]	10.7	29.2[a]	8.46	9.14[a]	3.12	13.6[a]	2.33	31.9[a]	8.76	27.7[a]	7.39	31.3[a]	18.8
Total CA	0.44	0.03	3.05[a]	0.54	1.16[a]	0.70	4.00[a]	1.02	5.07[a]	2.27	6.47[a]	2.25	1.96[a]	0.39	2.44[a]	0.85	1.65[a]	0.23	3.54[a]	1.11	2.70[a]	0.59	5.55[a]	3.09
% DCA	31.1	0.68	16.2[a]	1.27	19.9[a]	3.26	13.1[a]	0.95	7.99[a]	2.22	9.01[a]	1.86	15.6[a]	1.43	18.9	4.23	15.7[a]	1.22	14.9[a]	1.43	7.93[a]	2.68	17.4[a]	3.07
% HDCA	0.07	0.01	0.02[a]	0.00	0.06	0.02	0.02[a]	0.01	0.03	0.02	0.01	0.00	0.02	0.00	0.03	1.21	0.05	0.01	0.06	0.02	0.04	0.03	0.05	0.04
% MDCA	0.64	0.04	0.19[a]	0.04	0.38	0.08	0.16[a]	0.03	0.15[a]	0.07	0.18[a]	0.06	0.22[a]	0.05	0.38	0.16	0.49	0.21	0.16[a]	0.05	0.07[a]	0.01	1.34	0.98
Total G-amidated	7.59	0.29	44.8[a]	9.11	11.6[a]	4.52	50.8[a]	8.26	210[a]	60.4	106[a]	24.0	61.8[a]	13.5	16.3[a]	4.58	26.0[a]	4.01	78.1[a]	24.5	49.2[a]	14.9	86.8[a]	58.2
Total unsulfated	0.94	0.05	7.69[a]	1.43	1.62[a]	0.38	7.94[a]	1.21	17.6[a]	8.66	6.21[a]	1.13	6.06[a]	1.44	3.82[a]	1.21	4.64[a]	0.65	13.0[a]	3.16	3.58[a]	0.58	13.0[a]	9.22
Total sulfated	8.35	0.31	45.6[a]	8.73	12.1[a]	4.96	54.1[a]	8.59	219[a]	62.0	117[a]	26.5	65.9[a]	14.7	21.6[a]	6.11	25.2[a]	4.04	77.9[a]	24.2	52.4[a]	16.0	92.9[a]	60.2
Total di-OH	7.30	0.29	41.0[a]	7.98	10.6[a]	4.41	49.05[a]	7.97	214[a]	62.50	111[a]	25.9	60.9[a]	13.7	15.9[a]	4.72	23.2[a]	3.87	71.9[a]	23.0	50.3[a]	16.0	91.0[a]	61.0
Total tri-OH	0.82	0.04	8.61[a]	1.63	1.82[a]	0.80	8.59[a]	1.54	12.48[a]	6.02	9.28[a]	2.44	4.83[a]	0.76	5.24[a]	1.83	4.27[a]	0.66	13.8[a]	3.62	3.92[a]	0.65	10.8[a]	6.50
Total non-12α-OH	5.67	0.20	41.8[a]	7.46	10.3[a]	4.52	50.8[a]	7.85	224[a]	66.9	113[a]	26.5	62.3[a]	14.2	19.4[a]	5.59	23.8[a]	3.74	75.7[a]	22.1	51.1[a]	16.1	94.7[a]	66.1
12α-OH/non12α-OH	0.65	0.02	0.37[a]	0.05	0.51	0.08	0.31[a]	0.02	0.15[a]	0.05	0.29[a]	0.05	0.33[a]	0.04	0.40	0.07	0.34[a]	0.02	0.28[a]	0.03	0.22[a]	0.05	0.37[a]	0.06
% 12α-OH	36.7	0.62	22.8[a]	1.28	31.1	2.9	21.3[a]	1.0	10.8[a]	2.69%	18.8[a]	2.6	22[a]	1.69	27.1	3.18	23.5[a]	1.14	20.6[a]	1.55	16.2[a]	2.88	24.8[a]	2.8
% non-12α-OH	63.3	0.62	77.2[a]	1.28	68.9	2.9	78.7[a]	1.0	89.2[a]	2.69	81.2[a]	2.6	78.0[a]	1.69	72.9	3.18	76.5[a]	1.14	79.4[a]	155	83.8[a]	2.88	75.2[a]	2.8
Total primary	3.34	0.15	35.6[a]	6.23	8.58[a]	4.93	38.6[a]	5.47	41.1[a]	15.6	48.6[a]	12.6	34.1[a]	8.91	14.4[a]	4.33	17.9[a]	2.70	45.7[a]	12.1	31.6[a]	7.89	42.1[a]	23.9
Primary/secondary	0.69	0.03	3.70[a]	0.55	1.52[a]	0.59	4.33[a]	0.60	0.30	0.10	2.88[a]	0.68	2.09[a]	0.60	1.26	0.26	2.28[a]	0.30	2.32[a]	0.40	6.43[a]	2.09	1.70[a]	0.43
% Primary	36.7	0.70	60.2[a]	1.99	47.0[a]	3.93	60.9[a]	1.7	18.0[a]	2.97	51.6[a]	5.09	50.1[a]	2.77	50.3	5.43	52.8[a]	2.21	56.1[a]	3.25	68.2[a]	5.80	49.3[a]	4.6
% Secondary	63.3	0.70	39.8[a]	1.99	53.0[a]	3.93	39.1[a]	1.7	82.0[a]	2.97	48.4[a]	5.09	49.9[a]	2.77	49.7	5.43	47.2[a]	2.21	43.9[a]	3.25	31.8[a]	5.80	50.7[a]	4.6

[1]Bile acids with receiver operating characteristic (ROC)-areas under the ROC curve > 0.7 were included in this table.

[a]Significant difference between each specific liver disease subtype *vs* controls ($P < 0.05$).

BA: Bile acids; G: Glycine; CDCA: Chenodeoxycholic acid; CA: Cholic acid; DCA: Deoxycholic acid; HDCA: Hyodeoxycholic acid; MDCA: Murideoxycholic acid.

Both sulfated and unsulfated BA were higher in patients (Table 4), but % sulfation was slightly higher in medium- compared with low-MELD and in decompensated compared with compensated patients (Tables 5 and 6). The upregulation of sulfation of BA by SULT2A1 in patients with liver diseases is thought as a compensatory response to eliminate and detoxify the accumulated toxic BA[8-13,54,55]. However, it is also possible that sulfation activity in these patients may eventually decrease due to exhaustion or defects of these recovery mechanisms. Therefore, while liver insults can be remediated by upregulating BA sulfation under normal conditions and in milder forms of liver diseases, but subjects who fail to upregulate this defensive mechanism or exhaust it under more severe forms of the diseases are at higher risk of developing the disease and/or may have worse prognosis[26]. Another explanation for the preferential accumulation of BA-sulfates could be related to the inhibition of their canalicular transport into bile by efflux transporters, mainly the multidrug resistance-associated proteins 2-4 (MRP 2-4). These transporters preferentially transport divalent amidated and conjugated (sulfated and glucuronidated) BA[56-59]. MRPs including MRP2 activity is known to be compromised in various cholestatic liver diseases due downregulation of their expression and/or membrane localization[60-62], which may lead to the preferential retention of their substrates including BA-sulfates in the liver and systemic circulation.

CYP8B1 catalyzes 12α-hydroxylation of the di-OH CDCA to the tri-OH CA. The CA/CDCA or the 12α-OH/non-12α ratios are used as probes to measure CYP8B1 activity[63-65]. The 12α-OH/non-12α-OH ratio was 50% lower in patients compared with controls (Table 4). Also, both ratios were lower in medium-MELD *vs* low-MELD as well as decompensated *vs* compensated patients (Tables 5 and 6). This indicates that CYP8B1 activity, which exclusively takes place in the liver[66,67], may be compromised during liver diseases in general and is further compromised with disease severity. Also, CDCA has a much higher affinity to BSEP than CA and other 12α-OH BA[49,68]. Therefore, when BSEP activity is compromised in the more severe liver diseases, it is expected to lead to the preferential accumulation of its high-affinity substrates including CDCA, which will also decrease the CA/CDCA and 12α-OH/non-12α ratios.

Many BA concentrations and indices demonstrated AUC > 0.7 supporting their potential as biomarkers for the diagnosis of liver diseases (Supplementary Table 3). We identified three potential cut-off values, which achieve a good balance between specificity and sensitivity (Supplementary Table 5). BA indices have higher AUC values than the absolute BA concentrations, which indicates that BA indices are more accurate in distinguishing between controls and patients.

We found correlation between the risk of developing a liver disease and many BA indices using logistic regression analysis ($P < 0.05$). The univariate logistic regression associated with a 20% change from the mean value for the absolute BA concentrations ranged from 1.11 to 1.18, whereas it was as high as 2.72 for BA indices (Table 7). This suggests that BA indices are more sensitive than absolute BA concentrations in terms of predicting larger magnitudes of the risk of developing a liver disease.

All the above analyses demonstrate that BA indices can serve as a global marker to differentiate the pooled cholestatic liver disease population from controls in this study. In addition, we have divided the patients into different individual disease groups and performed similar analyses in these groups vs. controls, for the individual diseases. Most BA indices with ROC-AUC > 0.7 were significantly different between controls *vs* most of the individual liver disease subtypes (Table 8). In particular, hepatitis C and cirrhosis were the largest subpopulations in our study, and all global diagnostic BA indices from the pooled patients vs. control analyses ($P < 0.05$ and ROC-AUC > 0.7) were also specific diagnostic markers for these two particular liver diseases vs. controls ($P < 0.05$).

We have found a significant correlation between BA indices and non-BA parameters, except creatinine ($P > 0.05$) (Supplementary Table 8). However, BA indices, in general, outperformed non-BA parameters as biomarkers for liver diseases on many levels. Non-BA parameters were 0.76-fold to 2.5-fold higher (Table 9), whereas BA indices were as high as approximately 12-fold higher (total UDCA) in patients compared to controls (Table 4). Similarly, the magnitude of change within the MELD groups, compensation status, and among individual diseases were all much higher in BA *vs* non-BA.

This study has the following limitations: (1) Severity of the liver diseases were assessed using MELD score, compensation status, and a panel of liver enzymes. However, liver histological evaluation was not included because it is not a routine practice to perform liver histology on all patients, but rather for specific patients as required by the hepatologists. And (2) we have enough subjects in this study to

Table 9 Summary of non-bile acids parameters

Non-BA parameters	Controls		Patients										ROC[1]
			Pooled		Low-MELD		Medium-MELD		Compensated		Decompensated		AUC
	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE	
Creatinine (mg/dL)	0.87	0.01	0.99	0.05	0.93	0.07	1.33[b]	0.16	1.05	0.15	1.05	0.06	0.539
Albumin (g/dL)	3.96	0.02	3.61[a]	0.03	3.61	0.03	2.82[b]	0.10	3.69	0.04	3.03[c]	0.06	0.713
INR	0.99	0.01	1.18[a]	0.02	1.11	0.01	1.63[b]	0.10	1.15	0.03	1.36[c]	0.03	0.758
Protime (s)	13.4	0.10	10.2[a]	0.33	13.6	0.13	19.4[b]	0.98	11.2	0.52	13.7[c]	0.64	0.591
AST (U/L)	22.8	0.34	53.2[a]	2.31	52.1	2.59	79.2[b]	10.4	52.6	3.97	61.7	4.85	0.876
ALT (U/L)	21.0	0.46	51.0[a]	2.60	51.0	3.24	46.0	5.54	49.0	4.09	40.6	3.55	0.825
Bilirubin (mg/dL)	0.62	0.03	1.58[a]	0.09	1.31	0.05	5.02[b]	0.68	1.42	0.12	3.04[c]	0.29	0.804
AST/ALT	1.15	0.01	1.22	0.02	1.21	0.03	1.79[b]	0.09	1.21	0.04	1.61[c]	0.05	0.500
MELD	7.13	0.10	10.3[a]	0.24	9.07	0.16	18.9	0.42	9.54	0.37	14.0[c]	0.46	0.747
APRI	NA	NA	0.93	0.06	1.05	0.07	2.44[b]	0.42	0.94	0.08	1.63[c]	0.18	NA

[1]Areas under the receiver operating characteristic curve from receiver operating characteristic analysis of pooled patients *vs* controls.
[a]Significant difference between patients *vs* controls ($P < 0.05$).
[b]Significant difference between medium-model for end-stage liver disease *vs* low-model for end-stage liver disease groups ($P < 0.05$).
[c]Significant difference between decompensated *vs* compensated patients ($P < 0.05$).
NA: Not available; BA: Bile acids; MELD: Model for end-stage liver disease; INR: International normalized ratio; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; APRI: Aspartate aminotransferase/platelet ratio index; ROC: Receiver operating characteristic curve; AUC: Areas under the receiver operating characteristic curve.

perform solid statistics, but smaller number of subjects in many individual disease subgroups. Also, distribution of subjects between disease groups was unbalanced.

CONCLUSION

In summary, the results of this study demonstrated that total and all individual BA increased in patients with 11 different cholestatic diseases. However, the high inter-individual variability of BA absolute concentrations makes most of them statistically insignificant and prevent their utilization as diagnostic markers. In contrast, BA indices had much lower inter- and intra-individual variability, which allowed their use as diagnostic and prognostic markers for liver diseases. Furthermore, we have shown that several BA indices outperformed non-BA markers, currently used in the clinic, as

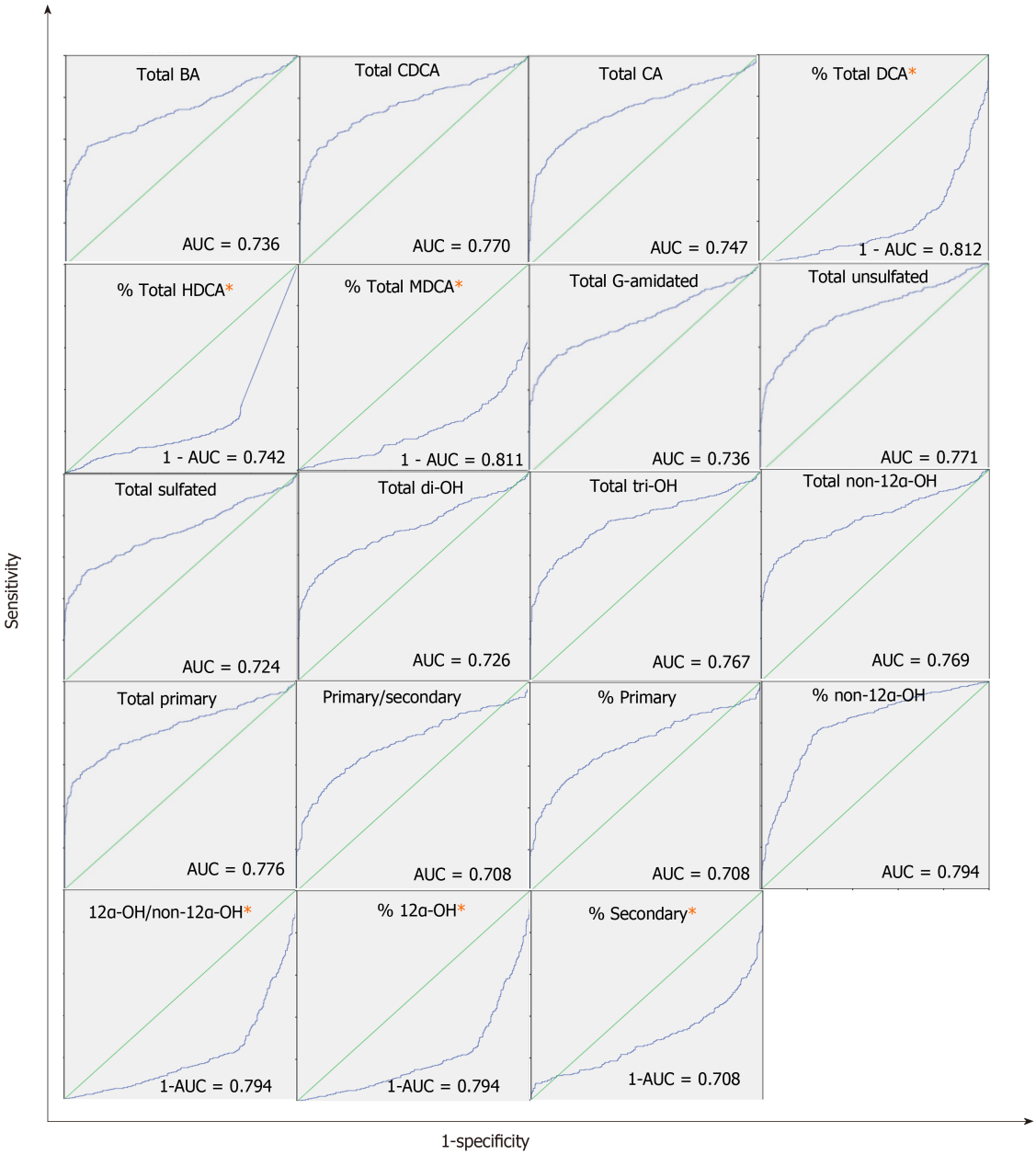

Figure 1 Receiver operating characteristics curves of bile acids concentrations and indices with area under the receiver operating characteristics curve > 0.7. The area under the receiver operating characteristics curve (AUC) for differentiating patients from healthy controls. The scale of both the Y-axis (sensitivity) and the X-axis (1-specificity) is 0-1. Bile acids (BA) indices are higher in patients vs. controls, and the positive actual state was patients except the ones annotated with "*", where BA indices were lower in patients compared to controls. For these BA indices, "1-AUC" instead of "AUC" was calculated. AUC: Area under the receiver operating characteristics curve; BA: Bile acids; CDCA: Chenodeoxycholic acid; CA: Cholic acid; DCA: Deoxycholic acid; HDCA: Hyodeoxycholic acid; MDCA: Murideoxycholic acid; G: Glycine.

diagnostic markers to differentiate our patient pool as well as individual cholestatic diseases against healthy controls.

The increase in the total BA concentration in patients can be attributed to specific changes in the BA pool composition. This increase primarily resulted from primary BA (CDCA, CA, and HCA), while the % of the secondary BA (LCA and DCA) were lower. This lead to about 4-fold increase in the primary/secondary BA ratio. Consequently, the BA pool has drastically shifted in patients from being 37% primary to approximately 50% primary BA. The increase in T-amidated BA was more profound than that of G-amidated BA, which lead to a marked increase in the % T-amidation. Furthermore, this trend of elevated primary and amidated BA was exacerbated with disease severity. This pattern can be a sign of less transformation of primary into secondary and less deconjugation of amidated BA by intestinal bacteria associated with more impairment of bile flow associated with more severe cholestatic diseases. % Sulfation was higher in patients with more severe forms of liver diseases indicating the

upregulation of sulfation in these patients as a compensatory response to detoxify BA accumulation. Finally, the increase in non-12α-OH was more profound than that of 12α-OH BA, which indicates that hepatic CYP8B1 activity is compromised in liver diseases in general and is further compromised with disease severity.

In the 2nd paper of this series, we have utilized BA indexes to build a survival model called "The Bile Acid Score", which we showed was able to predict the prognosis into adverse events including death and liver transplant in liver patients.

ARTICLE HIGHLIGHTS

Research background

Bile acids (BA) have been extensively investigated for decades as biomarkers for numerous hepatobiliary diseases. However, these efforts never translated into a widespread in the clinic, due to the extreme inter-and intra-individual variability of total and individual BA concentrations and the marked differences in the physiological and pathological properties of the different individual BA. To this end, we have developed the concept of "BA indices", which demonstrated their use as diagnostic biomarkers for cholestatic liver diseases.

Research motivation

Biomarkers currently used in the clinic are not specific to the liver or bile duct injurie. BA were extensively investigated for decades as biomarkers for numerous hepatobiliary diseases. This could be attributed to the marked differences in the physiological and pathological properties of the different individual BA. BA indices have much lower variability than the absolute BA concentrations used to calculate them. Indeed, we have demonstrated that BA indices offered numerous advantages over absolute total and individual BA concentrations including low inter- and intra-individual variability and were resistant to covariate influences such as age, gender, body mass index, food consumption, and moderate alcohol consumption.

Research objectives

The objective of this project was to discover and validate diagnostic biomarkers of cholestatic liver diseases based on the urinary BA profile. We have developed the concept of "BA indices", which are ratios calculated from the absolute concentrations of individual BA and their metabolites. BA indices have much lower variability than the absolute BA concentrations used to calculate them, which enabled their use as diagnostic biomarkers for cholestatic liver diseases.

Research methods

We analyzed urine samples by liquid chromatography-tandem mass spectrometry and compared the urinary BA profile between patients with hepatobiliary diseases *vs* healthy controls by statistical analysis (independent sample-*t*-test, Mann-Whitney test, Mixed effects models, by pairwise comparisons using Bonferroni's adjustment, receiver operating characteristic curve analyses, Univariate and multivariate logistic regression analysis).

Research results

The results of this study demonstrated that total and all individual BA increased in patients with 11 different cholestatic diseases. However, the high inter-individual variability of BA absolute concentrations makes most of them statistically insignificant and prevent their utilization as diagnostic markers. In contrast, BA indices had much lower inter- and intra-individual variability, which allowed their use as diagnostic and prognostic markers for liver diseases. Furthermore, we have shown that several BA indices outperformed non-BA markers, currently used in the clinic, as diagnostic markers to differentiate our patient pool as well as individual cholestatic diseases against healthy controls.

Research conclusions

BA indices demonstrated high area under the receiver operating characteristic curves, and changes of BA indices were associated with the risk of having a liver disease as determined by the logistic regression analysis, which demonstrated their use as diagnostic biomarkers for cholestatic liver diseases.

Research perspectives

We have developed survival models based on BA indices to predict the prognosis of hepatobiliary diseases which is illustrated in the second paper of this series.

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ORIGINAL ARTICLE

Retrospective Cohort Study

Elderly patients (≥ 80 years) with acute calculous cholangitis have similar outcomes as non-elderly patients (< 80 years): Propensity score-matched analysis

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Abstract

BACKGROUND

Acute cholangitis (AC) is a disease spectrum with varying extent of severity. Age ≥ 75 years forms part of the criteria for moderate (Grade II) severity in both the Tokyo Guidelines (TG13 and TG18). Aging is associated with reduced physiological reserves, frailty, and sarcopenia. However, there is evidence that age itself is not the determinant of inferior outcomes in elective and emergency biliary diseases. There is a paucity of reports comparing clinical outcomes amongst elderly patients *vs* non-elderly patients with AC.

AIM

To investigate the effect of age (≥ 80 years) on AC's morbidity and mortality using propensity score matching (PSM).

METHODS

This is a single-center retrospective cohort study of all patients diagnosed with calculous AC (January 2016 to December 2016) and ≥ 80 years old (January 2012 to December 2016) at a tertiary university-affiliated teaching hospital. Inclusion criteria were patients who were treated for suspected or confirmed AC secondary to biliary stones. Patients with AC on a background of hepatobiliary malignancy, indwelling permanent metallic biliary stents, or concomitant pancreatitis were excluded. Elderly patients were defined as ≥ 80 years old in our study. A 1:1 PSM analysis was performed to reduce selection bias and address confounding factors.

Informed consent statement: This study was conducted using data collected from an institutional board approved standing database (National Healthcare Group Domain Specific Review Board, Ref No.: 2017/00200). Informed consent was hence not obtained from the included patients. Collected data were de-identified and were only accessible to members of the study team with no subsequent patient contact for data collection purposes. The study team made no attempts to access patients' medical records *via* the national electronic health record system.

Conflict-of-interest statement: All of the authors declare no conflicts of interest.

Data sharing statement: The data used in this study is not publicly available due to institutional policies. However, requests may be made to the corresponding author for access to de-identified data.

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Study variables include comorbidities, vital parameters, laboratory and radiological investigations, and type of biliary decompression, including the time for endoscopic retrograde cholangiopancreatography (ERCP). Primary outcomes include in-hospital mortality, 30-d and 90-d mortality. Length of hospital stay (LOS) was the secondary outcome.

RESULTS

Four hundred fifty-seven patients with AC were included in this study (318 elderly, 139 non-elderly). PSM analysis resulted in a total of 224 patients (112 elderly, 112 non-elderly). The adoption of ERCP between elderly and non-elderly was similar in both the unmatched (elderly 64.8%, non-elderly 61.9%, P = 0.551) and matched cohorts (elderly 68.8% and non-elderly 58%, P = 0.096). The overall in-hospital mortality, 30-d mortality and 90-d mortality was 4.6%, 7.4% and 8.5% respectively, with no statistically significant differences between the elderly and non-elderly in both the unmatched and matched cohorts. LOS was longer in the unmatched cohort [elderly 8 d, interquartile range (IQR) 6-13, *vs* non-elderly 8 d, IQR 5-11, P = 0.040], but was comparable in the matched cohort (elderly 7.5 d, IQR 5-11, *vs* non-elderly 8 d, IQR 5-11, P = 0.982). Subgroup analysis of patients who underwent ERCP demonstrated the majority of the patients (n = 159/292, 54.5%) had delayed ERCP (> 72 h from presentation). There was no significant difference in LOS, 30-d mortality, 90-d mortality, and in-hospital mortality in patients who had delayed ERCP in both the unmatched and matched cohort (matched cohort: in-hospital mortality [n = 1/42 (2.4%) *vs* 1/26 (3.8%), P = 0.728], 30-d mortality [n = 2/42 (4.8%) *vs* 2/26 (7.7%), P = 0.618], 90-d mortality [n = 2/42 (4.8%) *vs* 2/26 (7.7%), P = 0.618], and LOS (median 8.5 d, IQR 6-11.3, *vs* 8.5 d, IQR 6-15.3, P = 0.929).

CONCLUSION

Mortality is indifferent in the elderly (≥ 80 years old) and non-elderly patients (< 80 years old) with AC.

Key Words: Cholangitis; Choledocholithiasis; Cholelithiasis; Aged 80 and over; Geriatrics; Cholangiopancreatography; Endoscopic retrograde

Core Tip: There is a paucity of data on mortality outcomes amongst elderly *vs* non-elderly patients with acute cholangitis. The overall in-hospital mortality, 30-d mortality and 90-d mortality was 4.6%, 7.4% and 8.5% respectively, with no significant differences in both the unmatched and matched cohorts. Mortality was comparable in patients with delayed endoscopic retrograde cholangiopancreatography.

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INTRODUCTION

Gallstones are widely prevalent in the community, and patients with gallstones are at risk of complications like acute cholecystitis, acute pancreatitis, and acute cholangitis (AC). AC results from an obstructed biliary system with sepsis, and resulting endotoxic shock is associated with a mortality risk of up to 20%[1]. AC is a disease spectrum ranging from mild AC, which may respond to conservative management with medical therapy, to severe AC, which requires urgent biliary decompression in addition to fluid resuscitation and antibiotics[2]. Tokyo Guidelines (TG13 and TG18) are widely accepted internationally and form the basis for diagnosis, severity

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stratification, and management of patients with AC[3]. In AC, age determines the severity stratification, and age ≥ 75 years is a criterion for moderate (Grade II) severity in both the TG13 and TG18 guidelines[3]. Aging is associated with reduced cardiac output, impaired gas exchange, reduction in vital capacity, decline in lean body mass, creatinine clearance reduction, hepatic drug metabolism impairment, frailty, and sarcopenia[4]. Due to functional metabolic decline, multiple comorbidities, and atypical presentation with potential diagnostic delays, age contributes to inferior outcomes[5]. Age is an independent predictor of mortality in lower respiratory tract infections, urinary tract infections, gastrointestinal infections and biliary infections[5-8]. Age is also a predictor of disease severity with higher morbidity and mortality risk[9].

However, there is evidence that age itself is not the determinant of inferior outcomes in elective and emergency biliary diseases[10,11]. Endoscopic retrograde cholangiopancreatography (ERCP) have been demonstrated to be safe with good outcomes in elderly patients[11,12]. In a study including 149 acute cholecystitis patients treated with emergency laparoscopic cholecystectomy (LC), Amirthalingam *et al*[13] showed that patient comorbidities and not age determine outcomes. In a study reporting 85 patients with a median age of 83 years (interquartile range 80-89) and admitted to intensive care unit (ICU) with a diagnosis of AC, Novy *et al*[14] reported malnutrition [odds ratio (OR) = 34.5, 95% confidence interval (CI): 1.4-817.9] and sequential organ failure assessment (SOFA) score at 48 h (OR by unit 0.7, 95%CI: 0.5-0.9) were associated with higher 6-mo mortality. Further, aging may impact other clinically relevant non-mortality outcomes such as length of hospital stay (LOS). In a prospective study including 124 patients with acute hepatobiliary sepsis and a median age of 64.5 years, Mak *et al*[15] have reported that age predicts LOS. There is a paucity of comparative data reporting mortality and LOS amongst elderly and non-elderly patients with AC. Also, aging is associated with the confounding effect of comorbidity. This, along with heterogeneity of evidence reporting outcomes in patients with diverse etiology of AC, leaves a lacuna in the scientific literature on the real impact of age on patients with AC due to stone disease. Our hypothesis is, age ≥ 80 years old is associated with higher mortality in patients with AC. This propensity score-matched study aims to investigate if mortality is higher in the elderly (≥ 80 years old) patients with AC as compared to non-elderly (< 80 years old).

MATERIALS AND METHODS

This is a single-center retrospective cohort study of all patients diagnosed with calculous AC (January 2016 to December 2016) and ≥ 80 years old AC patients (January 2012 to December 2016) at a tertiary university-affiliated teaching hospital. We included patients treated for a suspected or confirmed AC diagnosis due to biliary stones[16]. Patients with AC on a background of hepatobiliary malignancy, indwelling permanent metallic biliary stents, or concomitant pancreatitis were excluded. The severity grading of AC in the TG13 included age greater than 75 years as a risk factor, which was retained in TG18[17]. Due to a higher sample of elderly patients, the overall cohort's median age was > 80 years, so we defined elderly as ≥ 80 years old. Non-elderly was defined as patients < 80 years old. Our local institutional review board (National Healthcare Group Domain Specific Review Board, No. 2017/00200) approved this study. This study's conduct is per the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement for retrospective cohort studies[18].

Study variables and outcomes

The patient demographics and clinical outcomes were studied. Patient demographics included age, gender, and comorbidities. Comorbidities included diabetes mellitus, ischemic heart disease, chronic obstructive pulmonary disease, asthma, chronic renal failure, and biliary disease history. Previous history of biliary colic, acute cholecystitis, AC, and acute biliary pancreatitis were collectively defined as history of biliary disease. Presenting symptoms at admission included abdominal pain, fever, vomiting, jaundice, and hypotension. Hypotension was defined as admission systolic blood pressure < 90 mmHg. Laboratory data included white blood cell count, platelet count, creatinine, prothrombin time, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, albumin, gamma-glutamyl transferase, and total bilirubin levels. The shock index (SI) was defined as heart rate divided by the respective systolic blood pressure on arrival in triage[19,20]. Abnormal SI was defined as SI < 0.5 or > 0.7). In patients undergoing ERCP and cholecystectomy, procedure-related data and outcomes

were collected. Delayed ERCP was defined as ERCP > 72 h from admission. The primary outcomes of this study were in-hospital mortality, 30-d mortality and 90-d mortality. In-hospital mortality was defined as any deaths which occurred during the same hospital admission, regardless of the duration from admission. The 30-d and 90-d mortality were defined as any deaths (including both patients who were still inpatient and those who were discharged) within 30 d and 90 d from admission. The secondary clinical outcome was LOS.

Treatment protocol

Patients who presented with septic shock were managed according to the Surviving Sepsis Campaign Guidelines for Management of Severe Sepsis and Septic Shock, 2012[21]. The definite diagnosis of AC was based on the TG13 Guidelines, namely, evidence of systemic inflammation (fever, chills, or laboratory data), cholestasis (jaundice or laboratory data), and imaging of the biliary tree (dilatation, stricture, stone, or stent)[16]. The severity was graded as mild, moderate, or severe as per TG13 guidelines[16]. Out unit was involved in TG07 classification, and we were early adopters of the TG13 system. Thus, the majority of patients had TG13 stratification done prospectively. Patients that were included before the TG13 publication were retrospectively assigned TG13 diagnosis and severity stratification. Blood cultures were taken for all patients included in our study. Broad-spectrum empiric intravenous antibiotics were administered based on local antibiogram and in compliance with the World Society of Emergency Surgery guidelines for optimal and rational use of antibiotics in intra-abdominal sepsis[22,23]. Patients with mild AC, patients who declined invasive intervention, and patients who were responsive to antibiotics alone were managed conservatively. Urgent biliary drainage was performed for patients with moderate and severe AC. The endoscopists' discretion and resources determined the timing of biliary drainage. ERCP was the first-line modality for biliary drainage. A diclofenac suppository is inserted routinely for post-ERCP acute pancreatitis prophylaxis. Percutaneous transhepatic biliary drainage (PTBD) was offered when ERCP was not feasible or contraindicated. Complete stone removal or temporary placement of biliary stents was performed at the endoscopists' discretion. Index admission cholecystectomy was reserved for patients with mild AC and subject to surgeon preference.

Statistical analysis

A 1:1 propensity score matching (PSM)[24] was performed by the first author (Chan KS). PSM was performed at a ratio of 1:1 using a caliper width of 0.2 of the standard deviation of the logit of the propensity score[25]. Patients were adjusted for 15 factors. Seven factors: clinical presentation (fever and hypotension) and laboratory investigations (white blood cell count, platelets, bilirubin, international normalized ratio, and albumin) impact clinical outcomes and thus were adjusted[16,26]. Eight factors were statistically significant ($P < 0.1$) during comparison of the initial demographics between the elderly and non-elderly: gender, comorbidities (ischemic heart disease, chronic renal impairment, and history of biliary disease), clinical presentation (abdominal pain, jaundice), and laboratory investigations (gamma-glutamyl transferase and creatinine), and thus were adjusted. Standardized mean difference (SMD) and Hansen and Bowers were used to assess for covariate and global imbalance, respectively[27].

Categorical values were described as percentages and analyzed by the chi-square test. Continuous variables were expressed as median (interquartile range, IQR) and analyzed by the Mann-Whitney *U* test, respectively. Statistical significance was determined by $P < 0.05$. All statistical analyses were performed with SPSS version 25.0 (SPSS Inc., Chicago, III., United States) and R software (R-3.3.3). The statistical review was performed by one of the co-authors qualified in biomedical statistics (Shelat VG).

RESULTS

Patient demographics and clinical profile

Five hundred fifty-six patients were managed for AC during the study period. Ninety-nine AC patients were excluded due to underlying malignancy. Four hundred fifty-seven patients met the inclusion: 318 (69.6%) elderly *vs* 139 (30.4%) non-elderly. The overall cohort's median age was 82.4 years (IQR 77.6-85.3), with female predominance (n = 252/457, 55.1%). About half (n = 240/457, 52.5%) of patients had a biliary disease history. One hundred and eighty (39.4%) patients had positive blood cultures, and

Escherichia coli was the most common pathogen (n = 129/180, 71.7%). Figure 1 summarizes the microbiology of patients who had positive blood cultures. One hundred and ninety-eight (43.3%) and 126 (27.6%) patients had Grade II and Grade III AC, respectively. When the data of overall cohort was analyzed according to the timing of ERCP (≤ 72 h *vs* > 72 h from admission), there was no difference in the ERCP timing for patients with at least Grade II AC [≤ 72 h, n = 88/201 (43.8%) *vs* > 72 h, n = 113/201 (52.2%), P = 0.368].

PSM with a 1:1 ratio resulted in 224 patients (elderly 112, non-elderly 112). Before PSM, 5 of 15 unmatched variables had SMD > 0.25; following PSM, all of the variables reached an SMD < 0.25 (Table 1 and Figure 2), suggesting an adequate and improved balance. Hansen and Bowers test for global significance also did not demonstrate statistical significance in the matched cohort (matched cohort: χ^2: 4.73, P = 0.994; unmatched cohort: χ^2: 67.4, P < 0.001). Baseline demographics in both the unmatched and matched cohorts are summarized in Table 1. The adoption of biliary drainage procedures was similar between elderly and non-elderly patients in the unmatched cohort. Eleven (3.5%) and 2 (1.4%) elderly and non-elderly respectively received urgent biliary drainage. However, in the matched cohort, elderly patients were more likely to undergo PTBD than non-elderly patients (11.6% *vs* 4.5%, OR 2.81, P = 0.049). Incidence of index admission cholecystectomy and interval cholecystectomy was also comparable between elderly and non-elderly patients in the unmatched cohort. However, in the matched cohort, elderly patients were less likely to undergo index admission cholecystectomy (1.8% *vs* 10.7%, OR 0.15, P = 0.006).

Clinical outcomes

The overall in-hospital mortality, 30-d mortality and 90-d mortality was 4.6%, 7.4% and 8.5% respectively; this was comparable between elderly *vs* non-elderly in both unmatched and matched cohorts. Peri-operative outcomes are summarized in Table 2. In the unmatched cohort, elderly patients had a statistically significant longer LOS (median 8 d, IQR 6-13 *vs* 8 d, IQR 5-11, P = 0.040). However, after matching, LOS was similar (median 7.5 d, IQR 5-11 *vs* 8 d, IQR 5-11, P = 0.982).

Table 3 summarizes the outcomes of patients who underwent ERCP. In the unmatched subgroup of patients who underwent ERCP and had delayed ERCP (> 72 h from admission) (elderly n = 121, non-elderly n = 38), the primary and secondary outcomes were indifferent between elderly and non-elderly patients respectively: in-hospital mortality [n = 2/121 (1.7%) *vs* 1/38 (2.6%), P = 0.699], 30-d mortality [n = 9/121 (7.4%) *vs* 2/38 (5.3%), P = 0.645], 90-d mortality [n = 11/121 (9.1%) *vs* 2/38 (5.3%), P = 0.453], and LOS (median 10 d, IQR 7-15 *vs* 8 d, IQR 6-12, P = 0.103). These outcomes remain indifferent after PSM matching: in-hospital mortality [n = 1/42 (2.4%) *vs* 1/26 (3.8%), P = 0.728], 30-d mortality [n = 2/42 (4.8%) *vs* 2/26 (7.7%), P = 0.618], 90-d mortality [n = 2/42 (4.8%) *vs* 2/26 (7.7%), P = 0.618], and LOS (median 8.5 d, IQR 6-11.3 *vs* 8.5 d, IQR 6-15.3, P = 0.929).

In the unmatched cohort, an abnormal SI was not associated with ERCP [abnormal SI: 178/282 (63.1%) *vs* normal SI: 114/175 (65.1%), P = 0.662]. This was observed in both the elderly [abnormal SI: 125/194 (64.4%) *vs* normal SI: 81/124 (65.3%), P = 0.871] and the non-elderly [abnormal SI: 53/88 (60.2%) *vs* normal SI: 33/51 (64.7%), P = 0.600]. There was no difference after PSM matching on the association of abnormal SI with ERCP: abnormal SI: 90/139 (64.7%) *vs* normal SI: 52/85 (61.2%), P = 0.590. This was true in both the elderly [abnormal SI: 48/66 (72.7%) *vs* normal SI: 29/46 (63%), P = 0.277] and the non-elderly [abnormal SI: 42/73 (57.5%) *vs* normal SI: 23/39 (59%), P = 0.883]. Subgroup analysis of patients with an abnormal SI on triage did not show any significant differences in outcomes between elderly and non-elderly patients (Table 4).

DISCUSSION

In this single-center propensity score-matched study, patients ≥ 80 years old with AC due to biliary stone disease had similar mortality compared to patients < 80 years old. With an increase in life expectancy globally, the elderly population is also increasing. In the elderly population where there is an increased prevalence of gallstones in the elderly population, biliary events including AC are also more common. The elderly poses a unique challenge due to underlying comorbidity, frailty, sarcopenia, functional decline, cognitive decline, and diminished reserves to withstand stress[4]. With diminished physiological reserves, sepsis resulting from AC poses a mortality risk, and our mortality outcomes are acceptable, considering mortality risk of up to 20% in patients with AC[1]. Our reported mortality is comparable to mortality of less than 11%

Table 1 Patient demographics and clinical profile

	Overall cohort, *n* = 457				PSM cohort, *n* = 224			
	Elderly, *n* = 318	Non-elderly, *n* = 139	*P* value	SMD	Elderly, *n* = 112	Non-elderly, *n* = 112	*P* value	SMD
Age, yr	84.0 (82.1, 86.6)	67.9 (57.1, 77.2)	< 0.001		84.3 (82.1, 87.3)	66.6 (55.6, 76.4)	< 0.001	
Gender[1], male (%)	132 (41.5)	73 (52.5)	0.029	0.221	57 (50.9)	53 (47.3)	0.593	0.071
Co-morbidities, *n* (%)								
Diabetes mellitus	124 (39)	55 (39.6)	0.908		44 (39.3)	47 (42)	0.683	
Ischemic heart disease[1]	87 (27.4)	27 (19.4)	0.071	0.188	22 (19.6)	23 (20.5)	0.868	0.022
Chronic renal impairment[1]	61 (19.2)	17 (12.2)	0.069	0.191	17 (15.2)	14 (12.5)	0.562	0.077
COPD and/or asthma	18 (5.7)	4 (2.9)	0.201		8 (7.1)	3 (2.7)	0.122	
History of biliary disease[1]	182 (57.2)	58 (41.7)	0.002	0.313	50 (44.6)	53 (47.3)	0.688	0.054
Clinical presentation								
Abdominal pain[1]	197 (61.9)	109 (78.4)	0.001	0.365	85 (75.9)	85 (75.9)	1.000	< 0.001
Fever[1]	141 (44.3)	67 (48.2)	0.446	0.077	50 (44.6)	52 (46.4)	0.788	0.036
Vomiting	142 (44.7)	63 (45.3)	0.895		51 (45.5)	50 (44.6)	0.893	
Jaundice[1]	48 (15.1)	38 (27.3)	0.002	0.302	27 (24.1)	23 (20.5)	0.521	0.085
Hypotension[1,2]	18 (5.7)	12 (8.6)	0.238	0.115	10 (8.9)	6 (5.4)	0.299	0.138
Laboratory investigations								
WBC[1] (10^9/L)	12.4 (8.9, 16.1)	12.1 (8.3, 15.9)	0.551	0.113	12.2 (8.6, 15.4)	12.1 (8.0, 16.2)	0.745	0.100
Platelets[1] (10^9/L)	192 (150, 250)	216 (166, 280)	0.047	0.131	193 (160, 252)	209 (162, 280)	0.308	0.101
Creatinine[1] (μmol/L)	103 (81, 138)	89 (68, 116)	< 0.001	0.094	103 (80, 136)	86 (67, 119)	0.003	0.088
Albumin[1] (g/L)	32 (28, 35)	35 (29, 38)	< 0.001	0.396	33 (29, 36)	34 (29, 38)	0.186	0.150
Bilirubin[1] (μmol/L)	54 (33, 84)	60 (34, 96)	0.226	0.096	65 (42, 93)	58 (33, 93)	0.287	0.104
ALT (IU/L)	133 (61, 247)	143 (68, 295)	0.330		142 (82, 244)	123 (59, 263)	0.294	
AST (IU/L)	160 (78, 366)	140 (72, 314)	0.165		176 (93, 365)	150 (74, 345)	0.149	
ALP (IU/L)	209 (130, 346)	188 (117, 314)	0.149		208 (137, 346)	184 (111, 291)	0.136	
GGT[1] (IU/L)	242 (129, 435)	327 (158, 562)	0.006	0.292	286 (165, 504)	286 (133, 523)	0.591	0.022
INR[1]	1.13 (1.02, 1.30)	1.20 (1.10, 1.30)	0.890	0.112	1.15 (1.00, 1.30)	1.20 (1.10, 1.30)	0.506	0.038
Microbiology, positive (%)	132 (41.5)	48 (34.5)	0.160		44 (39.3)	36 (32.1)	0.265	
Escherichia coli	99 (75)	30 (62.5)	0.100		33 (75)	24 (66.7)	0.413	
Klebsiella pneumoniae	32 (24.2)	14 (29.2)	0.503		16 (36.4)	12 (33.3)	0.777	
Enterobacter spp	3 (2.3)	1 (2.1)	0.939		0 (0)	1 (2.8)	0.266	
Pseudomonas aeruginosa	1 (0.8)	0 (0)	0.550		0 (0)	0 (0)	-	
Enterococcus spp	1 (0.8)	1 (2.1)	0.453		1 (2.3)	1 (2.8)	0.886	
Citrobacter spp	1 (0.8)	0 (0)	0.545		1 (2.3)	0 (0)	0.357	
Aeromonas spp	1 (0.8)	0 (0)	0.545		0 (0)	0 (0)	-	
CT scan, *n* (%)	108 (34)	52 (37.4)	0.477		43 (38.4)	45 (40.2)	0.784	
Cholelithiasis	75 (69.4)	31 (59.6)	0.218		21 (48.8)	14 (31.1)	0.089	
Biliary dilation	47 (43.5)	18 (34.6)	0.283		30 (69.8)	26 (57.8)	0.243	
Choledocholithiasis	63 (58.3)	18 (34.6)	0.005		27 (62.8)	14 (31.1)	0.003	
MRCP, *n* (%)	157 (49.4)	73 (52.5)	0.536		61 (54.5)	55 (49.1)	0.422	

Cholelithiasis	113 (72)	38 (52.1)	0.003	37 (60.7)	35 (63.6)	0.741
Biliary dilation	93 (59.2)	50 (68.5)	0.178	41 (67.2)	26 (47.3)	0.030
Choledocholithiasis	103 (65.6)	39 (53.4)	0.077	41 (67.2)	23 (41.8)	0.006
Shock Index, abnormal[3]	194 (61)	88 (63.3)	0.641	66 (58.9)	73 (65.2)	0.335
TG13 severity grading	2 (2, 3)	2 (1, 2)	< 0.001	2 (1, 3)	2 (1, 2)	0.016
Grade I	67 (21.1)	66 (47.5)		31 (27.7)	46 (41.1)	
Grade II	152 (47.8)	46 (33.1)		49 (43.8)	46 (41.1)	
Grade III	99 (31.1)	27 (19.4)		32 (28.6)	20 (17.9)	

All continuous variables were expressed as median (interquartile range) unless specified. All categorical variables were expressed as *n* (%) unless otherwise specified.

[1]Propensity score matching was performed for these variables due to potential and/or significant effects on clinical outcomes, or due to significant differences in demographics between the two study groups.

[2]Hypotension was defined as systolic blood pressure of < 90 mmHg.

[3]Shock index was defined as heart rate divided by the respective systolic blood pressure during triage, where the normal range is 0.5 to 0.7 (inclusive).

ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; AST: Aspartate aminotransferase; COPD: Chronic obstructive pulmonary disease; CT: Computed tomography; GGT: Gamma-glutamyl transferase; INR: International normalized ratio; MRCP: Magnetic resonance cholangiopancreatography; PSM: Propensity score matching; SMD: Standardized mean difference; TG13: Tokyo Guidelines 2013; WBC: White blood cell.

Table 2 Clinical outcomes between elderly *vs* non-elderly patients

	Overall cohort, *n* = 457				PSM cohort, *n* = 224			
	Elderly, *n* = 318	Non-elderly, *n* = 139	OR, 95%CI	*P* value	Elderly, *n* = 112	Non-elderly, *n* = 112	OR, 95%CI	*P* value
Initial management								
ERCP	206 (64.8)	86 (61.9)	1.13 (0.75, 1.71)	0.551	77 (68.8)	65 (58)	1.59 (0.02, 2.75)	0.096
Percutaneous transhepatic biliary drainage	25 (7.9)	6 (4.3)	1.89 (0.76, 4.72)	0.166	13 (11.6)	5 (4.5)	2.81 (0.97, 8.17)	0.049
Conservative	98 (30.8)	49 (35.3)	0.82 (0.54, 1.25)	0.351	29 (25.9)	43 (38.4)	0.56 (0.32, 0.99)	0.045
Subsequent management								
Index admission cholecystectomy	16 (5.0)	13 (9.4)	0.51 (0.24, 1.10)	0.081	2 (1.8)	12 (10.7)	0.15 (0.03, 0.69)	0.006
Interval cholecystectomy	20 (6.3)	11 (7.9)	0.78 (0.36, 1.68)	0.525	7 (6.3)	10 (8.9)	0.68 (0.25, 1.86)	0.449
Length of hospital stay, days	8 (6, 13)	8 (5, 11)	-	0.040	7.5 (5, 11)	8 (5, 11)	-	0.982
In-hospital mortality	16 (5.0)	5 (3.6)	1.42 (0.51, 3.96)	0.500	6 (5.4)	5 (4.5)	1.21 (0.36, 4.09)	0.757
30-d mortality	27 (8.5)	7 (5)	1.75 (0.74, 4.12)	0.195	8 (7.1)	7 (6.3)	1.15 (0.40, 3.30)	0.789
90-d mortality	31 (9.7)	8 (5.8)	1.77 (0.79, 3.95)	0.160	8 (7.1)	8 (7.1)	1.00 (0.36, 2.77)	1.000

All continuous variables were expressed as median (interquartile range) unless specified. All categorical variables were expressed as *n* (%) unless otherwise specified. ERCP: Endoscopic retrograde cholangiopancreatography; CI: Confidence interval; OR: Odds ratio; PSM: Propensity score matching.

cited in more recent studies[28,29]. The higher mortality compared to some reports may be due to advanced age or co-morbidity associated with ageing[30]. With regards to the exact cause of mortality, we did not collect separate data, and this remains a limitation of our study. However, locally, our institution tracks procedure-related mortality separately; ERCP-related mortality is < 1% locally. Further, it is difficult to distinguish ERCP-related complications such as post-ERCP cholangitis from the index-admission sepsis. Due to the retrospective nature of our study, it is difficult to establish a cause-

Table 3 Subgroup analysis of patients who had endoscopic retrograde cholangiopancreatography on outcomes in elderly *vs* and non-elderly patients

	Overall cohort, *n* = 292				PSM cohort, *n* = 142			
	Elderly, *n* = 206	Non-elderly, *n* = 86	OR, 95%CI	*P* value	Elderly, *n* = 77	Non-elderly, *n* = 65	OR, 95%CI	*P* value
Timing of ERCP from presentation				**0.012**			-	0.247
Within 24 h	15 (7.3)	16 (18.6)			9 (11.7)	12 (18.5)		
24-48 h	36 (17.5)	13 (15.1)			14 (18.2)	11 (16.9)		
48-72 h	34 (16.5)	19 (22.1)			12 (15.6)	16 (24.6)		
>72 h	121 (58.7)	38 (44.2)			42 (54.6)	26 (40)		
Stone(s) removed	102 (49.5)	44 (51.2)	0.94 (0.57, 1.55)	0.797	36 (46.8)	30 (46.2)	1.02 (0.53, 1.99)	0.943
Stent placed	89 (43.2)	38 (44.2)	0.96 (0.58, 1.60)	0.877	35 (45.5)	30 (46.2)	0.97 (0.50, 1.89)	0.934
Length of hospital stay, d	9 (7, 13)	8 (5, 11)	-	0.016	8 (5, 12)	8 (5, 12)	-	0.546
In-hospital mortality	2 (1)	1 (1.2)	0.83 (0.08, 9.31)	0.882	1 (1.3)	1 (1.5)	0.84 (0.05, 13.73)	0.904
30-d mortality	13 (6.3)	4 (4.7)	1.38 (0.44, 4.36)	0.581	3 (3.9)	4 (6.2)	0.62 (0.13, 2.87)	0.536
90-d mortality	16 (7.8)	4 (4.7)	1.73 (0.56, 5.32)	0.337	3 (3.9)	4 (6.2)	0.62 (0.13, 2.87)	0.536

All continuous variables were expressed as median (interquartile range) unless specified. All categorical variables were expressed as *n* (%) unless otherwise specified. ERCP: Endoscopic retrograde cholangiopancreatography; CI: Confidence interval; OR: Odds ratio; PSM: Propensity score matching.

Table 4 Subgroup analysis of patients who had abnormal shock index on triage on outcomes in elderly *vs* non-elderly patients

	Overall cohort, *n* = 282				PSM cohort, *n* = 139			
	Elderly, *n* = 194	Non-elderly, *n* = 88	OR, 95%CI	*P* value	Elderly, *n* = 66	Non-elderly, *n* = 73	OR, 95%CI	*P* value
Length of hospital stay, d	8 (6-13)	8 (6-10.8)		0.379	8 (5-12)	6 (5-10)		0.217
In-hospital mortality	10 (5.2)	3 (3.4)	1.54 (0.41, 5.74)	0.517	3 (4.5)	3 (4.1)	1.11 (0.22, 5.71)	0.900
30-d mortality	19 (9.8)	4 (4.5)	2.28 (0.75, 6.91)	0.136	5 (7.6)	4 (5.5)	1.41 (0.36, 5.50)	0.616
90-d mortality	20 (10.3)	5 (5.7)	1.91 (0.62, 5.26)	0.205	5 (7.6)	5 (6.8)	1.12 (0.31, 4.04)	0.869

All continuous variables were expressed as median (interquartile range) unless specified. All categorical variables were expressed as *n* (%) unless otherwise specified. CI: Confidence interval; OR: Odds ratio; PSM: Propensity score matching.

effect relationship.

The principles of management of AC are early diagnosis, resuscitation, risk stratification, compliance to sepsis bundle, and source control[21]. Risk stratification is essential for resource allocation, patient and caregiver counselling, and timely proactive interventions. Source control is best achieved with endoscopic biliary decompression, *i.e.*, ERCP. The traditional systemic inflammatory response criteria lack specificity in hepatobiliary sepsis, and thus alternative indices are for risk stratification and prognostication of outcomes[15]. The SI (heart rate/systolic blood pressure) is a validated tool[19,31]. Yussof *et al*[31] demonstrated abnormal SI predicted mortality of severe sepsis in the emergency department. Our study however demonstrated that patients who had abnormal SI were equally likely to undergo

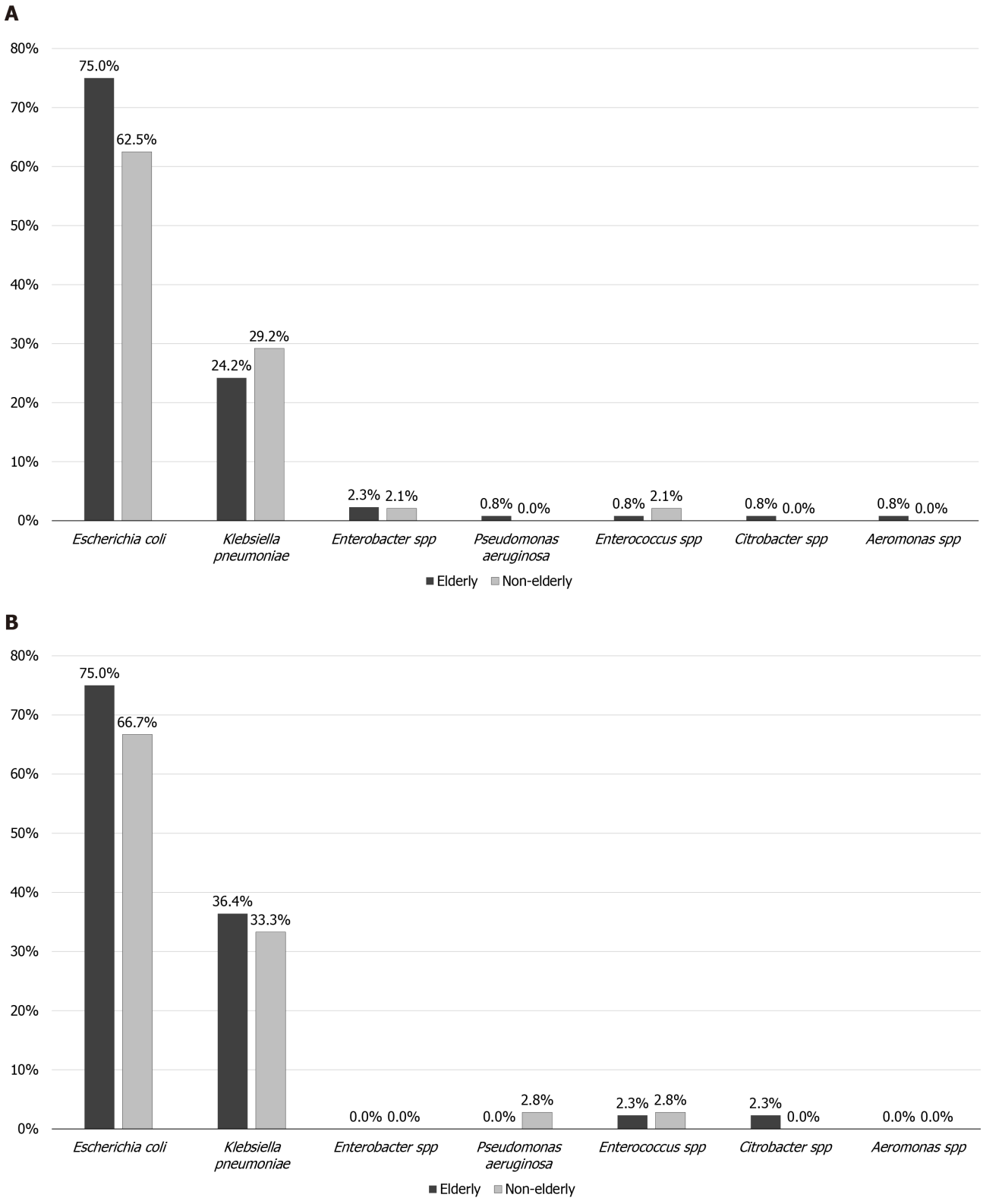

Figure 1 Microbiology of elderly and non-elderly patients who had positive blood cultures. A: Unmatched cohort; B: Matched cohort.

ERCP, and outcomes were comparable between elderly and non-elderly patients. SI is not reflective of the severity of sepsis as it does not take into account tissue perfusion indices and altered mental state. The decision for ERCP at the time of admission was based on the severity of AC and resources. Thus, SI does not predict the need for ERCP. Also, ERCP may occasionally be delayed in patients with abnormal SI in an attempt to resuscitate first. ERCP is an invasive procedure with approximately 10% risk of complications. Elderly patients undergoing ERCP are at higher risk of complications such as pancreatitis, hemorrhage, perforation, cardiorespiratory

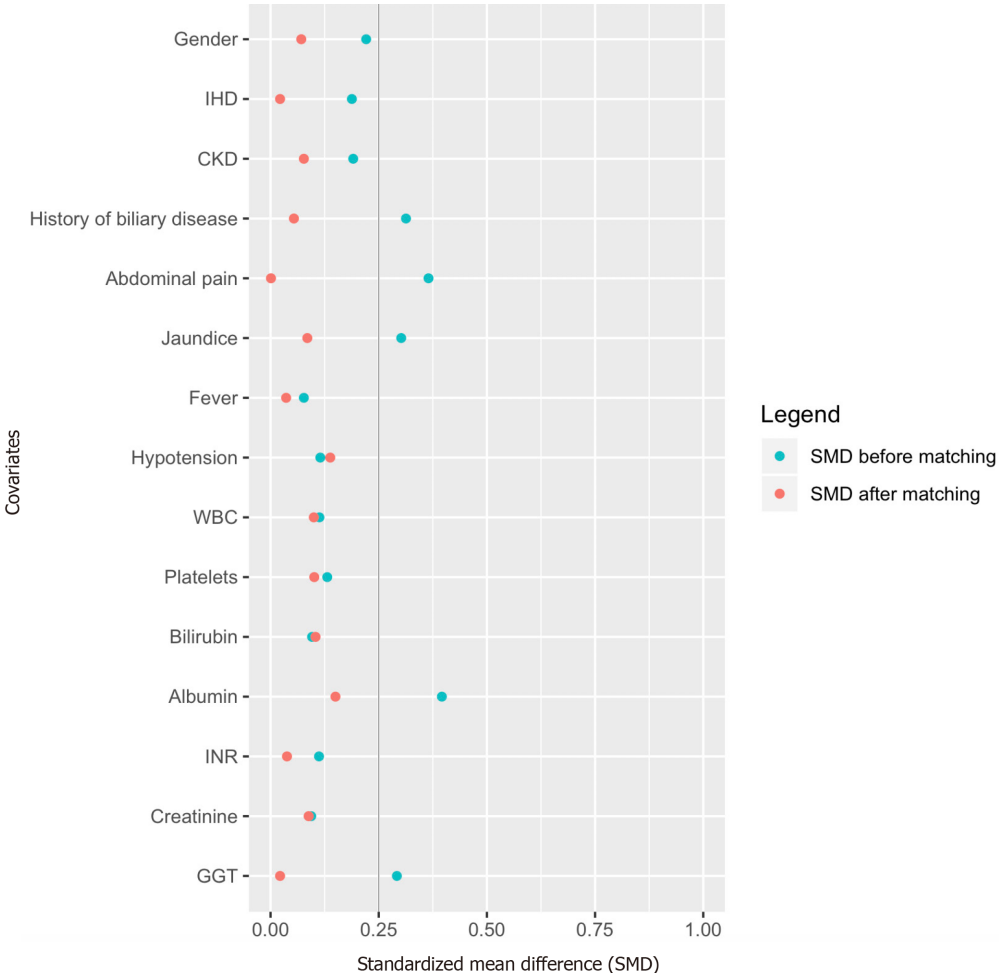

Figure 2 Plot of standardized mean difference in covariates: before propensity score matching (blue) and after propensity score matching (red). Standardized mean difference of < 0.25 indicates adequate balance. IHD: Ischemic heart disease; CKD: Chronic kidney disease; WBC: White blood cell; INR: International normalized ratio; GGT: Gamma-glutamyl transferase.

complications, and mortality[32]. This increased morbidity and mortality are attributed to underlying comorbidity and lower physiological reserves of the elderly[33].

However, several studies have shown no relationship between comorbidities and ERCP-related complications, except liver cirrhosis[34]. Many authors have demonstrated the safety and efficacy of ERCP in elderly patients[35,36]. In a single-center retrospective study reporting on efficacy and safety of ERCP in elderly patients with AC, Tohda *et al*[37] reported that patients ≥ 80 years old were more likely to have periampullary diverticulum (24.5% *vs* 13.3%), but equal technical success rates (95.1% *vs* 95.2%) and frequency of ERCP-related complications (6.9% *vs* 6.7%) as compared to patients < 80 years age. The authors reported a lower rate of post-ERCP pancreatitis in the elderly than non-elderly (1.0% *vs* 3.8%). We used PSM analysis to reduce the confounding effect of comorbidities on mortality outcomes, thus reducing the selection bias. We did not specifically compare procedure-related morbidity between elderly *vs* non-elderly and showed comparable LOS and mortality in both the unmatched and matched cohorts between elderly and non-elderly patients. Our experience shows that both stent insertion for biliary decompression and definitive stone removal can be safely performed. In particular, patient physiology, coagulopathy, and endoscopist experience are determinants of ERCP outcomes. Regarding the timing of ERCP, most authors agree that urgent ERCP should be done at the next available opportunity, and in clinical practice, timing is determined by local resources as well as clinical status. The majority of authors recommend ERCP within 24-72 h of admission[38]. Delay in ERCP in AC could influence patients' outcomes, and many authors define delay variably as the time to ERCP of more than 48-72 h since admission. Khashab *et al*[39] defined delay in ERCP as > 72 h after admission and reported that it was associated with prolonged LOS (OR 19.8, 95%CI: 2.18-178, $P = 0.008$). Navaneethan *et al*[40] defined delay in ERCP as > 48 h after admission and reported that it was associated with an increased risk of 30-d readmission. We defined delay as > 72 h after admission and did

not find any difference in clinical outcomes between elderly and non-elderly patients in both the unmatched and matched cohort. Khashab *et al*[39] demonstrated that delayed ERCP and age are associated with worse composite clinical outcomes (death, persistent organ failure and admission to ICU). However, as our 90-d mortality only had thirteen patients with delayed ERCP, it was not possible to perform subgroup analysis of age on clinical outcomes. It is possible that worse outcomes are associated with delay in ERCP but independent of age.

In addition, it is essential for patients with haemodynamic instability to be adequately resuscitated with airway management, prompt administration of vasopressor after volume replacement, and early engagement of critical care specialist or anesthetist, followed by prompt and early biliary decompression[41]. A recent study by Novy *et al*[14] in 2020, which analyzed the outcomes of 85 patients ≥ 75 years old with severe AC and admitted to ICU, showed that the majority (76%) of the ICU patients had ERCP within 24 h, which was attributed to the ease of access to facilities. Institutions with availability of ERCP services should consider early ERCP synchronized with resuscitation measures as delaying ERCP is associated with poor clinical outcomes[39]. Despite a policy for early ERCP, Novy *et al*[14] reported ICU mortality of 18%. This highlights that there are other determinants of mortality in critically ill patients. It is important to note that there is an inherent selection bias for elderly patients included in the study; patients not eligible for ICU admission may have more inferior pre-morbid status and deemed not suitable based on medical futility, or may have had advanced care planning performed and decided that ICU admission is unlikely to provide benefit for the patient[42]. Moreover, ICU admission implies the need for vasopressor therapy or intubation, which reflects the severity of the disease. We did not differentiate our patients based on their need for ICU admission or otherwise; or the use of vasopressor therapy. There is a paucity of data related to causative organisms and their impact on AC's clinical outcomes compared to other hepatobiliary diseases, such as acute cholecystitis or pyogenic liver abscesses[43]. Microbiology of patients with AC was also consistent with existing studies, where *Escherichia coli* and *Klebsiella pneumoniae* were the most typical organisms[44].

An alternative to biliary decompression is the use of PTBD. Our study demonstrated a significantly higher number of elderly patients who underwent PTBD compared to non-elderly patients [n = 13 (11.6%) *vs* n = 5 (4.5%), OR 2.81, P = 0.049] in the matched cohort. ERCP is traditionally the gold standard management for AC and has been proven to be safe and effective in the elderly population[36,37]. PTBD is regarded as a second-line treatment for patients who failed ERCP, with altered biliary anatomy, or were contra-indicated for ERCP. However, unlike ERCP which requires the use of moderate sedation or general anaesthesia, PTBD only requires the use of local anaesthesia. Despite the safety of ERCP in elderly patients, elderly patients are at higher risk of complications from the use of sedation[45]. Weighing the risks and benefits of endoscopic biliary decompression *vs* the use of sedation is also essential in the management of AC. Patient and/or family members may opt for PTBD which is deemed to be "less invasive" without the need for moderate sedation/general anaesthesia.

Following the acute management of AC, cholecystectomy should be offered to patients to prevent future recurrences. In our experience, non-elderly patients are more likely to undergo index admission LC (Matched cohort: P = 0.006). Five out of 12 patients in the non-elderly group who underwent index admission LC in the matched cohort did not receive ERCP. It is likely that in addition to age, underlying comorbidity and personal choices impact the decision for surgery. These findings are similar to a single-center retrospective study of Discolo *et al*[46]. In an eight-year study including 151 cholecystectomies for AC, Discolo *et al*[46] reported a more than 61% rate of index admission cholecystectomy, and patients with age > 75 years were more likely to receive delayed cholecystectomy (41.4% *vs* 21.5%, P = 0.01). The authors also showed that TG severity grading did not impact the decision for index admission cholecystectomy (P = 0.46). Furthermore, there was no difference in average operative time (P = 0.36), open conversion (P = 0.34), and intra-operative complications (P = 0.28) based on the timing of cholecystectomy. We did not perform subgroup analysis on postoperative outcomes in patients who underwent index admission cholecystectomy given the small sample size. In general, index admission cholecystectomy could reduce the risk of recurrent biliary events; however, more evidence is needed in patients with AC. We have previously reported our views on a policy of 'universal cholecystectomy', *i.e.*, patients with a diagnosis that requires cholecystectomy (*e.g.*, acute cholecystitis, AC, or acute biliary pancreatitis) procedure should receive index admission surgery unless contraindicated for general anesthesia or patient refusal[47].

The important issue that surfaces from our study is, if age should be considered as part of a risk stratification tool for the severity of AC. Age is usually included in severity classifications as a surrogate marker for functional capacity and extent of comorbidities. The use of other surrogate markers such as the clinical frailty scale or Charlson co-morbidity index may be a better predictor of disease severity in AC[48]. In reality however, age serves as a useful tool in view of its ease of use as well as age-associated reduced functional reserves that are not associated with any co-morbidity. While clinical outcomes are not determined by age in patients with AC in our study; based on available literature, we advocate that age should continue to remain as one of the component variables that determines disease severity in patients with AC.

There are several limitations of our study. A retrospective study is inherently prone to selection bias, and thus cause-effect cannot be established. PSM helps to reduce this bias, and such analysis ranks higher than traditional observational studies[24]. To the best of our knowledge, this is the first study using PSM to compare outcomes of AC secondary to biliary stones between elderly and non-elderly patients. PSM analysis cannot account for unknown confounding variables, and only a randomized controlled trial can overcome this bias. Our study included patients treated in 2012, *i.e.*, before the TG13 guidelines, and we retrospectively assigned TG13 criteria with possible reporting bias. We did not study the effect of polypharmacy, frailty, and Charlson's comorbidity index on AC outcomes. In a large population study over a decade in the Korean general population, Min *et al*[49] have reported that the use of proton pump inhibitor is associated with increased AC risk (hazard ratio 5.75, 95%CI: 4.39-7.54). We also did not evaluate comorbidities like cerebrovascular accident and liver cirrhosis, as data was not available for all the patients. Our study used the age of 80 years old as a cut-off compared to 75 years, used in TG13/18 guidelines. Existing studies evaluating the safety of ERCP in elderly patients have used a variety of cut-offs for age, ranging from 80 years old to 90 years old[35-37]. In addition, use of 75 years as a cut-off will reduce our sample size and impact the statistical power of study (96 patients < 75 years and 361 patients ≥ 75 years compared to 139 patients < 80 years and 318 patients ≥ 80 years respectively). Nevertheless, this difference in age cut-off reduces our study's generalizability from being considered an accurate validation study of TG13/18 guidelines. We also did not categorize which patients with history of biliary disease had prior ERCP and papillotomy. It is possible that elderly patients were more likely to have prior ERCP and papillotomy, and this could impact results of our study. We also did not collect data on disease or procedure-related morbidity and causes of mortality.

CONCLUSION

Elderly patients (≥ 80 years old) with AC have similar outcomes as compared to non-elderly patients (< 80 years old). In a subgroup of patients who underwent ERCP or with delayed ERCP, clinical outcomes are comparable between the elderly and non-elderly. Age alone may not predict the outcomes of AC and its use in the Tokyo Guidelines should be re-evaluated.

ARTICLE HIGHLIGHTS

Research background

Acute cholangitis (AC) is a disease spectrum with varying extent of severity. Age ≥ 75 years forms part of the criteria for moderate (Grade II) severity in the Tokyo Guidelines (TG13 and TG18). Aging is associated with reduced physiological reserves, frailty, and sarcopenia. However, there is evidence that age itself is not the determinant of inferior outcomes in elective and emergency biliary diseases.

Research motivation

Endoscopic retrograde cholangiopancreatography is deemed to be safe in elderly patients with AC. There is paucity of data on outcome determinants in elderly patients with AC. This era of ageing population prompted our interest to study the impact of age alone on outcomes of AC through the use of propensity score matching.

Research objectives

Our primary outcomes are in-hospital mortality, 30-d mortality and 90-d mortality. Secondary outcome is morbidity (length of hospital stay).

Research methods

This is a single-center retrospective cohort study of all patients diagnosed with calculous AC (January 2016 to December 2016) and ≥ 80 years old (January 2012 to December 2016) at a tertiary university-affiliated teaching hospital. Elderly was defined as ≥ 80 years old while non-elderly was defined as < 80 years old.

Research results

Four hundred fifty-seven patients with AC were included in this study (318 elderly, 139 non-elderly). Propensity score matching analysis resulted in a total of 224 patients (112 elderly, 112 non-elderly). The overall in-hospital mortality, 30-d mortality and 90-d mortality were 4.6%, 7.4% and 8.5% respectively, with no statistically significant differences between the elderly and non-elderly in both the unmatched and matched cohorts. Length of hospital stay was longer in the unmatched cohort [elderly 8 d, interquartile range (IQR) 6-13 *vs* non-elderly 8 d, IQR 5-11, P = 0.040], but was comparable in the matched cohort (elderly 7.5 d, IQR 5-11 *vs* non-elderly 8 d, IQR 5-11, P = 0.982).

Research conclusions

Mortality is indifferent in the elderly (≥ 80 years old) and non-elderly patients (< 80 years old) with AC.

Research perspectives

Age alone may not predict the outcomes of AC and its use in the Tokyo Guidelines should be re-evaluated.

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ORIGINAL ARTICLE

Retrospective Study

Retrospective analysis of complications related to endoscopic retrograde cholangio-pancreatography in patients with cirrhosis *vs* patients without cirrhosis

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Abstract

BACKGROUND

There is minimal objective data regarding adverse events related to endoscopic retrograde cholangio-pancreatography (ERCP) in patients with cirrhosis compared to those without cirrhosis and even fewer data comparing complications among cirrhosis patients based on severity of cirrhosis.

AIM

To determine if patients with cirrhosis are at increased risk of adverse events related to ERCP: mainly pancreatitis, bleeding, perforation, cholangitis, and mortality; And to see if higher Child-Pugh (CP) score and Model for End-Stage Liver Disease (MELD) score are associated with higher post-ERCP complications.

METHODS

We performed a retrospective analysis of 692 patients who underwent ERCP and analyzed the impact of cirrhosis etiology, gender, type of sedation used during procedure, interventions performed, and co-morbidities on the rate of complications in cirrhosis patients as compared to non-cirrhosis patients.

RESULTS

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Overall complications were higher in those with cirrhosis as compared to those without cirrhosis (P = 0.015 at significance level of 0.05). CP class, especially CP class C, was shown to be associated with a significantly higher rate of ERCP complications as compared to CP class A and CP class B (P = 0.010 at significance level of 0.05).

CONCLUSION
The results of our study reaffirm that liver cirrhosis has an impact on the occurrence of complications during ERCP. Our study shows that CP class seems to be more reliable as compared to MELD score in predicting complications of ERCP in cirrhosis patients.

Key Words: Cirrhosis; Complications; Advanced endoscopy; Endoscopic retrograde cholangio-pancreatography

Core Tip: What is previously known is that endoscopic retrograde cholangio-pancreatography is associated with a risk of adverse events. What is new in this manuscript is that complications are increased in patients with cirrhosis as compared to patients without cirrhosis. Statistical significance was demonstrated in patients classified as Child-Pugh (CP) Class C as compared to CP Classes A and B.

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INTRODUCTION

Endoscopic retrograde cholangio-pancreatography (ERCP) is a procedure utilized for the management of pancreatobiliary disorders, including but not limited to choledocolithiasis, biliary strictures, pancreatitis, and cholangitis[1]. However, like all procedures, there is an associated risk of adverse events, such as post-ERCP pancreatitis, bleeding, infection, perforation, and even death[2].

Patients with chronic liver disease and cirrhosis often require ERCP. However, because of hepatic synthetic dysfunction and portal hypertension, patients with cirrhosis have a much higher risk of developing adverse events and complications after invasive procedures[3]. Despite this, there remains a scarce amount of data investigating complications associated with ERCP in patients with cirrhosis as compared to patients without cirrhosis. There is even less information regarding adverse effects among patient with cirrhosis based upon cirrhosis severity.

Thus, our study aims to add to the limited body of knowledge regarding complications of ERCP in patients with cirrhosis. We hypothesized that patients with an underlying diagnosis of cirrhosis are at elevated risk of complications associated to ERCP, including mortality, pancreatitis, bleeding, perforation, and cholangitis. A secondary objective was to examine our hypothesis that a higher Child-Pugh (CP) score and/or Model for End-Stage Liver Disease (MELD) score is related to a greater number of post-ERCP complications in cirrhosis.

MATERIALS AND METHODS

This study was a retrospective review of all patients who underwent ERCP at a University hospital in Syracuse, NY, United States from 2012-2019. The project was presented to the Institutional Review Board and approved prior to its initiation. Chart review of 692 patients who underwent ERCP between January 1, 2012 and December 31, 2019 was conducted. Of the 692 patients, 174 patients had a diagnosis of cirrhosis at

the time of ERCP, and 518 patients did not. Demographics, co-morbidities [including chronic obstructive pulmonary disease (COPD), congestive heart failure (CHF), hypertension (HTN), diabetes mellitus (DM), chronic kidney disease (CKD), and hyperlipidemia (HLD)], indication for procedure, type of sedation used, type of intervention(s) performed, and complications within a 30-d period were analyzed for all subjects. Of the 174 patients with cirrhosis, we also recorded cirrhosis etiology and calculated their MELD score and CP class.

Statistical analysis of the complication rates in the groups with and without cirrhosis was performed using a chi-squared test, and fishers exact test when there were < 5 individuals in a category. Pearson's chi square test is sufficient when testing the impact of a single factor on binary outcome. Of those with cirrhosis, the complication rates in subjects grouped by Child score A, B, and C, as well as MELD score, were also compared using a chi-squared or fishers exact test. A P value of < 0.05 was considered statistically significant. Odds ratios with 95% confidence intervals were derived from logistic regression as a supportive method in confirming the findings of Child score significance.

RESULTS

A total number of 692 patients were included in our study. Of the 692, 174 had an underlying diagnosis of cirrhosis while 518 did not. Mean patient age was 58.6 years. Overall, there was a higher rate of complications in those with cirrhosis as compared to those without cirrhosis (P = 0.015) (Table 1). There was no statistical significance comparing the specific types of complications across the two groups (P = 0.897), including bleeding, pancreatitis, cholangitis, perforation, mortality, or other.

CP and MELD score

Complications in subjects with cirrhosis grouped by CP class are shown in Table 2. CP class, especially CP class C, was shown to be associated with a significantly higher rate of ERCP complications as compared to CP class A and CP class B (P = 0.010). In other words, a statistically significant proportion of cirrhosis patients with CP class A or class B are less likely to develop complications than those in CP class C (Figure 1). The odds ratios 0.342 with (0.132, 0.882) as 95% confidence interval for group A *vs* group C and 0.251 with (0.096, 0.6253) as 95% confidence interval for group B *vs* group C, as derived from logistic regression support the above conclusion (Table 3).

Complications in subjects with cirrhosis grouped by MELD score are shown in Table 4. There was no statistical significance when comparing complications in patients with cirrhosis with a MELD score of < 15 *vs* > 15 (P = 0.949). Thus, CP class was more reliable than MELD score in terms of predicating complications in cirrhosis.

Etiology of cirrhosis

We also analyzed the complication occurrence in cirrhosis patients based on underlying etiology. This included: Alcohol, hepatitis C, and non-alcoholic fatty liver disease. Etiology of cirrhosis did not have a significant difference in respect to complications related to ERCP (Table 5).

Gender

Gender did not have a statistically significant effect on complications between cirrhosis and non-cirrhosis patients (Table 5 and 6).

Anesthesia type

Type of anesthesia used during the ERCP did not have any statistically significant difference regarding complications between both cirrhosis and non-cirrhosis patients (Table 5 and 6).

Type of intervention

We collected data on whether the ERCP was for diagnostic or therapeutic purposes, as well as the types of intervention performed during the ERCP (Table 7 and 8). In non-cirrhosis patients, a "Diagnostic ERCP" showed a higher risk for complications (P = 0.039). Otherwise, type of intervention done did not have any statistically significant effect on complication occurrence between cirrhosis and non-cirrhosis patients.

Table 1 Complication status and different types of complications in group of subjects with/without cirrhosis, *n* (%)

	With cirrhosis (*n* = 174)	Without cirrhosis (*n* = 518)	*P* value
Any complication?			0.015[a]
No	133 (78.70)	448 (86.49)	
Yes	36 (21.30)	70 (13.51)	
Complications			0.897[1]
Bleeding	2 (5.56)	7 (10.00)	
Pancreatitis	11 (30.56)	25 (35.71)	
Cholangitis	2 (5.56)	5 (7.14)	
Perforation	1 (2.78)	2 (2.86)	
Mortality attributed to ERCP	0 (0.00)	1 (1.43)	
Other mortality	5 (13.89)	12 (17.14)	
Other	15 (41.67)	18 (25.71)	

[a]$P < 0.05$.
[1]Fishers exact test (used when < 5 individuals in a category). ERCP: Endoscopic retrograde cholangio-pancreatography.

Table 2 Child score of cirrhosis patients (*n* = 174) with or without any complication, *n* (%)

	A	B	C	*P* value
Any complication?				0.010[a]
No	46 (80.70)	56 (84.85)	20 (58.82)	
Yes	11 (19.30)	10 (15.15)	14 (41.18)	

[a]$P < 0.05$.

Table 3 Odds ratio estimates for Child-Pugh classes and Wald confidence intervals

Odds ratio	Estimate	95%CI	Limits
Child A *vs* B	1.363	0.532	3.491
Child A *vs* C	0.342	0.132	0.882
Child B *vs* C	0.251	0.096	0.653

Comorbidities

It was noted whether the patient had any of these comorbidities at the time of ERCP: COPD, CHF, HTN, DM, CKD, and HLD. In cirrhosis patients, COPD and HTN demonstrated significantly higher rates of complications (P = 0.009 and 0.003 correspondingly) (Table 9). In patients without cirrhosis, statistically significant complication rates were only demonstrated in those with an underlying diagnosis of COPD (P = 0.003) (Table 10).

DISCUSSION

In this retrospective cohort study of 692 patients, 174 with cirrhosis and 518 without cirrhosis, we found that the overall occurrence of complications was increased in those with cirrhosis to a statistically significant level. In subgroup analysis of CP class and MELD score, we found that CP class C was associated with higher risk of complications, and that CP class was a more reliable predictor of complications than MELD score. The years of experience amongst the advanced endoscopists ranged from approximately five to thirty years, with each performing approximately one-hundred

Table 4 Model for End-Stage Liver Disease score of cirrhosis patients (*n* = 174) with or without any complication, *n* (%)

< 10	10-15		> 15	*P* value
Any complication?				0.626
No	56 (74.67)	41 (82.00)	25 (78.13)	
Yes	19 (25.33)	9 (18.00)	7 (21.88)	
	< 10		≥ 10	
Any complication?				0.381
No	56 (74.67)		66 (80.49)	
Yes	19 (25.33)		16 (19.51)	
	≤ 15		> 15	
Any complication?				0.949
No	97 (77.60)		25 (78.13)	
Yes	28 (22.40)		7 (21.88)	

Table 5 Cirrhosis etiology, gender, and type of anesthesia effects on complication occurrence in the group of subjects with cirrhosis (*n* = 174), *n* (%)

Any complication?	No	Yes	*P* value
Alcohol etiology			0.192
No	65 (74.1)	22 (25.29)	
Yes	68 (82.93)	14 (17.07)	
HEPC etiology			
No	112 (78.87)	30 (21.13)	0.899
Yes	21 (77.78)	6 (22.22)	
NAFLD etiology			
No	123 (77.85)	35 (22.15)	0.461[1]
Yes	10 (90.91)	1 (9.09)	
Gender			
Female	51 (76.12)	16 (23.88)	0.507
Male	82 (80.39)	20 (19.61)	
Type of Anesthesia			
General Anesthesia	105 (78.36)	29 (21.64)	0.271
MAC	4 (57.14)	3 (42.86)	
Moderate conscious sedation	23 (85.19)	4 (14.81)	

[1]Fishers exact test (used when < 5 individuals in a category). HEPC: Hepatitis C; NAFLD: Non-alcoholic fatty liver disease; MAC: Monitored anesthesia care.

procedures per year.

There remains a scarcity in the literature regarding complications and adverse events after ERCP in cirrhosis patients, particularly those incorporating CP class and MELD score or type of intervention as predictors. A retrospective matched case-control study by Navaneethan *et al*[4] showed a higher risk of ERCP-associated hemorrhage in cirrhosis patients *vs* non-cirrhosis patients[4]. Similarly, Inamdar *et al*[5] found a higher rate of post-ERCP pancreatitis and bleeding in cirrhosis patients compared to non-cirrhosis patients. Furthermore, in subgroup analysis, compensated cirrhosis patients and non-cirrhosis patients had a similar complication profile as compared to decompensated cirrhosis patients except for a 2.2% higher rate of

Table 6 Gender and type of anesthesia effects on complication occurrence in the group of non-cirrhosis subjects (*n* = 518), *n* (%)

Any complication?	No	Yes	*P* value
Gender			0.692
Female	264 (85.99)	43 (14.01)	
Male	184 (87.20)	27 (12.80)	
Type of Anesthesia			
General Anesthesia	308 (85.56)	52 (14.44)	0.511
MAC	131 (89.12)	16 (10.88)	
Moderate conscious sedation	9 (81.82)	2 (18.18)	

MAC: Monitored anesthesia care.

Table 7 Type of Intervention in cirrhosis patients (*n* = 174) with or without any complication, *n* (%)

Any complication?	No	Yes	*P* value
Diagnostic ERCP			0.737[1]
No	122 (78.21)	34 (21.79)	
Yes	11 (84.62)	2 (15.38)	0.192
Sphincterotomy/sphincteroplasty			
No	86 (81.90)	19 (18.10)	
Yes	47 (73.44)	17 (26.56)	
Biliary intervention (stent, sweeping, dilatation, brushing)			1.000[1]
No	17 (80.95)	4 (19.05)	
Yes	116 (78.38)	32 (21.62)	
Spyglass			0.098[1]
No	128 (80.00)	32 (20.00)	
Yes	5 (55.56)	4 (44.44)	
Pancreatic intervention			1.000[1]
No	17 (80.95)	4 (19.05)	
Yes	116 (78.38)	32 (21.62)	
Manometry			
No	133 (78.7)	36 (21.3)	
Yes	0 (0)	0 (0)	

[1]Fishers exact test (used when < 5 individuals in a category). ERCP: Endoscopic retrograde cholangio-pancreatography.

pancreatitis[5]. More recently, Leal *et al*[6] reaffirmed a higher rate of adverse events after ERCP in cirrhosis *vs* non-cirrhosis patients[6]. In our study, no statistical significance was calculated when comparing the specific types of adverse events across the two groups, including bleeding, pancreatitis, cholangitis, perforation, mortality, or other. There have been other studies, such as ours, that demonstrated similar outcomes between groups[7]. Importantly, there remains a lack of conclusive evidence warranting further studies.

Data regarding the relationship of ERCP complications and CP class or MELD score are even more limited and contradictory. For instance, Adler *et al*[8] demonstrated that CP class A was associated with a lower risk of ERCP adverse events compared to class B and C combined[8]. Jagtap *et al*[9] found that overall post-ERCP adverse events were increased in patients with CP class C and MELD score > 18[9]. Li *et al*[10] demonstrated that CP class C was associated with a statistically significant higher risk of post-ERCP

Table 8 Type of Intervention in non-cirrhosis patients (*n* = 518) with or without any complication, *n* (%)

Any complication?	No	Yes	*P* value
Diagnostic ERCP			0.039[1]
No	422 (87.37)	61 (12.63)	
Yes	26 (74.29)	9 (25.71)	
Sphincterotomy/sphincteroplasty			0.252
No	262 (85.06)	46 (14.94)	
Yes	186 (88.57)	24 (11.43)	
Biliary intervention (stent, sweeping, dilatation, brushing)			0.133
No	70 (81.40)	16 (18.60)	
Yes	377 (87.47)	54 (12.53)	
Spyglass			0.118[1]
No	430 (87.04)	64 (12.96)	
Yes	18 (75.00)	6 (25.00)	
Pancreatic intervention			0.133
No	70 (81.40)	16 (18.60)	
Yes	377 (87.47)	54 (12.53)	
Manometry			0.252[1]
No	447 (86.63)	69 (13.37)	
Yes	1 (50.00)	1 (50.00)	

[1]Fishers exact test (used when < 5 individuals in a category). ERCP: Endoscopic retrograde cholangio-pancreatography.

bleeding, however showed no difference in bleeding between cirrhosis and non-cirrhosis patients[10]. Similarly, multiple studies have found higher rates of post-ERCP bleeding in CP class C compared to class A and B[11,12]. Our analysis correlates with these findings. However, Zhang *et al*[13] found no association of rates of adverse events with respect to CP class, and instead demonstrated MELD score as a more reliable predictor of higher rates of complications[13]. Interestingly, our study demonstrated a statistically significant proportion of cirrhosis patients with CP class A or class B were less likely to develop complications than those in CP class C. Our study demonstrated that MELD score was not reliable in predicting complications. Whereas our findings correlate with some of the already published studies, it takes research a step further by investigating the impact of cirrhosis etiology, gender, type of sedation used during procedure, interventions performed, and co-morbidities on the rate of complications of ERCP in cirrhosis patients as compared to non-cirrhosis patients.

Our study had several limitations. This includes its retrospective design and moderate sample size. Several patients did not have all the necessary lab values and information on the day of the documented ERCP. In these cases, we had to use the necessary data points obtained at the date closest to their ERCP to calculate MELD scores and CP class. Similarly, many of the data points we collected relied on accurate and complete physician documentation, which can have significant variance. In our data collection, we could not include all comorbidities of each patient, and therefore chose to include six common ones that can affect risk of procedural complications. We encourage that further studies include a broader scope of comorbidities, such as immunocompromising diseases, *etc*. Furthermore, we did not analyze specific pancreatic duct stenting, use of indomethacin, or coagulopathy in respect to outcome. Lastly, we only considered complications that occurred within the span of 30 d of ERCP. The clinical course of a cirrhosis patient who has undergone an invasive procedure may be more complex and indirect complications may occur further down the line.

Table 9 Comorbidities in cirrhosis patients (*n* = 174) with or without any complication, *n* (%)

Any complication?	No	Yes	*P* value
Chronic obstructive pulmonary disease			0.009[a]
No	114 (82.61)	24 (17.39)	
Yes	19 (61.29)	12 (38.71)	
Congestive heart failure			0.572[1]
No	116 (77.85)	33 (22.15)	
Yes	17 (85.00)	3 (15.00)	
Essential hypertension			0.003[a]
No	42 (66.67)	21 (33.33)	
Yes	91 (85.85)	15 (14.15)	
Diabetes mellitus			0.515
No	89 (80.18)	22 (19.82)	
Yes	44 (75.86)	14 (24.14)	
Chronic kidney disease			0.478[1]
No	124 (79.49)	32 (20.51)	
Yes	9 (69.23)	4 (30.77)	
Hyperlipidemia			
No	95 (76.00)	30 (24.00)	0.149
Yes	38 (86.36)	6 (13.64)	

[a]$P < 0.05$.
[1]Fishers exact test (used when < 5 individuals in a category).

CONCLUSION

In conclusion, the results of our study reaffirm that liver cirrhosis has an impact on the occurrence of complications during ERCP. Our study shows that CP class seems to be more reliable as compared to MELD score in predicting complications of ERCP in cirrhosis patients. However, we are also aware that CP and MELD scores are complementary to each other while evaluating outcomes of any surgery in patients with cirrhosis. These findings should encourage clinicians to be aware of the increased risk when referring for, or performing, an ERCP on a patient with cirrhosis. It is imperative to perform a thorough risk-benefit assessment taking into consideration the extent of liver disease and comorbidities prior to ERCP, as doing so may improve clinical outcomes. Further studies, particularly prospective studies, are required to confirm this risk and further delineate the relationship between cirrhosis and complication risk during ERCP.

Table 10 Comorbidities in non-cirrhosis patients (*n* = 518) with or without any complication, *n* (%)

Any complication?	No	Yes	*P* value
Chronic obstructive pulmonary disease			0.003[a]
No	421 (87.71)	59 (12.29)	
Yes	26 (70.27)	11 (29.73)	
Congestive heart failure			0.782[1]
No	424 (86.53)	24 (85.71)	
Yes	24 (85.71)	4 (14.29)	
Essential hypertension			0.071
No	237 (89.10)	29 (10.90)	
Yes	210 (83.67)	41 (16.33)	
Diabetes mellitus			0.652
No	350 (86.85)	53 (13.15)	
Yes	98 (85.22)	17 (14.78)	
Chronic kidney disease			0.827
No	413 (86.58)	64 (13.42)	
Yes	35 (85.37)	6 (14.63)	
Hyperlipidemia			0.531
No	350 (86.00)	57 (14.00)	
Yes	98 (88.29)	13 (11.71)	

[a]$P < 0.05$.
[1]Fishers exact test (used when < 5 individuals in a category).

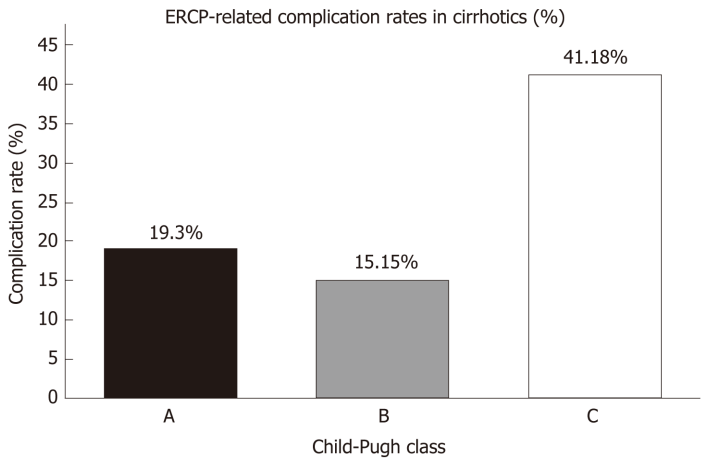

Figure 1 Endoscopic retrograde cholangio-pancreatography-related complications rates in cirrhotic patients based on Child-Pugh class.
ERCP: Endoscopic retrograde cholangio-pancreatography.

ARTICLE HIGHLIGHTS

Research background

Endoscopic retrograde cholangio-pancreatography (ERCP) is associated with a risk of adverse events. There remains a scarce amount of data investigating complications associated with ERCP in patients with cirrhosis as compared to patients without cirrhosis.

Research motivation

Our aim was to determine if patients with cirrhosis are at increased risk of complications associated with ERCP and if a higher Child-Pugh (CP) score and Model for End-Stage Liver Disease (MELD) score are linked to higher post-ERCP adverse events. Findings should encourage clinicians to be aware of the increased risk when referring for, or performing, an ERCP on a patient with cirrhosis.

Research objectives

Our primary aim was to determine if patients with an underlying diagnosis of cirrhosis are at elevated risk of complications compared to patients without cirrhosis, specifically pancreatitis, bleeding, perforation, cholangitis, and mortality. Our study takes previous research a step further by investigating the impact of cirrhosis etiology, gender, type of sedation used during procedure, interventions performed, and co-morbidities on the rate of complications of ERCP.

Research methods

This was a retrospective analysis in which a statistical analysis of the complication rates in the groups with and without cirrhosis was performed using a chi-squared test, and fishers exact test when there were < 5 individuals in a category. Odds ratios with 95% confidence intervals were derived from logistic regression as a supportive method in confirming the findings of Child score significance.

Research results

The results of our study reaffirm that liver cirrhosis has an impact on the occurrence of complications during ERCP. Our study demonstrated a statistically significant proportion of cirrhosis patients with CP class A or class B were less likely to develop complications than those in CP class C. Our study demonstrated that MELD score was not reliable in predicting complications.

Research conclusions

Complications are increased in patients with cirrhosis, especially those in CP Class C.

Research perspectives

Further studies, particularly prospective studies, are required to confirm the risk of performing an ERCP on a patient with cirrhosis, and further delineate the relationship between cirrhosis and complication risk during ERCP.

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Retrospective Study

Fatal arterial hemorrhage after pancreaticoduodenectomy: How do we simultaneously accomplish complete hemostasis and hepatic arterial flow?

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Abstract

BACKGROUND

Although arterial hemorrhage after pancreaticoduodenectomy (PD) is not frequent, it is fatal. Arterial hemorrhage is caused by pseudoaneurysm rupture, and the gastroduodenal artery stump and hepatic artery (HA) are frequent culprit vessels. Diagnostic procedures and imaging modalities are associated with certain difficulties. Simultaneous accomplishment of complete hemostasis and HA flow preservation is difficult after PD. Although complete hemostasis may be obtained by endovascular treatment (EVT) or surgery, liver infarction caused by hepatic ischemia and/or liver abscesses caused by biliary ischemia may occur. We herein discuss therapeutic options for fatal arterial hemorrhage after PD.

AIM

To present our data here along with a discussion of therapeutic strategies for fatal arterial hemorrhage after PD.

METHODS

We retrospectively investigated 16 patients who developed arterial hemorrhage after PD. The patients' clinical characteristics, diagnostic procedures, actual treatments [transcatheter arterial embolization (TAE), stent-graft placement, or surgery], clinical courses, and outcomes were evaluated.

Review Board of Shiga General Hospital, Moriyama, Japan.

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RESULTS

The frequency of arterial hemorrhage after PD was 5.5%. Pancreatic leakage was observed in 12 patients. The onset of hemorrhage occurred at a median of 18 d after PD. Sentinel bleeding was observed in five patients. The initial EVT procedures were stent-graft placement in seven patients, TAE in six patients, and combined therapy in two patients. The rate of technical success of the initial EVT was 75.0%, and additional EVTs were performed in four patients. Surgical approaches including arterioportal shunting were performed in eight patients. Liver infarction was observed in two patients after TAE. Two patients showed a poor outcome even after successful EVT. These four patients with poor clinical courses and outcomes had a poor clinical condition before EVT. Fourteen patients were successfully treated.

CONCLUSION

Transcatheter placement of a covered stent may be useful for simultaneous accomplishment of complete hemostasis and HA flow preservation.

Key Words: Pancreaticoduodenectomy; Endovascular treatment; Stent-graft; Covered stent; Transcatheter arterial embolization; Arterioportal shunting

Core Tip: Arterial hemorrhage after pancreaticoduodenectomy is fatal. This hemorrhage is caused by pseudoaneurysm rupture, and the gastroduodenal artery stump and hepatic artery are frequent culprit vessels. Simultaneous accomplishment of complete hemostasis and hepatic artery flow preservation is difficult after pancreaticoduodenectomy. Although complete hemostasis may be obtained by transcatheter arterial embolization or surgery, liver infarction and/or abscesses may occur. We here evaluate our experience including actual treatments (transcatheter arterial embolization, stent-graft placement, or surgery), and discuss therapeutic strategies. Transcatheter placement of a covered stent is useful for simultaneous accomplishment of complete hemostasis and hepatic arterial flow preservation.

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INTRODUCTION

The mortality rate after pancreaticoduodenectomy (PD) is currently < 5%[1-8] because surgical procedures and perioperative management techniques have been well established[1,9-11]. However, postoperative complications still remain a matter of concern[1,2,4,6,8,11-13]. Although arterial hemorrhage after PD is not frequent, it is fatal. Its mortality rate reportedly ranges from 10% to 60%[1,2,4,7,12,14-23], and it easily results in shock and coagulopathy[1,18,23]. Arterial hemorrhage is mainly caused by pseudoaneurysm rupture of a splanchnic artery[18,24], and the gastroduodenal artery (GDA) stump, common hepatic artery (CHA), and proper hepatic artery (PHA) are the most frequent culprit vessels[1-3,6,18,25-28]. Diagnostic and treatment strategies should be decided on a case-by-case basis[18,28,29].

Arterial flow, especially in the liver, is modified after PD (Figure 1). Briefly, the hepatopetal flow of the hepatic artery (HA) depends on the blood supply from the celiac artery (*e.g.*, the CHA and PHA), not from the superior mesenteric artery (SMA) [*e.g.*, the inferior pancreaticoduodenal artery (IPDA) and retrograde-flowing GDA] and collateral circulation (*e.g.*, hepatopetal collaterals *via* the inferior phrenic artery)[1,28,30,31]. This leads to a simple question: How do we simultaneously accomplish complete hemostasis and HA flow preservation? Endovascular treatment (EVT) [*e.g.*,

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transcatheter arterial embolization (TAE) and stent-graft placement] are currently available[1,6,13,16,18,19,32-40], and surgical arterioportal shunting has therapeutic potential for the arterial blood supply[41,42].

TAE provides complete hemostasis[1,2,13,19,20,23,39,43], although this approach increases the risk of severe complications associated with liver infarction caused by hepatic ischemia[1,13,18,19,23,30,32,33,39] and/or liver abscesses caused by biliary ischemia[6,34,35]. In contrast, transcatheter placement of a stent-graft (bare or covered stent) preserves HA flow[1,13,16,18,36-38,40], although technical failure of hemostasis may rarely occur[1]. From the viewpoint of cost-effectiveness, EVT is more advantageous than conventional surgery[29].

Complete hemostasis of fatal hemorrhage and preservation of HA flow should be simultaneously obtained; however, this may be difficult after PD because the hepatopetal arterial supply has been modified (Figure 1). In the present study, we retrospectively investigated our treatments for fatal arterial hemorrhage after PD and evaluated our own results. We also herein discuss the safety and feasibility of transcatheter stent-graft placement and especially validate the therapeutic potential of using a covered (not bare) stent for simultaneous accomplishment of complete hemostasis and HA flow preservation.

MATERIALS AND METHODS

Patients

This study focused on the postoperative state after PD (Figure 1); therefore, patients who underwent other surgeries (*e.g.*, distal pancreatectomy or gastrectomy) were excluded from further analysis. During a 14-year period (from January 2007 to December 2020), 291 PDs were performed in our institution. Fatal arterial hemorrhage occurred in 16 patients who underwent PD, and these patients were enrolled in this study. The patients' mean age at the time of PD was 73.4 ± 7.7 years, and the patients comprised 11 men and 5 women. The types of PD and postoperative complications are summarized in Table 1. The median follow-up duration after PD was 1.34 years [range, 14 d (death) to 9.55 years].

The clinical features, management strategy, and outcome of arterial hemorrhage were evaluated.

Surgical procedures of PD

The surgical procedures of PD have been described in detail elsewhere[9,44]. Lymphadenectomy and nerve dissection were performed in patients with malignancies in accordance with the Japanese guideline[45]. Briefly, the GDA from the celiac artery and IPDA from the SMA were cut after double ligation using a locking loop knot. Inherent reconstructions during subtotal stomach-preserving PD were performed by the modified Child's method with Braun's anastomosis. During pancreaticojejunostomy, an intraductal lost stent (pancreatic duct tube, 5 Fr, burled, MD41515; Sumitomo Bakelite Co., Ltd., Tokyo, Japan) was placed, and duct-to-jejunal anastomosis was performed with interrupted polydioxanone sutures (4-0 PDS II, violet, RB-1, Z712D; Ethicon, Inc., Cincinnati, OH, United States). Adequate approximation of the pancreatic stump and jejunal wall was ensured with interrupted polyvinylidene fluoride sutures (4-0 ASSP504-0IIN, ASFLEX, 75 cm; Kono Seisakusho Co., Ltd., Ichikawa, Chiba, Japan). choledochojejunostomy was performed with interrupted polydioxanone sutures. A linear stapler was employed for gastrojejunostomy, and the entry hole was closed by hand suturing in a layer-to-layer fashion. Braun's anastomosis was also performed by hand suturing in a layer-to-layer fashion.

Liver infarction caused by hepatic ischemia

Liver infarction was mainly diagnosed by imaging findings. A sudden increase in the serum aspartate aminotransferase concentration or a gradual increase in the total bilirubin concentration was used as supporting data[1].

Pancreatic leakage

Pancreatic leakage was diagnosed according to the criteria established by the International Study Group of Pancreatic Surgery[46].

TAE

TAE was performed as the EVT procedure in this study. We intend to arrest fatal

Table 1 Patients characteristics

Case number	Primary disease	Type of PD	Lymphadenectomy (categorization[1])	Nerve dissection	Associated pancreatitis	Pancreatic leakage	Postoperative complications	Hemorrhage oncet[2]	Sentinel bleeding	Symptoms	Sepsis	Shock	Liver ischemia
1	Insulinoma	SSpPD	No	No	No	Yes	-	7	No	Active bleeding from intraperitoneal drain	Yes	Yes	No
2	Gastric cancer	PD	Yes (D2)	No	No	Yes	-	20	No	Bleeding from wound	Yes	Yes	No
3	Gallbladder cancer	HPD	Yes (regional)	Yes	No	Yes	-	58	No	Hematemesis	No	No	No
4	Neuroendoicrine tumor	Lasparoscopic PD	No	No	No	Yes	-	18	Yes	Active bleeding from intraperitoneal drain	No	Yes	No
5	Bile duct cancer	PD	Yes (regional)	No	Yes	No	Digestive anastomotic failure	11	No	Active bleeding from intraperitoneal drain	No	No	No
6	Pancreatic cancer	SSpPD	Yes (D2)	Yes	Yes	Yes	-	22	No	Active bleeding from intraperitoneal drain	No	No	No
7	Bile duct cancer	SSpPD	Yes (regional)	Yes	No	Yes	-	14	No	Active bleeding from intraperitoneal drain	Yes	No	No
8	Gastric cancer	PD	Yes (D2+)	No	No	No	Ruptured suture (staple line)	32	Yes	Active bleeding fromintraperitoneal drain	Yes	Yes	No
9	Pancreatic cancer	SSpPD	Yes (D2)	Yes	Yes	No	-	6	Yes	Active bleeding from intraperitoneal drain	Yes	Yes	No
10	Pancreatic cancer	SSpPD	Yes (D2)	Yes	Yes	Yes	-	16	No	Melena	No	No	Yes
11	Pancreatic metastasis from renal cancer	SSpPD	No	No	No	Yes	-	30	Yes	Active bleeding from intraperitoneal drain	No	Yes	No
12	Ampullary cancer	SSpPD	Yes (D1)	No	No	Yes	-	6	Yes	Active bleeding from intraperitoneal drain	No	Yes	No
13	Pancreatic cancer	PD	Yes (D2)	Yes	Yes	Yes	-	14	No	Active bleeding from intraperitoneal drain	Yes	Yes	No
14	Intraductal papillary mucinous neoplasm	PpPD	No	No	No	Yes	-	22	No	Active bleeding from intraperitoneal drain	Yes	Yes	No
15	Pancreatic cancer	SSpPD	Yes (D1)	No	Yes	No	Biliary necrosis Ruptured cholangiojejunostomy	12	No	Active bleeding from intraperitoneal drain	Yes	Yes	No
16	Pancreatic cancer	SSpPD	Yes (D2)	Yes	Yes	Yes	-	28	No	Abdominal pain	Yes	Yes	Yes

[1]Intentional lymphadenectomy according to Japanese guidelines.
[2]Postoperative day after pancreaticoduodenectomy. HPD: Hepatopancreatoduodenectomy; PD: Pancreaticoduodenectomy; PpPD: Pylorus-preserving pancreaticoduodenectomy; SSpPD: Subtotal stomach-preserving pancreaticoduodenectomy.

hemorrhage by placement of microcoils (Deltaplush; Codman & Shurtleff, Inc., Raynham, MA, United States) in the pseudoaneurysm and/or culprit artery.

Stent-graft placement

The EVT procedures involved transcatheter placement of a stent-graft. In general, procedures of stent-graft placement were performed under local anesthesia. The target artery was dilated by a balloon catheter (Graftmaster; Abbott Laboratories, Chicago, IL, United States). Balloon catheter pressures was increased in manner of 2 atm per 5 s, and the maximum of intracatheter pressure was 15 atm (1520 kPa). A covered stent (Graftmaster; Abbott Laboratories), not a bare stent, was placed at the culprit artery. The size and length of covered stent was carefully decided on a case-by-case basis, based on angiographic findings after balloon dilation. We aimed to simultaneously obtain complete hemostasis of fatal hemorrhage and preservation of HA flow. The second overlapping stent-graft was implanted in an overlapping fashion, if needed. The actual procedure is shown in Figure 2.

Arterioportal shunting

An arterioportal shunt was surgically created (Figure 3). The ileocecal vein and artery were anastomosed in a side-to-side fashion using polypropylene suture. Thereafter, the hepatopetal flow of the portal vein (PV) was well oxygenated.

Ethical approval

This retrospective study was approved by the ethics review committee for clinical studies of our institution. The study was performed in accordance with the ethical guidelines of the Declaration of Helsinki. All patients involved in this study provided written informed consent authorizing the use and disclosure of their protected health information.

Statistical analysis

All results are shown as mean ± SD or median (range). Survival rates were calculated using the Kaplan-Meier method. All calculations were performed using statistical software (SPSS Inc., Chicago, IL, United States).

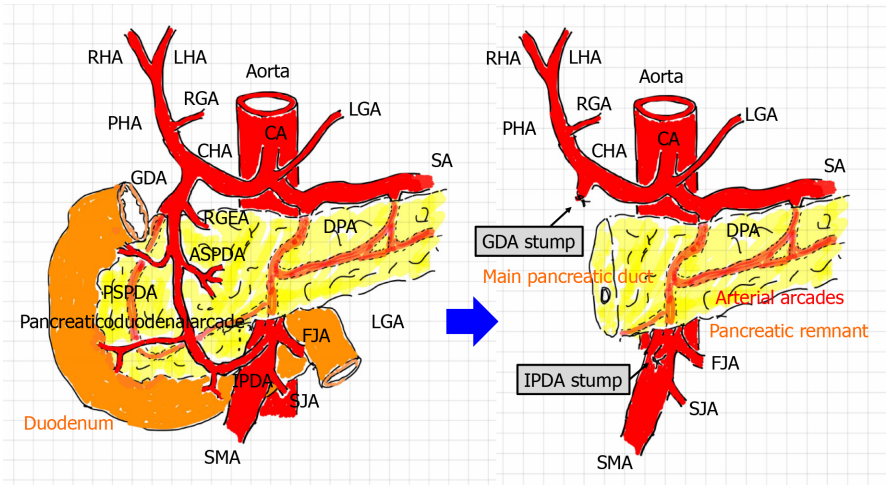

Figure 1 Arterial flow before and after pancreaticoduodenectomy. During pancreaticoduodenectomy (PD), the gastroduodenal artery (GDA) from the celiac artery and inferior pancreaticoduodenal artery (IPDA) from the superior mesenteric artery are ligated and then cut. Additionally, the pancreaticoduodenal arcade is resected. Hence, arterial flow to the liver is modified after PD. A hepatopetal blood supply from the GDA and IPDA *via* the pancreaticoduodenal arcade can no longer be expected. The hepatic artery flow depends on the celiac artery. Lymphadenectomy and nerve dissection for treatment of malignancies might render visceral arteries vulnerable to postoperative wall injuries. Arterial arcades still remain in the pancreatic remnant. ASPDA: Anterior superior pancreaticoduodenal artery; CA: Celiac artery; CHA: Common hepatic artery; DPA: Dorsal pancreatic artery; FJA: First jejunal artery; GDA: Gastroduodenal artery; HA: Hepatic artery; IPDA: Inferior pancreaticoduodenal artery; LGA: Left gastric artery; LHA: Left hepatic artery; PHA: Proper hepatic artery; RGA: Right gastric artery; RGEA: Right gastroepiploic artery; RHA: Right hepatic artery; PSPDA: Posterior superior pancreaticoduodenal artery; SA: Splenic artery; SJA: Second jejunal artery; SMA: Superior mesenteric artery.

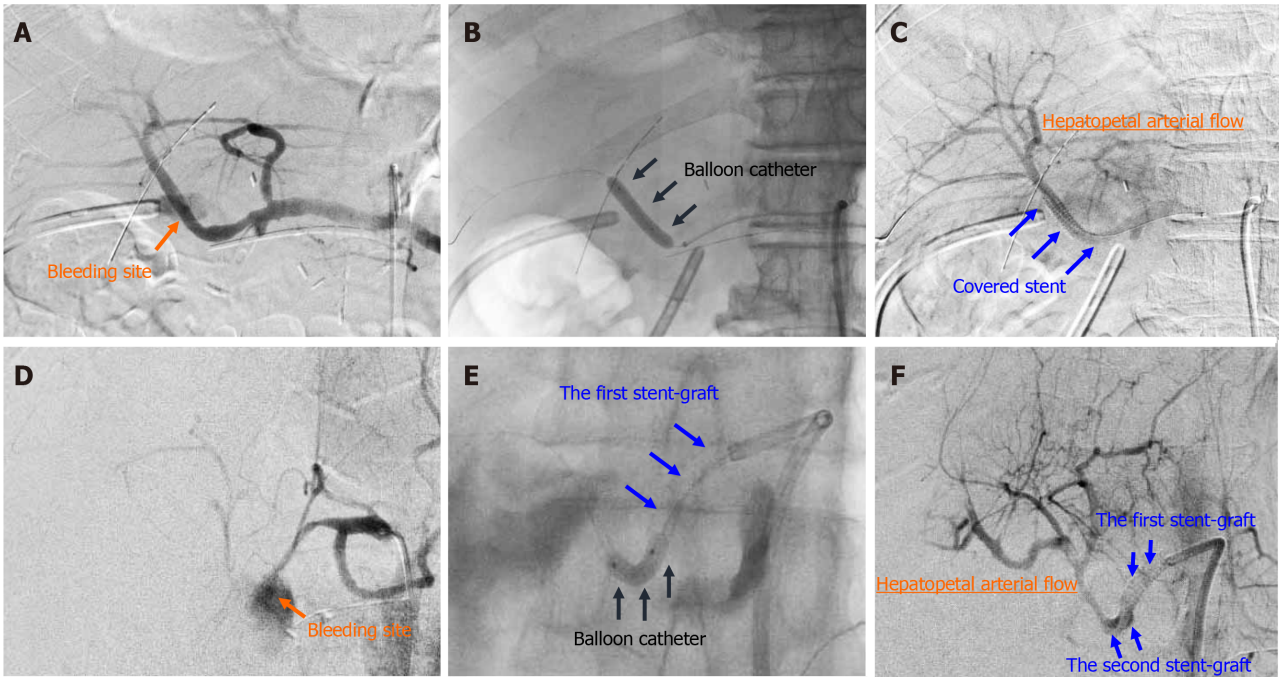

Figure 2 Actual procedures of transcatheter placement of covered stent. Actual procedures in patient 7 and patient 16 are shown. A: Patient 7, diagnostic angiography clearly detected the bleeding sites; B: Patient 7, the target artery was dilated by a balloon catheter; C: Patient 7, a covered stent was placed at the culprit artery; D: Patient 16, diagnostic angiography clearly detected the bleeding sites; E: Patient 16, the target artery was dilated by a balloon catheter; F: Patient 16, the second overlapping stent-graft was implanted in an overlapping fashion. The hepatopetal arterial flow resumed (C and F). Hence, complete hemostasis and preservation of hepatic artery flow were simultaneously obtained.

RESULTS

Institutional frequency of arterial hemorrhage after PD

The overall frequency of arterial hemorrhage after PD was 5.5% (16 of 291 patients who underwent PD in our institution).

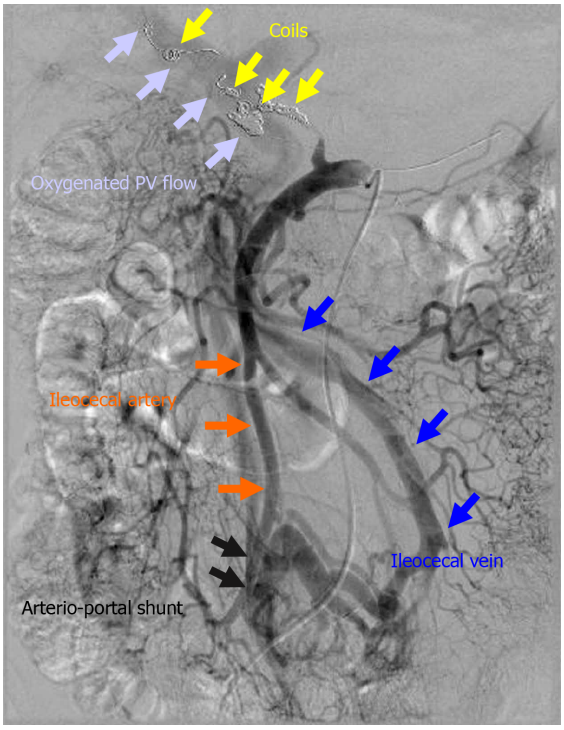

Figure 3 Actual finding of arterioportal shunting. The ileocecal vein and artery were anastomosed in a side-to-side fashion. In a patient in whom the initial endovascular treatment failed (patient 14), hemostasis was completed by additional transcatheter arterial embolization, and liver infarction subsequently occurred. Therefore, an arterioportal shunt was surgically created to oxygenate the portal vein flow. In this case, arterioportal shunting minimized progression to fatal liver infarction due to hepatic ischemia and refractory liver abscess due to biliary ischemia. PV: Portal vein.

Patients' characteristics and PD procedures

The primary diseases and surgical procedures are summarized in Table 1. Thirteen (81.3%) patients had malignancies. Lymphadenectomy and/or nerve dissection was performed in 12 (75.0%) patients.

Associated pancreatitis and pancreatic leakage

Associated pancreatitis occurred in seven patients, and nine (56.3%) patients had a soft pancreatic remnant (*i.e.*, pancreatic remnant without associated pancreatitis) (Table 1). Pancreatic leakage was observed in 12 (75.0%) patients (Table 1).

Hemorrhage onset, symptoms, and patients' conditions before EVT

The clinical characteristics at hemorrhage onset are summarized in Table 1. Hemorrhage onset occurred at a median of 18 d (range, 6-58 d) after PD. Sentinel bleeding was observed in 5 (31.3%) patients. Arterial hemorrhage was externalized through the intraperitoneal drain or wound in 13 (81.3%) patients and through the digestive tract in 2 (16.7%) patients. Sepsis, shock (including an unstable hemodynamic state), and liver infarction were observed in 9 (56.3%), 11 (68.8%), and 2 (16.7%) patients, respectively. Notably, four patients with poor clinical courses after EVT (Patients 13-16) had a poor clinical condition before EVT (Table 1).

Bleeding site, image findings, and definitive diagnosis

The most common and second most common sites of bleeding were the GDA stump (7 patients, 43.8%) and HA (4 patients, 25.0%), respectively (Table 2). Computed tomography (CT) angiography was the diagnostic modality in 13 (81.3%) patients. The imaging findings of CT angiography and angiography are summarized in Table 2. The median time from hemorrhage onset to definitive diagnosis and the median time from hemorrhage onset to EVT were 0 d (range, 0-1 d) and 0 d (range, 0-14 d), respectively (Table 2).

Actual EVT procedures, technical success of EVT, and long-term results after EVT

The treated arteries and ranges are summarized in Table 3. The initial EVT procedures were stent-graft placement in 7 (43.8%) patients, TAE in 6 (37.5%) patients, and combined therapy involving stent-graft placement and TAE in 2 (16.7%) patients

Table 2 Definitive diagnosis

Case number	Bleeding site	Diagnostic modality	CT angiographic findings	Angiographic findings	Time from hemorrhage onset to definitive diagnosis (d)	Time from hemorrhage onset to EVT (d)
1	RGA	CT angiography	Extravasation	Extravasation	0	0
2	SA	CT angiography	Extravasation	Extravasation	0	14[1]
3	RHA	CT angiography	Enlargement of pseudoaneurysm	Pseudoaneurysm; Extravasation	1	0
4	Cholangiojejunostomy	Clinical findings[2]	None	None	0	0
5	DPA	CT angiography	Extravasation	Extravasation	0	0
6	GDA stump	CT angiography	Extravasation	Extravasation	0	0
7	RHA	CT angiography	Extravasation	Extravasation	0	0
8	GDA stump	CT angiography	Pseudoaneurysm; Extravasation	Pseudoaneurysm	0	0
9	DPA	CT angiography	Extravasation	Extravasation; Pseudoaneurysm	0	0
10	PHA	CT angiography	Pseudoaneurysm	Obstruction of CHA; Pseudoaneurysm	0	1
11	RHA	CT angiography	Pseudoaneurysm	Pseudoaneurysm	0	0
12	GDA stump	Laparotomy[3]	None (hematoma only)	None (stenosis of CHA)	0	0
13	GDA stump	Angiography	Extravasation	Extravasation	0	0
14	GDA stump	CT angiography	Extravasation	Extravasation; Pseudoaneurysm	0	0
15	GDA stump	CT angiography	Minor extravasation	Extravasation; Pseudoaneurysm	0	1
16	GDA stump	CT angiography	Pseudoaneurysm; Extravasation	Pseudoaneurysm; Extravasation	0	0

[1]Hematemesis and endoscopic findings.
[2]Two surgical approaches were challenged during 14 d.
[3]Bleeding from the GDA was detected during laparotomy, even though computed tomography angiography and angiographic findings did not revealed extravasation. The endovascular treatment was done under the laparotomy. CHA: Common hepatic artery; CT: Computed tomography; DPA: Dorsal pancreatic artery; EVT: Endovascular treatment; GDA: Gastroduodenal artery; RGA: Right gastric artery; RHA: Right hepatic artery; PHA: Proper hepatic artery; SA: Splenic artery.

(Table 3).

The initial EVT failed and/or was incomplete in 4 (25.0%) patients, and the rate of technical success of the initial EVT was 75.0% (Table 3). The reasons for failed and/or incomplete EVT were stenosis in 2 patients, and subtle bleeding in one patient, and difficulty in packing in 1 patient (Table 3). Additional EVTs were performed in 4 (25.0%) patients (Table 3). Antiplatelet and/or anticoagulation agents were administered to 5 (31.3%) patients (Table 3), and these 5 patients continuously received medications even after discharge from our hospital.

Recanalization did not occur (0.0%) throughout the long-term follow-up after TAE (Table 3). Collateral circulation was observed in 2 (25.0%) of eight patients who underwent TAE (Table 3). Additionally, all implanted stent-grafts (100.0%) maintained their patency throughout the long-term follow-up after stent-graft placement (Table 3).

Surgical approaches including arterioportal shunting

Surgical approaches were utilized in eight patients and are summarized in Table 3. In one patient who underwent failed EVT (patient 10), hemostasis and ligation of the CHA were surgically performed under laparotomy. In one patient in whom the initial EVT failed (patient 14), hemostasis was completed by additional TAE, and liver infarction subsequently occurred. Therefore, an arterioportal shunt was surgically created to oxygenate the PV flow (Figure 3). In this case, arterioportal shunting

Table 3 Endovascular treatment

Case number	Treated artery (target and range)	TAE	Stent-graft placement			Technical success during EVT	Reasons for failed or incomplete EVT	Additional surgical approaches (day number[2])	Additional EVT (day number[2])	Antiplatelet and/or anticoagulation agents (number)	Long-term results of EVT		
											TAE		Stent-graft placement
			Stent type (number[1])	Size (mm)	Length (mm)						Collateral circulation (yr)[3]	Recanalization (yr)[3]	Patency (y)[3]
1	RGA	Coiling	-			Yes	-	No	No	No	No (4.39)	No (4.39)	-
2	CA; SA	-; Coiling	Covered stent (1); -	3.5; -	19; -	No	Stenosis	Hemostasis (-7 and -6)	Stent regrafting (+ 1); Coiling (+ 1)	No	-	-	-
3	PHA-LHA	-	Covered stent (1)	3.5	19	Yes	-	No	No	Yes (1)	-	-	Patent (0.72)
4	SMA branch; RHA	Coiling; -	-; Covered stent (1)	-; 3.5	-; 19	Yes	-	Lavage and cholangio-jejunal anastomosis (+ 7)	Stent regrafting (+ 28)	No	No (6.14); -	No (6.14); -	-; Patent (6.14)
5	SA branch	Coiling	-			Yes	-	No	No	Yes (1)	No (0.46)	No (0.46)	-
6	CHA-PHA	-	Covered stent (1)	3.5	19	Yes	-	No	No	No	-	-	Patent (0.93)
7	RHA	-	Covered stent (1)	3.5	19	Yes	-	Lavage and cholangio-jejunal anastomosis (+ 3)	No	No	-	-	Patent (1.27)
8	GDA	Coiling	-	-	-	No	Subtle bleeding[4]	No	No	No	No (0.24)	No (0.24)	-
9	DPA	Coiling	-	-	-	Yes	-	No	No	No	No (0.44)	No (0.44)	-
10	-	-	-	-	-	No	Stenosis	Hemostasis and ligation of CHA (± 0)	No	Yes (2)	-	-	Patent (1.95)
11	RHA	-	Covered stent (2)	3.0	19	Yes	-	Removal of hematoma (- 18)	Stent regrafting (+33)	Yes (2)	-	-	Patent (1.53)
12	CHA-PHA	-	Covered stent (1)	3.5	19	Yes	-	Removal of hematoma (± 0)	No	No	-	-	Patent (0.98)
13	CHA-PHA	Coiling	-	-	-	Yes	-	No	No	No	Yes (1.53)	No (1.53)	-
14	GDA	Coiling	-	-	-	No	Difficulty in packing	Arterio-portal shunting[5] (+ 4)	CHA coiling (+ 4)	Yes (1)	Yes (7.72)	No (7.72)	-
15	GDA	-	Covered stent (1)	3.5	19	Yes	-	No	No	No	-	-	Patent (0.00)

16	GDA	-	Covered stent (2)	2.6	19	Yes	-	Removal of hematoma (± 0)	No	No	-	-	Patent (0.01)

[1]The second stentgraft was implanted in overlapping fashion.
[2]The day number from the initial endovascular treatment (EVT).
[3]Findings in the latest dynamic image studies (time from the initial EVT).
[4]Subtle bleeding was observed at the end of EVT, but thereafter, complete hemostasis was fainally obtained.
[5]Shunt creation between the iliocecal artery and vein. CA: Celiac artery; CHA: Common hepatic artery; EVT: Endovascular treatment; GDA: Gastroduodenal artery; LHA: Left hepatic artery; RHA: Right hepatic artery; PHA: Proper hepatic artery; RGA: Right gastric artery; SA: Splenic artery; SMA: Superior mesenteric artery; TAE: Transcatheter arterial embolization.

minimized the patient's progression to fatal liver infarction due to hepatic ischemia and a refractory liver abscess caused by biliary ischemia.

Liver infarction due to hepatic ischemia

Liver infarction after EVT was observed in 2 (12.5%) patients (patients 13 and 14), and these patients underwent TAE (Tables 3 and 4). Complete hemostasis was obtained by TAE, but hepatopetal arterial flow was completely lost (Figure 4). Liver infarction due to hepatic ischemia subsequently occurred (Figure 4). In these patients, the serum aspartate aminotransferase concentration clearly increased after EVT (Figure 5). Fortunately, both patients successfully recovered from arterial hemorrhage after PD and liver infarction after TAE (Table 4).

Clinical course and outcome after EVT

The patients' clinical courses and outcomes after EVT are summarized in Table 4. Three patients (patients 2, 15, and 16) died during hospitalization, and the actual survival curves after PD and EVT are shown in Figure 6. The mean hospital stay after PD was 66.8 ± 27.7 d among 13 patients who achieved hospital discharge. Fourteen (87.5%) patients were successfully treated because the cause of death in 1 patient (patient 2) was unrelated to arterial hemorrhage (cancer-related death).

Two patients (patients 15 and 16) had a poor outcome even after successful EVT. These 2 patients had a poor clinical condition before EVT (Table 1). One patient (patient 15) had sepsis, shock, and disseminated intravascular coagulation before EVT and died of these conditions even after successful stent-graft placement (Tables 1 and 4). The other patient (patient 16) had sepsis, shock, and liver infarction before EVT (Table 1 and Figure 5) and finally died of liver failure even after successful stent-graft placement (Table 4).

DISCUSSION

In general, visceral artery pseudoaneurysms are rare but fatal[1,13,18,19,21,23,29,30]. The HA (

Table 4 Clinical course and outcome after endovascular treatment

Case number	Complication after EVT	Liver infarction after EVT	Hospital death (day number[1] and POD)	Clinical success[2]	Follow-up term (yr)	Cause of death	Prognosis (dead or alive)
1	-	No	No	Yes	5.57	Cancer-related death	Dead
2	-	No	Yes (+ 61 and 94)	Yes	0.26	Cancer-related death	Dead
3	-	No	No	Yes	0.72	Cancer-related death	Dead
4	-	No	No	Yes	8.36	-	Alive
5	-	No	No	Yes	0.56	Cancer-related death	Dead
6	Bleeding	No	No	Yes	1.04	Cancer-related death	Dead
7	-	No	No	Yes	1.34	Cancer-related death	Dead
8	-	No	No	Yes	0.52	Cancer-related death	Dead
9	-	No	No	Yes	0.47	Cancer-related death	Dead
10	-	No	No	Yes	1.95	-	Alive
11	-	No	No	Yes	1.69	-	Alive
12	-	No	No	Yes	1.46	-	Alive
13	-	Yes	No	Yes	1.74	Cancer-related death	Dead
14	Bleeding; Liver abscess	Yes	No	Yes	9.55	-	Alive
15	-	No	Yes (+ 1 and 14)	No	0.04	Bleeding, sepsis and DIC	Dead
16	-	No	Yes (+ 3 and 31)	No	0.08	Liver failure	Dead

[1]The day number from the initial EVT.
[2]Short-term clinical outcome. EVT: Endovascular treatment; DIC: Disseminated intravascular coagulation; POD: Postoperative day after pancreaticoduodenectomy.

i.e., the CHA, PHA, and lobular branches) is the second most frequent site of visceral pseudoaneurysms, and the splenic artery is generally the most common[47]. Pseudoaneurysms of the HA are usually iatrogenic[2,16,30,44] but may also be associated with localized infection or trauma[30]. Possible causes of intraoperative pseudoaneurysms include direct vascular injury during dissection or retraction, clamp injury to the vessel, or thermal injury *via* electrocautery[17,30]. Lymphadenectomy and/or nerve dissection for malignancy renders visceral arteries more vulnerable to further wall injuries[2,16,17,30,39,44,48]. Complications following PD commonly consist of localized infection, anastomotic failure, delayed gastric emptying, and gastrointestinal bleeding[2,6,8,9,29,30]. Although arterial hemorrhage after PD is not frequent, it is fatal[1,2,4,7,12,14-23]. The GDA stump is the most common site of arterial hemorrhage, and the CHA and PHA are the next most common sites[16,18,19,21,22,27]. Arterial hemorrhage of the SMA after PD has also been reported[49,50].

Pancreatic leakage compromises the arterial wall[2,6,16,17,19,22,24,29,30,39,44,51]. Pancreatic juice or localized infection gradually causes arterial wall erosions, resulting in pseudoaneurysms[2,6,16,17,22,24,29,39,44,51]. Pseudoaneurysm rupture causes sudden-onset, massive, and active hemorrhage[18]. Studies have shown a trend toward a higher prevalence of a soft pancreatic remnant in patients with arterial hemorrhage[2,6,44]. Leaving approximately 1 cm of the GDA stump, spreading an omental flap, and winding the HA by the round ligament of the liver have been suggested to minimize direct contact of pancreatic juice with adjacent vessels[18,22,51-53].

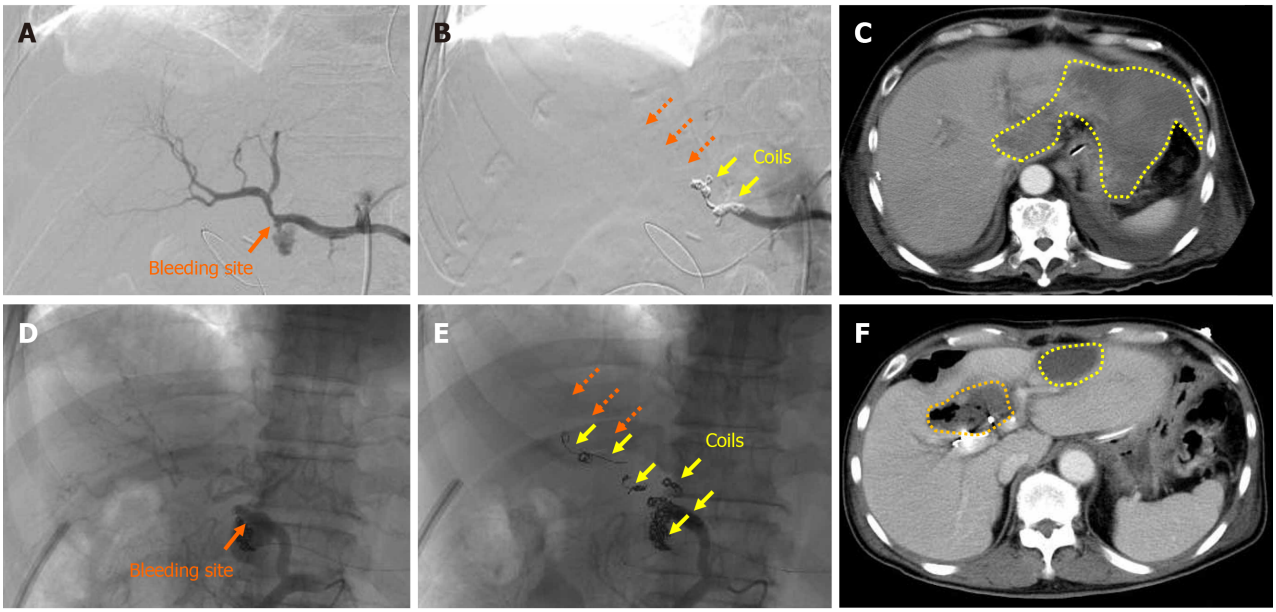

Figure 4 Liver infarction and abscess after transcatheter arterial embolization. Actual findings in patient 13 and patient 14 are shown. A: Patient 13, the bleeding sites were detected; B: Patient 13, complete hemostasis was obtained by transcatheter arterial embolization, but the hepatopetal arterial flow was completely lost (dotted orange arrows); C: Patient 13, the patient subsequently developed liver infarction due to hepatic ischemia (dotted yellow circles); D: Patient 14, the bleeding sites were detected; E: Patient 14, complete hemostasis was obtained by TAE, but the hepatopetal arterial flow was completely lost (dotted orange arrows); F: Patient 14, the patient subsequently developed liver infarction due to hepatic ischemia (dotted yellow circles) and a liver abscess due to biliary ischemia (dotted orange circle).

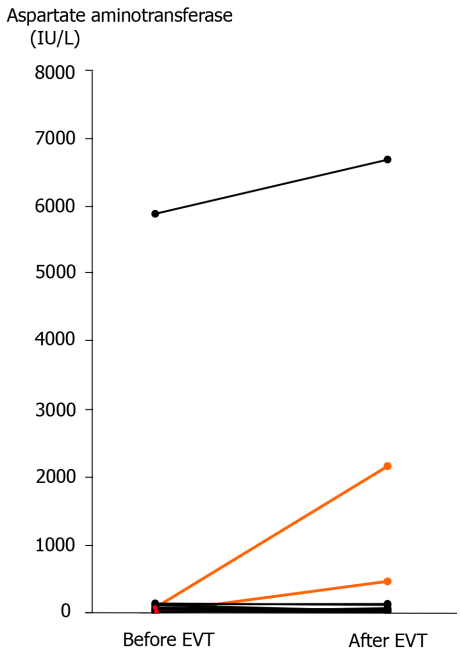

Figure 5 Serum aspartate aminotransferase concentration before and after endovascular treatment. Actual changes before and after endovascular treatment (EVT) are shown. In two patients who developed liver infarction after transcatheter arterial embolization (patients 13 and 14), the serum aspartate aminotransferase concentration was clearly elevated after EVT (orange lines). A high serum aspartate aminotransferase concentration was observed in a patient who had liver infarction before EVT (patient 16), and this patient finally died of liver failure even after successful stent-graft placement. EVT: Endovascular treatment.

Diagnostic procedures and imaging modalities are associated with certain difficulties[6,7,17,18,28,54]. Even based on laparotomy findings, definitive diagnosis may be difficult[54]. Bleeding from the digestive tract or intraperitoneal drain should be considered a warning because it is an important prelude to massive and active hemorrhage. The term "sentinel bleeding" was first coined in 1989 by Shankar and

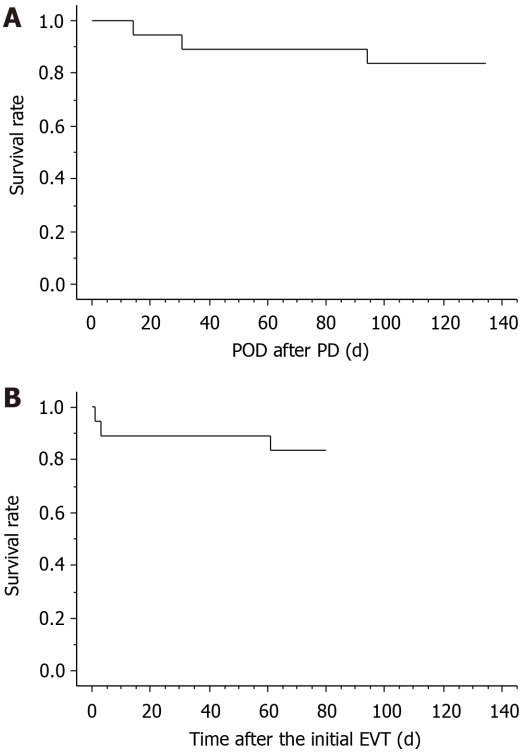

Figure 6 Short-term survival after pancreaticoduodenectomy and endovascular treatment. Three patients (patients 2, 15, and 16) died during hospitalization, and the actual survival curves after pancreaticoduodenectomy (PD) and endovascular treatment (EVT) are shown. Fourteen (87.5%) patients were successfully treated because the cause of death in one patient (patient 2) was unrelated to arterial hemorrhage (cancer-related death). A: PD; B: EVT. EVT: Endovascular treatment; PD: Pancreaticoduodenectomy; POD: Postoperative day.

Russell[55] and was further discussed by Brodsky and Turnbull in 1991[34]. The incidence of sentinel bleeding is approximately 30% to 80%[2,18]. Pseudoaneurysms can be detected by CT in patients with sentinel bleeding[6,7]. Sentinel bleeding should be regarded very seriously[2,4,6,18], even in asymptomatic patients with conservatively treated pancreatic leakage[6,56,57]. An accurate definitive diagnosis should be made immediately, before the patient's unstable hemodynamic state deteriorates[6,18,29]. Diagnostic digestive endoscopy delays adequate treatment in hemodynamically unstable patients because of pseudoaneurysm rupture[7,58]. The diagnostic potential of CT angiography[6,16,19,28,43] and diagnostic angiography[6,7,17,18,29,39,54] have been established. Diagnostic angiographic findings include extravasation of contrast medium, pseudoaneurysm formation, non-smooth arterial intima, local vascular spasm, stenosis, and distal arterial branch expansion[20,54]. Diagnostic angiography should be considered even in patients with suspected hemorrhage[6,7,17,18,29,39,54], and subsequent EVT should be adequately performed if necessary[1,7,16-18,30,54].

EVT represents the first-line treatment for arterial hemorrhage after PD[2,7,17,40,59-61]. Arterial hemorrhage easily results in unstable hemodynamic state[1,6,16,18,23,29,62], sepsis[2,6,7,13], and hepatic ischemia[6,28,39,63]. Prolonged hemorrhage leads to shock and coagulopathy[1,16,18,23], and further hemorrhage results in disseminated intravascular coagulation[1,16,18,23]. Complicated homeostasis is associated with a poor prognosis even after successful EVT[1,16,18], and the patient's condition before EVT is strongly associated with complications after EVT[1,16,29]. In fact, our two patients who had a poor clinical condition before EVT (*e.g.*, sepsis, shock, and liver infarction) finally died even after successful EVT (Tables 1 and 4). If a patient shows any signs of a suspected hemorrhage, EVT should be performed as soon as possible before the development of complicated homeostasis[1,2,16,18,23,30]. Concern exists regarding the placement of foreign bodies (*i.e.*, coils and stent-grafts) in the setting of infection or inflammation[40]. Intravascular stent infection can be a devastating complication, but it is very rare[40,64-66]. In fact, stent-grafts have been used to repair infected pseudoaneurysms[67,68]. Though pancreatic juice-related localized infection may associate with pseudoaneurysm and arterial wall erosion[2,6,16,17,22,24,29,39,44,51], we consider that stent-grafts can be placed even in suspicious infectious site.

Arterial hemorrhage after PD usually occurs after at least 1 d[24], and delayed hemorrhage generally occurs after 1 wk[6,58]. In one study, one-third of arterial

hemorrhages occurred 1 mo after PD[21]. Hence, delayed hemorrhage is common after PD[2,6,7-19,21,22,28,58,62], and the median or mean time point of hemorrhage onset ranges from 18 d to 21 d after PD[2,7,21,28]. Pancreatic leakage is a possible cause of delayed arterial hemorrhage[2,6,44], and delayed hemorrhage after PD carries a significantly higher mortality rate[2,6,7,21,28].

Because the GDA and IPDA were ligated and the pancreaticoduodenal arcade was resected during PD in the present study, hepatopetal blood supply *via* these arteries could no longer be expected (Figure 1). EVT may lead to severe complications (*e.g.*, hepatic ischemia, liver abscess formation, and PV stenosis)[1]. The EVT technique should be decided on a case-by-case basis[18,28,29]. Notably, the EVT procedure is strongly associated with complications after EVT[1,16]. Although TAE is technically easier than stent-graft placement[19], liver infarction secondary to hepatic ischemia frequently occurs[6,34,35]. Even a subtle ischemic change in the biliary tree results in intractable liver abscesses[6,34,35]. We also experienced a case of a refractory liver abscess due to biliary ischemia (patient 14) (Figure 4F). PV stenosis easily disturbs the hepatic parenchymal perfusion, resulting in liver infarction with a poor prognosis[23,69]. The rates of mortality and serious hepatic complications after EVT are approximately 20% to 50% and 20% to 80%, respectively[1,18,19,21,23,31,35,70-72].

TAE is advantageous for ensuring complete hemostasis[2,13,20,23,43,54,62,73-77], and the hemostatic rate is reportedly > 90%[17,20,23,54,71]. TAE is technically user-friendly at the most frequent site (*i.e.*, the GDA stump)[19,43], although both the proximal and distal sides of the GDA should be completely embolized[19]. To prevent recanalization and rebleeding, all arterial flows to the pseudoaneurysm should be completely interrupted[19,44,47,48]. Although the pancreaticoduodenal arcade is removed during PD, arterial arcades remain in the pancreatic remnant (Figure 1)[29,47]. Recanalization *via* the collateral circulation has been reported after TAE[29]; however, transcatheter techniques (*e.g.*, isolation, packing, and embolization) are available for various forms of pseudoaneurysms[29]. Notably, TAE is occasionally associated with serious hepatic complications caused by hepatic ischemia[1,16,23,30,32,33,69,70]. The liver has many potential collateral pathways that communicate with the adjacent arterial system[16,19,23,29,78,79], and a sudden complete block of HA flow immediately after surgery may induce an ischemic insult to the liver parenchyma[16,29,78,79]. Whether extrahepatic arteries (*e.g.*, the inferior phrenic artery and left gastric artery) provide sufficient hepatopetal collateral circulation to avoid fatal hepatic ischemia after TAE remains unclear[1,19,23,32]. Additionally, the liver can tolerate considerable TAE without significant liver infarction because it has a dual blood supply from the HA and PV[19,20,23,29]. TAE may cause liver infarction in patients with poor collateral circulation because of their postoperative status[29]. Approximately 30% to 80% of patients develop hepatic ischemia after TAE[69,71], and approximately 20% to 40% of patients progress to liver infarction[23,70,71]. The reported mortality rate ranges from 30% to 50%[19,31,35,72].

Simultaneous accomplishment of complete hemostasis and HA flow preservation is difficult[1,7,13,16,18,28,59]. Transcatheter placement of a covered stent may be of value in maintaining the patency of adjacent arteries, and stent-graft placement is an ideal technique to preserve HA flow[1,6,13,16,17,21,27-31,40,54,61,63,80-82]. If necessary, a second overlapping stent-graft can be implanted[40,83]. Actually, we placed a second stent in two patients (Table 3). Some researchers have described patients who underwent this EVT technique[30,31,63,80-82,84], and others have documented such cases in published case series[13,36,38,85]. The success rate of stent-graft placement reportedly ranges from 75% to 80% because the target arteries require a specialized stent size and/or exhibit narrowness and tortuosity[16,37,38,59]. The overall mortality and clinical outcomes are affected by the patients' conditions before stent-graft placement[16,29]. The use of antiplatelet agents or heparinization after stent-graft placement in the HA is still controversial[13,16,36,37,40,85]. Some clinicians do not use such agents in patients with an unstable hemodynamic state after arterial hemorrhage[37]. However, stent-graft placement in a patient with arterial hemorrhage after surgery carries a high risk of thrombosis because the damaged wall of the HA is surrounded by localized infection and/or massive hematoma, and the HA diameter is very small[16,40]. Hence, some clinicians use these agents after stent-graft placement[13,16,36,40,85].

Stent grafting is a technically difficult procedure and requires adaptation to vessels of various sizes[18,40]. However, this EVT technique is considered the most appropriate treatment method in patients with a favorable vascular anatomy. Stent-graft placement may fail for anatomical reasons (*e.g.*, tortuosity or variation)[13,17,40,59,84,86] or because of catheter-induced vasospasm or spontaneous thrombosis within the aneurysmal wall[17,87-89]. Actual reasons for failed or incomplete EVT in our institution were summarized in Table 3. Since stent-graft placement may technically failed due to tortuosity, variation, stenosis, vasospasm or thrombosis at the culprit

artery[13,17,40,59,84,86-89], preliminary dilatation by balloon catheter is indispensable even for self-expandable covered stent. The interventional radiologist who performs the procedure must have adequate experience and skill[59,62].

If EVT fails or is incomplete, the next management option for arterial hemorrhage is still a surgical approach[2,7,62]. Surgical exploration and complete hemostasis are difficult and hazardous because of postoperative adhesions and the patient's critical condition[17,18,48]. Surgical treatment is usually associated with a high mortality rate (29%-58%)[2,17-19,48]. Hepatopetal flow of the PV can be well oxygenated by creation of an arterioportal shunt, and some reports have described such cases[41,42]. The impact of a surgical approach involving arterioportal shunting on the prevention of liver infarction has been documented[90,91]. Although the clinical decision and optimal timing for the surgical approach of arterioportal shunting are still controversial, we consider that the presence of subtle clinical signs of progressive liver infarction after EVT is a clear indication for arterioportal shunting.

To our knowledge, most reports to date are limited to case reports or small series[13,30,31,36,38,63,80-82,84,85]. We acknowledge that this study has several limitations. The main limitation is that this was a retrospective study with a small number of patients from a single center. Of course, we have demonstrated our individual-tailored approach. Potential limitations due to bias and a small sample size are inherent to this type of study. This represents our experience in a single institution and our views may be affected by various biases. Hence, we understand that our conclusions must be drawn with extreme caution. However, we believe that transcatheter placement of a covered stent has therapeutic advantages for arterial hemorrhage after PD, with simultaneous accomplishment of complete hemostasis and HA flow preservation.

Actual therapeutic strategies for our patients who caused arterial hemorrhage after PD were summarized in Figure 7. We currently have an institutional therapeutic strategy for arterial hemorrhage after PD based on our own experiences: (1) CT angiography is performed if general condition is stable; (2) Diagnostic angiography is immediately performed even in a suspicious patient; and (3) Covered stent is subsequently placed at the culprit artery as the first line treatment.

CONCLUSION

In conclusion, transcatheter placement of a covered stent may be a powerful tool for simultaneous accomplishment of complete hemostasis and HA flow preservation, although arterial hemorrhage after PD is generally fatal.

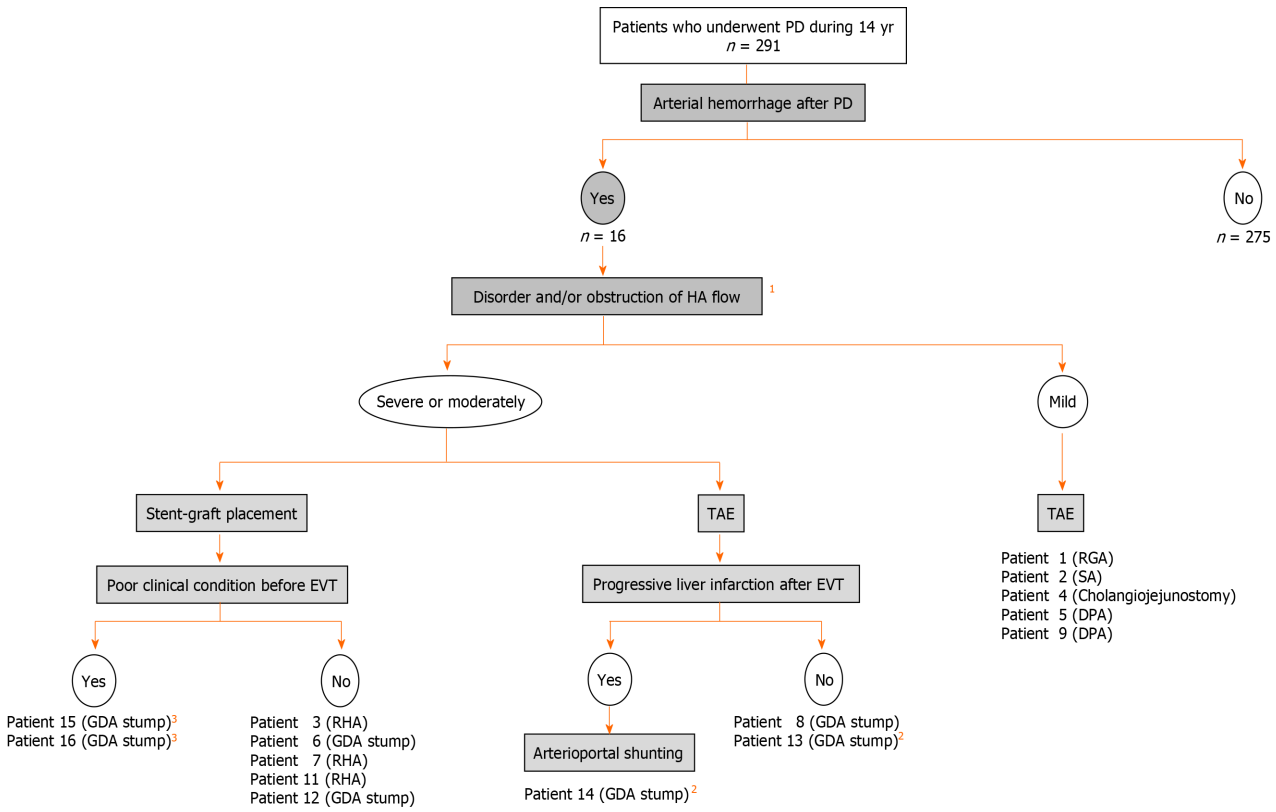

Figure 7 Flowchart of endovascular treatments and arterioportal shunting. Actual flowchart of our patients who caused arterial hemorrhage after pancreaticoduodenectomy was shown. [1]Image findings of computed tomography angiography and/or diagnostic angiography. [2]Patients with liver infarction after transcatheter arterial embolization. [3]Patients with poor outcome even after successful stent-graft placement. PD: Pancreaticoduodenectomy; HA: Hepatic artery; TAE: Transcatheter arterial embolization; EVT: Endovascular treatment; GDA: Gastroduodenal artery; RHA: Right hepatic artery; SA: Splenic artery; DPA: Dorsal pancreatic artery.

ARTICLE HIGHLIGHTS

Research background

Arterial hemorrhage after pancreaticoduodenectomy (PD) is fatal.

Research motivation

This hemorrhage is caused by pseudoaneurysm rupture, and the gastroduodenal artery stump and hepatic artery are frequent culprit vessels.

Research objectives

Simultaneous accomplishment of complete hemostasis and hepatic artery flow preservation is difficult after PD. Although complete hemostasis may be obtained by transcatheter arterial embolization or surgery, liver infarction and/or abscesses may occur.

Research methods

Arterial hemorrhage after PD is fatal. This hemorrhage is caused by pseudoaneurysm.

Research results

We here evaluate our experience including actual treatments (transcatheter arterial embolization, stent-graft placement, or surgery), and discuss therapeutic strategies.

Research conclusions

Transcatheter placement of a covered stent is useful for simultaneous accomplishment of complete hemostasis and hepatic arterial flow preservation.

Research perspectives

Therapeutic options for fatal arterial hemorrhage after PD is shown.

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ORIGINAL ARTICLE

Observational Study

Dried blood spot sampling as an alternative for the improvement of hepatitis B and C diagnosis in key populations

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Abstract

BACKGROUND

To achieve the elimination of hepatitis B and C, there is an urgent need to develop alternative strategies to increase the access of diagnosis, particularly among key populations such as people living with human immunodeficiency virus (HIV), individuals with coagulopathies and chronic kidney disease (CKD) patients.

AIM

Flores GL and Villar LM drafted the manuscript; Flores GL, Mota JC, Bastos FI and Villar LM critically revised the manuscript for intellectual content; All authors read and approved the final manuscript.

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To evaluate the use of dried blood spot (DBS) in the detection of hepatitis B virus (HBV) and hepatitis C virus (HCV) markers.

METHODS

A total of 430 individuals comprised of people living with HIV, coagulopathies and CKD provided paired serum and DBS samples. HBsAg, anti-HBc and anti-HCV were tested in those samples using a commercial electrochemiluminescence. Demographic and selected behavioral variables were evaluated to assess possible association with HBV and HCV positivity.

RESULTS

Using DBS, HBsAg prevalence varied from 3.9% to 22.1%, anti-HBc rates varied from 25.5% to 45.6% and anti-HCV positivity ranged from 15.9% to 41.2% in key populations. Specificities of HBV and HCV tests using DBS varied from 88.9% to 100%. The HBsAg assay demonstrated the best performance in CKD and coagulopathy individuals and the anti-HCV test had a sensitivity and specificity of 100% in people living with HIV. Accuracy of HBV and HCV detection in DBS varied from 90.2% to 100%. In the CKD group, HBsAg positivity was associated with infrequent use of condoms, and anti-HBc positivity was associated with sharing nail cutters/razors/toothbrushes. Anti-HCV reactivity was positively associated with a history of transplantation and length of time using hemodialysis in both specimens. In people living with HIV, only the male gender was associated with anti-HBc positivity in serum and DBS.

CONCLUSION

DBS with electrochemiluminescence are useful tools for the diagnosis and prevalence studies of hepatitis B and C among key populations and may increase the opportunity to foster prevention and treatment.

Key Words: Dried blood spot; Electrochemiluminescence; Hepatitis B; Hepatitis C; Key populations; Diagnosis

Core Tip: Dried blood spot (DBS) samples may be an alternative to serum to increase access and timeliness in the diagnosis of hepatitis B and C in key populations such as people living with human immunodeficiency virus, coagulopathies and chronic kidney disease. We found high accuracy for hepatitis B virus and hepatitis C virus detection using DBS. It was possible to observe similar hepatitis prevalence, demographic and clinical data related to hepatitis positivity in DBS and serum. DBS along with electrochemiluminescence could be used for diagnosis and prevalence studies of hepatitis B virus and hepatitis C virus among hard-to-reach populations.

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INTRODUCTION

Viral hepatitis is an important public health challenge with an estimated 257 million people living with chronic hepatitis B virus (HBV) and 71 million people living with chronic hepatitis C virus (HCV) worldwide[1,2]. HBV and HCV infection have a heterogeneous distribution in Latin America, where 7-12 million people have been infected with HBV and less than 2% are infected with HCV[3].

Some groups may be exposed more frequently to HBV and HCV infection mainly due to repeated exposure to contaminated blood that may occur during transfusions,

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hospitalizations, surgeries as well as other invasive procedures (including the management of chronic kidney disease (CKD) *via* hemodialysis) and last but not least coagulopathy individuals. In these groups, HBsAg prevalence varies from 3.9% to 7.0% and anti-HCV prevalence from 12.6% to 47.0%[4-8]. Another group at-risk for acquiring HBV and HCV is composed of people living with human immunodeficiency virus (HIV), as those viruses share common modes of transmission, such as sexual and parenteral transmission. Among people living with HIV, HBV prevalence varies from 2.8% to 10.3%, while HCV prevalence varies from 4.6% to 6.4%[3,7,9,10].

Diagnosis of infections with these viruses can be difficult in these at-risk groups, such as CKD individuals undergoing hemodialysis, coagulopathy individuals and people living with HIV, due to the difficulty of blood sample collection by venipuncture, their remote location and lack of health care.

In these real-life situations, biosecurity is an ever-present problem. In addition to difficulties affecting proper storage and transport of materials and samples, trained personnel are usually absent or scarce. Dried blood spot (DBS) samples could be a key alternative to serum obtained by venipuncture, which would increase access to diagnosis. These samples are easily collected using finger puncture and can be transported and stored at room temperature. Some studies have demonstrated the detection of HBV and HCV markers using DBS along with enzyme-linked immunosorbent assay in several groups, including monoinfected hepatitis patients and those coinfected with HIV[11-13].

Most studies aiming to detect hepatitis markers in DBS have employed enzyme immunoassays, but recently several laboratories have replaced manual or semimanual enzyme-linked immunosorbent assay with electrochemiluminescence (ECLIA). This technique is highly accurate, presents a low detection limit and delivers results quickly[14]. ECLIA has been used for detecting HBsAg and anti-HCV in DBS samples in monoinfected individuals with high sensitivity and specificity[15]. However, there is no information regarding the performance of ECLIA for the detection of HBV and HCV markers in DBS samples in key populations, such as individuals with coagulopathies, CKD patients and people living with HIV.

The main objective of this study was to investigate the putative influence of HIV infection as well as pathophysiological alterations in individuals with coagulopathies (hemophilia and von Willebrand disease) or CKD, vis-à-vis the performance of optimized ECLIA for the detection of HBsAg, anti-HBc and anti-HCV markers in DBS samples. This study also aimed to provide new data on the prevalence of these markers using DBS coupled with ECLIA.

MATERIALS AND METHODS

Study design and population

A cross-sectional study was conducted in different macro-regions of the country from June 2014 to March 2017. Basic sociodemographic data were collected using a standard questionnaire. While analyzing at-risk populations in Brazil presents inherent limitations in the sample-frames available, this study aimed to create a panel that was as broad as possible to maximize the use of individuals and samples obtained under the protocol.

Convenience samples include those as follows: Coagulopathy individuals, CKD patients and people living with HIV. Potential participants were recruited from hemodialysis clinics. Among them, coagulopathy individuals under follow-up in referral clinics as well as patients from HIV/AIDS services located in the northeastern and southeastern regions of Brazil were recruited.

These geographical domains correspond to an involuntary but insurmountable limitation. Although there are some data on the southernmost regions of Brazil from other research groups, data on the far north and central west locations represent a challenge in terms of budgetary constraints and logistics. To send research teams to such locations and to transport biological samples over such huge distances requires a sustained effort and costs comparable to travel across the whole territory of western Europe. Furthermore in Brazil, personnel (both technicians and ancillary personnel) and sample transportation remain a challenge due to a fractured and sometimes nonexistent aerial and terrestrial network.

Inclusion criteria for the selection of participants were as follows: Individuals of both sexes, aged 18 years or older, attending the healthcare centers involved in this study for their different medical conditions.

Three groups were included in this study: (1) Individuals with coagulopathies (hemophilia and von Willebrand disease) recruited from the coagulopathy outpatient clinic of the public Hematology and Hemotherapy Center of Ceará (Hemoce), located in Fortaleza city; (2) CKD patients on hemodialysis recruited from three private nephrology clinics that receive individuals from the public and private healthcare systems located in the states of Ceará and Rio de Janeiro; and (3) People living with HIV referred to the viral hepatitis ambulatory clinic (FIOCRUZ, RJ) from the gastroenterology outpatient clinic of the Gaffrée and Guinle Hospital (UNIRIO, RJ) infectious disease unit at Nova Iguaçu Hospital and the infectious disease outpatient clinic at the Clementino Fraga Filho Hospital (UFRJ).

Demographic characteristics and risk factors such as behavior, age, gender, marital status and education were defined using categories in the Brazilian Census and major national household surveys (*e.g.*, PNAD). All patients enrolled read and signed the informed consent form. The FIOCRUZ Ethics Committee approved this study (CAAE No. 34049514.7.3006.5258 e 34049514.7.3009.5051).

Laboratory tests

Paired serum and DBS samples were obtained by venipuncture. Whole blood (6 mL) was collected from each patient and 75 μL of this was applied to a 12 mm, preprinted circular disc on Whatman 903 protein protective card (Whatman, GE Healthcare, NJ, United States). To elute DBS samples, the 12 mm disc of filter paper was cut and transferred to a microtube containing 500 μL of 0.5% PBS/BSA for 18 h to 24 h[15]. The analysis of the serum samples was the gold standard for the detection of HBsAg, anti-HBc and anti-HCV. Serological markers were detected using a commercial ECLIA technique (Cobas E411, Roche, United States).

ECLIA in DBS samples

The ECLIA technique was used for the evaluation of HBsAg, anti-HBc and anti-HCV in DBS samples (Elecsys anti-HCV II, Elecsys HBsAg II and Elecsys anti-HBc II - Roche Diagnostics) following the manufacturer's instructions. In the anti-HCV and HBsAg assay, samples with sample/cutoff values < 1.0 were considered nonreactive, whereas for the anti-HBc assay, non-reactive samples should have an sample/cutoff value of > 1.0.

Statistical analysis

Absolute and estimated infection frequencies were calculated as well as mean and standard deviation of the patients' sociodemographic and clinical characteristics. For the association study, populations and markers were analyzed using the Chi-square test for homogeneity with a P value of 0.05. Variables with a proportion of missing values greater than 10.0% for each diagnostic test were excluded from the analysis. Unadjusted odds ratios (ORs) and respective 95% confidence intervals (95%CIs) were calculated for sociodemographic, behavioral and clinical variables as well as for each one of the diagnostic tests/seromarkers.

Associations were further analyzed using multiple logistic regression. Nonreactive samples were taken as the reference categories to which all other categories were cross-compared, yielding adjusted ORs and respective CIs. Only variables with statistical association at the level of 20% were entered into the multivariate models using a forward stepwise procedure. Maximum likelihood and the Wald test were used to assess the parsimony and fitness of intermediate models contemplating the exclusion or inclusion of different variables. Intermediate models were evaluated using the Hosmer-Lemeshow goodness-of-fit test using a 95%CI.

Taking ECLIA as the gold standard method for the sake of our analysis, sensitivity, specificity and positive and negative predictive values as well as accuracy were calculated for each biological outcome.

RESULTS

CKD patients

Among CKD patients (n = 284), HBsAg, anti-HBc and anti-HCV were detected in serum in 4.6%, 39.9% and 16.3% of individuals, respectively and were detected by DBS in 4.9%, 33.6% and 15.9% of individuals, respectively. Table 1 shows the sociodemographic and clinical characteristics of this population.

Table 1 Main sociodemographic and clinical characteristics of chronic kidney disease individuals, people living with human immunodeficiency virus and coagulopathy individuals

Variable		I_CKD	P_HIV	I_COAG
		n (%)	*n* (%)	*n* (%)
Gender	Female	101 (37.3)	37 (38.9)	1 (2.0)
	Male	170 (62.7)	58 (61.1)	50 (98.0)
Age	18-30	19 (7.0)	9 (9.5)	25 (50.0)
	30+	253 (93.0)	86 (90.5)	25 (50.0)
Marital status	Married	132 (49.3)	31 (35.2)	17 (33.3)
	Not married	136 (50.7)	57 (64.8)	34 (66.7)
Race	White	71 (27.6)	29 (46.0)	14 (29.2)
	Black	186 (72.4)	34 (54.0)	34 (70.8)
Length of education	Up to 8 yr	136 (51.1)	36 (38.7)	14 (27.5)
	9 or more	130 (48.9)	57 (61.3)	37 (72.5)
Acupuncture	Yes	21 (7.8)	9 (9.5)	15 (29.4)
	No	247 (92.2)	86 (90.5)	36 (70.6)
Tattoo	Yes	27 (10.1)	29 (31.2)	6 (12.0)
	No	240 (89.9)	64 (68.8)	44 (88.0)
Piercing	Yes	9 (3.4)	7 (7.5)	2 (4.1)
	No	259 (96.6)	86 (92.5)	47 (95.9)
Shared nail cutters/razor/toothbrush	Yes	190 (66.9)	70 (74.5)	22 (43.1)
	No	94 (33.1)	24 (25.5)	29 (56.9)
Blood or plasma transfusion	Yes	169 (63.3)	16 (17.2)	35 (70.0)
	No	98 (36.7)	77 (82.8)	15 (30.0)
Transfusion before 1994	Yes	34 (12.8)	7 (7.5)	25 (49.0)
	No	231 (87.2)	86 (92.5)	26 (51.0)
HBV vaccine	Yes	206 (72.5)	41 (43.2)	36 (70.6)
	No	78 (27.5)	54 (56.8)	15 (29.4)
Use of illicit drugs	Yes	18 (6.9)	22 (23.9)	6 (12.2)
	No	244 (93.1)	70 (76.1)	43 (87.8)
History of STI	Yes	61 (23.0)	44 (51.8)	10 (20.0)
	No	204 (77.0)	41 (48.2)	40 (80.0)
Alcohol consumption	Yes	48 (18.0)	31 (41.3)	30 (60.0)
	No	219 (82.0)	44 (58.7)	20 (40.0)
Condom use	Frequent	66 (25.6)	57 (64.0)	18 (39.1)
	Infrequent	192 (74.4)	32 (36.0)	28 (60.9)
Hemodialysis per week	3 times	236 (89.4)	-	-
	4 times or more	28 (10.6)	-	-
Hemodialysis time (mo)		76.1 (80.1)	-	-
Coagulopathy	Hemophilia	-	-	47 (92.2)
	von Willebrand 3	-	-	4 (7.8)
Type of hemophilia	Deficiency factor VIII	-	-	39 (84.8)
	Factor IV deficiency	-	-	7 (15.2)

Severity	Mild/moderate	-	-	13 (28.3)
	Serious	-	-	33 (71.7)
Inhibitory antibodies	Present	-	-	5 (11.4)
	Absent	-	-	39 (88.6)

I_CKD: Chronic kidney disease individuals; HBV: Hepatitis B virus; I_COAG: Coagulopathy individuals; P_HIV: People living with human immunodeficiency virus; STI: Sexually transmitted infection.

Most CKD patients were male (62.7%), over 30-years-old (93.0%), black (72.4%) and had up to 8 years of education (51.1%). The most risk behaviors were: Shared nail cutters/razors/toothbrushes (66.9%), previous transfusion of plasma or blood (63.3%), inconsistent use of condoms (74.4%) and the use of hemodialysis up to 3 times a week (89.5%).

HBV and HCV serological markers in DBS and serum were evaluated according to demographic and clinical data. Only statistically significant data are presented in Table 2. Infrequent use of condoms was associated with HBsAg positivity in serum and DBS (OR = 5.6 for serum and 4.4 for DBS). Sharing nail cutters/razors/toothbrushes was associated with anti-HBc positivity in serum and DBS (OR = 2.7 for serum and 2.6 for DBS). On the other hand, acupuncture and hemodialysis exposure was associated with anti-HBc detection in serum and a history of transplantation in DBS. Anti-HCV positivity was associated with a history of transplantation (OR = 2.8 for serum and DBS) and hemodialysis exposure (OR = 1.01 for both specimens).

People living with HIV

Among people living with HIV (n = 95), the mean age was 44.1 ± 11.4 years. Most individuals were male (61.1%), unmarried (64.8%), over 30-years-old (90.4%) and sharing nail cutters/razors/toothbrushes (74.5%) (Table 1). The prevalence of $HBsAg^+$ in serum/DBS was 21.0%/22.1%, of anti-HBc^+ was 40.0%/45.6% and anti-of HCV^+ was 25.5%/25.5%.

Table 3 shows the factors associated with the detection of HBV and HCV serological markers using DBS and serum in this group. Male gender (OR = 4.9) and blood transfusion (OR = 4.6) were associated with HBsAg reactivity in serum, while male gender was associated with anti-HBc positivity in serum (OR = 3.2) and DBS (OR = 2.9). No variable was associated with anti-HCV in this group.

Individuals with coagulopathy

Among coagulopathy patients (n = 51), the mean age was 31.3 ± 9.4 years and the main characteristics were: Male gender (98.0%), unmarried (66.7%), black (70.8%), had undergone blood or plasma transfusion (70.0%) and had severe hemophilia (71.7%) (Table 1).

The prevalence for each seromarker in serum was 3.9% for HBsAg, 31.4% for anti-HBc and 47.1% for anti-HCV. The prevalence for each seromarker from DBS was 3.9% for HBsAg, 25.5% for anti-HBc and 41.2% for anti-HCV. It was not possible to make a statistical analysis of this group due to the small size of the sample population.

Performance of ECLIA for HBV and HCV detection using DBS samples in high-risk groups

Among coagulopathy patients, HBsAg assay demonstrated the best performance (100% sensitivity and specificity) followed by anti-HBc (81.3% sensitivity and 100% specificity) and anti-HCV (83.3% sensitivity and 96.3% specificity). Among CKD patients, the best performance was observed for HBsAg (100% sensitivity and 99.6% specificity) followed by anti-HCV (93.5% sensitivity and 99.2% specificity) and anti-HBc (79.6% sensitivity and 97.1% specificity). Among people living with HIV, the best performance was observed for anti-HCV (100% sensitivity and specificity) followed by anti-HBc (97.2% sensitivity and 88.9% specificity) and HBsAg (85.0% sensitivity and 94.7% specificity).

Accuracy varied from 90.2% to 100% and incorrect classification was below 10% in all markers. Estimated prevalence varied between serum and DBS in coagulopathy patients, and CKD individuals showed low values of prevalence using DBS for anti-HBc and anti-HCV. In people living with HIV, estimated prevalence for HBsAg and anti-HBc were higher using DBS (Table 4).

Table 2 Bivariate analysis of sociodemographic and clinical characteristics according to hepatitis B virus and hepatitis C virus markers in chronic kidney disease individuals

Variable	Adjustment	HBsAg		Anti-HBc		Anti-HCV	
		DBS	Serum	DBS	Serum	DBS	Serum
Acupuncture	OR crude (95%CI)	-	-	-	4.0 (1.5-10.6)	-	-
	OR adjusted (95%CI)	-	-	-	5.1 (1.8-14.5)	-	-
Shared nail cutters/razor /toothbrush	OR crude (95%CI)	-	-	2.6 (1.4-4.6)	1.9 (1.1-3.2)	-	-
	OR adjusted (95%CI)	-	-	2.7 (1.5-4.8)	2.6 (1.5-4.7)	-	-
History of transplant	OR crude (95%CI)	-	-	2.9 (1.3-6.4)	-	5.8 (2.5-13.6)	5.8 (2.5-13.6)
	OR adjusted (95%CI)	-	-	2.7 (1.2-6.1)	-	2.8 (1.1-7.4)	2.8 (1.1-7.7)
Infrequent condom use	OR crude (95%CI)	5.6 (1.6-16.4)	4.4 (1.4-14.5)	-	-	-	-
	OR adjusted (95%CI)	5.6 (1.6-16.4)	4.4 (1.4-14.5)	-	-	-	-
Hemodialysis time (mo)	OR crude (95%CI)	-	-	-	1.01 (1.01-1.01)	1.01 (1.01-1.02)	1.01 (1.01-1.01)
	OR adjusted (95%CI)	-	-	-	1.01 (1.01-1.01)	1.01 (1.01-1.02)	1.01 (1.01-1.02)
Hemodialysis 4 times per week or more	OR crude (95%CI)	-	-	-	-	-	2.8 (1.1-6.9)
	OR adjusted (95%CI)	-	-	-	-	-	2.7 (1.1-7.4)

CI: Confidence interval; DBS: Dried blood spot; HBc: Hepatitis B core; HCV: Hepatitis C virus; OR: Odds ratio.

Table 3 Bivariate analysis of sociodemographic and clinical characteristics according to hepatitis B virus and hepatitis C virus markers in people living with human immunodeficiency virus

Variable	Adjustment	HBsAg		Anti-HBc		Anti-HCV	
		DBS	Serum	DBS	Serum	DBS	Serum
Male gender	OR crude (95%CI)	-	4.7 (1.3-17.4)	3.2 (1.3-7.8)	2.9 (1.1-7.3)	-	-
	OR adjusted (95%CI)	-	4.9 (1.2-19.2)	3.2 (1.3-7.8)	2.9 (1.1-7.3)	-	-
Blood or plasma transfusion	OR crude (95%CI)	-	4.2 (1.3-13.5)	-	-	-	-
	OR adjusted (95%CI)	-	4.6 (1.3-16.0)	-	-	-	-

CI: Confidence interval; DBS: Dried blood spot; HBc: Hepatitis B core; HCV: Hepatitis C virus; OR: Odds ratio.

DISCUSSION

To date, there are several studies reporting the importance of diagnosing hepatitis B and C in DBS samples[16-18]. However, the majority have focused only on HBsAg[13,16] and anti-HCV[11] along with manual assays. In the present study, an automated assay was evaluated for the detection of HBsAg, anti-HBc and anti-HCV in DBS samples from key populations demonstrating high sensitivities and specificities comparable to those observed in the general population[15]. These findings reinforce the importance of using DBS samples to reach these key populations in the diagnosis of viral hepatitis, which can be further facilitated using ECLIA.

Among CKD patients, HBsAg positivity in DBS or serum was associated with infrequent condom use, which was also found among young men enlisted in the Brazilian Army, demonstrating the importance of health campaigns with a focus on condom use[19]. Anti-HBc positivity in serum and DBS was associated with shared nail cutters/razor/toothbrush and highlights the discussion of the role of manicurists in the transmission of HBV. Villar *et al*[20] found a prevalence of 5.9% of anti-HBc in beauty professionals in southeast Brazil.

Anti-HCV positivity in serum and DBS was associated with a previous history of transplantation in CKD patients. A study that assessed the risk of transplant recipient infections showed that this will depend on the prevalence and incidence of HCV in a given population of donors and other risk exposures such as injecting drug use, men

Table 4 Test parameter values according to individuals with coagulopathies, chronic kidney disease and people living with human immunodeficiency virus

Diagnostic test parameters	I_COAG, *n* = 51			I_CKD, *n* = 284			P_HIV, *n* = 95		
	HBsAg	Anti-HBc	Anti-HCV	HBsAg	Anti-HBc	Anti-HCV	HBsAg	Anti-HBc	Anti-HCV
True positive (*n*)	2	13	20	13	90	43	17	35	24
True negative (*n*)	49	35	26	270	165	235	71	48	70
False positive (*n*)	0	0	1	1	5	2	4	6	0
False negative (*n*)	0	3	4	0	23	3	3	1	0
Sensitivity (%)	100	81.3	83.3	100	79.6	93.5	85.0	97.2	100
Specificity (%)	100	100	96.3	99.6	97.1	99.2	94.7	88.9	100
PPV (%)	100	100	95.2	92.9	94.7	95.6	81.0	85.4	100
NPV (%)	100	92.1	86.7	100	87.8	98.7	95.9	98.0	100
Correct classification (accuracy) (%)	100	94.1	90.2	99.6	90.1	98.2	92.6	92.2	100
Incorrect classification (%)	0	5.9	9.8	0.4	9.9	1.8	7.4	7.8	0
Estimated prevalence/serum (%)	3.9	31.4	47.1	4.6	39.9	16.3	21.1	40.0	25.5
Estimated prevalence/DBS (%)	3.9	25.5	41.2	4.9	33.6	15.9	22.1	45.6	25.5

DBS: Dried blood spot; HBc: Hepatitis B core; HCV: Hepatitis C virus; I_CKD: Chronic kidney disease individuals; I_COAG: Coagulopathy individuals; NPV: Negative predictive value; P_HIV: People living with human immunodeficiency virus; PPV: Positive predictive value.

who have sex with men, piercings and tattoos. These, among other risk factors, are already associated with transmission for the general population[21].

Among people living with HIV, HBsAg positivity was associated with male gender and blood and plasma transfusion using serum results, and anti-HBc positivity was associated with male gender using the results of both fluids. In Brazil, most HBV infected individuals were male (54.5%)[22], probably due to higher exposure to risk factors, such as promiscuity and drug use[23,24]. Although blood is screened for HBsAg and anti-HBc in blood banks in Brazil, molecular assays were only included in 2015. While rare, occult hepatitis B infection, mutations that escape vaccination and infected individuals occupying a certain immunological window could be potential donors of contaminated blood samples allowing HBV transmission[25,26].

DBS testing for HBsAg, anti-HBc and anti-HCV using ECLIA demonstrated high sensitivity and specificity in all groups. HBsAg testing demonstrated the best performance in coagulopathy individuals and CKD patients. Anti-HCV testing demonstrated higher efficiency in CKD individuals and people living with HIV and anti-HBc detection was more accurate in people living with HIV. The differences observed could be the result of different prevalences and risk behavior, such as multiple exposure to blood products that could interfere in the efficiency of the assay.

HBsAg and anti-HCV prevalence estimated by serum and DBS were similar in demonstrating that ECLIA along with DBS could be a potential tool for diagnosis of infected individuals in key populations. In contrast, anti-HBc prevalence varied by more than 5% between serum and DBS in all groups evaluated. In the present study, anti-HBc sensitivity varies from 79.6% to 97.2%, which is similar to findings in other studies that reported sensitivities from 76.9% to 97.6% using ECLIA or enzyme-linked immunosorbent assay for anti-HBc in the general population and people living with HIV[12,13,16]. Although there are differences found in anti-HBc prevalence in serum and DBS, there is an overlapping CI value for those specimens showing that DBS could be used for prevalence studies in key populations.

CONCLUSION

This study demonstrated the utility of HBsAg, anti-HBc and anti-HCV detection in DBS using ECLIA in high-risk populations. The use of DBS samples is much less

invasive, easier than venipuncture and could increase the access of diagnosis in people with limited social access as well as in people where it is difficult to draw blood. Automated assays such as ECLIA using DBS increases diagnostic speed, generating the diagnosis of many samples at once, which can be important during potential outbreaks in hemotherapy clinics for example. However, the anti-HBc marker should be used with due care, especially in the population of coagulopathy individuals and CKD patients, which due to multiple exposures may not show agreement with gold standard samples and therefore requires further study.

ARTICLE HIGHLIGHTS

Research background

Diagnosis of hepatitis B virus and hepatitis C virus (HCV) can be difficult in chronic kidney disease (CKD) individuals undergoing hemodialysis, coagulopathy individuals and people living with human immunodeficiency virus (HIV) due to the difficulty of blood sample collection by venipuncture, remote location and lack of health care.

Research motivation

There is no information regarding the performance of electrochemiluminescence (ECLIA) for the detection of hepatitis B virus and HCV markers in dried blood spot (DBS) samples in key populations, such as individuals with coagulopathies, CKD patients and people living with HIV.

Research objectives

To investigate the putative influence of HIV infection as well as pathophysiological alterations in individuals with coagulopathies (hemophilia and von Willebrand disease) or CKD in the performance of optimized ECLIA for the detection of HBsAg, anti-HBc and anti-HCV markers in DBS samples.

Research methods

The ECLIA technique was used for the evaluation of HBsAg, anti-HBc, and anti-HCV tests in DBS samples of CKD individuals undergoing hemodialysis, coagulopathy individuals and people living with HIV.

Research results

HBsAg detection presented sensitivities of 100% among coagulopathy and CKD patients and low sensitivity (85.0%) in people living with HIV. Anti-HBc detection had the best performance in people living with HIV followed by coagulopathy and CKD patients. Anti-HCV detection showed sensitivities above 83.0% in all groups. Specificities of these assays varied from 88.9% to 100%. Estimated prevalence was similar among serum and DBS except for the anti-HBc marker.

Research conclusions

This study demonstrated the utility of HBsAg, anti-HBc and anti-HCV detection in DBS using ECLIA in high-risk populations.

Research perspectives

Automated assays such as ECLIA using DBS increases diagnostic speed, generating the diagnosis of many samples at once, which can be important during potential outbreaks in hemotherapy clinics.

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CASE REPORT

Asymptomatic portal vein aneurysm: Three case reports

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Author contributions: De Sio I, Vitale LM and Niosi M performed the ultrasonography and interpreted the imaging findings; Priadko K reviewed the literature and drafted the manuscript; De Sio I contributed to manuscript drafting; Romano M was responsible for the revision and final approval of the manuscript; all authors issued final approval for the version to be submitted.

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Abstract

BACKGROUND

Portal vein aneurysm (PVA) is an uncommon vascular dilatation, showing no clear trend in sex or age predominance. Due to the low number of published cases and the lack of management guidelines, treatment of this condition remains a clinical challenge.

CASE SUMMARY

We present three cases of asymptomatic PVA; the first and second involve an extrahepatic manifestation, of 48 mm and 42.3 mm diameter respectively, and the third involves an intrahepatic PVA of 27 mm. All were diagnosed incidentally during routine check-up, upon ultrasonography scan. Since all patients were asymptomatic, a conservative treatment strategy was chosen. Follow-up imaging demonstrated no progression in the aneurysm dimension for any case.

CONCLUSION

As PVA remains asymptomatic in many cases, recognition of its imaging features is key to favourable outcomes.

Key Words: Extrahepatic portal vein aneurysm; Intrahepatic portal vein aneurysm; Asymptomatic; Ultrasonography imaging; Colour Doppler; Case report

Core Tip: Portal vein aneurysm (PVA) can be a congenital or acquired vascular malformation but in most cases is asymptomatic; as such, it remains underdiagnosed. We report on the features of PVA detected by ultrasonography, computed tomography and magnetic resonance imaging in three asymptomatic patients. Only one of our patients had a known predisposing factor (*i.e.*, liver cirrhosis). Throughout the

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surveillance period, our patients remained asymptomatic, with no dimensional changes in their PVAs. In reporting this case study, we highlight the need for PVA recognition and instituting a personalized management approach that takes into consideration factors predisposing to complications of this condition.

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INTRODUCTION

Portal vein aneurysm (PVA) is a vascular malformation that is rarely diagnosed but, if complications occur, can be life-threatening. The estimated number of reported cases is low, at approximately 200[1]. PVA is defined as a portal vein diameter exceeding 19 mm in cirrhotic patients and 15 mm in normal liver[1] and can be either congenital (due to vascular anomalies) or acquired (mostly due to cirrhosis and/or portal hypertension, that are present in approximately 28.0%-30.8% of cases)[2,3]. Several systematic reviews did not identify any sex-related predisposition[1,2]. Notably, among portal venous system aneurysms, those in the main extrahepatic portal vein appear to be the commonest[2]. The average mortality rate is 10% and this mostly involves patients who have undergone liver transplantation[2,4]. Incidental discovery of asymptomatic aneurysms normally occurs through abdominal imaging, such as with computed tomography (CT), magnetic resonance imaging (MRI), or ultrasonography.

The clinical management of PVA ranges from conservative follow-up, that lasts years, to surgical intervention, depending on the presence or absence of symptoms and complications, such as rupture, thrombosis and compression of adjacent organs[1,2].

In this article, we present clinical cases of three asymptomatic patients in whom PVA was an incidental finding. The clinical cases are accompanied by ultrasonography, CT and MRI images of asymptomatic extrahepatic PVAs and ultrasonographic images of intrahepatic PVA. We also provide a review of the relevant literature to advance the knowledge on this underdiagnosed condition.

CASE PRESENTATION

Chief complaints

Three patients, 81-year-old male, 52-year-old female and 73-year-old male respectively, presented to the outpatient clinic of our Unit of Diagnostic and Interventional Ultrasonography (Medical Center of the University Vanvitelli in Naples, Italy) for routine check-up for various pre-existing health issues.

History of past illness

Case 1: The 81-year-old patient's medical history included hepatitis B virus-associated well-compensated (*i.e.*, Child-Pugh classification stage A5) liver cirrhosis with portal hypertension and F1 oesophageal varices.

Case 2: Patient 2 had a previous history of dysmotility-like dyspepsia, for which the routine abdominal ultrasonography had been requested.

Case 3: Patient 3 was recovering from sepsis caused by infection of an aortal prosthesis and had no history of past illnesses relevant to the subsequent PVA finding.

Physical examination

Physical examination did not reveal any relevant signs in any of the patients.

Laboratory examinations

Blood testing of Case 2 affected by liver cirrhosis showed leucopenia, thrombocytopenia, and increased level of gamma globulins (2.3 g/dL; normal range: 0.7-1.6

g/dL) while blood testing of the other two patients yielded no abnormal findings.

Imaging examinations

Case 1: Ultrasound examination of patient 1 showed an extrahepatic aneurysmal dilatation of the portal vein (Figure 1A), with a maximal diameter of 48 mm. Colour Doppler examination showed the lesion to have the typical "Korean flag" appearance (Figure 1B), and a Doppler recording revealed flat venous flow (Figure 1C).

Case 2: Ultrasonographic examination of patient 2 detected extrahepatic aneurysmal dilatation of the portal vein (Figure 2A), with a maximal diameter of 42.3 mm (Figure 2B). Colour Doppler control examination showed a hepatopetal venous flow (Figure 2C) and a pulsating flow of venous type (Figure 2D). Considering the young age of the patient, second-level imaging techniques were performed. Abdominal CT (Figure 3) as well as contrast-enhanced MRI (Figure 4) confirmed the diagnosis of extrahepatic aneurysmal dilatation of portal vein.

Case 3: Abdominal ultrasonography of patient 3 showed an aneurysmal dilatation of the right branch of the portal vein (Figure 5A), with a maximal diameter of 27 mm (Figure 5B) and a typical "Korean flag" appearance (Figure 5C). No further diagnostic procedures were considered necessary.

FINAL DIAGNOSIS

Cases 1 and 2 were diagnosed with an acquired asymptomatic extrahepatic PVA while Case 3 was diagnosed with an acquired asymptomatic intrahepatic PVA.

TREATMENT

Due to lack of symptoms, ultrasonography surveillance every 6 mo was recommended. No specific treatment was prescribed.

OUTCOME AND FOLLOW-UP

For the 3-, 5- and 1-year of follow-up respectively, all the patients remained asymptomatic and no changes had been detected in the aneurysm measures.

DISCUSSION

PVA is a saccular or fusiform portal vein dilatation that was first described in 1956[5]. The commonest classifications divide PVAs into congenital or acquired, symptomatic or asymptomatic, and complicated or uncomplicated[2,6]. To date, the PVA reports in the literature are relatively scarce. A systematic review of 96 reports by Laurenzi *et al*[1] showed that the median age at diagnosis among 190 subjects was 52-year-old (0-89) with portal hypertension and liver cirrhosis discovered in 62 (32%) and 50 (26%) patients respectively with males and females being equally affected. Interestingly, the more recent studies describing PVA cases have shown weaker associations of the condition with chronic liver diseases or portal hypertension[7,8]. This is probably due to implementation and advancement of imaging techniques and of specific knowledge of specialists in the field. While chronic liver diseases remain the commonest acquired causal factors of PVA, other acquired cases are considered to originate from malignant invasion of the vein, inflammatory process due to pancreatitis, or trauma[6]. Most commonly, symptoms occur in patients with large extrahepatic aneurysmal dilatations while small aneurysms often remain asymptomatic[8,9]. Once thrombosed, PVA causes symptoms such as abdominal pain in 91%, fever in 53% and ascites in 38% of patients[10]. Authors noted that in symptomatic patients with or without portal hypertension, symptoms do not differ, except for gastrointestinal bleeding in patients suffering from elevated pressure in portal vein[1]. Unfortunately, no clear evidence exists helping to prospectively distinguish between aneurysms which will have a stable course *vs* those that are potentially complicated but it seems that unfavourable precursors of symptomatic and/or complicated disease are large size (> 3 cm), liver

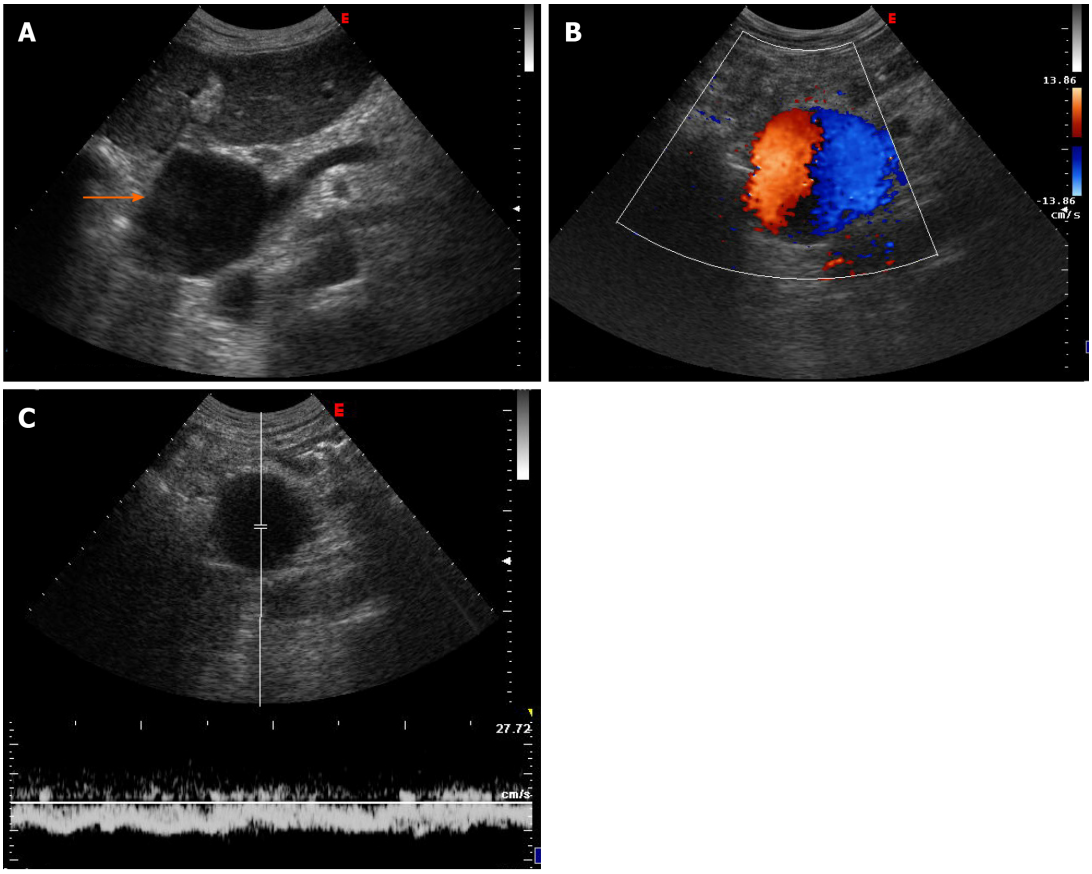

Figure 1 Abdominal ultrasonographic imaging of Case 1. A: Anechoic lesion corresponding to a notably dilated extrahepatic portal vein (arrow); B: Colour Doppler showed the "Korean flag" pathological sign in the dilated portal vein; C: Doppler recording showed flat venous flow.

and/or pancreatic diseases and thrombophilic risks. Koc *et al*[11] reported an incidence of thrombophilia in 4 out of 7 patients with thrombosed PVA, hence, pointing on an importance of thrombophilic screening in all the subjects with diagnosed PVA, even if asymptomatic at the beginning. Even though 18 cases of non-thrombosed PVAs exceeding 5 cm in their largest diameter were reported in the literature, with no anticoagulation taken before their diagnosis, many authors support a thrombophilic risk assessment[12]. While all of our patients were asymptomatic, only one (*i.e.*, the 81-year-old male) had predisposing factors to the formation of the portal aneurysm, namely hepatitis B virus-associated liver cirrhosis complicated with portal hypertension and oesophageal varices. A thorough examination of the other two patients did not reveal any predisposing risk factors. None of our patients had complications at the time of the first visit nor during the follow-up period. This is at odds with previous reports showing that abdominal pain occurs in approximately 50% of patients and upper gastrointestinal bleeding in less than 10%[2].

In general, complications of PVA vary depending on the location of the aneurysm and predisposing factors and include aneurysm thrombosis, rupture of the aneurysm, and compression of adjacent anatomical structures, such as the common bile duct, duodenum, or inferior vena cava[1,2]. Hence, complication risk assessment is a crucial management step that could help to avoid life-threatening outcomes of this condition. For patients with no risk factors for complications, a conservative strategy and follow-up surveillance using abdominal ultrasonography can be recommended, for up to 6 years or until resolution of the aneurysm[1,2,13]. While, in some studies, CT scan every 12 mo was the preferred surveillance strategy[14], the majority of published studies agree with ultrasonography being the preferred imaging technique for surveillance and monitoring of PVA growth, as it is relatively inexpensive and does not involve radiation exposure[15,16]. Ma *et al*[17] suggested a surveillance colour Doppler scanning as the method of choice for diagnosis and surveillance of aneurysms that are asymptomatic and do not increase in size over time while CT scan to be reserved for symptomatic lesions or indeterminate abdominal ultrasound scanning results. Moreover, ultrasonography is capable of differentiating a PVA from a hypervascular pancreatic mass[16], while contrast-enhanced CT and MRI are helpful in cases of

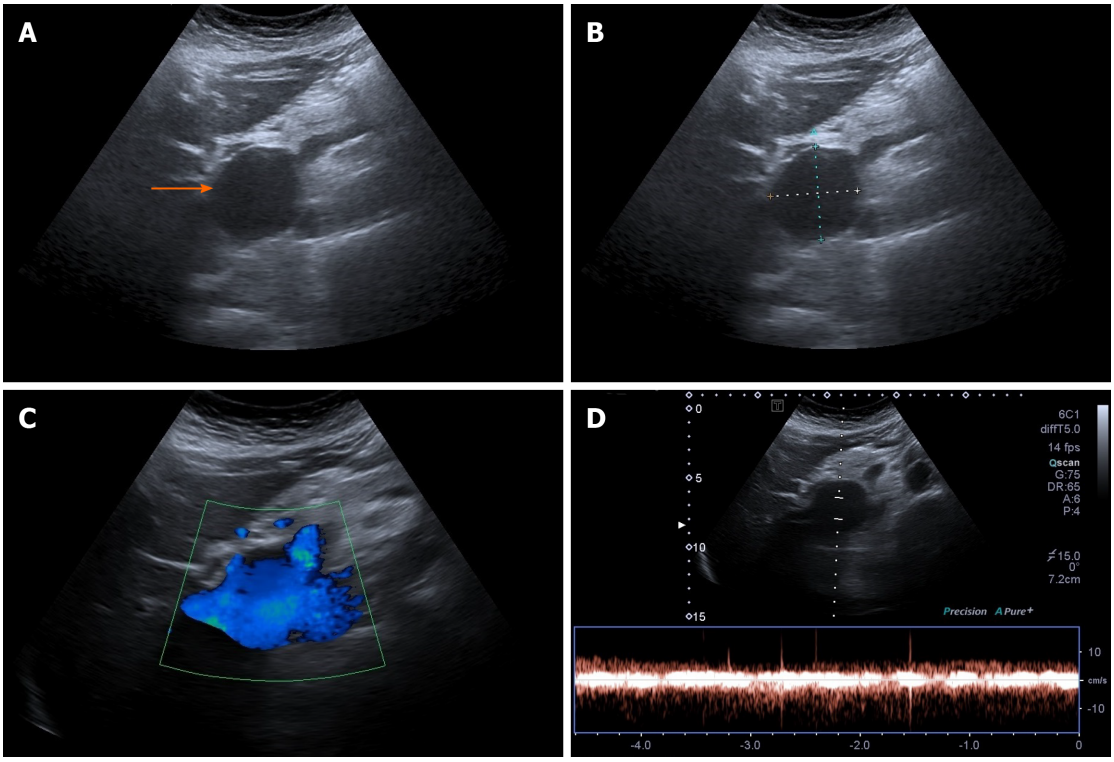

Figure 2 Abdominal ultrasonographic imaging of Case 2. A: Extrahepatic anechoic saccular lesion, indicating an aneurysmal dilation of the extrahepatic portal vein (arrow); B: The anechoic lesion was 42.3 mm at its maximal diameter; C: Colour Doppler showed hepatopetal venous flow in the extrahepatic aneurysmal dilated vessel; D: Doppler recording showed pulsating flow of venous type.

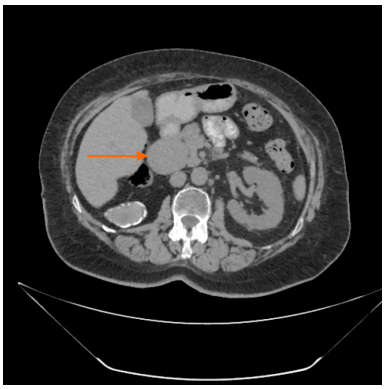

Figure 3 Abdominal computed tomographic scanning of Case 2. The axial image showed saccular extrahepatic aneurysmal dilatation of the portal vein (arrow).

diagnostic uncertainty between thrombosis and slow venous flow[18]. Second-level imaging techniques might also be helpful in differentiating compression of the surrounding viscera or rupture[15,18]. CT or MRI are, however, essential when planning surgical intervention[15,17].

After evaluation of our patients' health status, the conservative management strategy was chosen for each. In the long-term follow-up, none presented any change in aneurysmal dimension. Thus, it was decided to continue regular ultrasonographic examination. This decision was also supposed by studies that have shown partial or total regression of large PVAs over longer periods[13,14].

While the management strategy of large asymptomatic PVAs is still under debate, indications for active management are abdominal pain and occurrence of complications[1,2]. Surgical management depends on the aneurysm location and the presence of thrombi and portal hypertension. Aneurysmorrhaphy and aneurysmectomy are recommended in the absence of portal hypertension, while shunt procedures or liver transplantation are performed in case of portal hypertension[1,4,16]. Thrombolysis or thrombectomy are indicated in case of PVA thrombotic obstruction[2],

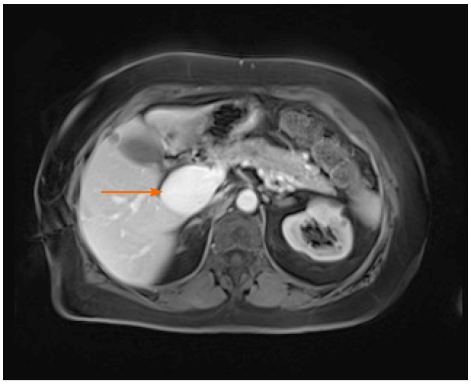

Figure 4 Contrast-enhanced magnetic resonance imaging of the abdomen of Case 2. The axial image showed saccular extrahepatic aneurysmal dilatation of the portal vein (arrow).

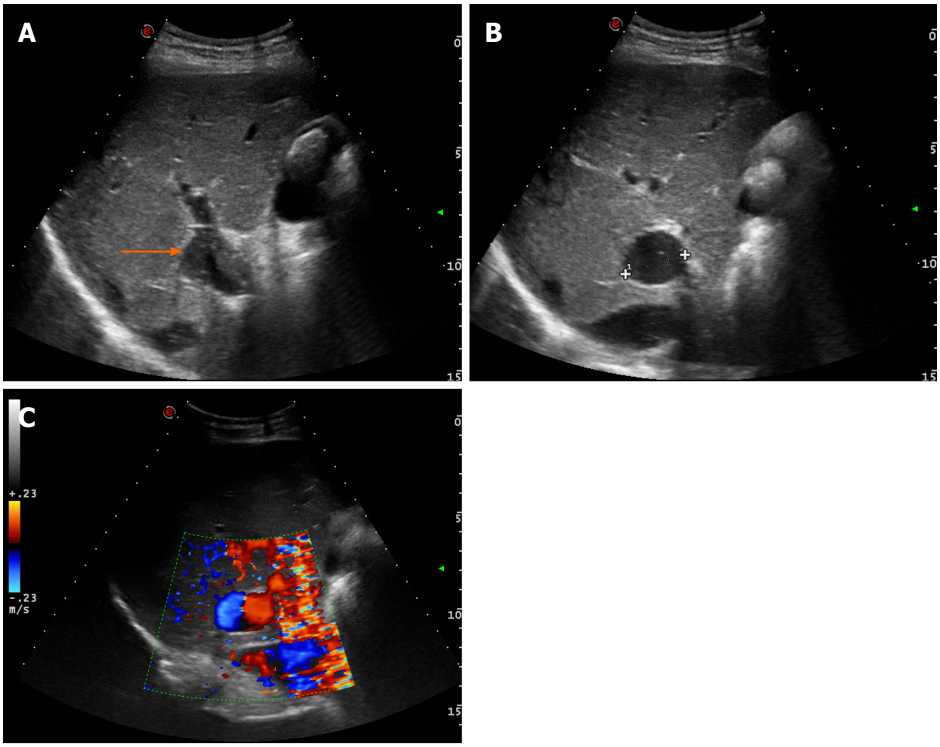

Figure 5 Abdominal ultrasonographic imaging of Case 3. A: Anechoic lesion of the right branch of the portal vein (arrow); B: The intrahepatic anechoic lesion was 27 mm at its maximal diameter; C: Colour Doppler showed the "Korean flag" pathological sign.

even though a case of conservative treatment was reported for a patient with PVA measuring 88 mm × 65 mm and complete thrombosis extending to the superior mesenteric and splenic veins[19].

CONCLUSION

Our cases, together with the review of the literature, support the concept that the management approach to PVA should be individualized, taking into account aneurysm size, complication risks, medical history, and presence of symptoms. Furthermore, our study highlights the need for gastroenterologists and radiologists to be familiar with PVA imaging features and those that facilitate differential diagnosis between PVA and other lesions, such as hypervascular abdominal masses[16].

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