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Jin-Lei Wang, Director

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World Journal of Hepatology
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-59080039
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Mechanisms of adaptation of the hepatic vasculature to the deteriorating conditions of blood circulation in liver cirrhosis

Dmitry Victorovich Garbuzenko, Nikolay Olegovich Arefyev, Dmitry Vladimirovich Belov

Dmitry Victorovich Garbuzenko, Nikolay Olegovich Arefyev, Department of Faculty Surgery, South Ural State Medical University, 454092 Chelyabinsk, Russia

Dmitry Vladimirovich Belov, Department of Hospital Surgery, South Ural State Medical University, 454092 Chelyabinsk, Russia

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Correspondence to: Dmitry Victorovich Garbuzenko, MD, PhD, Professor, Department of Faculty Surgery, South Ural State Medical University, Box 12317, 454092 Chelyabinsk, Russia. garb@inbox.ru
Telephone: +8-909-7459826
Fax: +8-351-2687772

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Abstract

PubMed, EMBASE, Orphanet, MIDLINE, Google Scholar and Cochrane Library were searched for articles published between 1983 and 2015. Relevant articles were selected by using the following terms: "Liver cirrhosis", "Endothelial dysfunction", "Sinusoidal remodeling", "Intrahepatic angiogenesis" and "Pathogenesis of portal hypertension". Then the reference lists of identified articles were searched for other relevant publications as well. Besides gross hepatic structural disorders related to diffuse fibrosis and formation of regenerative nodules, the complex morphofunctional rearrangement of the hepatic microvascular bed and intrahepatic angiogenesis also play important roles in hemodynamic disturbances in liver cirrhosis. It is characterized by endothelial dysfunction and impaired paracrine interaction between activated stellate hepatocytes and sinusoidal endotheliocytes, sinusoidal remodeling and capillarization, as well as development of the collateral microcirculation. In spite of the fact that complex morphofunctional rearrangement of the hepatic microvascular bed and intrahepatic angiogenesis in liver cirrhosis are the compensatory-adaptive reaction to the deteriorating conditions of blood circulation, they contribute to progression of disease and development of serious complications, in particular, related to portal hypertension.

Key words: Liver cirrhosis; Endothelial dysfunction; Sinusoidal remodeling; Intrahepatic angiogenesis; Pathogenesis of portal hypertension

Core tip: Besides gross hepatic structural disorders related to diffuse fibrosis and formation of regenerative nodules, the complex morphofunctional rearrangement

of the hepatic microvascular bed and intrahepatic angiogenesis play important roles in hemodynamic disturbances in liver cirrhosis. In spite of the fact that these changes of the hepatic vasculature are the compensatory-adaptive reaction to the deteriorating conditions of blood circulation, they contribute to the progression of disease and development of serious complications, in particular, related to portal hypertension.

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MORPHOFUNCTIONAL REARRANGEMENT OF THE HEPATIC MICROVASCULAR BED IN LIVER CIRRHOSIS

Besides gross hepatic structural disorders related to diffuse fibrosis and formation of regenerative nodules, the complex morphofunctional rearrangement of the hepatic microvascular bed in liver cirrhosis also contributes to the development of severe complications, in particular, associated with portal hypertension[1]. In this situation, the main place of resistance to portal blood flow is in pathologically modified sinusoids. Sinusoidal endothelial cells (SEC) become dysfunctional and among other features acquire a vasoconstrictor phenotype. It leads to increasing of SEC sensitivity to endogenous vasoconstrictors, such as endothelin, norepinephrine, angiotensin Ⅱ, vasopressin, leukotrienes, thromboxane A2. In contrast, the production of nitric oxide (the most studied vasodilator involved in the regulation of hepatic vascular tone) is reduced. The reason for this may be insufficient activity of endothelial nitric oxide synthase (eNOS) due to its increased interaction with caveolin-1. Furthermore, endotelin-1 activates G-protein-coupled receptor kinase-2 which directly interacts with and inhibits protein kinase B (Akt) phosphorylation and decreases the production of nitric oxide (NO)[2].

One of the main factors of sinusoidal endothelial dysfunction in cirrhosis is intrahepatic oxidative stress, which is associated with a decrease of eNOS expression and NO bioavailability. For example, cyclooxygenase attenuates Akt-eNOS signalization by stimulating thromboxane A2, which inhibits Akt phosphorylation in endothelial cells, as well as excessive activation of Rho-kinase. Asymmetric dimethylarginine, an endogenous inhibitor of NOS, causes uncoupling of NOS leading to generation of reactive nitrogen species such as peroxynitrite, and down-regulated tetrahydrobiopterin expression promotes that the eNOS cannot generate NO but instead produces O_2^-, thereby leading to further decreases in NO production. In addition, it was reported that a possible reason for the insufficient bioavailability of nitric oxide might be a reduction of superoxide dismutase ("an enzyme that saves NO") and increase of homocysteine level in the serum due to reduced expression of cystathionine-γ-lyase and cystathionine-β-syntase[3].

Activated hepatic stellate cells (HSCs) and its paracrine interaction with SEC play very important roles in the sinusoidal microcirculation in liver cirrhosis. In pathological conditions violation of the structure and function of HSCs accompanied by a loss of retinoids reserve and HSCs transformation into myofibroblasts. Activated HSCs start to perform the functions of pericytes. This is confirmed by the expression of its phenotypic markers such as α-smooth muscle actin, desmin, NG2, glial fibrillary acidic protein, as well as emergence or increase of receptors for growth factors, cytokines and endothelin, and a number of cell adhesion molecules on its surface[4].

HSCs, located in the subendothelial Disse spaces between the SEC and hepatocytes, are contacted because of the long branching cytoplasmic processes with nerve endings, which contains various neurotransmitters such as substance P, vasoactive intestinal peptide, somatostatin, cholecystokinin, neurotensin, NO, calcitonin gene-related peptide, and neuropeptide Y. Some vasoactive substances are able to regulate the tone of HSCs. Substances for instance endothelin-1, substance P, angiotensin Ⅱ, norepinephrine, prostaglandin F2, thromboxane A2, platelet activating factor (PAF) and thrombin can trigger HSC contractility. In contrast, vasoactive substances such as acetylcholine, vasoactive intestinal peptide, NO, carbon monoxide, hydrogen sulfide, prostaglandin E2, and adrenomedullin are known for the ability to relax HSCs[5].

Myosin Ⅱ is involved in the HSCs contraction, and Ca^{2+}-dependent and Ca^{2+}-independent pathways mediate this process. In a Ca^{2+}-dependent pathway, myosin light chains are phosphorylated by activated myosin light chain kinase, whose activation is induced in response to an increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and subsequent formation of the Ca^{2+}/calmodulin complex. In a Ca^{2+}-independent pathway, Rho kinase and protein kinase C inhibit the activity of myosin light chain phosphatase, an enzyme that dephosphorylates phosphorylated myosin light chains and induces relaxation[6].

Endothelin (ET) as a powerful endogenous vasoconstrictor modulates the tone of the HSCs. ET has three kinds of isoform, ET-1, 2, and 3, which are synthesized from ET by endothelin-converting enzymes. They interact with conjugated protein G receptors A and B types, which are well expressed in the HSCs. ET-1 is the most studied. The main site of its synthesis in liver

cirrhosis is activated HSCs. Stimulation of endothelin A receptors leads to its proliferation[7]. Angiotensin Ⅱ has a similar effect. In liver cirrhosis, HSCs increase its synthesis because of increased expression of angiotensin-converting enzyme. HSCs constriction may also be caused by decreased NO production and/or bioavailability in cirrhotic liver[8]. In contrast, carbon monoxide overproduction by Kupffer cells causes a dilation of the sinusoids and a decrease of hepatic vascular resistance (HVR) because of paracrine impact on HSCs and SEC[9].

Increased HSCs mobility and migration in liver cirrhosis are required to promote enhanced coverage of HSCs around an EC-lined sinusoid, contributing to the process of sinusoidal remodeling[10]. Changes in the structure of the HSCs membrane plays an important role in this process. Cellular locomotion requires dynamic but regulated actin remodeling to form membrane structures that facilitate cell extension. These include lamellipodia, which are membrane protrusions that form the leading edge toward directed cell migration, and filopodia, which are thin, actin filament-structured spikes emanating from the plasma membrane. Small guanosine triphosphatases from the Rho family including RhoA (Rho), Rac1 (Rac), and Cdc42 in turn, closely regulate formation of actin-based structures. Proved that if Rac contributes to HSC migration due to formation of filopodia, the Rho causes a resistant to the inhibitory action of NO and restores the chemotactic response to platelet-derived growth factor (PDGF) in the absence of a functional Rac[11].

A key molecule responsible for proliferation, migration, mobility and recruitment of HSCs is PDGF, which is secreted by endothelial cells and binds to its cognate PDGF receptor (PDGFR-β) on pericytes, in particular due to an ephrin-B2/EphB4 signaling pathway[12]. Moreover, activation of the PDGFR-β causes to stimulation of Raf-1 kinase, MEK kinase and extracellular-signal regulated kinase (ERK), which leads to the proliferation of the HSCs. Phosphatidylinositol 3-kinase activation is also necessary for both mitogenesis and chemotaxis induced by PDGF[13]. In addition, it is shown that the axonal guidance molecule neuropilin-1 contributes to the chemotactic response to PDGF too[14].

Activated HSCs are a rich source of polypeptides, eicosanoids and various other molecules with paracrine, juxtacrine, and autocrine signalization or chemoattractant activity, which include: (1) polypeptides which enhance cells proliferation in an autocrine and paracrine manner: Hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF), endothelin-1, insulin-like growth factor, transforming growth factor (TGF)-α, epidermal growth factor (EGF) and acidic fibroblast growth factor (aFGF); (2) members of the TGF-β family; (3) neurotrophins; and (4) hematopoietic growth factors such as erythropoietin[15].

When the liver is damaged, activated HSC proliferate and migrate to areas of inflammation and necrosis of hepatocytes, producing excessive amounts of extracellular matrix components. TGF-β1, PDGF, connective tissue growth factor and FGF regulate this process[16].

Overall there are three general sources of fibrogenic cells in the liver: (1) endogenous (resident) fibroblast or myofibroblast-like cells, mainly represented by HSCs, but also by portal fibroblast, vascular smooth muscle cells and pericytes; (2) the epithelial-mesenchymal transition that may occur in the liver as well as in other organs and lead to transdifferentiation of parenchymal cells; and (3) recruitment of fibrocytes from the bone marrow[17].

INTRAHEPATIC ANGIOGENESIS IN LIVER CIRRHOSIS

In 1983, Rappaport *et al*[18] were among the first who had described the collateral microcirculation in cirrhotic liver. Nowadays, pathological angiogenesis well characterized in experimental liver fibrosis, as well as in patients with chronic viral and autoimmune liver diseases and nonalcoholic steatohepatitis[19].

Angiogenesis is the complicated physiological process through which new blood vessels form from pre-existing vessels. It is accomplished by the activation of endothelial cells, expression in it proteases, destruction of the extracellular matrix, proliferation, migration of the endothelial cells and formation of high permeability primary vascular structures[20].

Molecular insights into the angiogenic process

The primary inducer of angiogenesis in physiological and pathological conditions is hypoxia. Cells respond to hypoxic stress through multiple mechanisms, including the stabilization of hypoxia-inducible factors (HIFs), which directly regulate the expression of angiogenic growth factors. The family of HIFs includes three α-subunits, which are associated with a common β-subunit (HIF-1β). HIF-1α appears to be ubiquitously expressed, whereas HIF-2α is detected in a more restricted set of cell types, including vascular endothelial cells, hepatocytes, type Ⅱ pneumocytes, and macrophages. A third mammalian HIF-α subunit, HIF-3α, has also been described, although its role in hypoxic responses is less well understood[21].

NADPH oxidase is an important mediator of angiogenic signaling pathways. It was noted that the increased NADPH oxidase expression because of NADPH oxidase subunit p 47phox phosphorylation leads to an increase in the reactive oxygen species (ROS) levels, contributing to HIF-1α induction, VEGF-receptors (VEGFR) activation and EGF-receptors transactivation[22].

The important role of miRNA has been shown recently in the regulation of cellular response to hypoxia. In particular, Let-7 and miR-103/107 favor the VEGF induction by targeting argonaute 1 protein[23].

The most studied angiogenic growth factors include

VEGF family consisting of five homologs: VEGF-A, B, C, D and placental growth factor (PlGF). VEGF stimulates both physiological and pathological angiogenesis. All members of this family are connected to different homologous receptors: VEGFR-1 (Flt-1), VEGFR-2 (KDR/Flk-1), VEGFR-3 (Flt-4), of which only the first and second responsible for angiogenic signals transmitting. Besides that, the binding of VEGA-A to VEGFR-2 and increasing vascular permeability through the nitric oxide are the mechanisms triggering angiogenesis and vasculogenesis.

PlGF, a homolog of VEGF binding VEGFR-1, enhances angiogenesis, but only in pathological conditions affecting, directly and indirectly, multiple cell types, including endothelial cells. In addition, it is assumed that PlGF, breaking the binding of VEGF with VEGFR-1, makes the binding of VEGF with VEGFR-2 more probable. Mass spectrometry studies showed that PlGF and VEGF each induce the phosphorylation of distinct tyrosine residues in VEGFR-1, further indicating that PlGF and VEGF transmit distinct angiogenic signals through VEGFR-1.

Different mechanisms are the basis of synergism between PlGF and VEGF. By activating VEGFR-1, PlGF induces an intermolecular cross talk between VEGFR-1 and VEGFR-2, which thereby is more response to VEGF. PlGF, as a subunit of PlGF/VEGF heterodimer, induces the formation of VEGFR-1/2 heterodimers, which transphosphorylate each other in an intramolecular reaction. By producing PlGF, endothelial cells are thus capable of enhancing their own responsiveness to VEGF but adjacent stromal or inflammatory cells may also release PlGF.

PlGF directly affects smooth muscle cells and fibroblasts, which express VEGFR-1, but may also indirectly influence its proliferation and migration through cytokine release from activated endothelial cells. Through these effects, PlGF recruits smooth muscle cells around nascent vessels, thereby stabilizing them into mature, durable and non-leaky vessels.

PlGF also mobilizes VEGFR-1 positive hematopoietic progenitor cells from the bone marrow and recruits, indirectly *via* upregulation of VEGF expression, VEGFR-2-positive endothelial progenitor cells to the ischemic tissue. PlGF is also chemoattractive for monocytes and macrophages, which express VEGFR-1[24].

FGF family members are also able to stimulate angiogenesis. Cellular response to FGFs occurs through specific binding FGF-receptor (FGFR), which has internal tyrosine kinase activity. FGFR dimerization is a prerequisite for phosphorylation and activation of signaling molecules with the participation of heparin-binding proteins. This causes migration, proliferation, cell differentiation and destruction of extracellular matrix. It should be noted that while VEGF family members are involved mainly in the formation of the capillaries, FGFs primarily involved in arteriogenesis[25].

Although the angiogenic effect of PDGF is not so expressed as in VEGF, PIGF and FGF, studies *in vivo* have shown that it may induce the formation of blood vessels and regulate their tone[26].

Tie-2 (Tek), an endothelial-specific receptor tyrosine kinase, and its ligands, the angiopoietins, have been identified as critical mediators of vascular development. Angiopoetin-1 induces endothelial cells migration, inhibits endothelial cells apoptosis and stimulates its formation, promoting stabilization of vessels. At the same time, NADPH oxidase is involved in the ang-1-mediated activation of Akt and mitogen-activated protein kinase (p42/p44 MAPK, or ERK2 and ERK1) and subsequent modulation of endothelial cell migration and angiogenesis[27]. In contrast, angiopoietin-2 causes vascular destabilization by shifting the endothelial cells from the stable state to the proliferative phenotype. However, it may also stimulate angiogenesis in the presence of VEGF[28].

Integrin $\alpha V\beta 3$ and $\alpha V\beta 5$ are adhesion receptors promoting angiogenesis by mediating migration and proliferation of endothelial cells and the formation of new blood vessels[29].

Endothelial-specific adhesion molecule vascular endothelial cadherin contributes to cell-cell junctions during neovascularization and controls the passage of molecules through the endothelial lining[30].

Thrombospondin-1 - one of the five known thrombospondins - is an adhesive protein that regulates the interaction of cells with each other and with the extracellular matrix. Its expression increases with the progression of liver cirrhosis and strongly correlated with the severity of fibrosis and angiogenesis. However, the precise role of thrombospondin-1 is not defined in this process. It may function as a promoter or inhibitor of angiogenesis that may depend on its concentration, the type of domain being activated and the type of receptors present on endothelial cells[31].

Angiostatin - a fragment of plasminogen, and endostatin - a fragment of the C-terminal part of the collagen ⅩⅧ α1-chain, inhibits the migration of human endothelial cells stimulated with FGF and VEGF and do not affect intracellular signaling pathways stimulated by FGF and VEGF[32].

Toll-like receptor 4, which recognizes bacterial lipopolysaccharide, is expressed by SECs involved in fibrosis-associated angiogenesis in cirrhotic liver. These properties are related through the cytosolic adapter protein MyD88, which is involved in the production of extracellular protease regulating the invasive ability of SECs[33].

Hepatic apelin system (apelin/APJ-receptor) - the connecting link between chronic inflammation and subsequent fibrogenic and angiogenic processes in liver cirrhosis. On the one hand, hypoxia and inflammation initiate expression of APJ, on the other profibrogenic activation of APJ mediates the induction of profibrogenic genes, HSCs proliferation and secretion of pro-angiogenic factors[34].

Aquaporin-1 is an integral membrane channel pro-

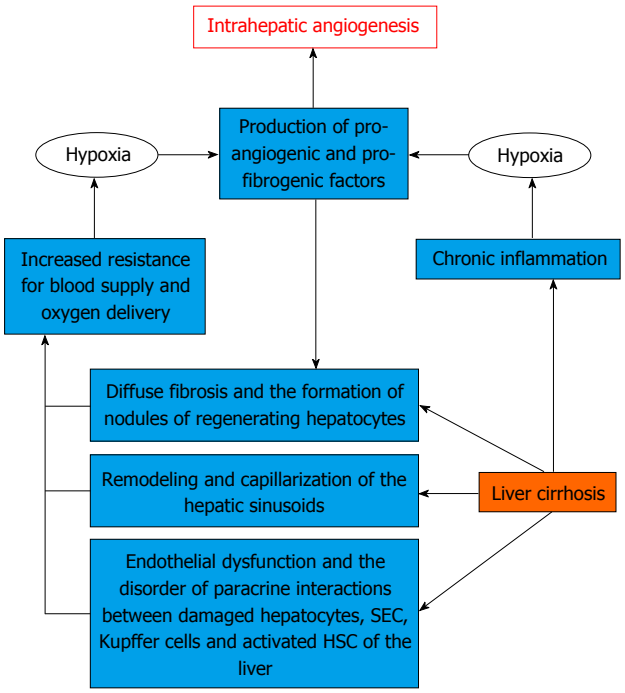

Figure 1 Two main ways of intraheptic angiogenesis in liver cirrhosis. SEC: Sinusoidal endothelial cells; HSC: Hepatic stellate cell.

tein, overexpressed in cirrhosis, that promotes angiogenesis by enhancing endothelial invasion[35].

It is known that chemokines from CXC family are involved in angiogenesis. ELR-positive chemokines stimulate this process, and ELR-negative suppress it[36].

Neuropilin-1 and neuropilin-2 are transmembrane glycoproteins with large extracellular domains that interact with both class 3 semaphorins, VEGF and the classical receptors for VEGF, VEGFR-1 and -2, mediating signal transduction. Neuropilin-1 expressed mainly by arterial endothelium, whereas neuropilin-2 is only expressed by venous and lymphatic endothelium. Both neuropilins are commonly over-expressed in regions of physiological and pathological angiogenesis, but the definitive role of neuropilins in angiogenic processes are not fully characterized[37].

Mechanisms of intrahepatic angiogenesis in liver cirrhosis

Hepatic angiogenesis may substantially differ from homologous processes in other organs or tissue on the basis of: (1) the rather unique phenotypic profile and functional role of activated HSCs and of other liver myofibroblasts; (2) the presence of two different microvascular structures described (*i.e.,* sinusoids lined by fenestrated endothelium *vs* large vessels lined by a continuous one); and (3) the existence of ANGPTL3, a liver-specific angiogenic factor.

There are two main ways of forming new blood vessels in liver cirrhosis[38] (Figure 1). One of them is associated with increased expression of pro-angiogenic growth factors, cytokines and matrix metalloproteinases on the background of chronic inflammation[39]. Proinflammatory mediators produced by Kupffer cells, mast cells and leukocytes may produce angiogenic response due to induction and increased transcriptional activity of HIF-1α[40].

It is believed that macrophages in the normal state are not directly involved in angiogenesis. In contrast, activated Kupffer cells contribute to the formation of new blood vessels through the production of cytokines, ROS and PAF in liver cirrhosis[41].

Kupffer cells also produce tumor necrosis factor-α (TNF-α), which induces the migration of cells and regulates apoptosis and angiogenesis[42]. The increase of ROS in the liver stimulates angiogenesis due to enhanced expression of TNF-α, NO, HIF-1 and VEGF[43]. PAF promotes the development of VEGF by the activation of nuclear transcription factor nuclear factor κB (NF-κB)[44]. Mast cells involved in the formation of new blood vessels through the production of heparin, histamine, tryptase, cytokines (TGF-β1, TNF-α, interleukins) and VEGF. They can also increase the number of SECs *in vitro*[45]. Soluble mediators, in particular, pro-inflammatory cytokines, growth factors, proteases, and products of oxidative stress regulate increased expression of chemokines in chronic inflammation of the liver. Leukocytes can thereby penetrate into the liver tissue where they produce angiogenic factors such as VEGF, PIGF, PDGF, FGF, TGF-β1, EGF, angiopoietin-2 and different interleukins[46].

On the one hand, hypoxia, caused by HIF-1α stimulation, activates the HSCs and leads to the production of various angiogenic and fibrogenic factors (PIGF, VEGF, NO, HGF, PDGF)[47], promoting angiogenesis and progression of hepatic fibrosis[48]. On the other hand, diffuse fibrosis, regenerative liver nodules and forming of sinusoidal capillarization cause an increase of HVR and impair the oxygen supply to the liver cells[49]. Accumulation of HIFs, in particular, HIF-1α, increases the VEGF, angiopoietin-1 and their receptors expression on activated HSCs. This leads to involvement and stimulation of SECs, stabilizing the newly formed vessels and providing them with strength[50]. In turn, SECs generate PDGF and TGF-β, helping to attract and migration of HSCs. This process includes ROS-mediated activation of ERK and c-Jun-NH2-terminal kinase (JNK) followed by a delayed- and HIF-1α-dependent up-regulation and release of VEGF[51].

Respectively there are two different phases of an angiogenic process occurring in the liver cirrhosis. Initially, the formation of blood vessels occurs in developing incomplete septa in which concomitant expression of VEGF, Flk-1, and Tie-2 is restricted by HSCs. In a later phase, angiogenesis occurs in large bridging septa and the expression of this proangiogenic panel is limited to endothelial cells and aims to stabilize the newly formed blood vessels[52]. Some of them occur mainly along areas of active inflammation and fibrous septa, probably favors inflammation, tissue repair, and gives rise to intrahepatic shunts. Some of them probably needed

for compensation of insufficient intrahepatic blood flow. Other forms intrahepatic shunts bypassing sinusoids and draining blood from the portal to the central venule. Although they perform the decompression role, they can lead to liver dysfunction due to declining oxygen delivery and nutrients to the liver tissues and limiting the free exchange between hepatocytes and sinusoids[53].

During last years it has been found that endothelial progenitor cells produced by stem cells of the bone marrow are capable of causing *in situ* neovascularization in both physiological and pathological conditions (post-natal vasculogenesis). In particular, they may play an important paracrine role in liver angiogenesis by stimulating resident SECs through as factors as PDGF and VEGF in liver cirrhosis[54]. However, their angiogenic ability is significantly reduced in patients in this category, especially with severe hepatic dysfunction. Perhaps this is because chronic inflammation stimulates the release of angiogenic factors by resident HSCs and SECs, and inhibits the endothelial progenitor cells mobilization into the bloodstream[55].

CONCLUSION

Endothelial dysfunction and impaired paracrine interaction between activated HSCs and SECs, as well as sinusoidal remodeling and capillarization play an important role in improving the HVR to portal blood flow, adding structural changes associated with diffuse fibrosis and regenerative nodules in liver cirrhosis. The development of intrahepatic angiogenesis can be regarded as a compensatory mechanism that is aimed at decompression of the portal system. However, the newly formed vessels carrying blood to bypass the sinusoids are unable to provide oxygen and nutrients to the liver tissue which leads to progression of the disease. A comprehensive assessment of morphological and functional changes of hepatic vessels in the liver cirrhosis might allow to develop some new correction methods of its specific hemodynamic disorders. Moreover, it could help to enhance the effectiveness of therapeutic interventions aimed at the prevention of portal hypertension complications.

REFERENCES

1 **Fernandez M**. Molecular pathophysiology of portal hypertension. *Hepatology* 2015; **61**: 1406-1415 [PMID: 25092403 DOI: 10.1002/hep.27343]

2 **García-Pagán JC**, Gracia-Sancho J, Bosch J. Functional aspects on the pathophysiology of portal hypertension in cirrhosis. *J Hepatol* 2012; **57**: 458-461 [PMID: 22504334 DOI: 10.1016/j.jhep.2012.03.007]

3 **Hu LS**, George J, Wang JH. Current concepts on the role of nitric oxide in portal hypertension. *World J Gastroenterol* 2013; **19**: 1707-1717 [PMID: 23555159 DOI: 10.3748/wjg.v19.i11.1707]

4 **Hellerbrand C**. Hepatic stellate cells--the pericytes in the liver. *Pflugers Arch* 2013; **465**: 775-778 [PMID: 23292551 DOI: 10.1007/s00424-012-1209-5]

5 **Ueno T**, Bioulac-Sage P, Balabaud C, Rosenbaum J. Innervation of the sinusoidal wall: regulation of the sinusoidal diameter. *Anat Rec A Discov Mol Cell Evol Biol* 2004; **280**: 868-873 [PMID: 15382014]

6 **Iizuka M**, Murata T, Hori M, Ozaki H. Increased contractility of hepatic stellate cells in cirrhosis is mediated by enhanced Ca2+-dependent and Ca2+-sensitization pathways. *Am J Physiol Gastrointest Liver Physiol* 2011; **300**: G1010-G1021 [PMID: 21393429 DOI: 10.1152/ajpgi.00350.2010]

7 **Takashimizu S**, Kojima S, Nishizaki Y, Kagawa T, Shiraishi K, Mine T, Watanabe N. Effect of endothelin A receptor antagonist on hepatic hemodynamics in cirrhotic rats. Implications for endothelin-1 in portal hypertension. *Tokai J Exp Clin Med* 2011; **36**: 37-43 [PMID: 21769771]

8 **Lugo-Baruqui A**, Muñoz-Valle JF, Arévalo-Gallegos S, Armendáriz-Borunda J. Role of angiotensin II in liver fibrosis-induced portal hypertension and therapeutic implications. *Hepatol Res* 2010; **40**: 95-104 [PMID: 19737316 DOI: 10.1111/j.1872-034X.2009.00581.x]

9 **Reynaert H**, Urbain D, Geerts A. Regulation of sinusoidal perfusion in portal hypertension. *Anat Rec* (Hoboken) 2008; **291**: 693-698 [PMID: 18484616 DOI: 10.1002/ar.20669]

10 **Lee JS**, Semela D, Iredale J, Shah VH. Sinusoidal remodeling and angiogenesis: a new function for the liver-specific pericyte? *Hepatology* 2007; **45**: 817-825 [PMID: 17326208]

11 **Lee JS**, Kang Decker N, Chatterjee S, Yao J, Friedman S, Shah V. Mechanisms of nitric oxide interplay with Rho GTPase family members in modulation of actin membrane dynamics in pericytes and fibroblasts. *Am J Pathol* 2005; **166**: 1861-1870 [PMID: 15920170]

12 **Semela D**, Das A, Langer D, Kang N, Leof E, Shah V. Platelet-derived growth factor signaling through ephrin-b2 regulates hepatic vascular structure and function. *Gastroenterology* 2008; **135**: 671-679 [PMID: 18570897 DOI: 10.1053/j.gastro.2008.04.010]

13 **Pinzani M**. PDGF and signal transduction in hepatic stellate cells. *Front Biosci* 2002; **7**: d1720-d1726 [PMID: 12133817]

14 **Cao S**, Yaqoob U, Das A, Shergill U, Jagavelu K, Huebert RC, Routray C, Abdelmoneim S, Vasdev M, Leof E, Charlton M, Watts RJ, Mukhopadhyay D, Shah VH. Neuropilin-1 promotes cirrhosis of the rodent and human liver by enhancing PDGF/TGF-beta signaling in hepatic stellate cells. *J Clin Invest* 2010; **120**: 2379-2394 [PMID: 20577048 DOI: 10.1172/JCI41203]

15 **Friedman SL**. Hepatic stellate cells: protean, multifunctional, and enigmatic cells of the liver. *Physiol Rev* 2008; **88**: 125-172 [PMID: 18195085 DOI: 10.1152/physrev.00013.2007]

16 **Seki E**, Brenner DA. Recent advancement of molecular mechanisms of liver fibrosis. *J Hepatobiliary Pancreat Sci* 2015; **22**: 512-518 [PMID: 25869468 DOI: 10.1002/jhbp.245]

17 **Svegliati-Baroni G**, De Minicis S, Marzioni M. Hepatic fibrogenesis in response to chronic liver injury: novel insights on the role of cell-to-cell interaction and transition. *Liver Int* 2008; **28**: 1052-1064 [PMID: 18783548 DOI: 10.1111/j.1478-3231.2008.01825.x]

18 **Rappaport AM**, MacPhee PJ, Fisher MM, Phillips MJ. The scarring of the liver acini (Cirrhosis). Tridimensional and microcirculatory considerations. *Virchows Arch A Pathol Anat Histopathol* 1983; **402**: 107-137 [PMID: 6420982]

19 **Elpek GÖ**. Angiogenesis and liver fibrosis. *World J Hepatol* 2015; **7**: 377-391 [PMID: 25848465 DOI: 10.4254/wjh.v7.i3.377]

20 **Folkman J**. Angiogenesis: an organizing principle for drug discovery? *Nat Rev Drug Discov* 2007; **6**: 273-286 [PMID: 17396134]

21 **Skuli N**, Majmundar AJ, Krock BL, Mesquita RC, Mathew LK, Quinn ZL, Runge A, Liu L, Kim MN, Liang J, Schenkel S, Yodh AG, Keith B, Simon MC. Endothelial HIF-2α regulates murine pathological angiogenesis and revascularization processes. *J Clin Invest* 2012; **122**: 1427-1443 [PMID: 22426208 DOI: 10.1172/JCI57322]

22 **Brandes RP**, Miller FJ, Beer S, Haendeler J, Hoffmann J, Ha T, Holland SM, Görlach A, Busse R. The vascular NADPH oxidase subunit p47phox is involved in redox-mediated gene expression.

Free Radic Biol Med 2002; **32**: 1116-1122 [PMID: 12031896]
23 **Chen Z**, Lai TC, Jan YH, Lin FM, Wang WC, Xiao H, Wang YT, Sun W, Cui X, Li YS, Fang T, Zhao H, Padmanabhan C, Sun R, Wang DL, Jin H, Chau GY, Huang HD, Hsiao M, Shyy JY. Hypoxia-responsive miRNAs target argonaute 1 to promote angiogenesis. *J Clin Invest* 2013; **123**: 1057-1067 [PMID: 23426184 DOI: 10.1172/JCI65344]
24 **Carmeliet P**. Manipulating angiogenesis in medicine. *J Intern Med* 2004; **255**: 538-561 [PMID: 15078497]
25 **Klein S**, Roghani M, Rifkin DB. Fibroblast growth factors as angiogenesis factors: new insights into their mechanism of action. *EXS* 1997; **79**: 159-192 [PMID: 9002232]
26 **Hellberg C**, Ostman A, Heldin CH. PDGF and vessel maturation. *Recent Results Cancer Res* 2010; **180**: 103-114 [PMID: 20033380 DOI: 10.1007/978-3-540-78281-0_7]
27 **Chen JX**, Zeng H, Lawrence ML, Blackwell TS, Meyrick B. Angiopoietin-1-induced angiogenesis is modulated by endothelial NADPH oxidase. *Am J Physiol Heart Circ Physiol* 2006; **291**: H1563-H1572 [PMID: 16679392]
28 **Pauta M**, Ribera J, Melgar-Lesmes P, Casals G, Rodríguez-Vita J, Reichenbach V, Fernandez-Varo G, Morales-Romero B, Bataller R, Michelena J, Altamirano J, Jiménez W, Morales-Ruiz M. Overexpression of angiopoietin-2 in rats and patients with liver fibrosis. Therapeutic consequences of its inhibition. *Liver Int* 2015; **35**: 1383-1392 [PMID: 24612347 DOI: 10.1111/liv.12505]
29 **Patsenker E**, Popov Y, Stickel F, Schneider V, Ledermann M, Sägesser H, Niedobitek G, Goodman SL, Schuppan D. Pharmacological inhibition of integrin alphavbeta3 aggravates experimental liver fibrosis and suppresses hepatic angiogenesis. *Hepatology* 2009; **50**: 1501-1511 [PMID: 19725105 DOI: 10.1002/hep.23144]
30 **Kevil CG**, Payne DK, Mire E, Alexander JS. Vascular permeability factor/vascular endothelial cell growth factor-mediated permeability occurs through disorganization of endothelial junctional proteins. *J Biol Chem* 1998; **273**: 15099-15103 [PMID: 9614120]
31 **Elpek GO**, Gokhan GA, Bozova S. Thrombospondin-1 expression correlates with angiogenesis in experimental cirrhosis. *World J Gastroenterol* 2008; **14**: 2213-2217 [PMID: 18407596]
32 **Eriksson K**, Magnusson P, Dixelius J, Claesson-Welsh L, Cross MJ. Angiostatin and endostatin inhibit endothelial cell migration in response to FGF and VEGF without interfering with specific intracellular signal transduction pathways. *FEBS Lett* 2003; **536**: 19-24 [PMID: 12586331]
33 **Jagavelu K**, Routray C, Shergill U, O'Hara SP, Faubion W, Shah VH. Endothelial cell toll-like receptor 4 regulates fibrosis-associated angiogenesis in the liver. *Hepatology* 2010; **52**: 590-601 [PMID: 20564354 DOI: 10.1002/hep.23739]
34 **Melgar-Lesmes P**, Pauta M, Reichenbach V, Casals G, Ros J, Bataller R, Morales-Ruiz M, Jiménez W. Hypoxia and proinflammatory factors upregulate apelin receptor expression in human stellate cells and hepatocytes. *Gut* 2011; **60**: 1404-1411 [PMID: 21450694 DOI: 10.1136/gut.2010.234690]
35 **Huebert RC**, Jagavelu K, Hendrickson HI, Vasdev MM, Arab JP, Splinter PL, Trussoni CE, Larusso NF, Shah VH. Aquaporin-1 promotes angiogenesis, fibrosis, and portal hypertension through mechanisms dependent on osmotically sensitive microRNAs. *Am J Pathol* 2011; **179**: 1851-1860 [PMID: 21854740 DOI: 10.1016/j.ajpath.2011.06.045]
36 **Sahin H**, Borkham-Kamphorst E, Kuppe C, Zaldivar MM, Grouls C, Al-samman M, Nellen A, Schmitz P, Heinrichs D, Berres ML, Doleschel D, Scholten D, Weiskirchen R, Moeller MJ, Kiessling F, Trautwein C, Wasmuth HE. Chemokine Cxcl9 attenuates liver fibrosis-associated angiogenesis in mice. *Hepatology* 2012; **55**: 1610-1619 [PMID: 22237831 DOI: 10.1002/hep.25545]
37 **Staton CA**, Kumar I, Reed MW, Brown NJ. Neuropilins in physiological and pathological angiogenesis. *J Pathol* 2007; **212**: 237-248 [PMID: 17503412]
38 **Fernández M**, Semela D, Bruix J, Colle I, Pinzani M, Bosch J. Angiogenesis in liver disease. *J Hepatol* 2009; **50**: 604-620 [PMID: 19157625 DOI: 10.1016/j.jhep.2008.12.011]
39 **Ehling J**, Bartneck M, Wei X, Gremse F, Fech V, Möckel D, Baeck C, Hittatiya K, Eulberg D, Luedde T, Kiessling F, Trautwein C, Lammers T, Tacke F. CCL2-dependent infiltrating macrophages promote angiogenesis in progressive liver fibrosis. *Gut* 2014; **63**: 1960-1971 [PMID: 24561613 DOI: 10.1136/gutjnl-2013-306294]
40 **Chaparro M**, Sanz-Cameno P, Trapero-Marugan M, Garcia-Buey L, Moreno-Otero R. Mechanisms of angiogenesis in chronic inflammatory liver disease. *Ann Hepatol* 2007; **6**: 208-213 [PMID: 18007549]
41 **Steib CJ**. Kupffer cell activation and portal hypertension. *Gut* 2011; **60**: 1307-1308 [PMID: 21708827 DOI: 10.1136/gut.2011.242560]
42 **Lochhead PA**, Gilley R, Cook SJ. ERK5 and its role in tumour development. *Biochem Soc Trans* 2012; **40**: 251-256 [PMID: 22260700 DOI: 10.1042/BST20110663]
43 **Dewhirst MW**, Cao Y, Moeller B. Cycling hypoxia and free radicals regulate angiogenesis and radiotherapy response. *Nat Rev Cancer* 2008; **8**: 425-437 [PMID: 18500244 DOI: 10.1038/nrc2397]
44 **Ko HM**, Seo KH, Han SJ, Ahn KY, Choi IH, Koh GY, Lee HK, Ra MS, Im SY. Nuclear factor kappaB dependency of platelet-activating factor-induced angiogenesis. *Cancer Res* 2002; **62**: 1809-1814 [PMID: 11912159]
45 **Franceschini B**, Ceva-Grimaldi G, Russo C, Dioguardi N, Grizzi F. The complex functions of mast cells in chronic human liver diseases. *Dig Dis Sci* 2006; **51**: 2248-2256 [PMID: 17103041]
46 **Marra F**. Chemokines in liver inflammation and fibrosis. *Front Biosci* 2002; **7**: d1899-d1914 [PMID: 12161342]
47 **Copple BL**, Bai S, Burgoon LD, Moon JO. Hypoxia-inducible factor-1α regulates the expression of genes in hypoxic hepatic stellate cells important for collagen deposition and angiogenesis. *Liver Int* 2011; **31**: 230-244 [PMID: 20880076 DOI: 10.1111/j.1478-3231.2010.02347.x]
48 **Lemoinne S**, Cadoret A, El Mourabit H, Thabut D, Housset C. Origins and functions of liver myofibroblasts. *Biochim Biophys Acta* 2013; **1832**: 948-954 [PMID: 23470555 DOI: 10.1016/j.bbadis.2013.02.019]
49 **Yokomori H**, Oda M, Yoshimura K, Hibi T. Enhanced expressions of apelin on proliferative hepatic arterial capillaries in human cirrhotic liver. *Hepatol Res* 2012; **42**: 508-514 [PMID: 22502744 DOI: 10.1111/j.1872-034X.2011.00945.x]
50 **Coulon S**, Heindryckx F, Geerts A, Van Steenkiste C, Colle I, Van Vlierberghe H. Angiogenesis in chronic liver disease and its complications. *Liver Int* 2011; **31**: 146-162 [PMID: 21073649 DOI: 10.1111/j.1478-3231.2010.02369.x]
51 **Novo E**, Povero D, Busletta C, Paternostro C, di Bonzo LV, Cannito S, Compagnone A, Bandino A, Marra F, Colombatto S, David E, Pinzani M, Parola M. The biphasic nature of hypoxia-induced directional migration of activated human hepatic stellate cells. *J Pathol* 2012; **226**: 588-597 [PMID: 21959987 DOI: 10.1002/path.3005]
52 **Novo E**, Cannito S, Zamara E, Valfrè di Bonzo L, Caligiuri A, Cravanzola C, Compagnone A, Colombatto S, Marra F, Pinzani M, Parola M. Proangiogenic cytokines as hypoxia-dependent factors stimulating migration of human hepatic stellate cells. *Am J Pathol* 2007; **170**: 1942-1953 [PMID: 17525262]
53 **Vanheule E**, Geerts AM, Van Huysse J, Schelfhout D, Praet M, Van Vlierberghe H, De Vos M, Colle I. An intravital microscopic study of the hepatic microcirculation in cirrhotic mice models: relationship between fibrosis and angiogenesis. *Int J Exp Pathol* 2008; **89**: 419-432 [PMID: 19134051 DOI: 10.1111/j.1365-2613.2008.00608.x]
54 **Kaur S**, Tripathi D, Dongre K, Garg V, Rooge S, Mukopadhyay A, Sakhuja P, Sarin SK. Increased number and function of endothelial progenitor cells stimulate angiogenesis by resident liver sinusoidal endothelial cells (SECs) in cirrhosis through paracrine factors. *J Hepatol* 2012; **57**: 1193-1198 [PMID: 22824816 DOI: 10.1016/j.jhep.2012.07.016]
55 **Chen CH**, Chang LT, Tung WC, Chen YL, Chang CL, Leu S, Sun CK, Tsai TH, Tsai IT, Chang HW, Yip HK. Levels and values

of circulating endothelial progenitor cells, soluble angiogenic factors, and mononuclear cell apoptosis in liver cirrhosis patients. *J Biomed Sci* 2012; **19**: 66 [PMID: 22809449 DOI: 10.1186/1-423-0127-19-66]

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ORIGINAL ARTICLE

Basic Study

Obese diet-induced mouse models of nonalcoholic steatohepatitis-tracking disease by liver biopsy

Maria Nicoline Baandrup Kristiansen, Sanne Skovgård Veidal, Kristoffer Tobias Gustav Rigbolt, Kirstine Sloth Tølbøl, Jonathan David Roth, Jacob Jelsing, Niels Vrang, Michael Feigh

Maria Nicoline Baandrup Kristiansen, Sanne Skovgård Veidal, Kristoffer Tobias Gustav Rigbolt, Kirstine Sloth Tølbøl, Jacob Jelsing, Niels Vrang, Michael Feigh, Gubra Aps, 2970 Hørsholm, Denmark

Jonathan David Roth, Intercept Pharmaceuticals, Inc., San Diego, CA 9212, United States

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Institutional animal care and use committee statement: All procedures involving animals were reviewed and approved by the Danish Committee for animal research and covered by a personal license for Jacob Jelsing (2013-15-2934-00784). All of the institutional and national guidelines for the care and use of laboratory animals were followed.

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Correspondence to: Michael Feigh, PhD, Gubra Aps, Hørsholm Kongevej 11B, 2970 Hørsholm, Denmark. mfe@gubra.dk
Telephone: +45-31522651

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Abstract

AIM: To characterize development of diet-induced nonalcoholic steatohepatitis (NASH) by performing liver biopsy in wild-type and genetically obese mice.

METHODS: Male wild-type C57BL/6J (C57) mice (DIO-NASH) and male *Lep*^{ob}/*Lep*^{ob} (*ob*/*ob*) mice (*ob*/*ob*-NASH) were maintained on a diet high in trans-fat (40%), fructose (22%) and cholesterol (2%) for 26 and 12 wk, respectively. A normal chow diet served as control in C57 mice (lean chow) and *ob*/*ob* mice (*ob*/*ob* chow). After the diet-induction period, mice were liver biopsied and a blinded histological assessment of steatosis and fibrosis was conducted. Mice were then stratified into groups counterbalanced for steatosis score and fibrosis stage and continued on diet and to receive daily PO dosing of vehicle for 8 wk. Global gene expression in liver tissue was assessed by RNA sequencing and bioinformatics. Metabolic parameters, plasma liver enzymes and lipids (total cholesterol, triglycerides) as well as hepatic lipids and collagen content were measured by biochemical analysis. Non-alcoholic fatty liver disease activity score (NAS) (steatosis/inflammation/ballooning

degeneration) and fibrosis were scored. Steatosis and fibrosis were also quantified using percent fractional area.

RESULTS: Diet-induction for 26 and 12 wk in DIO-NASH and *ob*/*ob*-NASH mice, respectively, elicited progressive metabolic perturbations characterized by increased adiposity, total cholesterol and elevated plasma liver enzymes. The diet also induced clear histological features of NASH including hepatosteatosis and fibrosis. Overall, the metabolic NASH phenotype was more pronounced in *ob*/*ob*-NASH *vs* DIO-NASH mice. During the eight week repeated vehicle dosing period, the metabolic phenotype was sustained in DIO-NASH and *ob*/*ob*-NASH mice in conjunction with hepatomegaly and increased hepatic lipids and collagen accumulation. Histopathological scoring demonstrated significantly increased NAS of DIO-NASH mice (0 *vs* 4.7 ± 0.4, $P < 0.001$ compared to lean chow) and ob/ob-NASH mice (2.4 ± 0.3 *vs* 6.3 ± 0.2, $P < 0.001$ compared to *ob*/*ob* chow), respectively. Furthermore, fibrosis stage was significantly elevated for DIO-NASH mice (0 *vs* 1.2 ± 0.2, $P < 0.05$ compared to lean chow) and *ob*/*ob* NASH (0.1 ± 0.1 *vs* 3.0 ± 0.2, $P < 0.001$ compared to *ob*/*ob* chow). Notably, fibrosis stage was significantly ($P < 0.001$) increased in *ob*/*ob*-NASH mice, when compared to DIO-NASH mice.

CONCLUSION: These data introduce the obese diet-induced DIO-NASH and *ob*/*ob*-NASH mouse models with biopsy-confirmed individual disease staging as a preclinical platform for evaluation of novel NASH therapeutics.

Key words: Nonalcoholic steatohepatitis; Liver biopsy; Diet-induced obesity; Nonalcoholic fatty liver disease; Fibrosis

Core tip: We characterize the development and progression of diet-induced nonalcoholic steatohepatitis (NASH) in a wild-type and a genetically obese mouse model. We confirm that a diet high in trans-fat, fructose and cholesterol, develops key histological hallmarks of NASH (steatosis, inflammation, ballooning degeneration) in conjunction with fibrosis. Concomitantly, marked alterations in NASH associated gene expression pathways can be evaluated by RNAseq analysis. In addition, we describe that performing a baseline liver biopsy enables individual disease staging for subsequent stratified randomization of animals into study groups. Finally, we show these models' utility for a chronic repeated dosing study to evaluate pharmacological intervention.

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INTRODUCTION

It is generally accepted that along with increasing rates of obesity, type 2 diabetes and metabolic syndrome, the incidence and prevalence of patients with nonalcoholic fatty liver disease (NAFLD) continues to rise[1-4]. NAFLD is considered the hepatic manifestation of the metabolic syndrome and covers a variety of pathologies ranging from simple hepatic steatosis (accumulation of triglycerides in hepatocytes) to nonalcoholic steatohepatitis (NASH), characterized by inflammation, cellular ballooning and fibrosis in varying degrees[1-3]. The pathogenesis of NASH is described by the "two-hit" hypothesis, the first hit being fat accumulation in hepatocytes, while the "second hit", *e.g.,* oxidative stress, apoptosis or mitochondrial dysfunction, causes development of inflammation and fibrosis[5].

There are currently no pharmacological agents specifically approved for the treatment of NASH and disease management is consequently focused on the correction of underlying risk factors (*e.g.,* obesity, insulin resistance and dyslipidemia)[1,6]. A likely contributor to the absence of therapeutics is the paucity of preclinical models resembling human NAFLD/NASH[6]. Historically, several animal models have been developed to represent the pathophysiology, morphological findings, biochemical changes, and clinical features of human NAFLD/NASH. These models are usually divided into two main categories: The diet-induced models and the genetically modified models (transgenic or knockout models)[1]. Some diet-induced models are based on *ad libitum* feeding of diets enriched with various combinations of fat, cholesterol and sugars (*e.g.,* fructose) thereby developing a metabolic phenotype reflected by adiposity and hepatosteatosis, albeit only presenting mild characteristics of NASH and typically lack of liver fibrosis[7,8]. Other dietary models involve feeding nutrient-deficient diets such as the methionine- and choline-deficient diet (MCD). Methionine and choline deficiency impairs liver β-oxidation and the production of very-low density lipoproteins (VLDL) hereby generating a "second hit"[1], eliciting a more severe fibrotic NASH phenotype within hepatic tissue[8,9]. However, these models fail to recapitulate a clinically relevant overall metabolic phenotype as MCD animals demonstrate pronounced weight loss and perturbed energy- and glucose homeostasis[10]. Recently, a novel wild-type diet-induced obese fibrotic NASH mouse model was introduced by Trevaskis *et al*[11], based on the ALIOS diet model[12]. This model, generated by feeding an *ad libitum* diet high in trans-fat, fructose and cholesterol to wild-type C57Bl/6J mice [the Amylin liver NASH model (AMLN)], displayed key hallmarks of clinical NASH[11]. The AMLN mouse model was further optimized by demonstrating a liver biopsy technique for

assessing individual steatosis, inflammation, ballooning degeneration and fibrosis staging, prior to a putative study intervention[6]. Not only does the baseline liver biopsy reduce biological variability by excluding mice that fail to develop NASH prior to initiating therapy, but it also allows for within-subject comparisons over time, thereby increasing statistical power[6].

For the genetically modified NASH models, several studies have implicated a role of individual genes involved in the development of NASH using deletion or overexpression models[7,9]. For example, mice that overexpress the transcription factor sterol regulatory element-binding proteins (SREBPs), a feedback regulatory system controlling intracellular levels of cholesterol and free fatty acids develop a hepatic phenotype resembling NASH. However, like MCD-fed mice, SREBP overexpression does not induce a metabolic profile consistent with obesity and insulin resistance[13]. In contrast, impairment of leptin signaling (*e.g.*, *db*/*db* mice) results in obesity, insulin resistance and diabetes[14]. Leptin-deficient mice (Lep^{ob}/Lep^{ob}) are predisposed to develop steatohepatitis, however, when maintained on regular rodent chow they do not develop fibrosis[11]. In fact, it was previously postulated that Lep^{ob}/Lep^{ob} mice are incapable of developing hepatic fibrosis[9]. This notion was dispelled by the observation that Lep^{ob}/Lep^{ob} mice maintained on the AMLN diet for at least 12 wk do in fact develop the key hallmarks of NASH, including fibrosis[11].

The present study assessed key NASH diagnostic characteristics (*e.g.*, steatosis score, inflammation, ballooning degeneration and fibrosis stage), metabolic endpoints and gene expression signatures in wild-type C57Bl/6J and Lep^{ob}/Lep^{ob} mice, fed the AMLN diet for a total of 34 and 20 wk, respectively, including an eight-week repeated vehicle dosing period. In addition, we demonstrate how a baseline liver biopsy allows for individual disease staging and for stratified randomization into experimental groups with reduced biological variability and for a clear cut analysis of individual response to pharmacological intervention.

MATERIALS AND METHODS

Animals and experimental set-up

All animal experiments were conformed to international accepted principles for the care and use of laboratory animals and were covered by a personal license for Jacob Jelsing (2013-15-2934-00784) issued by the Danish Committee for animal research.

Male C57Bl/6J (C57) and Lep^{ob}/Lep^{ob} (*ob*/*ob*) mice at 5 wk of age were obtained from JanVier (JanVier labs, France), and group housed 5 animals pr. cage under a 12/12 h dark-light cycle. Room temperature was controlled to 22 ℃ ± 1 ℃, with 50% ± 10% humidity. Animals had *ad libitum* access to diet high in fat (40%, of these 18% trans-fat), 40% carbohydrates (20% fructose) and 2% cholesterol (D09100301, Research Diet, United States) previously described as the AMLN diet[6], or regular rodent chow (Altromin 1324, Brogaarden, Denmark), and tap water. Both strains had *ad libitum* access to either the AMLN diet (DIO-NASH, n = 110; *ob*/*ob* NASH, n = 40) or chow (lean chow, n = 10; *ob*/*ob* chow, n = 10). After 26 (DIO-NASH) or 12 wk (*ob*/*ob*-NASH) a liver biopsy was performed for histological assessment of individual fibrosis and steatosis staging at baseline. Following biopsy procedure animals were single housed. An 8-wk vehicle intervention period was conducted in a representative subset of DIO-NASH and *ob*/*ob*-NASH mice, and their respective chow controls. Vehicle dosing consisted of once daily per oral dose of carboxymethyl cellulose (C57 and *ob*/*ob*) and subcutaneous injection with PBS (C57). The rationale was to mimic repeated dosing administration and animal handling in combination with AMLN diet-maintenance. After a total of 34 and 20 wk on AMLN diet for DIO-NASH and *ob*/*ob*-NASH mice, respectively, animals were euthanized and liver tissue collected for histological and biochemical analysis. Total animal numbers for each experiment is indicated in the figures and table.

Baseline liver biopsy after diet-induction

Mice were pretreated with enrofloxazin (Bayer, Germany) (5 mg/mL-1 mL/kg) one day before being biopsied. Prior to biopsy, mice were anesthetized with isoflurane (2%-3%) in 100% oxygen. A small abdominal incision in the midline was made and the left lateral lobe of the liver was exposed. A cone shaped wedge of liver tissue (50-100 mg) was excised from the distal portion of the lobe fixed in 4% paraformaldehyde for histology. The biopsy procedure previously described by Clapper *et al*[6] 2013 was refined using electrocoagulation of the cut surface of the liver by means of bipolar coagulation using ERBE VIO 100C electrosurgical unit (ERBE, United States). The liver was returned to the abdominal cavity, abdominal wall was sutured and skin stapled. Carprofen (Pfizer, United States) (5 mg/mL-0.01 mL/10 g) and enrofloxazin (5 mg/mL-1 mL/kg) were administered intraperitoneal at the time of surgery and at post-operation day one and two, to control postoperative pain relief and infection, respectively.

Hepatic gene expression changes

Gene expression changes were measured in a representative subset of DIO-NASH mice and *ob*/*ob*-NASH. Liver tissue was harvested from the left lateral lobe and snap frozen in liquid nitrogen. Tissue sections (about 50 mg) were homogenized in lysis buffer containing protease inhibitors and used for RNA extraction using NucleoSpin Plus RNA columns (Macherey-Nagel). The quantity of the RNA was analyzed using a Nano Drop 2000 spectrophotometer (Thermo Scientific, United States). RNAseq libraries were prepared with the KAPA poly-A kit (Kapa Biosystems, United States) and sequenced on the NextSeq 500 (Illumina, United States) (single-end, 75 bp reads). Reads were aligned to the GRCm38 Ensembl Mus musculus genome using STAR v.2.4.0[15] and feature counts were obtained using HTseq v.0.6.1[16], both with default parameters. Differential

expression analysis was performed with edgeR[17] and genes with a $P \leqslant 0.05$ after correction for multiple testing using the Benjamini and Hochberg method was regarded as significantly regulated. Pathway analysis of WikiPathways[18] was performed using the statistics module in PathVisio[19].

Body weight and body composition analysis

Body weight was intermittently monitored during the diet-induction period and once daily during the intervention period. Whole-body fat mass was analyzed at baseline (week -1) and week 8 of the intervention period by non-invasive EchoMRI scanning using Echo-MRI-900 (EchoMRI, United States). During the scanning procedure the mice were placed in a restrainer for 90-120 s.

Plasma biochemistry analysis

After diet-induction, a baseline blood sample was collected from the submandibular vein in non-fasted conscious animals and blood sampling was repeated following the intervention period. Plasma levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), triglycerides (TG) and total cholesterol (TC) were measured using the auto analyzer Cobas C-111 (Roche Diagnostics, Germany). Plasma levels of insulin were measured in duplicates using an AlphaLisa kit (Perkin Elmer), according to the manufacturer's instructions.

Oral glucose tolerance test

An oral glucose tolerance test (OGTT) was performed in week 4 of the intervention period. Animals were fasted for 4 h prior to OGTT. At $t = 0$ an oral glucose load [2 g/kg glucose 200 mg/mL, (Fresenius Kabi, Sweden)] was administered *via* a gastrically placed tube. Blood samples for measuring blood glucose (BG) were collected from the tail vein at $t = 0$, 15, 30, 60 and 120 min. Glucose area under the curve (AUC) calculations were determined as total AUC from the sampling period of 0 to 120 min.

Whole blood glucose analysis

Blood samples for BG analysis were collected into 10 μL heparinized glass capillary tubes and immediately suspended in buffer [0.5 mL of glucose/lactate system solution (EKF-diagnostics, Germany)] and analyzed for glucose using a BIOSEN c-Line glucose meter (EKF-diagnostics, Germany) according to the manufacturer's instructions.

Terminal hepatic hydroxyproline content

Formalin fixed (50 mg) liver tissue was homogenized in 500 μL water. Five hundred microliter concentrated HCl was added to the samples and hydrolyzed at 120 ℃ for three hours. Supernatants were transferred to a 96 well plate and wells were allowed to evaporate dry overnight. Total collagen content in the liver was measured by colorimetric determination of hydroxyproline residues by acid hydrolysis of collagen (Cat no. MAK008, Sigma Aldrich).

Terminal hepatic triglyceride and total cholesterol content

A liver piece (about 100 mg) was collected in FastPrep tubes and snap-frozen in liquid nitrogen. One milliliter 5%NH-40/ddH_2O solution (ab142227, Abcam) was added to the FastPrep tube. The tubes were homogenized in a FastPrep homogenizer and shaken for 2 × 60 s. After homogenization the samples were slowly heated to 80 ℃-100 ℃ in a heating block for three minutes. Samples were allowed to return to room temperature prior to a second round of heating. Next, samples were centrifuged for two minutes at top speed using a microcentrifuge to remove any insoluble material. TG and TC content in liver homogenates are measured in single determinations using auto analyzer Cobas C-111 with commercial kit (Roche Diagnostics, Germany) according to manufacturer's instructions.

Histology assessment and digital image analysis

Baseline liver biopsy and terminal samples were collected from the left lateral lobe (about 100 mg) and fixed overnight in 4% paraformaldehyde. Liver tissue was paraffin embedded and sectioned (3 μm thickness). To assess hepatic morphology and fibrosis, sections were stained with Hematoxylin and Eosin and Sirius Red, respectively, followed by analysis with Visiomorph software (Visiopharm, Denmark). Histological assessment and scoring was performed by a pathologist blinded to the study. NAFLD activity score (NAS) (steatosis/inflammation/ballooning degeneration) and fibrosis stage were performed using the clinical criteria outlined by Kleiner *et al*[20].

Statistical analysis

All data were analyzed using GraphPad Prism 5.0. The results are presented as mean ± standard error of the mean. Statistical significance was evaluated using One-way analysis of variance with Turkey's multiple comparison test, and for histological analysis using Kruskal-Wallis test with Dunn's multiple comparison test. $P < 0.05$ was considered statistical significant.

RESULTS

Male C57 and ob/ob mice developed adiposity and elevated plasma metabolic parameters after AMLN diet-induction

The overall study design is outlined in Figure 1A. Following a diet-induction period of 26 wk, C57 (DIO-NASH) mice demonstrated increased body weight (adiposity), when compared to lean chow animals. In the already obese *ob*/*ob* strain there was no additional effect on body weight noted in *ob*/*ob*-NASH mice relative to chow controls. Whereas all mice experienced slight weight loss following the biopsy, they returned

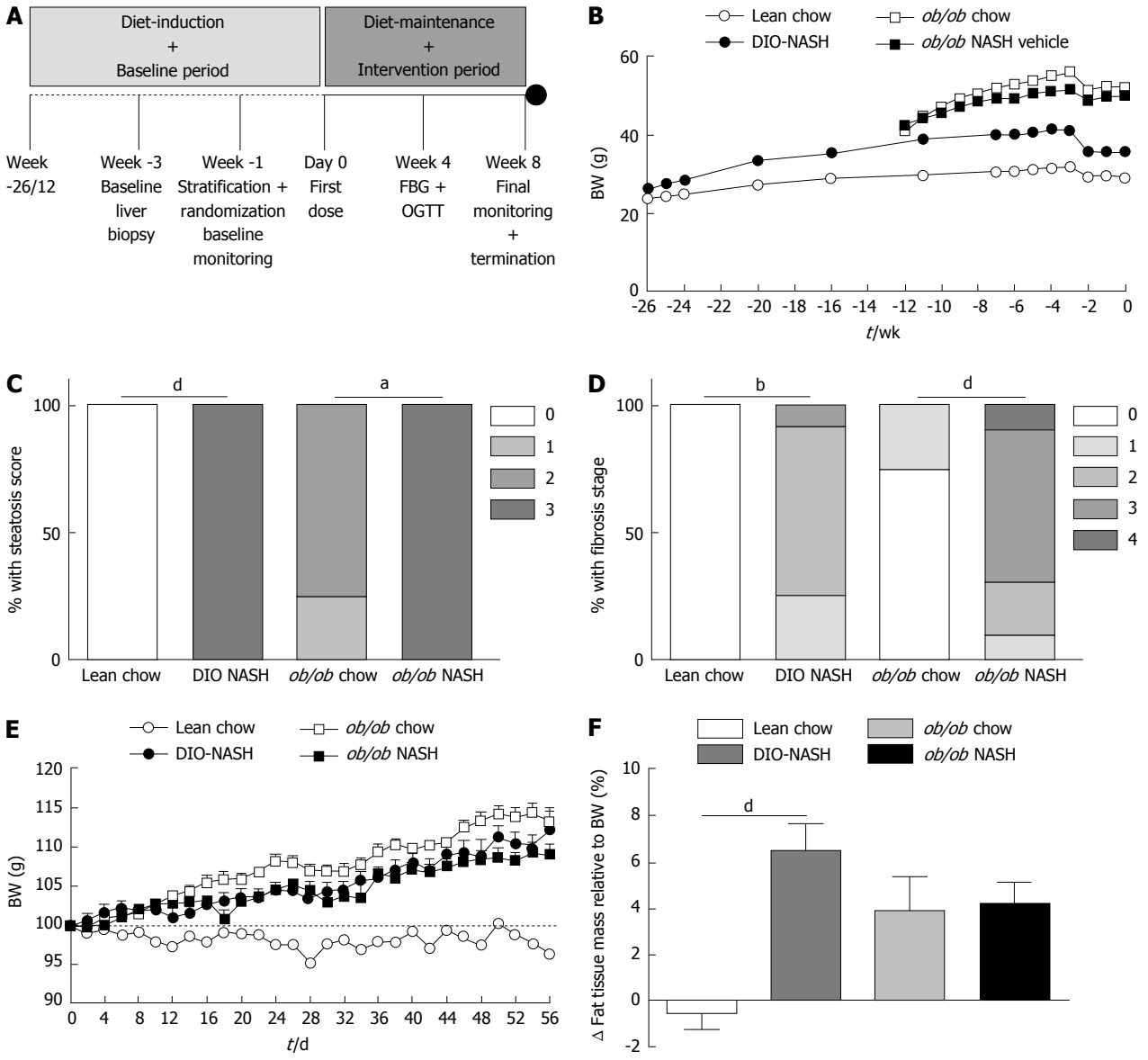

Figure 1 Study design, body weight regulation and liver biopsy-confirmed disease development. A: Study design of the DIO-NASH and *ob/ob* NASH mouse models; B: BW during diet-induction and baseline period (monitoring and biopsy-recovery); C and D: Liver biopsy-derived histopathological assessment of (C) steatosis score (0-3), and (D) fibrosis stage (0-4); E and F: Change in BW (E), and change in adiposity (F), during diet-maintenance and intervention period (repeated vehicle dosing). $^{a}P < 0.05$, $^{b}P < 0.01$, $^{d}P < 0.001$. The results are presented as mean ± SEM. Lean chow (n = 9), DIO-NASH (n = 12), *ob/ob* chow (n = 8), *ob/ob* NASH (n = 10). NASH: Nonalcoholic steatohepatitis; BW: Body weight; OGTT: Oral glucose tolerance test; FBG: Fed blood glucose; SEM: Standard error of the mean.

to a weight stable state within one week (Figure 1B). After diet-induction, DIO-NASH and *ob*/*ob*-NASH mice demonstrated a metabolic NAFLD phenotype, as reflected by elevated levels of plasma total cholesterol (TC) and liver enzymes ALT and AST, when compared to respective chow fed animals. Overall, the *ob*/*ob*-NASH mice demonstrated an accelerated and more pronounced metabolic phenotype, when compared to DIO-NASH mice (Table 1).

Male C57 and ob/ob mice demonstrated biopsy-proven hepatic steatosis and fibrosis after AMLN diet-induction

Histological assessments of biopsied liver tissue revealed that lean chow animals did not develop hepatic steatosis or fibrosis over the 26-wk diet-induction (Figure 1C and D). In contrast, DIO-NASH mice presented with high levels of steatosis (score 3) (Figure 1C) and fibrosis stage ranging from 1-3 (Figure 1D). The *ob*/*ob* chow phenotype displayed mild steatosis (score 1-2) and lack of or only mild fibrosis (stage 1) whereas all ob/ob-NASH mice displayed a steatosis score of 3 (Figure 1C) and a fibrosis stage ranging from 1-4 (Figure 1D).

Altered hepatic gene expression in male C57 and ob/ob mice after AMLN diet-induction

To characterize the effect of 26 wk diet-induction on global liver gene expression, the transcriptome of lean chow *vs* DIO-NASH mice were analyzed by RNAseq[21]. Principal component analysis identified a clear separation between the two groups along the first component,

Table 1 Effect of Amylin liver nonalcoholic steatohepatitis model diet on metabolic parameters, non-alcoholic fatty liver disease activity score/fibrosis stage, body weight/composition and liver weight

	Lean chow *n* = 9	DIO-NASH *n* = 12	*ob/ob* chow *n* = 8	*ob/ob* NASH *n* = 10
Baseline plasma ALT (U/L)	30.7 ± 0.8	133.6 ± 16.3	207.0 ± 58.7	577.4 ± 43.4[d,f]
Terminal plasma ALT (U/L)	31.5 ± 2.9	126.1 ± 19.8	249.7 ± 47.4	670.0 ± 59.0[d,f]
Baseline plasma AST (U/L)	46.5 ± 2.2	134.8 ± 11.6[b]	174.2 ± 41.1	436.7 ± 36.8[d,f]
Terminal plasma AST (U/L)	139.0 ± 28.2	213.8 ± 31.6	338.9 ± 87.7	552.6 ± 49.5[c,f]
Baseline plasma TC (mmol/L)	2.1 ± 0.1	6.8 ± 0.3[b]	3.5 ± 0.2	10.4 ± 0.9[d,f]
Terminal plasma TC (mmol/L)	2.3 ± 0.1	6.7 ± 0.4[b]	4.4 ± 0.2	10.8 ± 0.6[d,f]
Baseline plasma TG (mmol/L)	0.7 ± 0.1	0.9 ± 0.1	1.1 ± 0.2	0.8 ± 0.1
Terminal plasma TG (mmol/L)	0.8 ± 0.1	1.0 ± 0.1	1.3 ± 0.1	0.7 ± 0.1[d]
OGTT-AUC	1104 ± 49.7	1217 ± 39.9	1612 ± 173.4	1319 ± 61.4
Fasting blood glucose (mmol/L)	7.3 ± 0.3	7.6 ± 0.2	8.1 ± 0.3	7.6 ± 0.3
Plasma insulin (pmol/L)	30.6 ± 6.7	97.4 ± 18.3	1189 ± 94	567.3 ± 123[d,e]
Baseline steatosis score (0-3)	0	2.7 ± 0.3[b]	1.8 ± 0.2	2.7 ± 0.3
Baseline fibrosis stage (0-4)	0	1.8 ± 0.2	0.25 ± 0.2	2.7 ± 0.3[d,f]
Terminal NAFLD activity score (0-8)	0	4.7 ± 0.4[b]	2.4 ± 0.3	6.3 ± 0.2[d]
Terminal steatosis score (0-3)	0	2.8 ± 0.1[b]	2.1 ± 0.2	2.1 ± 0.2
Terminal inflammation score (0-3)	0	1.4 ± 0.2[b]	0.3 ± 0.2	2.4 ± 0.2[d]
Terminal ballooning degeneration score (0-2)	0	0.4 ± 0.1	0	0.9 ± 0.1[d]
Terminal fibrosis stage (0-4)	0	1.2 ± 0.2[a]	0.1 ± 0.1	3.0 ± 0.2[d,e]
Terminal steatosis (% area)	5.4 ± 0.5	33.9 ± 2.6[b]	29.5 ± 2.3	41.2 ± 1.0[c]
Terminal fibrosis (% area)	0.3 ± 0.1	1.1 ± 0.2	1.2 ± 0.2	4.9 ± 0.7[d,e]
Terminal BW (g)	28.0 ± 0.3	39.1 ± 1.1	59 ± 1.1	54.8 ± 0.8[e]
Terminal lean tissue mass (g)	14.6 ± 0.8	18.8 ± 0.5[b]	19.9 ± 0.9	17.3 ± 0.4[c]
Terminal lean tissue mass (% of BW)	49.6 ± 2.6	47.7 ± 1.5	33.6 ± 1.1	31.9 ± 0.6[f]
Terminal fat tissue mass (g)	1.5 ± 0.1	8.1 ± 0.7[b]	25.1 ± 0.7	22.5 ± 0.5[c,f]
Terminal fat tissue mass (% of BW)	5.1 ± 0.5	20.0 ± 1.3[b]	42.3 ± 0.8	41.5 ± 0.5[f]
Liver weight (g)	1.1 ± 0.1	2.5 ± 0.3[b]	2.9 ± 0.2	5.4 ± 0.2[d,f]
Liver weight (% of BW)	3.9 ± 0.3	6.3 ± 0.6[b]	4.9 ± 0.3	9.9 ± 0.3[d,f]

[a]$P < 0.05$ *vs* lean chow, [b]$P < 0.01$ *vs* lean chow, [c]$P < 0.05$ *vs ob/ob* chow, [d]$P < 0.01$ *vs ob/ob* chow, [e]$P < 0.05$ *vs* DIO-NASH and [f]$P < 0.01$ *vs* DIO-NASH. NASH: Nonalcoholic steatohepatitis; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; BW: Body weight; OGTT: Oral glucose tolerance test; AUC: Area under the curve.

indicating that the NASH diet markedly alters the overall gene expression profile (Figure 2A). We identified a total of 1378 differentially expressed genes, composed of 510 repressed and 868 induced genes (Figure 2B). To explore biological processes affected, sets of significantly altered signaling pathways were extracted (Figure 2C and D). Many of these pathways are consistent with the observed NASH phenotype including focal adhesion, toll-like receptor (TLR) signaling pathway, matrix metalloproteinases and inflammatory response pathway. Consistent with the identification of focal adhesion as the top affected pathways multiple collagen subtypes showed increased expression (Figure 2E).

Similar analyses were conducted on a subset of samples from *ob*/*ob*-NASH animals which confirmed the exaggerated expression levels of collagen types (Figure 2E). The pathway analysis also highlighted TLR signaling as one of the primary affected signaling processes, an observation supported by the increased expression of TLR4, which was recently demonstrated as an important pro-inflammatory mediator in the pathogenesis of NASH[22,23]. Notably, mRNA expression levels of a number of other TLR subtypes (TLR7, TLR8, TLR12 and TLR13) were upregulated to greater extent than TLR4 (Figure 2F). Furthermore, a large collection of pro-inflammatory factors ranging from chemokines, such as monocyte chemoattractant protein-1 (MCP-1), to chemokine receptors, such as C-C motif chemokine receptor-2 (Ccr2) and macrophage markers (*i.e.*, CD68, CD86, F4-80 and MAC-2) (Figure 2G) were significantly induced. Finally, in line with observed hepatosteatosis, expression levels of genes involved in triglyceride biosynthesis were significantly increased in C57 and ob/ob animals exposed to the AMLN diet. Conversely, cholesterol biosynthesis expression was significantly decreased in the DIO-NASH and *ob*/*ob*-NASH mice (Figure 2H).

Male C57 and ob/ob mice sustained adiposity and elevated plasma metabolic parameters after AMLN diet-maintenance and repeated dosing intervention period

During the intervention period with diet-maintenance and repeated vehicle dosing for a total of 8 wk, DIO-NASH mice progressively gained body weight (adiposity), when compared to lean chow animals. Leptin-deficient mice also gained fat mass during the 8-wk intervention period (Figure 1E and F). Fat gain was somewhat less in the *ob*/*ob*-NASH mice, however, these mice began the study with a higher % adiposity (37%, *n* = 10) relative to DIO-NASH mice (14%, *n* = 12). At study end (termination), DIO-NASH and *ob*/*ob*-NASH animals sustained the elevated levels of plasma liver enzymes and hypercholesterolemia, when compared to respective chow-fed mice (Table 1). In contrast, terminal plasma TG levels were unchanged in DIO-NASH mice, and were significantly decreased for *ob*/*ob*-NASH animals,

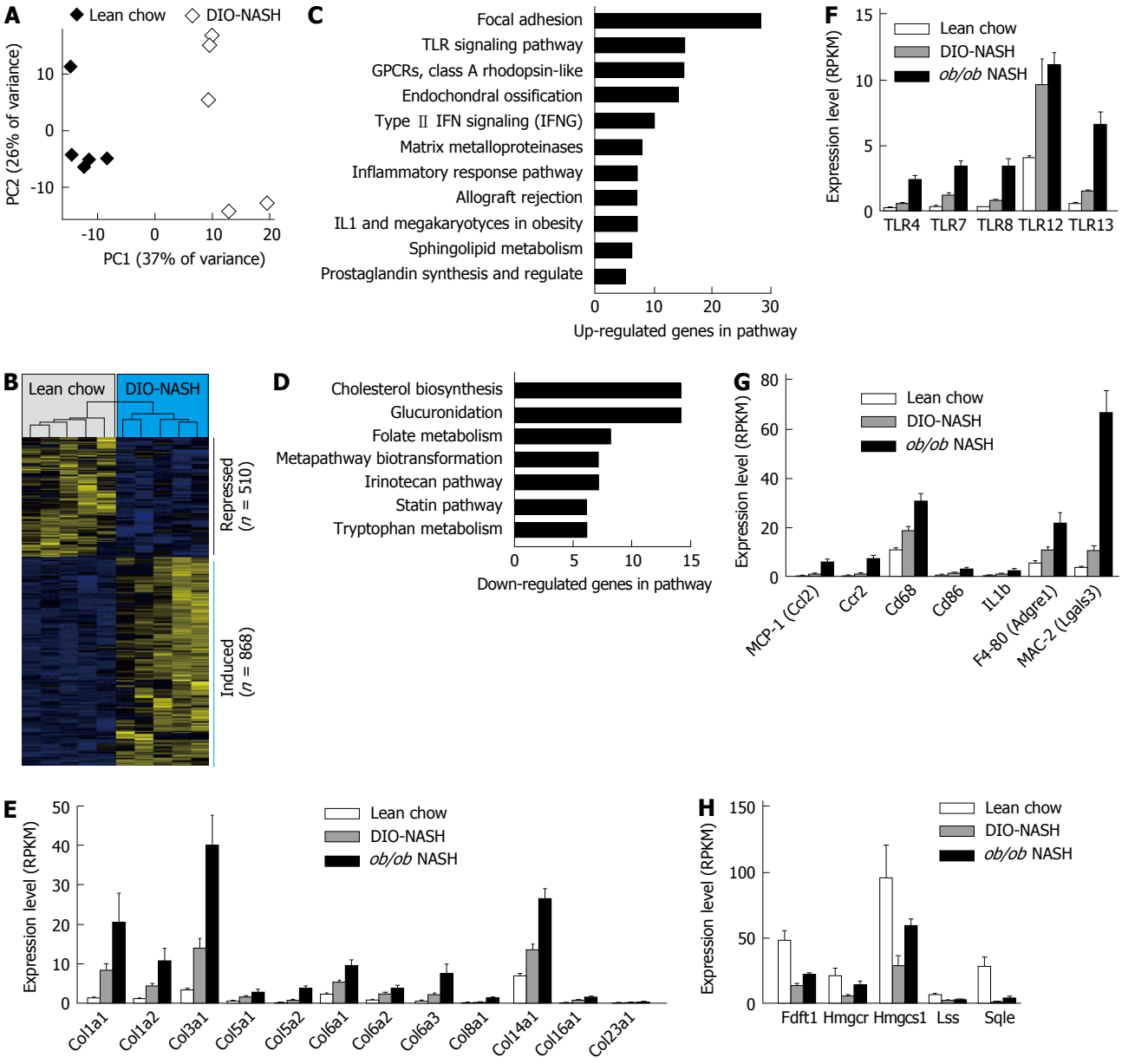

Figure 2 Gene expression analysis by RNAsequencing and bioinformatics. A: Principal component analysis of the 500 most variable genes, [principal component (PC)]; B: Hierarchical clustering of the differentially expressed genes, bars on the right indicate the induced and repressed genes; C: Pathways enriched for the induced (up-regulated) genes, filtered for $P < 0.01$ and $n > 4$; D: Same as (C) for the down-regulated genes; E-H: Expression levels of selected differentially expressed genes shown as mean ± SEM. Lean chow ($n = 5$), DIO-NASH ($n = 5$), *ob/ob* NASH ($n = 5$). NASH: Nonalcoholic steatohepatitis; BW: Body weight; TLR: Toll-like receptor; IFN: Interferon; IL1: Interleukine 1; MCP-1: Monocyte chemoattractant protein-1; GPCRs: G protein-coupled receptors; IFNG: Interferon gamma; RPKM: Reads per kilobase of transcript per million mapped reads; SEM: Standard error of the mean.

when compared to chow (Table 1). Collectively, terminal plasma levels of ALT, AST and TC were markedly elevated in *ob*/*ob*-NASH, when compared to DIO-NASH mice (Table 1).

An OGTT was performed four weeks into the intervention period. Fasting blood glucose and OGTT AUC for blood glucose were unchanged in DIO-NASH and *ob*/*ob*-NASH mice, as compared to respective chow fed animals (Table 1). Diet effects on glycemic status are supported by the elevation in plasma insulin levels of about 3-fold (NS) in DIO-NASH when compared to lean chow, whereas *ob*/*ob*-NASH showed a surprisingly decrease in plasma insulin levels at study end when compared to *ob*/*ob* chow animals (Table 1).

Male C57 and ob/ob mice demonstrated hepatomegaly with increased hepatic lipids and collagen content after AMLN diet-maintenance and repeated dosing intervention period

At study end, terminal liver weight was significantly increased in DIO-NASH and *ob*/*ob*-NASH mice, when compared to respective chow animals (Figure 3A). Additionally, liver weight of *ob*/*ob*-NASH was significantly higher than liver weight of DIO-NASH animals (Figure 3A). Both strains demonstrated increased deposition of liver TG and TC when compared to respective chow mice

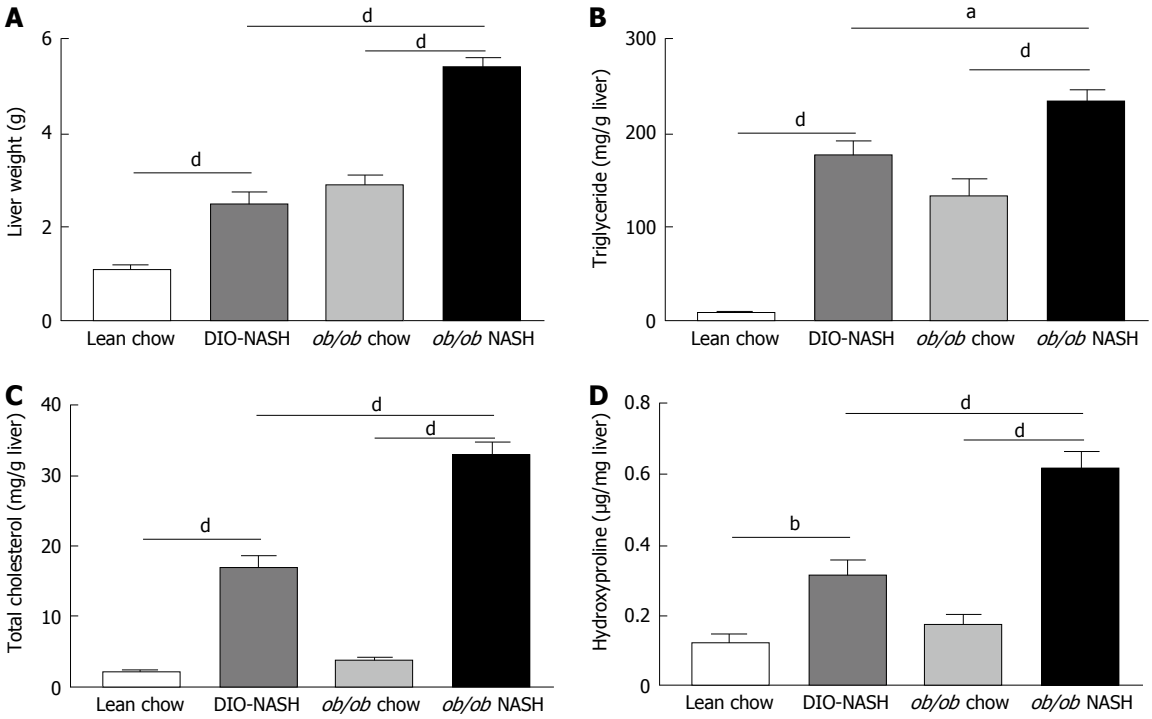

Figure 3 Liver weight and hepatic lipid and collagen content at study end. Liver weight at termination (A), hepatic triglyceride content (B), hepatic total cholesterol content (C), and hepatic hydroxyproline (collagen) content (D) at study end. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{d}P < 0.001$. The results are presented as mean ± SEM. Lean chow (n = 9), DIO-NASH (n = 12), *ob/ob* chow (n = 8), *ob/ob* NASH (n = 10). NASH: Nonalcoholic steatohepatitis; BW: Body weight; SEM: Standard error of the mean.

(Figure 3B and C). Furthermore, liver TG and TC content were significantly increased in *ob*/*ob*-NASH mice, when compared to DIO-NASH animals (Figure 3B and C). Notably, DIO-NASH and *ob*/*ob*-NASH mice showed elevated levels of liver hydroxyproline (collagen) content, when compared to chow controls (Figure 3D). Overall, levels of liver hydroxyproline content were higher in *ob*/*ob*-NASH animals relative to DIO-NASH mice (Figure 3D).

Histopathological scoring of liver steatosis, inflammation and ballooning degeneration after AMLN diet-maintenance and repeated dosing intervention period in male C57 and ob/ob mice

Blinded histological assessment of NAS was performed on hematoxylin and eosin stained terminal hepatic tissue (Table 1). No evidence of steatosis, inflammation and ballooning degeneration was observed in lean chow controls (Figure 4A). In *ob*/*ob* chow mice steatosis was categorized as pronounced microvesicular with mild microvesicular steatosis (Figure 4B). Despite increased steatosis when maintained on chow diet, neither ballooning degeneration nor inflammation was observed in *ob*/*ob* chow animals (Figure 4B). In contrast, DIO-NASH mice developed micro- and macro-vesicular steatosis, with inflammation and ballooning degeneration (Figure 4C). Similarly, *ob*/*ob*-NASH mice developed micro- and macro-vesicular steatosis, and more pronounced inflammation and ballooning degeneration (Figure 4D). Thus, the NASH phenotypes of both strains of mice were clearly reflected in significantly increased NAS, when compared to respective chow animals (Figure 4E). Finally, image analysis confirmed hepatic steatosis in DIO-NASH and *ob*/*ob*-NASH mice (Figure 4F).

Histopathological scoring of liver fibrosis after AMLN diet-maintenance and repeated dosing intervention period in male C57 and ob/ob mice

Fibrosis stage was assessed by blinded histological evaluation using Sirius red staining of terminal liver tissue (Table 1). Hepatic fibrosis was not observed in lean chow or *ob*/*ob* chow mice (Figure 5A and B). In contrast, fibrosis was observed in DIO-NASH mice (Figure 5C) and to a greater extent in *ob*/*ob*-NASH animals who progressed to bridging fibrosis (Figure 5D). Fibrosis was most evident at tissue margins, but also penetrated into the tissue (Figure 5A-D). The fibrotic phenotypes of the DIO-NASH and *ob*/*ob*-NASH mice were mirrored by an increase in fibrosis stage compared to respective chow animals (Figure 5E). Increases in fibrosis stage were reflected by our image analyses showing an increase in % fractional area of Sirius Red (Figure 5F). Notably, the *ob*/*ob*-NASH animals were more fibrotic than DIO-NASH mice (Figure 5E and F).

DISCUSSION

In the present study two obese mouse models of diet-induced NASH were evaluated; the C57 DIO-NASH and the *ob*/*ob*-NASH. We confirm that a diet high in trans-fat, fructose and cholesterol produces a metabolic NASH phenotype with elevated plasma liver enzymes,

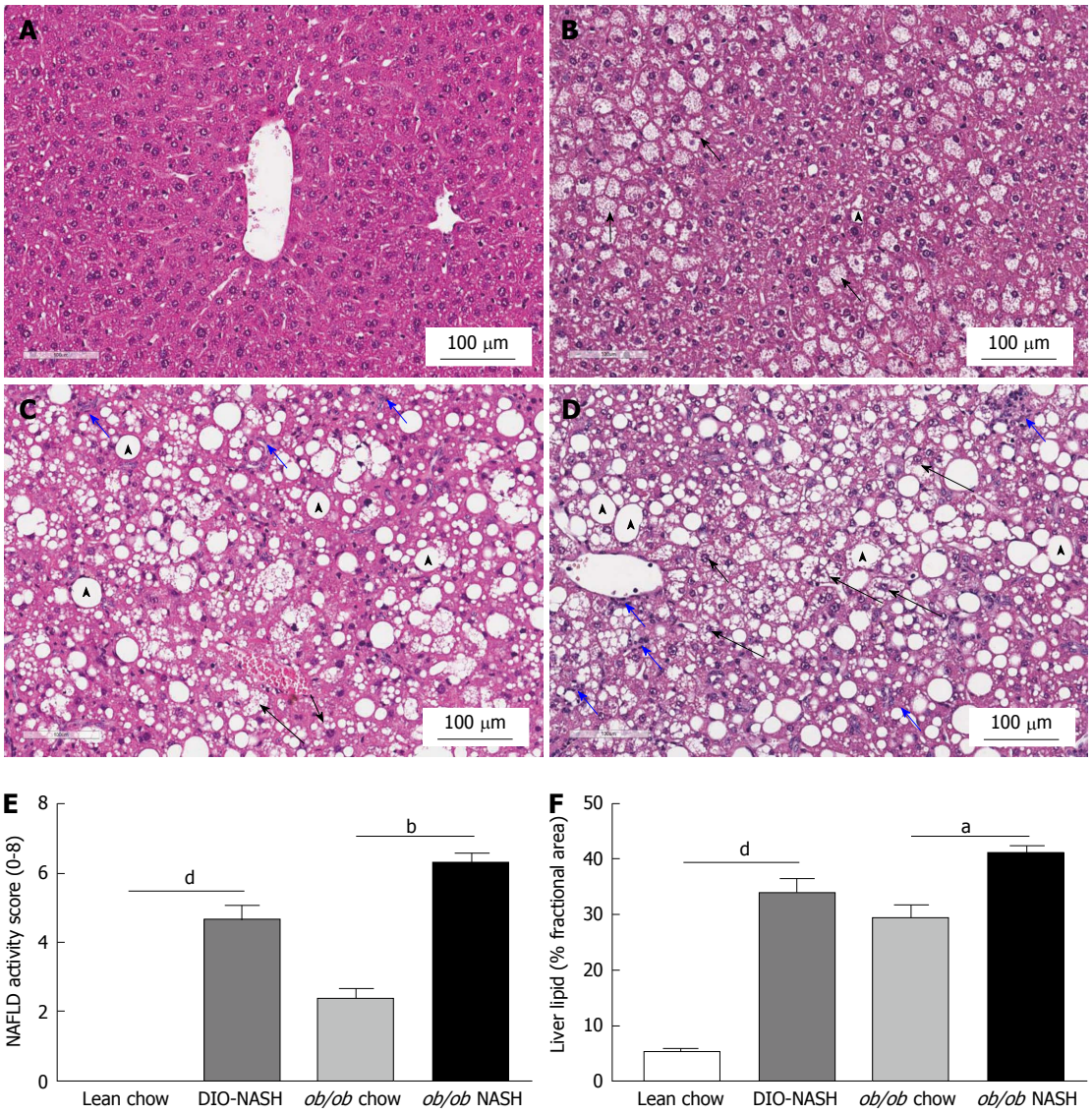

Figure 4 Histological assessment of non-alcoholic fatty liver disease activity score and liver lipid at study end. Representative H and E stained sections from; lean chow (A), *ob/ob* chow (B), DIO-NASH (C) and *ob/ob* NASH (D) mouse models. NAFLD activity score (steatosis, inflammation and ballooning degeneration) performed by a blinded pathologist at study end (E), and quantitatively image analysis of steatosis (% area) from H and E staining using visiomorph software (F). Macrovesicular steatosis indicated by arrowheads, microvesicular steatosis indicated by short black arrows, inflammation indicated by short blue arrows, ballooning degeneration indicated by long black arrows. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{d}P < 0.001$. The results are presented as mean ± SEM. Lean chow (n = 9), DIO-NASH (n = 12), *ob/ob* chow (n = 8), *ob/ob* NASH (n = 10). NASH: Nonalcoholic steatohepatitis; NAFLD: Nonalcoholic fatty liver disease; SEM: Standard error of the mean.

hepatomegaly and recapitulates multiple clinical features including key hallmarks of NASH (steatosis, inflammation, ballooning degeneration and fibrosis). These changes were associated with marked alterations in associated gene expression pathways implicated in NASH and development of fibrosis. The mouse models are also suitable for pharmacological intervention studies, with a paired baseline liver biopsy procedure enabling individual disease stage before a repeated dosing period as is customary in NASH preclinical studies. Whereas all mice experienced slight weight loss following the biopsy, they returned to a weight stable state within one week moreover DIO-NASH and *ob/ob*-NASH sustained hepatomegaly, hepatic steatosis, inflammation, ballooning degeneration and fibrosis following repeated dosing intervention for a total of 8 wk.

DIO-NASH and *ob/ob*-NASH mice developed key hallmarks of fibrotic NASH including marked hepatosteatosis with evident inflammation and ballooning degeneration, as assessed by a clinical-derived histological NAS and fibrosis stage classification system developed by Kleiner *et al*[20]. This is in line with recent findings by Clapper *et al*[6] and Honda *et al*[24] in C57 AMLN mice, thus supporting the wild-type C57 DIO-NASH mouse as a suitable preclinical model for diet-induced obesity and NASH. In addition, the genetically obese ob/ob mouse model was also recently demonstrated to exhibit fibrotic NASH when fed AMLN diet[11,25]. In the leptin-deficient model superimposing the NASH diet high in trans-fat, fructose and cholesterol represents a "second hit" in development of preclinical NASH. The present study demonstrates that the NAS

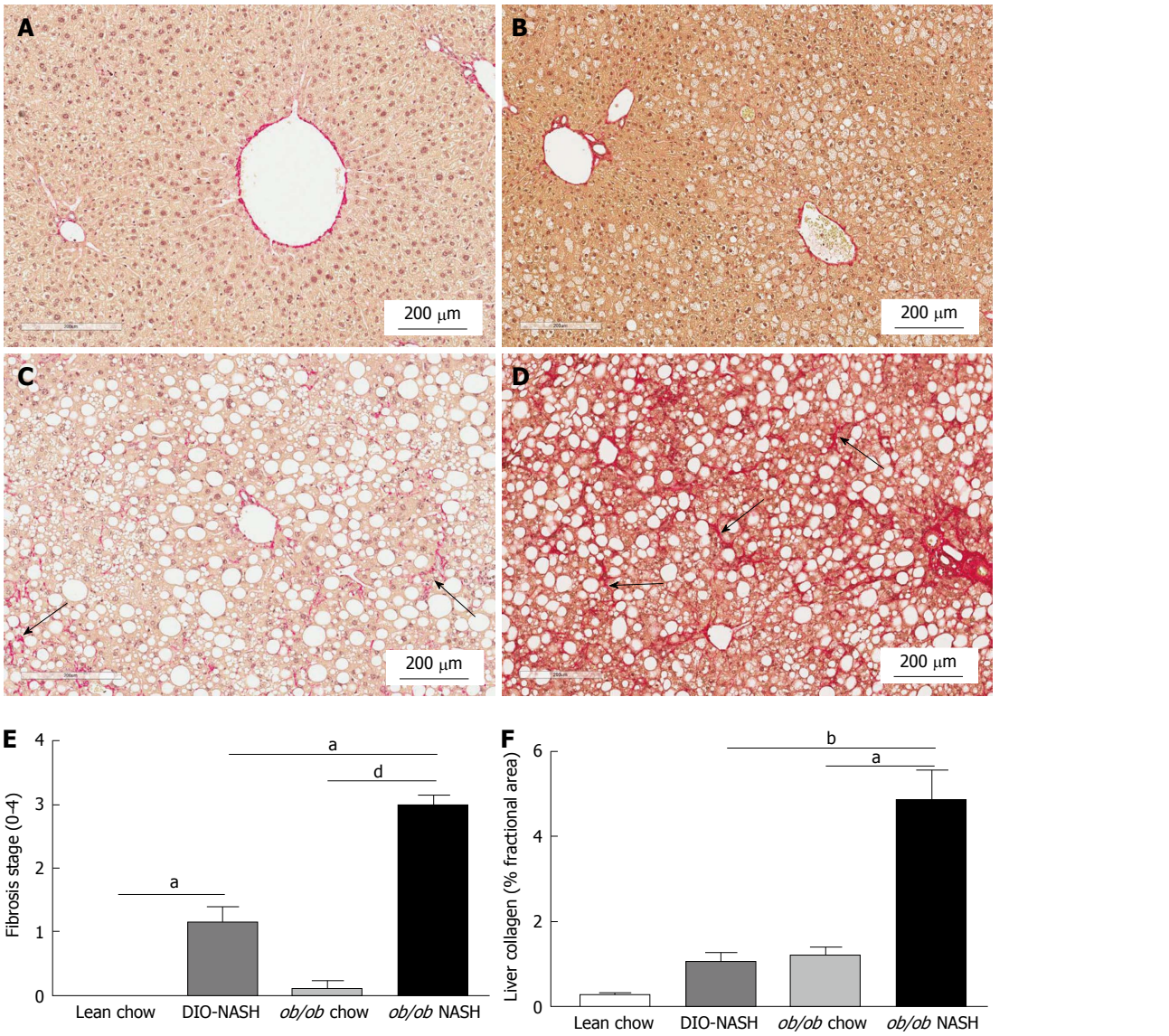

Figure 5 Histological assessment of fibrosis stage and liver collagen at study end. Representative sirius red stained sections from; lean chow (A), *ob/ob* chow (B), DIO-NASH (C) and *ob/ob* NASH (D) mouse model. Liver fibrosis stage performed by a blinded pathologist at study end (E), and quantitatively image analysis of collagen (% area) from sirius red staining using visiomorph software (F). Fibrous band formation indicated by black arrows. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{d}P < 0.001$. The results are presented as mean ± SEM. Lean chow (n = 9), DIO-NASH (n = 12), *ob/ob* chow (n = 8), *ob/ob* NASH (n = 10). NASH: Nonalcoholic steatohepatitis; SEM: Standard error of the mean.

and fibrosis stage can also be integrated in analyses of the *ob*/*ob*-NASH mouse - and in conjunction with the excessive accumulation of hepatic lipids and collagen content - introduces an accelerated and aggressive diet-induced NASH phenotype relative to the C57 DIO-NASH model.

In accordance with histological observations of hepatic inflammation, mRNA analyses revealed within inflammatory pathways that toll-like receptors and downstream pro-inflammatory effectors (*e.g.*, Il1b, MCP-1) were markedly upregulated after the diet-induction period in DIO-NASH and *ob*/*ob*-NASH. In line with previous findings in diet-induced NASH mouse models[26], we observed increased expression of TLR4, a key receptor in fibrogenic development as demonstrated in high-fat diet-induced TLR4 knockout[22], and bile duct ligation models[23]. In addition, TLR4 KO in *ob*/*ob* mice was protective against NASH development as evinced by reduced NAS compared to regular *ob*/*ob* mice[27]. Interestingly, we observed higher expression levels of four additional TLRs: TLR7, TLR8, TLR12 and TLR13, which could be of relevance in future elucidation of the pathogenesis of NASH and for pharmacological intervention.

CCR2 mRNA levels were also increased in *ob*/*ob*-NASH mice. CCR2 has been implicated in the development of liver fibrosis, with $Ccr2^{-/-}$ mice showing reduced fibrosis following bile duct ligation or CCl_4 exposure[28]. CCR2 is a functional receptor for MCP-1, and is involved in the migration of macrophages during obesity[29]. Together, the impact on TLR signaling and macrophage abundance indicates an accelerated inflammatory NASH

phenotype in the *ob*/*ob*-genotype. Furthermore, markers of macrophage infiltration CD68 and F4-80, were up-regulated in DIO-NASH and to a larger extent in *ob*/*ob*-NASH mice, hereby corroborating the histological finding of increased inflammation in the two models.

In accordance with histological observations of hepatic fibrosis, our mRNA analyses also revealed increased expression of fibrillary collagens. Of particular interest is the increased diet- and strain-induced regulation of type Ⅰ, Ⅲ and type Ⅳ collagen, as these are known to be abundantly increased in liver fibrosis[30,31]. Interestingly, we also report altered expression of type Ⅰ collagen α1 and α2 chain, type Ⅲ collagen α1, as well as type XIV collagen α1, which could be of relevance in development/progression from NASH to fibrosis and future design of anti-fibrotic agents.

Pathway analyses also shed light on the transcriptional regulation of the main enzymes involved in triglyceride and cholesterol biosynthesis induced by the AMLN diet in DIO-NASH and *ob*/*ob*-NASH mice. Expression of these enzymes are all regulated by SREBP transcription factors, with SREBP1 regulating triglyceride synthesis and SREBP2 regulating cholesterol synthesis[32]. We report significantly increased levels of total cholesterol in plasma and livers of DIO-NASH and *ob*/*ob*-NASH mice compared to respective chow groups. Interestingly, the gene markers for biosynthesis of cholesterol in the liver appear to be dramatically reduced for DIO-NASH and *ob*/*ob*-NASH, presumably to compensate for the intake of high level of cholesterol in the diet. However, to our surprise the same was not observed for the triglyceride synthesis as the main lipid enzymes showed increased expression (data not shown), albeit plasma levels of triglycerides were significantly decreased for *ob*/*ob*-NASH mice compared to *ob*/*ob* chow. This could be caused by impairment in VLDL secretion from the liver[6], as relative triglyceride content in the liver was significantly increased in livers of *ob*/*ob*-NASH compared to levels in livers from *ob*/*ob* chow. Dysfunctional VLDL synthesis and secretion has been suggested to be a key factor in the progression of simple steatosis to NASH[33]. The mechanism(s) of action involved in the perturbed lipid metabolism awaits further investigations.

In conclusion, the diet-induced DIO-NASH and *ob*/*ob*-NASH mouse models demonstrate metabolic and histological key hallmarks of NASH. A clinically-derived histopathological scoring system can be applied in the DIO-NASH and *ob*/*ob*-NASH mouse models, thereby introducing a preclinical platform for evaluation of novel NASH therapeutics. Finally, a liver biopsy procedure at baseline allows for evaluation of individual disease staging prior to pharmacological intervention hereby reducing biological variability.

COMMENTS

Background

Nonalcoholic steatohepatitis (NASH) is an emerging liver disease with increasing prevalence. There are currently no pharmacological agents specifically approved for the treatment of NASH and disease management is consequently focused on the correction of underlying risk factors such as obesity, insulin resistance and dyslipidemia.

Research frontiers

The lack of approved therapeutics has to some degree been attributed to the failure of animal models to faithfully represent the clinical condition (*e.g.*, disease progression and metabolic background) and the way NASH is assessed clinically (paired biopsies and validated histological methods). Hence, novel diet-induced NASH models that develop the appropriate metabolic phenotype with improved liver sampling methods are highly desirable as a preclinical platform for exploring novel NASH treatments.

Innovations and breakthroughs

The authors describe and characterize a wild-type C57 and a genetically (*ob*/*ob*) obese diet-induced mouse model of NASH and confirm previous findings demonstrating key hallmarks of metabolic deregulation and fibrotic NASH using biochemical, histological and gene expression endpoints. Notably, a liver biopsy-confirmed and clinically-derived histological NASH scoring and fibrosis staging are being performed in *ob*/*ob* mice, which the authors are the first to report. Finally, the utility of the diet-induced NASH mouse models for pharmacological investigations is being demonstrated by performing a chronic intervention period with repeated dosing following a baseline liver biopsy procedure.

Applications

A baseline liver biopsy performed after diet-induction allows for individual disease staging for stratification and randomization into study groups and for evaluation of novel NASH therapeutics.

Peer-review

The results of this study demonstrated a useful animal model for evaluation the disease progression and treatment of NASH. The data were appropriately presented and interpreted. The manuscript was well prepared.

REFERENCES

1 **Ariz U**, Mato JM, Lu SC, Martínez Chantar ML. Nonalcoholic steatohepatitis, animal models, and biomarkers: what is new? *Methods Mol Biol* 2010; **593**: 109-136 [PMID: 19957147 DOI: 10.1007/978-1-60327-194-3]

2 **Adams LA**, Sanderson S, Lindor KD, Angulo P. The histological course of nonalcoholic fatty liver disease: a longitudinal study of 103 patients with sequential liver biopsies. *J Hepatol* 2005; **42**: 132-138 [PMID: 15629518 DOI: 10.1016/j.jhep.2004.09.012]

3 **Zeevaart JG**, Wang L, Thakur VV, Leung CS, Tirado-Rives J, Bailey CM, Domaoal RA, Anderson KS, Jorgensen WL. Optimization of azoles as anti-human immunodeficiency virus agents guided by free-energy calculations. *J Am Chem Soc* 2008; **130**: 9492-9499 [PMID: 18588301 DOI: 10.1021/ja8019214]

4 **Farrell GC**, Larter CZ. Nonalcoholic fatty liver disease: from steatosis to cirrhosis. *Hepatology* 2006; **43**: S99-S112 [PMID: 16447287 DOI: 10.1002/hep.20973]

5 **Day CP**, James OF. Steatohepatitis: a tale of two "hits"? *Gastroenterology* 1998; **114**: 842-845 [PMID: 9547102 DOI: 10.1016/S0016-5085(98)70599-2]

6 **Clapper JR**, Hendricks MD, Gu G, Wittmer C, Dolman CS, Herich J, Athanacio J, Villescaz C, Ghosh SS, Heilig JS, Lowe C, Roth JD. Diet-induced mouse model of fatty liver disease and nonalcoholic steatohepatitis reflecting clinical disease progression and methods of assessment. *Am J Physiol Gastrointest Liver Physiol* 2013; **305**: G483-G495 [PMID: 23886860 DOI: 10.1152/ajpgi.00079.2013]

7 **Sanches SC**, Ramalho LN, Augusto MJ, da Silva DM, Ramalho FS. Nonalcoholic Steatohepatitis: A Search for Factual Animal Models. *Biomed Res Int* 2015; **2015**: 574832 [PMID: 26064924 DOI: 10.1155/2015/574832]

8 **Machado MV**, Michelotti GA, Xie G, Almeida Pereira T, Boursier

J, Bohnic B, Guy CD, Diehl AM. Mouse models of diet-induced nonalcoholic steatohepatitis reproduce the heterogeneity of the human disease. *PLoS One* 2015; **10**: e0127991 [PMID: 26017539 DOI: 10.1371/journal.pone.0127991]

9 **Takahashi Y**, Soejima Y, Fukusato T. Animal models of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. *World J Gastroenterol* 2012; **18**: 2300-2308 [PMID: 22654421 DOI: 10.3748/wjg.v18.i19.2300]

10 **Starkel P**, Leclercq IA. Animal models for the study of hepatic fibrosis. *Best Pract Res Clin Gastroenterol* 2011; **25**: 319-333 [PMID: 21497748 DOI: 10.1016/j.bpg.2011.02.004]

11 **Trevaskis JL**, Griffin PS, Wittmer C, Neuschwander-Tetri BA, Brunt EM, Dolman CS, Erickson MR, Napora J, Parkes DG, Roth JD. Glucagon-like peptide-1 receptor agonism improves metabolic, biochemical, and histopathological indices of nonalcoholic steatohepatitis in mice. *Am J Physiol Gastrointest Liver Physiol* 2012; **302**: G762-G772 [PMID: 22268099 DOI: 10.1152/ajpgi.00476.2011]

12 **Tetri LH**, Basaranoglu M, Brunt EM, Yerian LM, Neuschwander-Tetri BA. Severe NAFLD with hepatic necroinflammatory changes in mice fed trans fats and a high-fructose corn syrup equivalent. *Am J Physiol Gastrointest Liver Physiol* 2008; **295**: G987-G995 [PMID: 18772365 DOI: 10.1152/ajpgi.90272.2008]

13 **Nakayama H**, Otabe S, Ueno T, Hirota N, Yuan X, Fukutani T, Hashinaga T, Wada N, Yamada K. Transgenic mice expressing nuclear sterol regulatory element-binding protein 1c in adipose tissue exhibit liver histology similar to nonalcoholic steatohepatitis. *Metabolism* 2007; **56**: 470-475 [PMID: 17379003 DOI: 10.1016/j.metabol.2006.11.004]

14 **Chen H**, Charlat O, Tartaglia LA, Woolf EA, Weng X, Ellis SJ, Lakey ND, Culpepper J, Moore KJ, Breitbart RE, Duyk GM, Tepper RI, Morgenstern JP. Evidence that the diabetes gene encodes the leptin receptor: identification of a mutation in the leptin receptor gene in db/db mice. *Cell* 1996; **84**: 491-495 [PMID: 8608603 DOI: 10.1016/S0092-8674(00)81294-5]

15 **Dobin A**, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, Batut P, Chaisson M, Gingeras TR. STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* 2013; **29**: 15-21 [PMID: 23104886 DOI: 10.1093/bioinformatics/bts635]

16 **Anders S**, Pyl PT, Huber W. HTSeq--a Python framework to work with high-throughput sequencing data. *Bioinformatics* 2015; **31**: 166-169 [PMID: 25260700 DOI: 10.1101/002824]

17 **Robinson MD**, McCarthy DJ, Smyth GK. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* 2010; **26**: 139-140 [PMID: 19910308 DOI: 10.1093/bioinformatics/btp616]

18 **Kutmon M**, Riutta A, Nunes N, Hanspers K, Willighagen EL, Bohler A, Mélius J, Waagmeester A, Sinha SR, Miller R, Coort SL, Cirillo E, Smeets B, Evelo CT, Pico AR. WikiPathways: capturing the full diversity of pathway knowledge. *Nucleic Acids Res* 2016; **44**: D488-D494 [PMID: 26481357 DOI: 10.1093/nar/gkv1024]

19 **Kutmon M**, van Iersel MP, Bohler A, Kelder T, Nunes N, Pico AR, Evelo CT. PathVisio 3: an extendable pathway analysis toolbox. *PLoS Comput Biol* 2015; **11**: e1004085 [PMID: 25706687 DOI: 10.1371/journal.pcbi.1004085]

20 **Kleiner DE**, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, Ferrell LD, Liu YC, Torbenson MS, Unalp-Arida A, Yeh M, McCullough AJ, Sanyal AJ. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* 2005; **41**: 1313-1321 [PMID: 15915461 DOI: 10.1002/hep.20701]

21 **Wang Z**, Gerstein M, Snyder M. RNA-Seq: a revolutionary tool for transcriptomics. *Nat Rev Genet* 2009; **10**: 57-63 [PMID: 19015660 DOI: 10.1038/nrg2484]

22 **Sutter AG**, Palanisamy AP, Lench JH, Esckilsen S, Geng T, Lewin DN, Cowart LA, Chavin KD. Dietary Saturated Fat Promotes Development of Hepatic Inflammation Through Toll-Like Receptor 4 in Mice. *J Cell Biochem* 2016; **117**: 1613-1621 [PMID: 26600310 DOI: 10.1002/jcb.25453]

23 **Seki E**, De Minicis S, Osterreicher CH, Kluwe J, Osawa Y, Brenner DA, Schwabe RF. TLR4 enhances TGF-beta signaling and hepatic fibrosis. *Nat Med* 2007; **13**: 1324-1332 [PMID: 17952090 DOI: 10.1038/nm1663]

24 **Honda Y**, Imajo K, Kato T, Kessoku T, Ogawa Y, Tomeno W, Kato S, Mawatari H, Fujita K, Yoneda M, Saito S, Nakajima A. The Selective SGLT2 Inhibitor Ipragliflozin Has a Therapeutic Effect on Nonalcoholic Steatohepatitis in Mice. *PLoS One* 2016; **11**: e0146337 [PMID: 26731267 DOI: 10.1371/journal.pone.0146337]

25 **Griffett K**, Welch RD, Flaveny CA, Kolar GR, Neuschwander-Tetri BA, Burris TP. The LXR inverse agonist SR9238 suppresses fibrosis in a model of non-alcoholic steatohepatitis. *Mol Metab* 2015; **4**: 353-357 [PMID: 25830098 DOI: 10.1016/j.molmet.2015.01.009]

26 **Rivera CA**, Adegboyega P, van Rooijen N, Tagalicud A, Allman M, Wallace M. Toll-like receptor-4 signaling and Kupffer cells play pivotal roles in the pathogenesis of non-alcoholic steatohepatitis. *J Hepatol* 2007; **47**: 571-579 [PMID: 17644211 DOI: 10.1016/j.jhep.2007.04.019]

27 **Sutter AG**, Palanisamy AP, Lench JH, Jessmore AP, Chavin KD. Development of steatohepatitis in Ob/Ob mice is dependent on Toll-like receptor 4. *Ann Hepatol* 2015; **14**: 735-743 [PMID: 26256903]

28 **Seki E**, de Minicis S, Inokuchi S, Taura K, Miyai K, van Rooijen N, Schwabe RF, Brenner DA. CCR2 promotes hepatic fibrosis in mice. *Hepatology* 2009; **50**: 185-197 [PMID: 19441102 DOI: 10.1002/hep.22952.CCR2]

29 **Weisberg SP**, Hunter D, Huber R, Lemieux J, Slaymaker S, Vaddi K, Charo I, Leibel RL, Ferrante AW. CCR2 modulates inflammatory and metabolic effects of high-fat feeding. *J Clin Invest* 2006; **116**: 115-124 [PMID: 16341265 DOI: 10.1172/JCI24335]

30 **Bataller R**, Brenner DA. Liver fibrosis. *J Clin Invest* 2005; **115**: 209-218 [PMID: 15690074 DOI: 10.1172/JCI200524282]

31 **Friedman SL**. Mechanisms of hepatic fibrogenesis. *Gastroenterology* 2008; **134**: 1655-1669 [PMID: 18471545 DOI: 10.1053/j.gastro.2008.03.003]

32 **Horton JD**, Goldstein JL, Brown MS. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. *J Clin Invest* 2002; **109**: 1125-1131 [PMID: 11994399 DOI: 10.1172/JCI200215593]

33 **Fujita K**, Nozaki Y, Wada K, Yoneda M, Fujimoto Y, Fujitake M, Endo H, Takahashi H, Inamori M, Kobayashi N, Kirikoshi H, Kubota K, Saito S, Nakajima A. Dysfunctional very-low-density lipoprotein synthesis and release is a key factor in nonalcoholic steatohepatitis pathogenesis. *Hepatology* 2009; **50**: 772-780 [PMID: 19650159 DOI: 10.1002/hep.23094]

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ORIGINAL ARTICLE

Retrospective Study

Hepatocellular carcinoma after locoregional therapy: Magnetic resonance imaging findings in falsely negative exams

David Becker-Weidman, Jesse M Civan, Sandeep P Deshmukh, Christopher G Roth, Steven K Herrine, Laurence Parker, Donald G Mitchell

David Becker-Weidman, Sandeep P Deshmukh, Christopher G Roth, Laurence Parker, Donald G Mitchell, Department of Radiology, Thomas Jefferson University, Philadelphia, PA 19107, United States

Jesse M Civan, Steven K Herrine, Division of Gastroenterology and Hepatology, Department of Medicine, Thomas Jefferson University, Philadelphia, PA 19107, United States

Author contributions: Becker-Weidman D designed the study, collected the data, and drafted the manuscript; Civan JM developed the concept, collected the data and drafted the manuscript; Deshmukh SP and Roth CG interpreted MRI images and edited the manuscript; Herrine SK developed the concept and edited the manuscript; Parker L performed statistical analysis and edited the manuscript; Mitchell DG developed the concept, interpreted MRI images, and edited the manuscript.

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Correspondence to: Donald G Mitchell, MD, Department of Radiology, Thomas Jefferson University, 132 S 10th St, Main Building, Room 1094, Philadelphia, PA 19107, United States. donald.mitchell@jefferson.edu
Telephone: +1-215-9554809
Fax: +1-215-9558270

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Abstract

AIM: To elucidate causes for false negative magnetic resonance imaging (MRI) exams by identifying imaging characteristics that predict viable hepatocellular carcinoma (HCC) in lesions previously treated with locoregional therapy when obvious findings of recurrence are absent.

METHODS: This retrospective institutional review board-approved and Health Insurance Portability and Accountability Act-compliant study included patients who underwent liver transplantation at our center between 1/1/2000 and 12/31/2012 after being treated for HCC with locoregional therapy. All selected patients had a contrast-enhanced MRI after locoregional therapy within 90 d of transplant that was prospectively interpreted as without evidence of residual or recurrent

tumor. Retrospectively, 2 radiologists, blinded to clinical and pathological data, independently reviewed the pre-transplant MRIs for 7 imaging features. Liver explant histopathology provided the reference standard, with clinically significant tumor defined as viable tumor ⩾ 1.0 cm in maximum dimension. Fisher's exact test was first performed to identify significant imaging features.

RESULTS: Inclusion criteria selected for 42 patients with 65 treated lesions. Fourteen of 42 patients (33%) and 16 of 65 treated lesions (25%) had clinically significant viable tumor on explant histology. None of the 7 imaging findings examined could reliably and reproducibly determine which treated lesion had viable tumor when the exam had been prospectively read as without evidence of viable HCC.

CONCLUSION: After locoregional therapy some treated lesions that do not demonstrate any MRI evidence of HCC will contain viable tumor. As such even patients with a negative MRI following treatment should receive regular short-term imaging surveillance because some have occult viable tumor. The possibility of occult tumor should be a consideration when contemplating any action which might delay liver transplant.

Key words: Hepatocellular carcinoma; Transarterial chemoembolization; Tumor recurrence; Locoregional therapy; Imaging surveillance

Core tip: Hepatocellular carcinoma (HCC) is often treated with locoregional therapy such as transarterial chemoembolization as a bridge to transplantation. Detecting residual or recurrent tumor within these treated lesions is challenging and some treated lesions that do not demonstrate any magnetic resonance imaging (MRI) evidence of HCC will contain foci of viable tumor. Regular, short-term imaging surveillance is clinically important for patients being considered for liver transplantation even when prior MRIs have been negative and the possibility of a false negative MRI exam needs to be considered when managing these patients.

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INTRODUCTION

The American College of Radiology developed the liver imaging reporting and data system (LI-RADS) to standardize how hepatocellular carcinoma (HCC) is diagnosed[1]. These criteria were validated in untreated lesions and therefore do not apply to lesions after treatment with locoregional therapy. Although certain imaging findings are associated with the presence of viable HCC in a treated lesion there is currently no formal system to assess the probability of viable tumor.

Magnetic resonance imaging (MRI) is commonly used status post locoregional therapy to evaluate for recurrent or residual viable tumor. Because the hallmark of HCC is avid arterial-phase enhancement, dynamic imaging following gadolinium-based contrast administration should be a core component of the examination. Arterial-phase enhancement following locoregional therapy has a reported sensitivity and specificity of 82% to 100% and 79% to > 90% respectively[2,3]. Subtle arterial-phase enhancement can be obscured in treated lesions as they often demonstrate heterogeneous high signal on T1-weighted images due to the presence of blood products (Figure 1). HCC is a very cellular tumor[4] and will typically restrict the diffusion of water molecules giving it high signal on diffusion-weighted imaging (DWI) and corresponding low signal on the computer generated apparent diffusion coefficient map. Diffusion restriction following locoregional therapy has a reported sensitivity and specificity of 61% to 75% and 88% to > 90% respectively[2,3]. Identifying restricted diffusion in treated areas is complicated by the fact that these areas typically demonstrate high signal on T2-weighted images due to fibrosis (Figure 2), appearing as T2 shine through on DWI. Signal intensity on T2-weighted images is not typically helpful as it is affected by treatment and can be variable, although it is typically mildly to moderately hyperintense. Signal intensity on precontrast T1-weighted images is quite variable and generally not helpful. HCC is usually hypointense or isointense but can be hyperintense with intratumoral fat.

Unresectable HCC is often treated with locoregional therapy to decrease disease burden and as a bridge to transplant. In these patients accurate assessment of tumor response is integral to directing patient care. False negative MRI exams are due to a number of factors including technical limitations and inherent MR signal alteration of the treated areas. In addition there is no formal system for evaluating treated lesions. The goal of this study was to retrospectively determine which MRI features were most predictive of histological findings of residual or recurrent HCC in a population that does not demonstrate obvious recurrence.

MATERIALS AND METHODS

Patient and lesion inclusion criteria

This retrospective study was approved by our institutional review board and was compliant with the Health Insurance Portability and Accountability Act. Our study included patients with HCC who underwent liver transplantation at our center between 1/1/2000 and 12/31/2012 after being treated with locoregional therapy. Inclusion criteria selected patients that had a

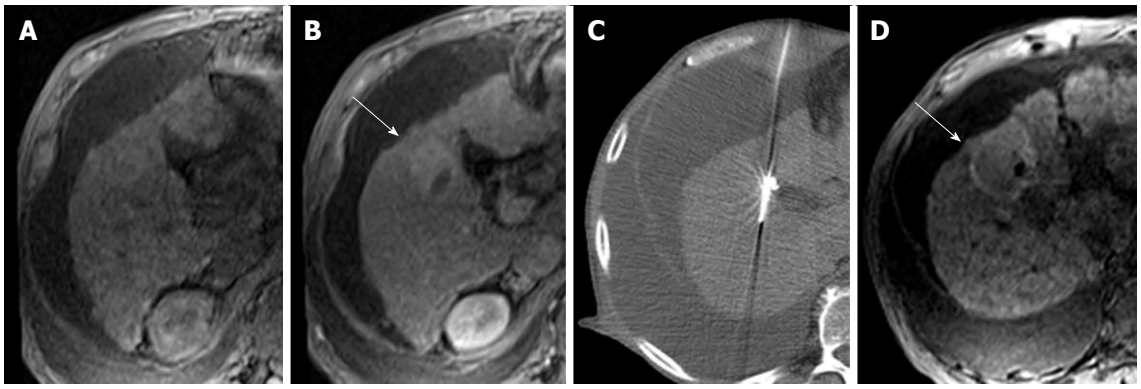

Figure 1 Treatment-related signal alterations on T1-weighted images. A 59-year-old man with HCV cirrhosis and a segment Ⅴ LI-RADS 5B lesion measuring up to 3.6 cm. Fat-saturated T1-weighted precontrast (A) and arterial-phase (B) images demonstrate a 3.6 cm enhancing focus of viable tumor (B, arrow) within an area previously treated with TACE. Mass could not be visualized upon selective angiography so repeat TACE was not performed. Non-contrast CT from a radiofrequency ablation procedure (C) demonstrates an electrode positioned within the tumor. One month later a fat-saturated T1-weighted precontrast image (D) demonstrates peripheral high signal related to hemorrhage from coagulative necrosis (D, arrow). LI-RADS: Liver imaging reporting and data system; TACE: Transarterial chemoembolization; HCV: Hepatitis C virus.

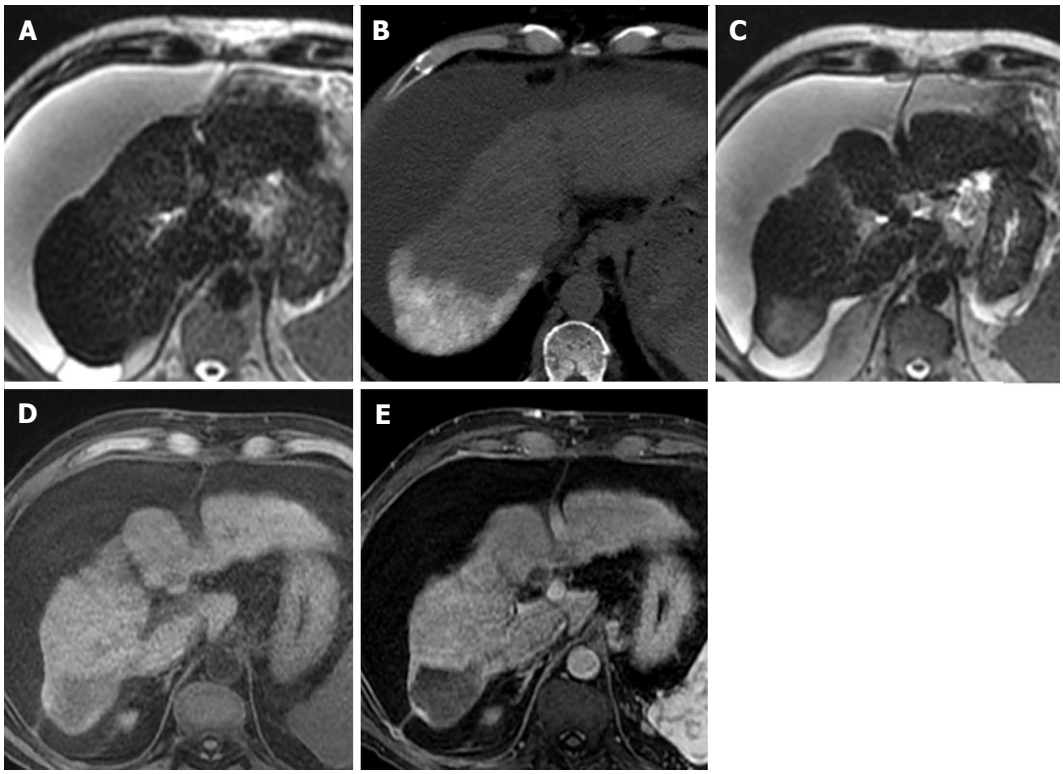

Figure 2 Treatment-related signal alterations on T2-weighted images. A 63-year-old man with HCV/EtOH cirrhosis and a segment Ⅶ LI-RADS 5B lesion measuring 2.4 cm × 1.8 cm. T2-weighted image (A) just superior to the segment Ⅵ lesion before treatment demonstrates a cirrhotic liver with homogeneous low signal intensity. Non-contrast CT (B) performed immediately after a TACE procedure shows high attenuation Lipiodol® in segment Ⅶ confirming that the appropriate segment was treated. T2-weighted image (C) from an MRI performed one month later shows high signal intensity in the area that was treated due to fibrotic change. Precontrast (D) and arterial-phase images (E) from that exam demonstrate that the treated area is completely necrotic. LI-RADS: Liver imaging reporting and data system; TACE: Transarterial chemoembolization; HCV: Hepatitis C virus; CT: Computed tomography; MRI: Magnetic resonance imaging.

contrast-enhanced MRI after locoregional therapy within 90 d of transplant that was prospectively interpreted as without evidence of residual or recurrent tumor. Patients were identified through our electronic medical record.

While HCC is a radiologic diagnosis, subcentimeter lesions cannot be designated as "definite" HCC by either the American Association for the Study of Liver Diseases or the LI-RADS criteria in recognition of the fact that early tumors may not demonstrate hypervascularity and technical limitations preclude adequate assessment of lesions below this threshold[1,5]. Therefore, we considered foci of HCC identified on explant significant for the purpose of our study only if it measured ⩾ 1.0 cm in maximal diameter.

Foci of viable tumor detected histologically on explant, distinct from a previously treated lesion were

not included in our analysis. These foci were treated as incidental findings as our current analysis regards the MRI findings in lesions previously identified as HCC subsequently undergoing treatment.

MR image analysis

All MRI data sets were reviewed on a workstation equipped with image review software (iSite, version 3.6; Philips, Andover, MA). Retrospective image interpretations were performed independently by two body MRI specialists, each with more than 10 years of experience. The study coordinator, a radiology resident, prepared the exams for review by correlating the lesions described in the explant pathology report with the treated lesions on the MRI, marking the lesions to be evaluated with an arrow. Exams were reviewed in random order by the interpreting radiologists, who were blinded to all other patient history, including pathology and other imaging results.

Each liver lesion was assessed by the interpreting radiologist for the presence or absence of: Arterial-phase nodular enhancement, arterial-phase non-nodular enhancement, gradual enhancement, partial or complete T1 signal hypointensity, partial or complete T2 signal hyperintensity, lipid as determined by comparison of in-phase and opposed-phase images, and restricted diffusion if DWI was performed. Findings were recorded in prepared data sheets.

Gross pathology and histopathologic analysis

All explanted livers were received as surgical resection specimens. Each explant was serially sectioned in contiguous slices at 5 mm intervals, and processed for routine Hematoxylin and Eosin stains. These slides were prospectively reviewed for the presence of viable HCC and the pathology report produced was used to retrospectively correlate the histologic findings with the pretransplant MRI.

Statistical analysis

Statistical review of the study was performed by a biomedical statistician. Statistical software (SAS version 9.4; SAS Institute, Cary, NC) was used for all data analysis. Fisher's exact test was first performed to identify significant imaging features in a bivariate analysis followed by a step-wise logistic regression if more than one variable was significant. The significance threshold was set at a *P*-value of 0.05 and any variable with $P > 0.05$ was removed from the model and determined to be insignificant. The agreement level between readers was measured by using *k* coefficient. We defined *k* values for level of agreement as follows: 0.81-0.99, almost perfect agreement; 0.61-0.80, substantial agreement; 0.41-0.60, moderate agreement; 0.21-0.40, fair agreement; and 0.01-0.20, slight agreement[6].

RESULTS

Patients

A search of our electronic medical record showed that 488 patients had a liver transplant at our center between 1/1/2000 and 12/31/2012, of which 167 (34.2%) had HCC, all of whom were treated with locoregional therapy prior to transplant. Of these patients, 84 (50.3%) had findings suspicious for recurrent or residual HCC on the pre-transplant MRI, 24 (14.4%) underwent locoregional treatment between the pre-transplant MRI and transplant, 16 (9.6%) had the pre-transplant MRI over 90 d before transplant, and 1 (0.6%) did not receive intravenous contrast and were excluded from our study. Patient accrual details are presented in Figure 3. The final cohort of 42 patients (mean age, 59 years; age range, 46-73 years) included 34 men (mean age, 59 years; age range, 46-73 years) and 8 women (mean age, 59 years; age range, 53-70 years). Patients had cirrhosis secondary to hepatitis C ($n = 29$), hepatitis C and alcohol abuse ($n = 5$), alcohol abuse ($n = 4$), nonalcoholic steatohepatitis ($n = 1$), or an unknown cause ($n = 3$). MRI was performed an average of 40 d before transplant (range, 1-89 d).

Prior to transplant 33 (79%) patients were treated with transarterial chemoembolization (TACE) only, 3 (7%) were treated with radiofrequency ablation only, 2 (5%) were treated with radioactive embolization only, 1 (2%) was treated with bland transarterial embolization only, and 3 (7%) were treated with TACE and radiofrequency ablation.

Reference histopathologic analysis

The 42 patients who met our inclusion criteria included 18 (43%) who had no viable tumor, 10 (24%) who had viable tumor that was considered clinically insignificant, and 14 (33%) who had at least one focus of clinically-significant viable tumor on explant pathology. Two patients had two foci of clinically-significant viable tumor. The explant Pathology report mentioned a single lesion in 27 patients (64%), 2 lesions in 9 patients (21%), 3 lesions in 3 patients (7%), 4 lesions in 2 patients (5%), and 5 lesions in 1 patient (3%) for a total of 65 treated lesions. Sixteen treated lesions (25%) had clinically significant viable tumor (mean size, 1.5 cm; range, 1.0-3.5 cm), 13 treated lesions (20%) had a focus of tumor < 1.0 cm (mean size, 0.5 cm; range, 0.1-0.9 cm), and 36 (55%) treated lesions had no viable tumor.

MR image and statistical analysis

Of the 42 patients, 15 received gadoextate disodium (Eovist) (36%), 13 received gadopentate dimeglumine (Magnevist) (31%), 8 received gadobutrol (Gadavist) (19%), and 6 received gadobenate dimeglumine (MultiHance) (14%). DWI was only performed in 19 patients (45%) as DWI was not included as a part of our routine MRI exam of the abdomen until 2011.

For reader #1 "arterial-phase non-nodular enhancement" and "partial or complete T1 signal hypointensity" were significant predictors of viable tumor. For reader #2 "partial or complete T2 signal hyperintensity" was the sole significant predictor of viable tumor.

Table 1 *P*-values and Kappa values associated with the 7 different imaging features

	P-values		Kappa values
	Reader #1	Reader #2	
Arterial-phase nodular enhancement	0.44	0.14	0.37 (fair agreement)
Arterial-phase non-nodular enhancement	0.25	0.008[b]	0.23 (fair agreement)
Gradual enhancement	0.47	0.15	0.10 (slight agreement)
Partial or complete T1 signal hypointensity	0.21	0.001[b]	0.15 (slight agreement)
Partial or complete T2 signal hyperintensity	0.047[a]	0.47	0.07 (slight agreement)
Lipid	0.56	0.44	-0.03 (no agreement)
Restricted diffusion	N/A[1]	N/A[1]	N/A[1]

[a,b]*P*-values reached significance; [1]Could not be assessed due to collinearity. N/A: Not applicable.

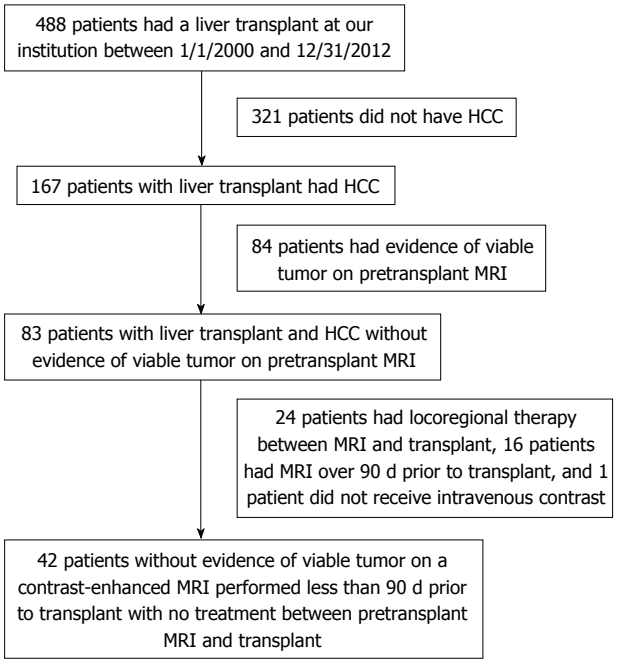

Figure 3 Patient accrual flowchart. HCC: Hepatocellular carcinoma; MRI: Magnetic resonance imaging.

There was fair agreement for arterial-phase nodular enhancement and non-nodular enhancement (k = 0.37, 0.23 respectively); slight agreement for gradual enhancement, partial or complete T1 signal hypointensity, and partial or complete T2 signal hyperintensity (k = 0.07, 0.10, 0.15 respectively); and no agreement for the presence of lipid (k = -0.03). The *P*-values and kappa values are presented in Table 1.

Patient outcomes

There was a single post-transplant recurrence in the 18 patients without viable tumor (mean length of followup, 4.9 years; range, 1.1-9.0 years). There was a single recurrence in the 10 patients with clinically insignificant cancer (mean length of followup, 5.2 years; range, 2.6-13.4 years). There was a single recurrence in the 14 patients with clinically significant viable tumor (mean length of followup, 3.4 years; range, 0.2-7.1 years). One patient with only 0.2 years of followup died from a stroke.

DISCUSSION

Retrospective review of true negative and false negative MR exams did not identify any subtle findings that can reliably and reproducibly indicate the presence of viable HCC in studies that were prospectively interpreted as negative. T1 and T2 signal intensity are highly variable after locoregional therapy and are not reliable indicators of viable tumor. Delayed enhancement is often seen after treatment and indicates fibrosis. Arterial-phase enhancement is associated with viable tumor but may be subtle or absent and has a reported sensitivity of as low as 82%[2]. In other words, some patients who do not have evidence of HCC on MRI will have viable tumor on explant pathology.

A limitation of this study was the low level of agreement between readers. This can be partially explained due to the low number of "positive" imaging features. When there is a low base rate a small number of discordant findings will have a disproportionately large effect on Cohen's kappa coefficient[7]. Agreement regarding enhancement characteristics is only slight to fair because any case that demonstrated obvious enhancement was prospectively read as suspicious for viable HCC and excluded from our study. The only cases that remained were those that demonstrated subtle enhancement. Agreement for signal intensity on T1- and T2-weighted images is only slight due to the inherent difficulty in classifying a highly heterogeneous area. That being said the low kappa value limits the value and reliability of any imaging finding that was positively associated with viable HCC. Therefore, we do not propose that signal intensity on unenhanced T1-weighted or T2-weighted images is predictive of viable tumor. It is possible that non-nodular arterial-phase enhancement is predictive of viable tumor but this finding was not reliable enough in our study for clinical use.

Another limitation was the inconsistency of the explant pathology reports. Some pathology reports measured the size of the viable component or state the percentage of necrosis within the measured treated lesion, whereas, other reports used subjective terminology such as "partially necrotic" or "largely necrotic" which made exact measurement of the viable component difficult. Another problem was that some of

the treated lesions demonstrated partial diffuse necrosis and contained only microscopic islands of tumor. These lesions are considered viable by histology but impossible to identify by imaging.

Noting the limitations above it is clear that occasionally treated lesions without evidence of viable HCC by MRI can contain foci of viable tumor. This supports the utilization of regular short-term imaging surveillance even when prior MRIs have been negative and is clinically important for patients being considered for liver transplantation. For example, a decision to delay transplant to allow treatment of underlying chronic viral hepatitis C should be made with caution, without over-reliance on a sense of security suggested by surveillance imaging with no definite evidence of viable HCC. The possibility of a false negative MRI exam needs to be considered when managing patients after locoregional therapy.

COMMENTS

Background

Unresectable hepatocellular carcinoma (HCC) is often treated with locoregional therapy to decrease disease burden and as a bridge to transplant. After locoregional therapy magnetic resonance imaging (MRI) interpretation can be more difficult due to a number of factors. Several imaging findings have been shown to correlate with the presence of viable HCC in this setting including diffusion restriction and arterial-phase enhancement.

Research frontiers

Liver imaging reporting and data system (LI-RADS) was not developed to be applied to treated lesions and as such theses lesions are designated "LR-treated". Further investigation into the imaging characteristics of treated lesions could lead to the development of a version of LI-RADS that can be applied to these lesions.

Innovations and breakthroughs

In this study, the authors demonstrate that treated lesions can harbor foci of viable HCC but demonstrate no MRI findings.

Applications

The research supports the utilization of regular short-term imaging surveillance in patients with HCC treated with locoregional therapy even when prior MRIs have been negative due to the possibility of a false negative exam.

Terminology

Diffusion-weighted imaging: MRI sequence that measures random Brownian motion of water molecules within a voxel of tissue; LI-RADS: Set of terminology developed by the American College of Radiology to standardize the reporting of imaging findings of liver lesions; Locoregional therapy: Transarterial and/or local ablative therapy.

Peer-review

This is an interesting manuscript providing information for an easily neglected field. It is thus of value to be considered for publication.

REFERENCES

1 **Mitchell DG**, Bruix J, Sherman M, Sirlin CB. LI-RADS (Liver Imaging Reporting and Data System): summary, discussion, and consensus of the LI-RADS Management Working Group and future directions. *Hepatology* 2015; **61**: 1056-1065 [PMID: 25041904 DOI: 10.1002/hep.27304]

2 **Goshima S**, Kanematsu M, Kondo H, Yokoyama R, Tsuge Y, Shiratori Y, Onozuka M, Moriyama N. Evaluating local hepatocellular carcinoma recurrence post-transcatheter arterial chemoembolization: is diffusion-weighted MRI reliable as an indicator? *J Magn Reson Imaging* 2008; **27**: 834-839 [PMID: 18383261 DOI: 10.1002/jmri.21316]

3 **Mannelli L**, Kim S, Hajdu CH, Babb JS, Clark TW, Taouli B. Assessment of tumor necrosis of hepatocellular carcinoma after chemoembolization: diffusion-weighted and contrast-enhanced MRI with histopathologic correlation of the explanted liver. *AJR Am J Roentgenol* 2009; **193**: 1044-1052 [PMID: 19770328 DOI: 10.2214/AJR.08.1461]

4 **Taouli B**, Vilgrain V, Dumont E, Daire JL, Fan B, Menu Y. Evaluation of liver diffusion isotropy and characterization of focal hepatic lesions with two single-shot echo-planar MR imaging sequences: prospective study in 66 patients. *Radiology* 2003; **226**: 71-78 [PMID: 12511671 DOI: 10.1148/radiol.2261011904]

5 **Bruix J**, Sherman M. Management of hepatocellular carcinoma: an update. *Hepatology* 2011; **53**: 1020-1022 [PMID: 21374666 DOI: 10.1002/hep.24199]

6 **Viera AJ**, Garrett JM. Understanding interobserver agreement: the kappa statistic. *Fam Med* 2005; **37**: 360-363 [PMID: 15883903]

7 **Sim J**, Wright CC. The kappa statistic in reliability studies: use, interpretation, and sample size requirements. *Phys Ther* 2005; **85**: 257-268 [PMID: 15733050]

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Redefining Budd-Chiari syndrome: A systematic review

Naomi Shin, Young H Kim, Hao Xu, Hai-Bin Shi, Qing-Qiao Zhang, Jean Paul Colon Pons, Ducksoo Kim, Yi Xu, Fei-Yun Wu, Samuel Han, Byung-Boong Lee, Lin-Sun Li

Naomi Shin, Young H Kim, Department of Radiology, University of Massachusetts Medical School, Worcester, MA 01655, United States

Hao Xu, Qing-Qiao Zhang, Department of Interventional Radiology, the Affiliated Hospital of Xuzhou Medical College, Xuzhou 221006, Jiangsu Province, China

Hai-Bin Shi, Yi Xu, Fei-Yun Wu, Lin-Sun Li, Department of Radiology, the First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, Jiangsu Province, China

Jean Paul Colon Pons, Ducksoo Kim, Department of Radiology, Boston University School of Medicine, Boston, MA 02118, United States

Samuel Han, Department of Gastroenterology, University of Massachusetts Medical School, Worcester, MA 01655, United States

Byung-Boong Lee, Department of Surgery, George Washington University School of Medicine, Washington, DC 20037, United States

Author contributions: Shin N, Kim YH, Xu H, Shi HB, Zhang QQ and Xu Y designed the research; Shin N, Colon Pons JP, Kim D, Xu Y, Wu FY, Lee BB and Li LS conducted the research; Shin N, Kim YH, Xu H and Han S wrote the paper.

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Correspondence to: Young H Kim, MD, PhD, Associate Professor, Department of Radiology, University of Massachusetts Medical School, 55 Lake Avenue North, Worcester, MA 01655, United States. young.kim@umassmemorial.org
Telephone: +1-508-3342087
Fax: +1-508-8564910

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Abstract

AIM: To re-examine whether hepatic vein thrombosis (HVT) (classical Budd-Chiari syndrome) and hepatic vena cava-Budd Chiari syndrome (HVC-BCS) are the same disorder.

METHODS: A systematic review of observational studies conducted in adult subjects with primary BCS, hepatic vein outflow tract obstruction, membranous obstruction of the inferior vena cava (IVC), obliterative hepatocavopathy, or HVT during the period of January 2000 until February 2015 was conducted using the following databases: Cochrane Library, CINAHL, MEDLINE, PubMed and Scopus.

RESULTS: Of 1299 articles identified, 26 were included in this study. Classical BCS is more common in women with a pure hepatic vein obstruction (49%-74%). HVC-BCS is more common in men with the obstruction often located in both the inferior vena cava and hepatic veins (14%-84%). Classical BCS presents with acute abdominal pain, ascites, and hepatomegaly. HVC-BCS presents with chronic abdominal pain and abdominal

wall varices. Myeloproliferative neoplasms (MPN) are the most common etiology of classical BCS (16%-62%) with the JAK2V617-F mutation found in 26%-52%. In HVC-BCS, MPN are found in 4%-5%, and the JAK2V617-F mutation in 2%-5%. Classical BCS responds well to medical management alone and 1st line management of HVC-BCS involves percutaneous recanalization, with few managed with medical management alone.

CONCLUSION: Systematic review of recent data suggests that classical BCS and HVC-BCS may be two clinically different disorders that involve the disruption of hepatic venous outflow.

Key words: Budd-Chiari; Hepatic vein outflow tract obstruction; Membranous obstruction of the inferior vena cava; Obliterative hepatocavopathy; Hepatic vein thrombosis

Core tip: With improved diagnostic methods, the terminology for Budd-Chiari syndrome (BCS) has expanded discordantly. This systematic review discusses recent population studies of BCS and proposes the delineation of two clinically unique syndromes.

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INTRODUCTION

Budd-Chiari syndrome (BCS) was originally described as a rare vascular disorder that encompasses an array of symptoms due to obstruction of hepatic blood outflow at the level of the hepatic veins or hepatic portion of the inferior vena cava (IVC)[1]. The symptoms resulting from this type of occlusion of the hepatic outflow, "classical BCS", were first described by Budd[2,3] in 1845 and later by Hans Chiari in 1899. With the advancement of diagnostic and therapeutic techniques, providers have expanded upon these initial characterizations[4]. Historically, identifying the precise location of the obstruction was challenging, leading to the propagation of simplified descriptions. The precise location of the obstruction(s) is however clinically and prognostically significant. As Valla[5] proposed, the clinical manifestations of BCS (the selective group of symptoms that characterize the syndrome) can be explained by the location of the obstruction: Within the hepatic veins *vs* within the IVC at the level of the hepatic ostia. Over time, in order to incorporate novel and more detailed findings associated with BCS, the lexicon has evolved discordantly. The lexicon now includes a myriad of ambiguous terms or eponyms: Budd's disease, Chiari's disease, Chiari's syndrome, Rokitansky's disease, von Rokitansky disease, Hepatic vein outflow tract obstruction, membranous obstruction of the IVC, obliterative hepatocavopathy, Hepatic vena cava disease, Budd-Chiari syndrome with occlusion of hepatic vein, or hepatic vein thrombosis[6-8]. These eponyms have been used at some point during the course of further discovery; this disarray of terms, some of which are unclear and nonspecific, reflects not only the heterogeneous presentation of BCS, but also the possibility of distinct entities within this syndrome.

The currently accepted definition of primary BCS is hepatic outflow obstruction regardless of the cause or level of obstruction[6,9]. The obstruction can range from the small hepatic veins to the orifice of the IVC into the right atrium. Sinusoidal obstruction syndrome is excluded from this definition[6,9]. Secondary BCS is defined as a hepatic venous outflow obstruction due to compression or invasion by extravascular lesions, including benign or malignant diseases such as abscesses, hepatocellular carcinomas, and renal cell carcinomas, or secondary to cardiac or pericardial diseases[6,9].

In 1998, Okuda *et al*[4] proposed that primary hepatic venous thrombosis (classical BCS) and thrombosis of the IVC at the level of the IVC were two separate syndromes. Recent studies continue to suggest a clear division within the definition of "primary BCS" based on the location of the obstructive lesion[4,10]. Obstruction of the hepatic veins or "classical BCS" appears to be more common in Western patient populations and usually has a known etiology[11,12], acute onset of symptoms, and a greater severity of symptoms requiring a different therapeutic approach than obstruction of the IVC at the level of the hepatic veins[4,13,14]. In comparison with "classical BCS", hepatic vena cava (HVC)-BCS appears to be more common in East Asian patient populations, and is more often idiopathic or due to membranous obstruction. HVC-BCS more commonly presents with a chronic onset of less severe symptoms, thus requiring a different therapeutic approach than "classical BCS"[15]. The location, size, and chronicity is clinically important as it dictates the patient's symptoms and directs the therapeutic approach for patient management[10].

Precedence

Historically, hepatic sinusoidal obstruction syndrome (SOS) or veno-occlusive disease was included under the general term BCS[1,16-18]. SOS is specifically defined as obstruction of the sinusoids or hepatic veins resulting from sinusoidal wall injury. Several distinct clinical characteristics differentiate SOS from BCS and the two conditions are now considered separate entities as the distinct etiology and pathophysiology of SOS necessitates different management strategies. SOS is caused by pyrrolizidine alkaloid toxicity, whereas BCS is caused by multifactorial prothrombotic condition(s) or membranous obstruction of the IVC and/or HV[18]. Pyrrolizidine alkaloids include over 150 compounds that

occur naturally in several plant families[18]. Historically, they were ingested in indigenous herbal teas or inadvertently *via* crop contamination in developing countries. Currently, pyrrolizidine alkaloids are used as myeloablative regimens for patients preparing for hematopoietic stem cell transplantation. Thus, SOS almost exclusively affects hematopoietic stem cell transplant patients, while BCS can affect a wide range of patient populations[9]. Clinically, both SOS and BCS can present with abdominal pain, portal hypertension, jaundice, and non-cirrhotic ascites. Management of SOS is challenging and involves preventive measures (avoiding pyrrolizidine alkaloids in susceptible patients) and a few interventional therapeutic options (defibrotide, heparin, shunt procedures, *etc.*). In contrast, management of BCS ranges from medical management (*e.g.*, anticoagulation) to interventional procedures (angioplasty, stents, shunt procedures, *etc.*)[19].

Due to the low incidence of "BCS" in many countries, published data tended to include only small case series. Recently, there have been an increasing number of larger observational studies (both retrospective and prospective), particularly from Asia (China) and Europe. Advancing imaging technologies, such as computed tomography (CT) angiography, magnetic resonance (MR) angiography, Doppler ultrasound (US), and angiography have allowed for better identification and delineation of this disease. This may signal the start of prospective, randomized, controlled therapeutic trials which can differentiate classical BCS from HVC-BCS and their management strategies. Other investigators have suggested various novel classification systems, including those that forego the eponym "Budd-Chiari" altogether[8-16]. However, given that both classical and HVC-BCS reflect an obstruction in hepatic venous outflow, we propose a clarification of the general BCS term into classical BCS and HVC-BCS.

MATERIALS AND METHODS

A systematic literature search yielded 818 results in the PubMed database; 428 in the Scopus database; 18 in the CINAHL database; and 17 in the Cochrane database. All duplicates were removed. After 18 additional studies (from the references within included studies) were added, 1178 study abstracts were screened. Of these, 591 were excluded because of the publication type and/or subject (reviews, case reports including less than 20 patients, non-human studies, or studies not on BCS (*e.g.,* Chiari malformations, acute liver failure, *etc.*). The full text articles of the remaining 587 studies were acquired to determine eligibility.

Inclusion criteria

Clinical trials and observational studies (prospective or retrospective) conducted in predominantly adult subjects with primary BCS were included in this study. All of the included studies needed to explicitly delineate diagnostic methods for BCS (namely standard imaging studies such as US, CT, MR imaging, or venography) and to explicitly describe inclusion and exclusion criteria to ensure the focus on primary BCS (*vs* secondary BCS). For multiple studies published from the same institution(s) within a close time frame, we reviewed years of subject recruitment, methodology specifics, and results. In addition, we also investigated if there were possible overlapping subjects and/or results. Only the most recent eligible studies were included in this review, unless distinctly specific and separate findings were previously reported[20,21].

Of the 587 studies, the following were excluded: 390 were missing key clinical information (*e.g.*, clear inclusion and exclusion criteria) or focused on a subpopulation within the BCS population (*e.g.*, only BCS patients requiring liver transplantation, *etc.*); 71 studies were not limited to primary BCS; 86 studies were not mainly focused on BCS, but rather broader topics associated with BCS (*e.g.*, causes of liver transplantation, *etc.*); 17 studies were older versions of recently published subject populations with similar study aims. Twenty-six studies were included for analysis in this review (Figure 1).

RESULTS

Epidemiology

Many observational studies have recently been published on "BCS" (Table 1). For clarity and compromise, only the terms classical BCS and HVC-BCS will be used to differentiate between the two types of BCS in this review. After considering the location of the obstruction and clinical manifestations of the subjects, studies were grouped as majority-classical BCS or majority-HVC-BCS studies in Table 1. It has previously been suspected that classical BCS is more likely to present in women with a pure hepatic vein obstruction[9,13]. This review continues to support this observation as 13 of the 14 included studies reported a higher incidence of classical BCS in women; 55%-76% of the reported population is female. In addition, recent studies continue to report pure obstruction in the majority of cases 49%-85%. Most studies reported pure hepatic vein obstruction in > 71% of patients (Table 1). Compared with classical BCS, HVC-BCS is more common in men (51%-66%) and more likely to present with an IVC obstruction with or without involvement of the HVs (69%-100%) (Table 1).

Clinical manifestations in classical BCS vs HVC-BCS

Classical BCS typically presents with an acute onset of symptoms with most studies reporting the duration of symptoms < 6 mo (Table 2) with 60%-85% of patients having an acute presentation of symptoms; however, one study from Egypt designated 80% of their 94 patients as chronic, but the definitions of chronic *vs* acute were not explicitly delineated[22]. Classical BCS typically presents with abdominal pain (45%-86% of patients), ascites (76%-100%), and hepatomegaly (43%-83%) (Table 2). In comparison, HVC-BCS typically presents

Table 1 Epidemiology of classical Budd-Chiari syndrome and hepatic vena cava-Budd Chiari syndrome

Ref.	Country	Publication date	Recruitment years	*n*	Age (median)	Gender		Location of obstruction (%)		
						M (%)	F (%)	HV	IVC	Both
Janssen *et al*[27]	The Netherlands	2000	1984-1997	43	40	16 (37)	27 (63)			
Perelló *et al*[40]	Spain	2002	1990-2000	21	36[1]	5 (24)	16 (76)	17 (81)	0 (0)	4 (19)
Colaizzo *et al*[30]	Italy	2008	1997-2006	32	35	9 (28)	23 (72)			
Darwish Murad *et al*[24]	Europe	2009	2003-2005	163	38	70 (43)	93 (57)	80 (49)	4 (2)	79 (48)
Xavier *et al*[31]	Brazil	2010	2000-2008	31	33	11 (35)	20 (65)			
Sakr *et al*[22]	Egypt	2011	2009-2011	94	28.8[1]	36 (38)	58 (62)	70 (74)	3 (3)	16 (17)
Deepak *et al*[29]	India	2011	2006-2009	20	36.6	14 (70)	6 (30)	17 (85)	1 (5)	2 (10)
Rautou *et al*[37]	France	2011	1995-2005	94	38[1]	34 (36)	60 (64)	73 (78)		13 (14)
Raszeja-Wyszomirska *et al*[45]	Poland	2012	2004-2011	20	38	9 (45)	11 (55)			
Westbrook *et al*[32]	United Kingdom	2012	1985-2008	66	36	27 (41)	39 (59)			
D'Amico *et al*[34]	Italy	2013	2005-2011	31	46	14 (45)	17 (55)			
Harmanci *et al*[42]	Turkey	2013	1989-2011	62	42.8[1]	26 (42)	36 (58)	35 (56)	8 (14)	19 (30)
Nozari *et al*[47]	Iran	2013	1989-2012	55	29[1]	22 (40)	33 (60)			
Pavri *et al*[38]	United States	2014	2008-2013	47	42.4	16 (34)	31 (66)			
Faraoun *et al*[25]	Algeria	2015	2008-2012	176	33[1]	75 (43)	101 (57)	125 (71)	0 (0)	51 (29)
De *et al*[23]	India	2001	1992-1998	40	35.2[1]	26 (65)	14 (35)	N/A	23 (72)	9 (28)
Xu *et al*[41]	China	2004	1983-2003	1360	33.2[1]	833 (61)	527 (39)	2 (0)	1358 (100)[2]	
Ebrahimi *et al*[46]	Iran	2011	2002-2008	21	42[1]	11 (52)	10 (48)	6 (29)	12 (57)	3 (14)
Park *et al*[51]	South Korea	2012	1988-2008	67	47	34 (51)	33 (49)	5 (7)	56 (84)	6 (9)
Qi *et al*[35]	China	2013	1999-2011	169	38.3[1]	66 (52)	61 (48)	53 (31)	20 (12)	96 (57)
Cheng *et al*[13]	China	2013	2010-2011	145	46	90 (6)	55 (38)	45 (31)	8 (6)	92 (63)
Qi *et al*[36]	China	2014	2012-2012	25	35.7[1]	14 (56)	11 (44)	4 (16)	0 (0)	21 (84)
Zhou *et al*[26]	China	2014	2006-2010	338	41.7[1]	209 (62)	129 (38)	45 (13)	8 (2)	285 (84)
Gao *et al*[49]	China	2015	2008-2012							
R				98	36[3]	62 (63)	36 (37)	31 (32)	26 (27)	41 (42)
NR				373	45[3]	193 (52)	180 (48)	82 (22)	169 (45)	122 (33)

[1]Mean values; [2]No differentiation between IVC alone *vs* both IVC and hepatic vein; [3]Provided median ages for the two groups separately. R: Recurrence of disease, NR: Non-recurrence of the disease; HV: Hepatic vein; IVC: Inferior vena cava; M: Male; F: Female; N/A: Not available.

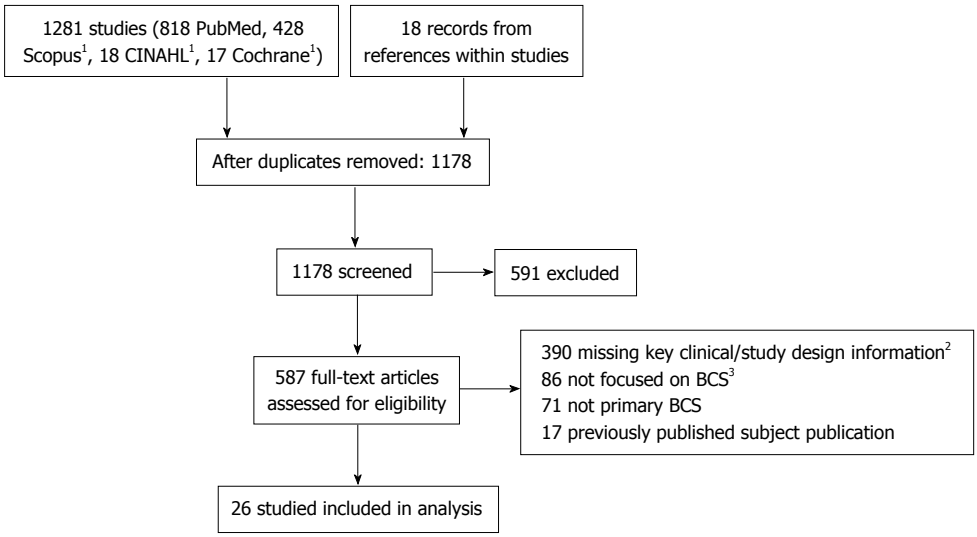

Figure 1 Flow diagram of studies selection. [1]Searches conducted with MEDLINE results removed; [2]Studies missing key clinical information including clear inclusion and exclusion criteria, clear diagnostic parameters, *etc*., and studies that investigated subpopulations (*e.g*., BCS patients requiring liver transplantation, BCS patients without MPN, *etc*.); [3]Studies focused on other categories (*e.g*., causes of liver failure). BCS: Budd-Chiari syndrome; MPN: Myeloproliferative neoplasms.

with chronic onset of symptoms (75%-86% of patients), with an average duration of symptoms prior to diagnosis ranging from 44-96 mo. Nine to seventy percent of patients (most studies reporting < 29%) with HVC-BCS present with abdominal pain, 32%-90% with ascites, and 28%-95% with hepatomegaly. Splenomegaly, abdominal wall varices, lower extremity varices, and discoloration are more commonly associated with HVC-BCS (Table 2)[13,23]. The severity of disease depends upon both the extent of disease (the number of occluded vessels, complete or incomplete occlusion), the presence of associated symptoms (refractory ascites, portal vein thrombosis, *etc*.), and the chronicity of symptoms. Patients with the chronic variation of disease generally have several milder episodes of vague symptoms (abdominal pain or leg swelling), providing sufficient time for

Table 2 Signs and symptoms in classical Budd-Chiari syndrome and hepatic vena cava-Budd Chiari syndrome

	Classical BCS									HVC-BCS						
	Perelló *et al*[40]	Darwish Murad *et al*[24]	Sakr *et al*[22]	Rautou *et al*[46]	Raszeja-Wyszomirska *et al*[45]	Westbrook *et al*[32]	D'Amico *et al*[34]	Harmanci *et al*[42]	Nozari *et al*[47]	De *et al*[23]	Xu *et al*[41]	Ebrahimi *et al*[46]	Qi *et al*[35]	Cheng *et al*[13]	Gao *et al*[49] R *vs* NR	
Country	Spain	Europe	Egypt	France	Poland	United Kingdom	Italy	Sweden	Iran	India	China	Iran	China	China	China	
n (%)	21	163	94	94	20	66	31	62	55	40	1360	21	169	145	98	373
Abdominal pain	18 (86)	99 (61)	78 (83)			36 (55)		28 (45)	33 (60)	28 (70)	122 (9)	5 (29)		30 (21)		
Ascites	18 (86)	135 (83)	80 (85)	73 (78)	20 (100)	57 (87)			42 (76)	30 (75)	914 (67)	19 (90)	95 (56)	77 (53)	76 (78)	224 (60)
Hepatomegaly	9 (43)	109 (67)	78 (83)						33 (60)	38 (95)	1124 (83)	8 (38)		40 (28)	61 (62)	165 (44)
Splenomegaly		85 (52)	48 (51)						19 (34)	26 (65)	683 (50)			113 (78)		
Abdominal wall varices			39 (41)							38 (95)	821 (60)		50 (30)	73 (50)		
Esophageal varices		45 (58)[1]		53 (56)			18 (58)									
Lower extremity edema			46 (49)					28 (45)		28 (70)		14 (67)	86 (51)	76 (52)		
Jaundice	10 (48)		36 (38)						10 (18)	15 (38)	116 (9)					
Encephalopathy	1 (5)	15 (9)	29 (31)	7 (7)		32 (48)						12 (57)	1 (1)			
Bleeding episodes	1 (5)	8 (5)	15 (16)		5 (25)		7 (23)			6 (15)	162 (12)		25 (15)	31 (21)		
Duration of symptoms	1.4[2]	< 1						1-6	6				44	96		
Chronic, > 6 mo		23 (14)	75 (80)[3]					25 (40)	21 (38)	30 (75)				125 (86)		
Acute, < 6 mo		138 (85)	18 (19)					37 (60)	34 (62)					20 (14)		

[1]77 patients underwent EGD; [2]Mean, not median. Most studies reported median duration of symptoms in months; when the median was not available, the mean is reported; [3]No definition of "chronic" was provided. BCS: Budd-Chiari syndrome; HVC: Hepatic vena cava; R: Recurrence of BCS; NR: Non-recurrence of BCS.

the development of collateral vessels[13,24]. In contrast, acute onset and/or significant obstruction (*e.g.*, complete occlusion of several hepatic veins) increases the risk of acute hepatic failure.

Obstruction characteristics

Patients with classical BCS typically have an obstructing thrombus within the hepatic veins (Figure 2)[5,24]. In contrast, patients with HVC-BCS typically have a membranous or segmental obstruction involving the IVC[24] (proximal to the ostia of the hepatic veins), but the obstruction can extend into, or secondarily involve the hepatic veins themselves (Figure 3). Observational studies continue to reflect this difference between classical BCS and HVC-BCS patients; several studies from Europe to northern Africa consistently describe a thrombotic obstruction (87%-95%) limited to the hepatic veins (49%-85%) and rarely describe a membranous obstruction (1%-5%) located at only the IVC (0%-14%). However, in HVC-BCS patients, many studies report a membranous obstruction (30%-61%) only located at the IVC (57%-72%) or both the IVC and HV (63%-84%). Obstructions in HVC-BCS patients are not commonly isolated in the hepatic veins (0%-31%)[24-26]. The development of collateral circulation takes time; given that the chronic form of BCS is more commonly associated with HVC-BCS, it is not surprising that the development of collateral circulation is more typical with HVC-BCS patients (63%-65%) than with classical BCS patients (Table 3).

Etiology

Uncovering the etiology of BCS can be challenging. In classical BCS, however, thrombotic risk factors are consistently identified in the majority of patients[1]. Findings reported in recent studies continue to report myeloproliferative neoplasms [MPN, previously called myeloproliferative disorders (MPD)] as the most common etiology of classical BCS; 9 out of 14 studies found that it is the most common cause of classical BCS affecting 16%-62% of patients, with many reporting between 41%-62% (Table 4). The most commonly observed MPNs include polycythemia vera (PV) and essential thrombocythemia (ET) found in 18%-43% and 6%-14% of classical BCS patients, respectively. The JAK2V617-F mutation is a sensitive marker for MPN and has been observed in 26%-52% of patients with classical BCS[27-34]. In contrast, in several large Chinese studies,

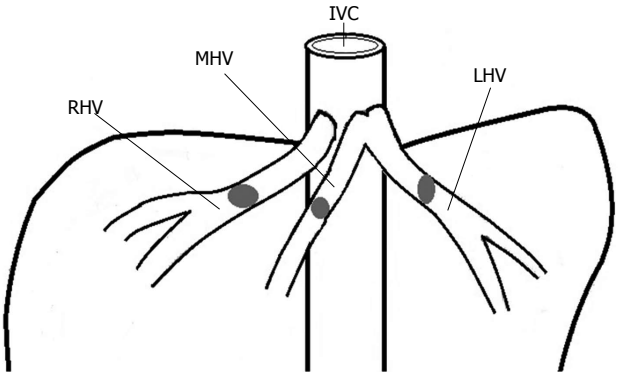

Figure 2 Classical Budd-Chiari syndrome - Occlusions are within the hepatic veins themselves and usually thrombi. RHV: Right hepatic vein; MHV: Middle hepatic vein; LHV: Left hepatic vein; IVC: Inferior vena cava.

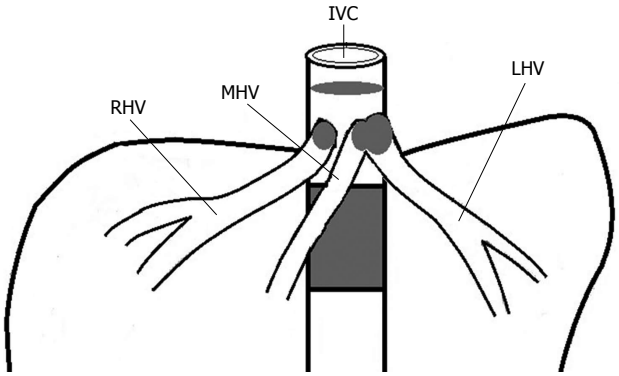

Figure 3 Hepatic vena cava-Budd Chiari syndrome - Occlusions are thin or thick (membranous or segmental) and within the inferior vena cava and occlusion can extend into the hepatic veins and generally involve the ostia to the inferior vena cava. RHV: Right hepatic vein; MHV: Middle hepatic vein; LHV: Left hepatic vein; IVC: Inferior vena cava.

MPN were only found in 4%-5% of patients (PV in 2% and ET in 1%-2%) and the JAK2V617-F mutation in only 0%-5% of patients diagnosed with primary HVC-BCS (Table 4)[13,35,36].

Hereditary prothrombotic conditions such as factor Ⅴ Leiden mutation (FVL), prothrombin (PT) 20210A mutation, protein C deficiency (PCD), protein S deficiency (PSD), antithrombin deficiency (ATD), plasminogen activator inhibitor [PAI-1 (4G-4G)], and the 5,10-methlenetetrahydrofolate reductase enzyme mutation (MTHFRC677T) often also play a significant role in the development of classical BCS. Following MPNs, the FVL mutation is the second most common cause of classical BCS and was found in 2%-53% of patients. Thrombophilic conditions also may contribute to the development of classical BCS. Mutations in PT were found in 2%-8% of patients with classical BCS *vs* 0% of patients with HVC-BCS. PCD, PSD, and ATD were found in 3%-26%, 1%-9% and 3%-15% of patients with classical BCS, respectively, *vs* 0% of patients with HVC-BCS (Table 4). Interestingly, this pattern is not apparent with MTHFRC677T mutations; these mutations were found in 26%-52% of patients with classical BCS and 71%-72% of patients with HVC-BCS. Less common but established prothrombotic or associated conditions further include antiphospholipid antibodies (classical BCS: 3%-29% *vs* HVC-BCS: 0%-17%), hyperhomocysteinemia (10%-18% *vs* 21%-50%), and paroxysmal nocturnal hemoglobinuria (0%-19% *vs* 0%-4%). Several systemic conditions (classical BCS: 5%-24% *vs* HVC-BCS: 1%-19%) including connective tissue disorders such as systemic lupus erythematosus (5%-12% *vs* 1%) are generally associated more frequently with classical BCS. Hormonal factors such as oral contraceptives, pregnancy, or puerperium can also increase the risk of thrombosis as can local insults such as recent surgery. Of these numerous differences between classical BCS and HVC-BCS, one consistent difference is the greater influence of hormonal changes (be it oral contraceptive use or pregnancy) in classical BCS patients (4%-52% of the female population) (Table 4)[29,37-39].

Membranous obstruction of the IVC (and/or HV) is consistently listed as the etiology of a significant number of HVC-BCS patients (52%-61%). In classical BCS patients, membranous obstruction is rare (1%) or rarely explicitly delineated, except in one study of 23 consecutive patients diagnosed with BCS in Germany where 5 patients (22%) were found to have a membranous obstruction of the IVC[24]. Furthermore, despite comprehensive work-up, an etiologic factor is often not identified in HVC-BCS patients (19%-29% *vs* classical BCS: 5%-30%) (Table 4).

Data from recent studies continues to support the possibility of two different types of BCS with separate etiologies: Classical BCS, where thrombophilic risk factors and often multiple concomitant factors are common *vs* HVC-BCS, where thrombophilic risk factors are uncommon, but membranous obstruction and idiopathic hepatic venous outflow obstruction are more common.

Management and outcomes

Treatment and prognosis of BCS depends on a few key factors: Acuity of symptoms, location and extent of the obstruction, and etiology[24]. In 2013, Seijo *et al*[11] outlined a step-wise management approach for BCS patients from the analysis of the extended follow-up data of 157 patients from 9 European countries. This management approach starts with medical management alone (*e.g.*, salt-restriction, anticoagulation, diuretics), including concomitant management of any underlying etiological processes. Diagnostic work-up for classical BCS patients generally includes hematologic work-up for MPN, JAK2V617F mutation screening for MPN[29-31,33], testing for FVL mutation[28], and the aforementioned thrombophilic risk factors. In addition, some studies recommend continued monitoring of JAK2-mutation-positive-patients for occult MPNs[5,9,33]. In general, the medical management of classical BCS patients involves anticoagulation and ascites management with diuretics. Patients with MPN require additional aspirin and cyto-

Table 3 Obstruction characteristics: Location, type, and associated findings

	Classical BCS						HVC-BCS				
	Perelló *et al*[40]	Darwish Murad *et al*[24]	Sakr *et al*[22]	Deepak *et al*[29]	Harmanci *et al*[42]	Faraoun *et al*[25]	De *et al*[23]	Xu *et al*[41]	Ebrahimi *et al*[46]	Cheng *et al*[13]	Zhou *et al*[26]
Country	Spain	Europe	Egypt	India	Turkey	Algeria	India	China	Iran	China	China
n (%)	21	163	94	20	62	176	40	1360	21	145	338
Obstruction location											
HV only	17 (81)	80 (49)	70 (74)	17 (85)	35 (56)	125 (71)	N/A	2 (0)	6 (29)	45 (31)	45 (13)
IVC only	0 (0)	4 (2)	3 (3)	1 (5)	8 (14)	0 (0)	23 (72)	1358 (100)	12 (57)	8 (6)	8 (2)
Both HV and IVC	4 (19)	79 (48)	16 (17)	2 (10)	19 (30)	51 (29)	9 (28)		3 (14)	92 (63)	285 (84)
HV thrombosis	20 (95)				54 (87)	170 (97)		DNS		15 (10)	
IVC thrombosis	3 (14)	DNS	DNS	DNS	27 (44)	DNS		123 (9)			
IVC web/membrane	1 (5)		2 (1)	DNS	DNS	DNS	12 (30)	717 (53)	11 (52)	89 (61)	
Collateral circulation										92 (63)	220 (65)
Benign regenerative nodules										36 (25)[1]	79 (23)

[1]Described as "benign nodules", not benign regenerative nodules. DNS: Study mentions generally, but, does not provide specific counts; BCS: Budd-Chiari syndrome; HV: Hepatic vein; HVC: Hepatic vena cava; IVC: Inferior vena cava.

reductive medications (*e.g.*, hydroxyurea). Patients with autoimmune diseases (*e.g.*, antiphospholipid syndrome, Behçet's disease, *etc.*) require additional corticosteroids and/or immunosuppressive drugs. If patients fail medical management, therapy is then escalated to minimally invasive procedures including percutaneous transluminal angioplasty (PTA) and/or thrombolysis. If patients fail to respond to these measures, developing refractory ascites, variceal bleeding, or liver failure, they are then treated with transjugular intrahepatic portosystemic shunt (TIPS) or other shunt operations[11,40,41], with liver transplantation as a final option[32]. Such an approach appears to result in good long-term survival (Table 5)[11].

Recent studies continue to support that medical management alone can be appropriate for classical BCS patients; 33%-54% of the classical BCS patients treated with medical management alone have good outcomes. In contrast, only 0%-7% of HVC-BCS patients are treated with medical management alone. While both classical and HVC-BCS patients benefit from interventional therapy, the specific interventions are different. Classical BCS patients commonly undergo TIPS (classical BCS: 4%-62% *vs* HVC-BCS: 1%-4.5%) and liver transplantation (9%-55% *vs* 0%-1%). In a study of 62 predominantly classical BCS patients from Turkey, none of the patients underwent liver transplantations, but that was due to a lack of donor availability[42]. In contrast, first line management of HVC-BCS with percutaneous re-canalization (with or without stent deployment) has good outcomes[43]. In one large study from China, Han *et al*[44] found that all 187 consecutively diagnosed primary BCS patients at one institution were eligible for percutaneous recanalization, regardless of the location of the obstruction. Recent studies report that HVC-BCS patients undergo PTA more frequently compared to classical BCS patients (HVC-BCS: 43%-92% *vs* classical BCS: 3%-18%). After percutaneous recanalization, patients are anticoagulated with an international normalized ratio goal of 2-3 for a minimum of 6-8 mo per standard post-endovascular intervention management guidelines[44].

Median follow-up for both classical BCS and HVC-BCS patients were similar (classical BCS: 17-58 mo and HVC-BCS: 12-103 mo). Both groups of patients fared well with their respective management strategies. One-year and five-year survival was 79%-96% and 56%-79% among classical BCS patients, respectively. One-year and five-year survival for HVC-BCS patients was 67%-99% and 75%-86%, respectively (Table 5). Poor prognostic factors for classical BCS patients include: Severe BCS (*e.g.*, ascites requiring diuretics or paracentesis, pleural effusion, higher Clichey Prognositc index score), older age, cirrhosis at diagnosis of BCS, and chronic kidney disease[37,38]. Development of cirrhosis or hepatocellular carcinoma (HCC) are poor prognostic factors for HVC-BCS patients[23].

DISCUSSION

This systematic literature review highlights the numerous differences between classical BCS and HVC-BCS. Despite the growing cognizance of this difference[4,5] and despite

Table 4 Risk factors and/or etiologies of classical Budd-Chiari syndrome and hepatic vena cava-Budd Chiari syndrome

	Classical BCS														HVC-BCS			
	Perelló *et al*[40]	Smalberg *et al*[39]	Colaizzo *et al*[30]	Xavier *et al*[31]	Sakr *et al*[22]	Deepak *et al*[29]	Rautou *et al*[37]	Raszeja-Wyszomirska *et al*[45]	Westbrook *et al*[32]	D'Amico *et al*[34]	Seijo *et al*[11]	Harmanci *et al*[42]	Nozari *et al*[47]	Pavri *et al*[38]	Ebrahimi *et al*[46]	Qi *et al*[35]	Cheng *et al*[13]	Qi *et al*[36]
Country	Spain	Netherlands	Italy	Brazil	Egypt	India	France	Poland	United Kingdom	Italy	Europe	Turkey	Iran	United States	Iran	China	China	China
n (%)	21	40	32	31	94	20	94	20	66	31	157	62	55	47	21	169	145	25
MPN (%)	13 (62)	13 (33)	13 (41)	5 (16)		8 (40)	51 (59)	8 (40)	37 (56)	17 (55)	52 (33)	19 (31)	9 (16)			7 (4)[9]	5 (5)[10]	
JAK2V617-F	N/A	7 (41)		8 (26)	18 (29)[4]	8 (40)			34 (52)							4 (2)	5 (5)[10]	0 (0)
PV	9 (43)										28 (18)			14 (30)		3 (2)	2 (2)[10]	
ET	3 (14)										12 (8)			3 (6)		1 (1)	2 (2)[10]	
FVL	2 (10)	5 (15)	6 (19)	3 (10)	34 (53)[5]	5 (25)	15 (19)	1 (5)	1 (2)	9 (29)	19 (12)	15 (30)	10 (18)	4 (9)		0 (0)	0 (0)[11]	0 (0)
PT 20210A		2 (8)	1 (3)	1 (3)	3 (5)[6]		6 (8)		1 (2)	1 (3)	5 (3)	1 (2)				0 (0)	0 (0)[11]	0 (0)
Protein C deficiency		2 (7)[2]			4 (4)	2 (10)	6 (12)	3 (15)	2 (3)		5 (3)	16 (31)	12 (20)	2 (4)			0 (0)[12]	
Protein S deficiency		2 (7)[2]			1 (1)	1 (5)	5 (9)				3 (2)	5 (10)	3 (6)	1 (2)			0 (0)[12]	
AT deficiency					4 (4)	3 (15)	3 (4)				4 (3)	6 (15)	3 (6)				0 (0)[12]	
PAI-1 (4G-4G)										17 (55)								
MTHFRC677T					31 (52)[6]					8 (26)		19 (39)				96 (71)		18 (72)
HH						2 (10)					29 (18)					64 (50)	30 (21)	
PNH	4 (19)	2 (9)	1 (3)		2 (2)	1 (5)	8 (12)	1 (5)	0 (0)		15 (10)	1 (2)		3 (6)		1 (1)	0 (0)[13]	1 (4)
OCP, pregnancy, or puerperium[1]		13 (52) (OC)[3]	1 (4) (OC)[3]	7 (35)	19 (33)		21 (35) (OC)[3]			4 (24)	35 (39)	4 (11)	3 (9)	2 (6) (OC)[3]			2 (4)	
Systemic diseases or local factors		2 (5)			12 (13)[7]				3 (5)[7]		37 (24)	8 (13)[7]	5 (9) autoimmune		4 (19)[8]	2 (1)	1 (1)	
NAD/idiopathic	1 (5)				8 (9)			6 (30)		2 (6)		6 (10)	10 (18)		6 (29)		28 (19)	
Web/membrane															11 (52)		89 (61)	
MOVC																	6 (4)	
MOVC + HV	1 (5)																60 (41)	
MOHV																	23 (16)	

[1]Percentage of total female patients; [2]Patients on anticoagulation were not tested; [3]Number of women on OC only, no specific information on number of pregnant women; [4]Out of 62 tested; [5]Out of 64 tested; [6]Out of 60 tested; [7]Mainly Behcet's disease; [8]Behcet's disease (2), Hepatitis C (1), Leukemia (1); [9]2 of these 7 patients were diagnosed with latent MPN; [10]In this study, most (105 patients), but, not all were tested; percentages are out of 105; [11]Out of 96 patients were tested; [12]Out of 80 patients tested; [13]Out of 83 patients tested. BCS: Budd-Chiari syndrome; HV: Hepatic vein; HVC: Hepatic vena cava; ET: Essential thrombocythemia; PNH: Paroxysmal nocturnal hemoglobinuria; OCP: Oral contraception; MOVC: Membranous obstruction of IVC; NAD: No associated disease/etiology found; MPN: Myeloproliferative neoplasms; FVL: Factor V Leiden mutation; HH: Hyperhomocysteinemia.

the abundance of recent publications on BCS, there is still a paucity of large, randomized clinical trials with regard to classical and/or HVC-BCS. The vast heterogeneity of these recent publications with regards to recruitment of solely primary *vs* secondary BCS, of solely classical or HVC-BCS, and of patients who have not previously been recruited and described, may lead to disparate and skewed patient populations that preclude certain data analyses and conclusions. Thus, this review specifically sought out studies that recruited BCS subjects that were representative of the indigenous BCS patient population. We attempted to minimize selection bias by excluding studies that focused on a particular subgroup of patients (*e.g.*, BCS patients requiring liver transplantation, exclusion of patients with specific previously diagnosed etiologies, *etc.*). We also attempted to minimize multiple representations of the same patient population by comparing recruitment periods from studies that were similar in geographic location (institution, city, and country) or similar in authorship. The selected studies thereby provide an unadulterated presentation of the differences between classical *vs* HVC-BCS according to geography, patient demographics, location of obstruction, and treatment strategies and outcomes. The examination of these differences is important as they impact the diagnostic work-up and the therapeutic management strategies for individual patients and healthcare communities alike.

Table 5 Management and outcomes in classical Budd-Chiari syndrome and hepatic vena cava-Budd Chiari syndrome

	Classical BCS								HVC-BCS					
	Perelló *et al*[40]		Rautou *et al*[37]	Raszeja-Wyszomirska *et al*[45]	Westbrook *et al*[32]	Harmanci *et al*[42]	Seijo *et al*[11]	Nozari *et al*[47]	Pavri *et al*[38]	De *et al*[23]	Xu *et al*[41]	Ebrahimi *et al*[46]	Park *et al*[51]	Cheng *et al*[13]	Gao *et al*[49]
Country	Spain		France	Poland	United Kingdom	Turkey	Europe	Iran	United States	India	China	Iran	South Korea	China	China
n (%)	21		94	20	66	62	157	55	47	40	1360	21	67	145	471
Medical management	21 (100)		94 (100)	20 (100)	61 (92)	61 (98)	139 (89)	55 (100)	⩾ 40 (85)			12 (57)	32 (48)		
Medical management only (%)[1]	7 (33)						69 (54) 49 (71) alive				0 (0)			4 (3)	31 (7)
Interventional therapy		14 (67)			34 (52)[5]		88 (56) 72 (82) alive	10 (18)		23 (58)	1360 (100) 1318 alive	9 (43)		141 (97)	440 (93)
PTA	1 (5)		17 (18)			2 (3) in IVC	22 (14)			23 (58)		9 (43)	27 (40)	134 (92)	
Shunt operation		2 (10)			32 (48.5)[6]			2 (4)			330 (24)	3 (14)	4 (5.9)		
TIPS[2]		13 (62)	28 (30)	2 (10)		4 (6)	62 (39)[3]	2 (4)	21 (45)				3 (4.5)	1[2]	
Liver transplantation			15 (16)	10 (50)	36 (55)	0 (0)[8]	20[4] (13)	5 (9)	8 (17)		0 (0)			0 (0)[12] 2 (1)[12]	
Median follow-up (in months)	58[13]	38[13]	43	17	40-73[9]	25.2[12]	50		32	56	81.6		103	12	19
Survival	7 (100)	12 (86)	74 (79)	15 (75)			95 (73)	65 mo[10]	37 (79)						
At 1 yr				80%	88%	96%			37 (79)			67%[7]		99%	401 (94)
At 5 yr			74 (79)		56%		95 (73)			75%			86%		
Mortality										10[11]				2 (1)	

[1]Medical management includes anticoagulation, diuretics, and medical treatment of any underlying causes; [2]After PTA failed; [3]Of the 22 patients who initially were treated with PTA/thrombolytics, 12 subsequently underwent TIPS and 2 underwent OLT; [4]Among the 62 patients who underwent TIPS, 4 subsequently underwent OLT; [5]Patients who failed medical management (namely anticoagulation with heparin and warfarin and diuretics) as defined by persistent transaminitis, resistant ascites or worsening hepatic function) moved on to receive stenting, shunting, or TIPS; [6]50% success rate (16/32); [7]Per study, 7 out of 21 patients died before hospital discharge; [8]No patients underwent liver transplantations due to a lack of donor availability; [9]Median follow-up post liver transplantation was 40 mo and median follow-up of patients with MPN was 73 mo; [10]Mean survival time was 65 mo; [11]10 patients died by the 5-yr follow-up period; [12]At the end of the follow-up period, 2 patients were waiting to receive OLT from another hospital; [13]Mean, not median values provided. Interventional therapy includes both endovascular and surgical procedures. OLT: Orthotopic liver transplant; TIPS: Transjugular intrahepatic portosystemic shunt; PTA: Percutaneous transluminal angioplasty; BCS: Budd-Chiari syndrome; HVC: Hepatic vena cava; IVC: Inferior vena cava.

Differentiation between classical and HVC-BCS is important because it dictates what constitutes comprehensive and appropriate diagnostic strategies. Given the likelihood of multiple pro-thrombotic risk factors contributing to the development of classical BCS, recommendations for extensive routine work-up for multiple possible etiologies include testing for MPN; JAK2V617F mutation screening for MPN[42,45]; continued monitoring of JAK2-mutation-positive-patients for occult MPN[5,9,24,31]; further testing for TET2 mutation when the JAK2 screening is negative[32]; FVL mutation[28]; PT 20210A mutation; protein C and S deficiencies; AT deficiency; PAI-1 and MTHFRC677T mutations[24]. Since more than one thrombophilic condition often manifests in classical BCS patients, such an extensive work-up is appropriate, but recent data continues to suggest that those same recommendations may not be appropriate for HVC-BCS patients[24]. For instance, screening for the JAK2V617F mutation is important for classical BCS patients because it has been reported to be a better diagnostic test for MPN when compared to traditional hematologic tests[42]. The JAK2V617F mutation has consistently been found in a significant number of "idiopathic" cases of classical BCS (although this is not observed in "idiopathic" HVC-BCS). In a study of 41 classical BCS patients from England, the JAK2V617F mutation was detected in 58.5% of idiopathic BCS cases[32]. Furthermore, 93% of the patients who later developed latent MPN were positive for the mutation suggesting that the JAK2V617F mutation is a highly sensitive marker to detect overt or covert MPN[33]. Given the possible geographic distribution of classical and HVC-BCS in regions with limited healthcare resources, a clear delineation of standard of care would benefit patients and providers alike. Historically, it has been speculated that there is association between lower standards of living and HVC-BCS. However, a recent prospective study including 53 consecutive BCS patients from Western India found no association between socioeconomic status and location of hepatic venous outflow obstruction although, a correlation between living in mud houses and IVC membranous obstruction was observed[28]. Therefore, according to this review, balancing the costs of diagnostic work-up for numerous potential genetic or acquired pro-thrombotic factors

with the actual benefit the patient may gain should be BCS-type specific.

Treatment and prognosis of BCS depends on a few key factors: Acuity and severity of the symptoms, location and the extent of the obstruction, and etiology of the obstruction[24]. While anticoagulation (initially with heparin and chronically with warfarin) is the initial treatment of choice for both classical BCS and HVC-BCS patients[45], the expected response and course of therapy differs dramatically. Classical BCS patients often present with acute thrombosis of the hepatic veins. This rapid blockage of hepatic venous outflow precludes the ability to adapt *via* the development of collateral circulation. It is not surprising then that acute fulminant liver failure (with its sequelae) is more common among classical BCS patients, thus requiring shunt operations and liver transplantations more frequently than in HVC-BCS patients[13]. In contrast, HVC-BCS patients generally present with chronic symptoms that may lead to the transformation of an old thrombus into a fibrous, membranous obstruction[7,25]. Depending on the thickness and the extent of the obstruction, early interventional therapy (most commonly PTA with or without stent deployment), is very effective and thus more commonly utilized among HVC-BCS patients[13,46]. The thrombotic nature of obstruction observed in classical BCS may explain why these obstructions are more susceptible and responsive to medical management (namely anticoagulation) alone. As noted in two long-term follow up studies by Perelló *et al*[40] and Darwish Murad *et al*[24] (with median follow-ups of 58 and 17 mo respectively) of predominantly classical BCS patients, 33%-44% of patients that were maintained on medical management alone had good outcomes: 100% and 44% (at 12 mo), respectively. In both studies, very few classical BCS patients (5%-9%) required percutaneous recanalization. In HVC-BCS patients, the role of anticoagulation is often adjunctive and temporary; the use of warfarin before angioplasty can improve outcomes in patients with IVC obstruction[47]. Few HVC-BCS patients are managed with medical management alone because of previously reported poor outcomes[38]. In terms of the pathophysiology, Simonetto *et al*[48] recently used a murine model to demonstrate that hepatic venous outflow obstruction as seen in congestive heart failure or veno-occlusive disease led to liver fibrosis not *via* an inflammatory pathway, but *via* sinusoidal thrombosis and mechanical strain, while also showing that anticoagulation may have a beneficial effect in decreasing fibrosis. This aids our understanding of the mechanism by which BCS and HVC-BCS can result in fibrosis, and emphasizes the need for relief of obstruction for proper management. Given the different presentations and treatment courses of the two entities, it would be relevant to further study the pathophysiology of these conditions to better optimize management.

Factors that contribute poor prognosis in classical BCS include: Increasing age, cirrhosis at the time of diagnosis, chronic kidney disease, and portal vein thrombosis[25,38]. The Child-Pugh and MELD scores also play an unclear role in terms of practical management, while asymptomatic patients generally have better prognoses[45,49]. For HVC-BCS patients, factors that contribute to poor prognosis include the development of cirrhosis and HCC. Recent studies have suggested that the risk of developing HCC in HVC-BCS patients (unlike classical BCS patients) is directly attributable to the disease *vs* Hepatitis B or C infections[7]. Furthermore, the incidence of developing HCC in HVC-BCS patients is similar to those of cirrhotic patients[50,51]. These findings suggest that HVC-BCS patients, unlike classic BCS patients, should be routinely monitored for the development of HCC. Specific interventions to address and reduce the high pressure gradient in BCS patients may reduce the risk of HCC development.

In conclusion, clarification in the terminology describing hepatic venous outflow obstruction would enable both clinicians and investigators to identify patients with comparable signs and symptoms, thus enabling the execution of sound (randomized and controlled) and separate research studies on pathogenesis, therapy, and prognosis of what seems to be two different etiologies of Budd-Chiari syndrome. As summarized in this review, recent studies continue to support that classical and HVC-BCS have distinct demographics, characteristics, etiologies, therapeutic strategies, and prognoses. To address gaps in knowledge within classical BCS and HVC-BCS patients, these differences should be acknowledged and future research should be performed on these two conditions separately.

COMMENTS

Background

Budd-Chiari syndrome (BCS) encompasses a wide array of symptoms that are caused by hepatic venous outflow tract obstruction and has been known by many different names. While reviewing the recent literature, this paper delineates the difference between primary hepatic venous thrombosis and thrombosis of the inferior vena cava (IVC), which have both previously been referred to as BCS.

Research frontiers

With the influx of new studies examining the wide spectrum of BCS, there has been a growing argument for the separation of primary hepatic venous thrombosis (classical BCS) and thrombosis of the IVC at the level of the IVC (hepatic vena cava-BCS) given the difference in their etiology and management.

Innovations and breakthroughs

This paper supports the clarification of terminology used to describe hepatic venous outflow obstruction, which will help guide future research and allow for more specific treatment modalities for this condition.

Applications

The evidence presented helps clinicians to understand the difference in etiologies of this syndrome and their influence in the management of the separate entities of this condition.

Terminology

Classical BCS refers to primary hepatic venous thrombosis. HVC-BCS refers to thrombosis of the IVC at the level of the IVC.

Peer-review

This review is well organized and comprehensive, has a good clinical message about BCS, and should be of great interest to the readers.

REFERENCES

1 **Valla DC**. Vascular disorders of the liver. *Acta Gastroenterol Belg* 2003; **66**: 294-297 [PMID: 14989053]
2 **Budd G**. On diseases of the liver. Philadelphia: Lea and Blanchard, 1846
3 **Budd G**. On diseases of the liver. Philadelphia: Blanchard and Lea, 1857
4 **Okuda K**, Kage M, Shrestha SM. Proposal of a new nomenclature for Budd-Chiari syndrome: hepatic vein thrombosis versus thrombosis of the inferior vena cava at its hepatic portion. *Hepatology* 1998; **28**: 1191-1198 [PMID: 9794901 DOI: 10.1002/hep.510280505]
5 **Valla DC**. The diagnosis and management of the Budd-Chiari syndrome: consensus and controversies. *Hepatology* 2003; **38**: 793-803 [PMID: 14512865 DOI: 10.1002/hep.1840380404]
6 **Janssen HL**, Garcia-Pagan JC, Elias E, Mentha G, Hadengue A, Valla DC. Budd-Chiari syndrome: a review by an expert panel. *J Hepatol* 2003; **38**: 364-371 [PMID: 12586305 DOI: 10.1016/S0168-8278(02)00434-8]
7 **Shrestha SM**. Liver cirrhosis and hepatocellular carcinoma in hepatic vena cava disease, a liver disease caused by obstruction of inferior vena cava. *Hepatol Int* 2009; **3**: 392-402 [PMID: 19669366 DOI: 10.1007/s12072-009-9122-5]
8 **Okuda K**. Membranous obstruction of the inferior vena cava (obliterative hepatocavopathy, Okuda). *J Gastroenterol Hepatol* 2001; **16**: 1179-1183 [PMID: 11903732 DOI: 10.1046/j.1440-1746.2001.02577.x]
9 **DeLeve LD**, Valla DC, Garcia-Tsao G. Vascular disorders of the liver. *Hepatology* 2009; **49**: 1729-1764 [PMID: 19399912 DOI: 10.1002/hep.22772]
10 **Valla DC**. Hepatic venous outflow tract obstruction etiopathogenesis: Asia versus the West. *J Gastroenterol Hepatol* 2004; **19**: S204-S211 [DOI: 10.1111/j.1440-1746.2004.03642.x]
11 **Seijo S**, Plessier A, Hoekstra J, Dell'era A, Mandair D, Rifai K, Trebicka J, Morard I, Lasser L, Abraldes JG, Darwish Murad S, Heller J, Hadengue A, Primignani M, Elias E, Janssen HL, Valla DC, Garcia-Pagan JC. Good long-term outcome of Budd-Chiari syndrome with a step-wise management. *Hepatology* 2013; **57**: 1962-1968 [PMID: 23389867 DOI: 10.1002/hep.26306]
12 **Cazals-Hatem D**, Vilgrain V, Genin P, Denninger MH, Durand F, Belghiti J, Valla D, Degott C. Arterial and portal circulation and parenchymal changes in Budd-Chiari syndrome: a study in 17 explanted livers. *Hepatology* 2003; **37**: 510-519 [PMID: 12601347 DOI: 10.1053/jhep.2003.50076]
13 **Cheng D**, Xu H, Lu ZJ, Hua R, Qiu H, Du H, Xu X, Zhang J. Clinical features and etiology of Budd-Chiari syndrome in Chinese patients: a single-center study. *J Gastroenterol Hepatol* 2013; **28**: 1061-1067 [PMID: 23425079 DOI: 10.1111/jgh.12140]
14 **Denninger MH**, Chaït Y, Casadevall N, Hillaire S, Guillin MC, Bezeaud A, Erlinger S, Briere J, Valla D. Cause of portal or hepatic venous thrombosis in adults: the role of multiple concurrent factors. *Hepatology* 2000; **31**: 587-591 [PMID: 10706547 DOI: 10.1002/hep.510310307]
15 **Qi X**, Zhang C, Han G, Zhang W, He C, Yin Z, Liu Z, Bai W, Li R, Bai M, Yang Z, Wu K, Fan D. Prevalence of the JAK2V617F mutation in Chinese patients with Budd-Chiari syndrome and portal vein thrombosis: a prospective study. *J Gastroenterol Hepatol* 2012; **27**: 1036-1043 [PMID: 22142461 DOI: 10.1111/j.1440-1746.2011.07040.x]
16 **Dilawari JB**, Bambery P, Chawla Y, Kaur U, Bhusnurmath SR, Malhotra HS, Sood GK, Mitra SK, Khanna SK, Walia BS. Hepatic outflow obstruction (Budd-Chiari syndrome). Experience with 177 patients and a review of the literature. *Medicine* (Baltimore) 1994; **73**: 21-36 [PMID: 8309360 DOI: 10.1097/00005792-199401000-00003]
17 **Ludwig J**, Hashimoto E, McGill DB, van Heerden JA. Classification of hepatic venous outflow obstruction: ambiguous terminology of the Budd-Chiari syndrome. *Mayo Clin Proc* 1990; **65**: 51-55 [PMID: 2296212 DOI: 10.1016/S0025-6196(12)62109-0]
18 **McDermott WV**, Ridker PM. The Budd-Chiari syndrome and hepatic veno-occlusive disease. Recognition and treatment. *Arch Surg* 1990; **125**: 525-527 [PMID: 2322120 DOI: 10.1001/archsurg.1990.01410160111022]
19 **Plessier A**, Rautou PE, Valla DC. Management of hepatic vascular diseases. *J Hepatol* 2012; **56** Suppl 1: S25-S38 [PMID: 22300463 DOI: 10.1016/S0168-8278(12)60004-X]
20 **Schein M**, Paladugu R. Redundant surgical publications: tip of the iceberg? *Surgery* 2001; **129**: 655-661 [PMID: 11391360 DOI: 10.1067/msy.2001.114549]
21 **Qi X**, Ren W, Liu L, Yang Z, Yang M, Fan D, Han G. Prevalence of covert duplicate publications in Budd-Chiari syndrome articles in China: a systematic analysis. *Am J Med* 2013; **126**: 633-9.e2 [PMID: 23787196 DOI: 10.1016/j.amjmed.2012.12.021]
22 **Sakr M**, Barakat E, Abdelhakam S, Dabbous H, Yousuf S, Shaker M, Eldorry A. Epidemiological aspects of Budd-Chiari in Egyptian patients: a single-center study. *World J Gastroenterol* 2011; **17**: 4704-4710 [PMID: 22180713 DOI: 10.3748/wjg.v17.i42.4704]
23 **De BK**, Biswas PK, Sen S, Das D, De KK, Das U, Mandal SK, Majumdar D. Management of the Budd-Chiari syndrome by balloon cavoplasty. *Indian J Gastroenterol* 2001; **20**: 151-154 [PMID: 11497174]
24 **Darwish Murad S**, Plessier A, Hernandez-Guerra M, Fabris F, Eapen CE, Bahr MJ, Trebicka J, Morard I, Lasser L, Heller J, Hadengue A, Langlet P, Miranda H, Primignani M, Elias E, Leebeek FW, Rosendaal FR, Garcia-Pagan JC, Valla DC, Janssen HL. Etiology, management, and outcome of the Budd-Chiari syndrome. *Ann Intern Med* 2009; **151**: 167-175 [PMID: 19652186 DOI: 10.7326/0003-4819-151-3-200908040-00004]
25 **Faraoun SA**, Boudjella Mel A, Debzi N, Afredj N, Guerrache Y, Benidir N, Bouzid C, Bentabak K, Soyer P, Bendib SE. Budd-Chiari syndrome: a prospective analysis of hepatic vein obstruction on ultrasonography, multidetector-row computed tomography and MR imaging. *Abdom Imaging* 2015; **40**: 1500-1509 [PMID: 25687630 DOI: 10.1007/s00261-015-0380-5]
26 **Zhou P**, Ren J, Han X, Wu G, Zhang W, Ding P, Bi Y. Initial imaging analysis of Budd-Chiari syndrome in Henan province of China: most cases have combined inferior vena cava and hepatic veins involvement. *PLoS One* 2014; **9**: e85135 [PMID: 24416352 DOI: 10.1371/journal.pone.0085135]
27 **Janssen HL**, Meinardi JR, Vleggaar FP, van Uum SH, Haagsma EB, van Der Meer FJ, van Hattum J, Chamuleau RA, Adang RP, Vandenbroucke JP, van Hoek B, Rosendaal FR. Factor V Leiden mutation, prothrombin gene mutation, and deficiencies in coagulation inhibitors associated with Budd-Chiari syndrome and portal vein thrombosis: results of a case-control study. *Blood* 2000; **96**: 2364-2368 [PMID: 11001884]
28 **Mohanty D**, Shetty S, Ghosh K, Pawar A, Abraham P. Hereditary thrombophilia as a cause of Budd-Chiari syndrome: a study from Western India. *Hepatology* 2001; **34**: 666-670 [PMID: 11584361 DOI: 10.1053/jhep.2001.27948]
29 **Deepak A**, Punamiya S, Patel N, Parekh S, Mehta S, Shah N. Prevalence of JAK29V617F) mutation in intra-abdominal venous thrombosis. *Trop Gastroenterol* 2011; **32**: 279-284 [PMID: 22696908]
30 **Colaizzo D**, Amitrano L, Tiscia GL, Iannaccone L, Gallone A, Grandone E, Guardascione MA, Margaglione M. Occurrence of the JAK2 V617F mutation in the Budd-Chiari syndrome. *Blood Coagul Fibrinolysis* 2008; **19**: 459-462 [PMID: 18600100 DOI: 10.1097/MBC.0b013e3283049662]
31 **Xavier SG**, Gadelha T, Pimenta G, Eugenio AM, Ribeiro DD, Gomes FM, Bonamino M, Zalcberg IR, Spector N. JAK2V617F mutation in patients with splanchnic vein thrombosis. *Dig Dis Sci* 2010; **55**: 1770-1777 [PMID: 19690956 DOI: 10.1007/s10620-009-0933-y]
32 **Westbrook RH**, Lea NC, Mohamedali AM, Smith AE, Orr DW, Roberts LN, Heaton ND, Wendon JA, O'Grady JG, Heneghan MA, Mufti GJ. Prevalence and clinical outcomes of the 46/1

haplotype, Janus kinase 2 mutations, and ten-eleven translocation 2 mutations in Budd-Chiari syndrome and their impact on thrombotic complications post liver transplantation. *Liver Transpl* 2012; **18**: 819-827 [PMID: 22467227 DOI: 10.1002/lt.23443]

33 **Goulding C**, Uttenthal B, Foroni L, Duke V, Traore A, Kottaridis P, Hoffbrand AV, Patch D, McNamara C. The JAK2(V617F) tyrosine kinase mutation identifies clinically latent myeloproliferative disorders in patients presenting with hepatic or portal vein thrombosis. *Int J Lab Hematol* 2008; **30**: 415-419 [PMID: 19046316 DOI: 10.1111/j.1751-553X.2007.00973.x]

34 **D'Amico M**, Sammarco P, Pasta L. Thrombophilic Genetic Factors PAI-1, MTHFRC677T, V Leiden 506Q, and Prothrombin 20210A in Noncirrhotic Portal Vein Thrombosis and Budd-Chiari Syndrome in a Caucasian Population. *Int J Vasc Med* 2013; **2013**: 717480 [PMID: 24455271 DOI: 10.1155/2013/717480]

35 **Qi X**, Wu F, Ren W, He C, Yin Z, Niu J, Bai M, Yang Z, Wu K, Fan D, Han G. Thrombotic risk factors in Chinese Budd-Chiari syndrome patients. An observational study with a systematic review of the literature. *Thromb Haemost* 2013; **109**: 878-884 [PMID: 23447059 DOI: 10.1160/TH12-10-0784]

36 **Qi X**, Wu F, Fan D, Han G. Prevalence of thrombotic risk factors in Chinese Budd-Chiari syndrome patients: results of a prospective validation study. *Eur J Gastroenterol Hepatol* 2014; **26**: 576-577 [PMID: 24694738 DOI: 10.1097/MEG.0000000000000056]

37 **Rautou PE**, Douarin L, Denninger MH, Escolano S, Lebrec D, Moreau R, Vidaud M, Itzykson R, Moucari R, Bezeaud A, Valla D, Plessier A. Bleeding in patients with Budd-Chiari syndrome. *J Hepatol* 2011; **54**: 56-63 [PMID: 20889223 DOI: 10.1016/j.jhep.2010.06.019]

38 **Pavri TM**, Herbst A, Reddy R, Forde KA. Budd-Chiari syndrome: a single-center experience. *World J Gastroenterol* 2014; **20**: 16236-16244 [PMID: 25473178 DOI: 10.3748/wjg.v20.i43.16236]

39 **Smalberg JH**, Darwish Murad S, Braakman E, Valk PJ, Janssen HL, Leebeek FW. Myeloproliferative disease in the pathogenesis and survival of Budd-Chiari syndrome. *Haematologica* 2006; **91**: 1712-1713 [PMID: 17145613]

40 **Perelló A**, García-Pagán JC, Gilabert R, Suárez Y, Moitinho E, Cervantes F, Reverter JC, Escorsell A, Bosch J, Rodés J. TIPS is a useful long-term derivative therapy for patients with Budd-Chiari syndrome uncontrolled by medical therapy. *Hepatology* 2002; **35**: 132-139 [PMID: 11786969 DOI: 10.1053/jhep.2002.30274]

41 **Xu PQ**, Ma XX, Ye XX, Feng LS, Dang XW, Zhao YF, Zhang SJ, Zhao LS, Tang Z, Lu XB. Surgical treatment of 1360 cases of Budd-Chiari syndrome: 20-year experience. *Hepatobiliary Pancreat Dis Int* 2004; **3**: 391-394 [PMID: 15313675]

42 **Harmanci O**, Kav T, Peynircioglu B, Buyukasik Y, Sokmensuer C, Bayraktar Y. Long-term follow-up study in Budd-Chiari syndrome: single-center experience in 22 years. *J Clin Gastroenterol* 2013; **47**: 706-712 [PMID: 22495815 DOI: 10.1097/MCG.0b013e31824ffd63]

43 **Wang R**, Meng Q, Qu L, Wu X, Sun N, Jin X. Treatment of Budd-Chiari syndrome with inferior vena cava thrombosis. *Exp Ther Med* 2013; **5**: 1254-1258 [PMID: 23596497]

44 **Han G**, Qi X, Zhang W, He C, Yin Z, Wang J, Xia J, Xu K, Guo W, Niu J, Wu K, Fan D. Percutaneous recanalization for Budd-Chiari syndrome: an 11-year retrospective study on patency and survival in 177 Chinese patients from a single center. *Radiology* 2013; **266**: 657-667 [PMID: 23143028 DOI: 10.1148/radiol.12120856]

45 **Raszeja-Wyszomirska J**, Mieżyńska-Kurtycz J, Marlicz W, Lawniczak M, Wójcicki M. Primary Budd-Chiari syndrome - a single center experience. *Hepatogastroenterology* 2012; **59**: 1879-1882 [PMID: 22819909]

46 **Ebrahimi M**, Modaghegh MH, Esmaeilzadeh A. Presentation of hospital outcomes and different treatment methods of patients with budd-Chiari syndrome: a report from two tertiary hospitals in iran. *Med Princ Pract* 2011; **20**: 287-290 [PMID: 21455002 DOI: 10.1159/000323755]

47 **Nozari N**, Vossoghinia H, Malekzadeh F, Kafami L, Mirheidari M, Malekzadeh R. Long-term Outcome of Budd-Chiari Syndrome: A Single Center Experience. *Middle East J Dig Dis* 2013; **5**: 146-150 [PMID: 24829685]

48 **Simonetto DA**, Yang HY, Yin M, de Assuncao TM, Kwon JH, Hilscher M, Pan S, Yang L, Bi Y, Beyder A, Cao S, Simari RD, Ehman R, Kamath PS, Shah VH. Chronic passive venous congestion drives hepatic fibrogenesis via sinusoidal thrombosis and mechanical forces. *Hepatology* 2015; **61**: 648-659 [PMID: 25142214 DOI: 10.1002/hep.27387]

49 **Gao X**, Gui E, Lu Z, Ning X, Zu M, Zhang P, Sun G. Risk factors of recurrence among 471 Chinese patients with Budd-Chiari syndrome. *Clin Res Hepatol Gastroenterol* 2015; **39**: 620-626 [PMID: 25656980 DOI: 10.1016/j.clinre.2014.12.010]

50 **Gwon D**, Ko GY, Yoon HK, Sung KB, Kim JH, Lee SS, Lee JM, Ohm JY, Shin JH, Song HY. Hepatocellular carcinoma associated with membranous obstruction of the inferior vena cava: incidence, characteristics, and risk factors and clinical efficacy of TACE. *Radiology* 2010; **254**: 617-626 [PMID: 20093533 DOI: 10.1148/radiol.09090738]

51 **Park H**, Yoon JY, Park KH, Kim do Y, Ahn SH, Han KH, Chon CY, Park JY. Hepatocellular carcinoma in Budd-Chiari syndrome: a single center experience with long-term follow-up in South Korea. *World J Gastroenterol* 2012; **18**: 1946-1952 [PMID: 22563176 DOI: 10.3748/wjg.v18.i16.1946]

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