The clinical spectrum of Fontan-associated liver disease: results from a prospective multimodality screening cohort

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Abstract

Aims

Liver fibrosis and cirrhosis are a consequence of a Fontan physiology, and determine prognosis. It is unclear whether non-invasive assessment of liver pathology is helpful to provide clinically relevant information. The aims of this study were to assess the spectrum of Fontan-associated liver disease (FALD) and usefulness of non-invasive methods to assess biopsy confirmed liver fibrosis.

Methods

Hepatic screening of consecutive patients consisted of a blood panel, ultrasonography, elastography, contrast-enhanced MRI/CT, and liver biopsy (scored with Fontan specific fibrosis scores and collagen proportionate area; CPA). Fibrosis parameters, varices, ascites, and splenomegaly were measured on imaging.

Results

38/49 referred patients (27 ± 6.6 years, 73.7% male) underwent the complete screening protocol. Liver fibrosis on biopsy was present in all patients, and classified as severe (stage 3-4) in 68%. Median CPA was 22.5% (16.9-29.5) and correlated with individual fibrosis scores. ELF® and liver stiffness were elevated, but MELD-XI scores were low in all patients. Fibrosis severity neither correlated to ELF® and liver stiffness, nor to (semi-) quantitative fibrosis parameters on MRI/CT. Varices were present in 50% and hyper-enhancing nodules in 25% of patients, both independent of fibrosis stage, but varices were associated with higher CPA values.

Conclusion

The FALD spectrum includes both hepatic congestion and severe fibrosis, with signs of portal hypertension and hyper-enhancing nodules as significant manifestations. Routine imaging, transient elastography and serum biomarkers are unable to accurately assess severity of liver fibrosis in this cohort. Future research should focus on validating new diagnostic tools with biopsy as the reference standard.

Keywords (6): Fontan, Fontan-associated liver disease, liver fibrosis/cirrhosis, , liver biopsy, screening, nodules

Introduction

Structural hepatic changes are a recognized complication after palliative Fontan surgery of patients with severe congenital heart disease.[1, 2] The Fontan procedure establishes a unique hemodynamic physiology, resulting from a direct conduit between caval veins and pulmonary arteries.[3, 4] As a consequence, caval pressure rises resulting in elevated hepatic afterload, liver congestion, and increased risk of liver fibrosis.[5]

Multidisciplinary consensus recommends hepatological evaluation of patients for Fontan-associated liver disease (FALD), however specific guidelines how to commence screening are lacking.[8] Since liver fibrosis is mostly asymptomatic, clinical history and physical examination are of limited value. As in many other chronic liver diseases routine liver biochemistry is inaccurate to quantify Fontan-associated liver fibrosis.[9-11] Several non-invasive tools, such as transient elastography and fibrosis biomarkers have gained traction to diagnose and quantify fibrosis, but have only been evaluated to a limited extent in this population.[12-14] A liver biopsy is considered to be the gold standard for staging of hepatic fibrosis, and is used as the reference standard method in evaluations of biomarkers and non-invasive markers of liver fibrosis. However, biopsies are invasive and carry a certain risk of complications, in particular bleeding.[16] The exact bleeding risk is unknown, but could be high in the Fontan population in view of the frequent use of anticoagulants.[17] There are a number of studies that have evaluated the extent of liver disease in this population but they are hampered by retrospective design, selection bias, or the absence of histology as reference standard.[7, 12-14, 18-24] It is unclear which investigations contribute to true prognostic information that changes clinical management and outcomes.

The aims of this study were (1) to assess the clinical spectrum of FALD including signs of portal hypertension and hepatic nodules in asymptomatic patients with a Fontan physiology, and (2) to examine the usefulness of serum and image-based fibrosis markers to assess fibrosis, in comparison to liver biopsy as the reference standard.

Methods

Study population

All consecutive patients (18 years or older) with a Fontan physiology, monitored at the congenital cardiology department of the Radboud university medical centre were referred to the hepatology department for a prospective screening programme for liver disease. Inclusion took place from November 2015 to September 2017. The screening protocol consisted of routine biochemistry

testing, VO2max exercise test, cardiac and liver ultrasound, transient elastography, advanced imaging of the liver with MRI or CT, and liver biopsy. Patients were required to consent for the individual procedures. All procedures were performed on the same day, while the liver biopsy procedure and advanced imaging were performed within a range of three months. Data from clinical evaluations was collected through chart review. The study was approved by the institutional review board of the Radboud university medical centre (no. 2015-2132).

Clinical evaluation

All patients were simultaneously clinically evaluated at the departments of Hepatology and Cardiology. Patients were evaluated for risk factors for liver disease such as alcohol and drug use. Physical examination included hemodynamic parameters and signs of Fontan failure or liver disease such as peripheral edema, ascites, spider naevi, palmar erythema, and jaundice. Patients underwent cardiac ultrasonography, visually assessing global ventricle function, and a VO2 max test. [25, 26]

Liver biopsy and histological assessment

Ultra-sound guided percutaneous liver biopsy (16G, true-cut or suction biopsy) was performed under conscious sedation by experienced hepatologists (ET, JD). Liver tissue slides were stained with hematoxylin-eosin, elastica-Van Gieson, and picrosirius red. Slides were evaluated independently by two liver pathologists experienced in vascular liver disease (CB, TK). Adequate biopsy samples were defined as length ≥ 2cm and the presence of ≥ 11 portal tracts. Three fibrosis scores, previously reported to assess Fontan-associated liver fibrosis, were assessed: Gross architectural distortion score,[19] scoring overall fibrosis distribution and architectural changes (ranging 0-4); A four component score,[20] scoring portal fibrosis (0-4), sinusoidal fibrosis (0-4), sinusoidal dilation (0-3), portal inflammation (0-3); and the congestive heart failure fibrosis score (CHFS; 0-4).[27] See supplementary files for exact description of scores.

To obtain a comprehensive overview of total amount of fibrosis present, the percentage of total tissue area occupied by collagen (picrosirius red stain) or so-called collagen proportionate area (CPA) was calculated, as described before.[28] Steatosis was assessed with NAFLD activity score (0-3).[29]

Laboratory evaluation

Laboratory testing included routine liver biochemistry (alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphotase (ALP), gamma-glutamyl transferase (GGT), total bilirubin, and liver function tests (INR or prothrombin time, platelets, and albumin). Hepatitis B and C serology was tested. A commercial panel of direct fibrosis markers was assessed (Enhanced Liver Fibrosis Test®, Siemens, the Netherlands) consisting of hyaluronic acid, PIIINP, and TIMP-1. The score has a cut-off of ≥ 7.7 for the presence of fibrosis.[30] Furthermore the APRI, an indirect fibrosis score

was calculated from AST and platelets.[31] Von Willebrand factor (vWf) and VITRO score (VWF/platelet ratio), as surrogate markers for portal hypertension, were measured.[32, 33] MELD-XI score (developed to assess end-stage liver disease in patients on anticoagulants by excluding INR) was calculated.[34]

Liver ultrasonography and transient elastography

Dedicated liver ultrasonography (Hitachi Preius TM) was performed, and evaluation focused on aspect, pattern of parenchyma and liver surface, assessment of focal lesions, presence of ascites, and spleen size. Standardized Doppler flow measurements were performed on inferior caval vein (diameter, respiratory variability), hepatic veins (diameter, flow pattern), portal vein (flow direction and flow velocity), and hepatic artery (peak systolic flow velocity, end diastolic flow velocity, and resistance index).

Liver stiffness (in kPa) was measured by transient elastography with Fibroscan 502®, assessing the median of ≥10 measurements with an interquartile range/median ≤ 30%.

Spleen diameter (mm) and platelet count were incorporated in the platelet/spleen ratio, with a proposed cut-off of <909 for identification of patients at risk for presence of clinically relevant portal hypertension.[35]

Advanced imaging

A contrast-enhanced MRI was performed (Dotarem®; gadoterate meglumine contrast, Siemens, 3 tesla), or in case this was not possible a multiphase liver CT-scan (iodinated contrast, Toshiba, slide thickness 5 mm). For clinical CT and MRI protocols see supplementary methods. Liver radiologists and a specialized vascular liver radiologist (MR) assessed and semi-quantitatively scored imaging characteristics of fibrosis and portal hypertension: liver nodularity and dysmorphy, size of liver segment 1 and 4, ascites, splenomegaly, and varices. Ascites was defined as the presence of any amount of free fluid in the peritoneal cavity. Splenomegaly was defined as a spleen diameter > 12 cm. Spleen diameter was calculated as the largest spleen bipolar diameter at the splenic hilum. Varices were defined as the presence of dilated vessels (regardless of the size) in one of the 5 territories of porto-systems shunting, i.e. gasto-esophageal, spleno-renal, para-umbilical or parietal, mesenteric or peri-rectal, and retroperitoneal. Discrimination with systemic-pulmonary shunts was made following the different pathways.

A combination of liver peripheral atrophy with hyper signal intensity on T2-weigthed imaging and delayed enhancement (short 'periphery') and subcapsular intermingled enhanced septa (short 'reticulation') were scored on MRI. Apparent diffusion coefficient (ADC) and relative enhancement ratio (RER) were calculated on diffusion-weighted imaging, as a modification of methodology described before (see supplementary methods).[36] All focal liver lesions were described and when larger than 10 mm scored on the following characteristics: signal T1 and T2 intensity, diffusion-

weighted intensity, intensity on different contrast-enhanced phases, washout, fat content, capsule formation, and central scar. Alpha-fetoprotein (AFP) was measured in patients with focal liver lesions.

Statistical analyses

Continuous data are presented with mean and standard deviation (±SD), nominal data as count (n) and percentage (%), and ordinal or not normally distributed data with median and interquartile range (25^{th}-75^{th} percentile). Independent t-test, or non-parametric tests where applicable, were used to compare continuous variables. Chi-Square test was used to compare categorical variables and Mann-Whitney U test for ordinal variables. Correlations were tested with Pearson's correlation coefficient or Spearman's Rho, depending on the level of measurement. A two-sided level of $p < 0.05$ was considered statistically significant. All statistical analyses were carried out using SPSS software (version 22; SPSS Inc., Chicago, Illinois, USA).

Results

Clinical characteristics

A total of 49 patients with a Fontan physiology was referred for simultaneous cardiac and hepatologic screening. Eleven patients refused biopsy, so they were excluded from further analyses. The cohort consisted of 38 patients, prospectively assessed with the multimodality approach (see Flowchart). Mean age at time of screening was 27 ± 6.6 years, with 74% males. Average time after completion of the Fontan circulation was 21.4 ± 5.5 years. For all characteristics see Table 1.
Based on cardiac ultrasound assessment, 21 (55%) of patients had good systolic function of the systemic ventricle, 15 patients (40%) had mild dysfunction and two (5%) moderate dysfunction.[25]
The majority of patients were classified as NYHA I (90%) or NYHA II (10%). All patients had regular physical activity, but the mean VO_2max, as a marker of exercise tolerance, was 25.2 ± 5.8 ml/kg/min corresponding with 56 ± 12% of age and gender matched healthy individuals. [26]
Patients had a median BMI of 22.2 (20.3-24.6) kg/m^2 and a median alcohol consumption of 1 (0-4) units/week. None tested positive for hepatitis B or C. Physical examination of patients did not reveal any signs or symptoms suggestive for chronic liver disease. All, but one patient, were treated with anti-thrombotics. Eleven patients (29%) used platelet aggregation inhibitors, while 26/38 used oral anticoagulants (23/26 coumarin derivatives and 3/26 Direct Oral Anti-Coagulants).

Liver histology

All patients underwent percutaneous liver biopsy without any complications defined as severe pain,

bleeding, or any cause that required prolonged admission, additional diagnostic procedures, or treatment. Antithrombotic or anticoagulant medication could be temporarily interrupted without bridging. No thrombotic events occurred.

All biopsies were of sufficient quality. There was substantial interobserver agreement on portal fibrosis score between both pathologists (kappa=0.747, 95% CI: 0.615-0.879), but not on sinusoidal fibrosis score (kappa=0.104, 95% CI: -0.226-0.434).

The median gross architectural distortion score was 3 (2-4). Median score for portal fibrosis was 2 (2-4) and for sinusoidal fibrosis 2 (1-2). Median CHFS was 3 (2.75-4). Eleven (29%) of patients had stage 4; cirrhosis, on all three scores. There were no patients without fibrosis on any of the scores (Figure 1A). Median sinusoidal dilatation was 1 (.75-2) and median portal inflammation score was 0, with only three patients classified as mild to moderate inflammation (score 1-2). Steatosis was absent from all biopsies. Median collagen proportionate area (CPA) was 22.5% (16.9-29.5). CPA correlated with overall architectural distortion score (Rs=0.457), portal fibrosis score (Rs=0.524) and CHFS score (Rs=0.419; all p<.01), but not with sinusoidal fibrosis score (Rs=0.102, p=0.541). Gross architectural score, portal fibrosis score and CHFS all correlated significantly with each other (Rs>.800 and p<.001). Key histopathological features of FALD are depicted in Figure 1B.

For further analyses, patients were divided in two groups, based on stage of fibrosis by gross architectural distortion score: 16 patients (42%) had mild fibrosis (stage 1-2) and 22 (68%) had severe fibrosis (stage 3-4).

Non-invasive assessment of FALD

1) Liver biochemistry

Liver biochemistry did not differ between patients with mild and severe fibrosis (Table 2). To highlight, the majority of patients in both groups had an elevated GGT (80% of patients with mild fibrosis vs. 86% of patients with severe fibrosis p=.670).

Bilirubin was increased in 30% of patients (20% with mild fibrosis and 41% with severe fibrosis, p=.286). Platelets (10^09/L) were within normal range in most cases (73% in both groups). MELD-XI scores were similar between groups and almost all within normal range. None of the parameters correlated with CPA (data not shown).

2) Serum fibrosis markers

Median ELF score was above the threshold of 7.7 in all patients, suggesting the presence of fibrosis. Scores were similar between groups, median 9.03 (8.38-9.25) in mild and 9.20 (8.69-9.57) in severe fibrosis (see figure 2A). APRI was also comparable between groups (median 0.42 (0.35-0.53) in mild

and 0.44 (0.35-0.64) in severe fibrosis (p=.531)). ELF and APRI did not correlate with CPA (Rs=.242, p=.161 and Rs=.100, p=.555 respectively).

3) Ultrasonography and Elastography

Six patients with severe fibrosis (27%; five with cirrhosis and one with stage 3 on gross architectural score) had a heterogeneous liver aspect of the parenchyma, compared to none with mild fibrosis (p=.030). Median CPA was also significantly higher in patients who had a heterogeneous aspect of the liver on ultrasound (35% (27-37) vs. 22% (17-24), p=.026).

Portal vein flow was similar between patients with mild and severe fibrosis, see figure 2C. Portal vein flow and spleen size showed a moderate correlation with CPA (Rs=-.442, p=.005 resp. Rs=.452, p=.004). Ultrasound and Doppler flow parameters are shown in Table 3.

Fibroscan was successful in 36/38 patients. Median liver stiffness was 22.5 (8.8- 45.7) kPa. Liver stiffness was similar in mild (median 21.3 (14.3-29.1) kPa) and severe fibrotic patients (26.0 (15.1-28.9) kPa, p=.511, see figure 2B), and did not correlate with CPA (Rs=-.015, p=.931). Liver stiffness did not correlate to histological grade of sinusoidal dilatation (Rs=.-079, p=.648).

4) Advanced Imaging

Fibrosis

In 30/38 patients we performed an MRI, and eight patients were subjected to a CT-scan. Nodularity was a frequently observed feature, absent only in five patients, and was evenly distributed between patients with mild and severe fibrosis. Other semi-quantitative fibrosis features were also equally present in patients with mild and severe fibrosis (see Table 4). The combination of specific hallmarks of cirrhosis; an enlarged caudate lobe, atrophied segment 4 and surface nodularity were present in five patients: two patients with severe fibrosis (stage 3) and three patients with mild (stage 2) fibrosis. ADC and RER were similar between groups, and did not correlate with CPA (Rs=-.092, p=.627 and Rs=-.006, p=.972, respectively). None of the other histological component scores correlated with any of the imaging parameters (data not shown).

Assessment of portal hypertension

Varices were present in 19 (50%) patients, ascites in 22 (58%) and splenomegaly in 7 (18%) of patients. A combination of all three characteristics was found in five patients (see Table 5). 8/11 patients with cirrhosis (73%) had varices compared to 11/27 (41%) patients with a lower stage of fibrosis (p=.074). CPA was significantly higher in patients with varices than in patients without varices: median 24.2% (21.5-35.7) vs. 18.5% (14.2-24.6), p=0.015.

Liver stiffness was similar between patients with and without varices (mean 21.4 ± 11.3 kPa and 24.1 ± 9.1 kPa, respectively; p=.391). Platelet count and platelet/spleen ratio were significantly lower in

the group with varices (median 153 (125-175) 10^9/L and 1177 (892-1446), respectively) than in patients without varices (median 174 (147-222) 10^9/L, p=.020; and 1740 (1185-2009), p=.001, respectively). Of note, none of the patients reported clinical events such as variceal bleeding.

Hepatic nodules

Thirteen patients had several nodules smaller than 10 mm, while 13 had no nodules at all.
Nine patients had in total 25 focal hyper-enhancing hepatic lesions larger than 10 mm (median 1 nodule per patient, ranging from 1-7) (Table 6). The number of patients with nodules was comparable between patients with mild and severe fibrosis (n=3, 19% and n=6, 27% respectively, p=.706) and patients with or without varices (4 vs. 5 patients, p=1.000). Patients with severe fibrosis did have more nodules (median 4 nodules; range 1-7) than patients with mild fibrosis (all three patients had 1 nodule, p=.047). Delayed-phase washout was present in 9 nodules (36%) in 5 patients; one patient with cirrhosis (stage 4) had 4 nodules with wash-out. These lesions remained stable up to 12 months on follow-up. Two patients with stage 3 fibrosis had 1 and 2 nodules, respectively, and 2 patients with stage 1 and stage 2 fibrosis had one nodule.
None of these nodules had additional ancillary features such as hyperintensity on T2 and DWI images or fat content. The mean AFP was low in all patients (mean AFP 3.39 ± 1.29 ug/L).

Discussion

Key findings

The present study demonstrates (1) the presence of severe liver fibrosis, signs of portal hypertension and hyperenhancing nodules in a majority of asymptomatic patients with a Fontan physiology, and (2) the lack of correlation between several non-invasive diagnostic tools and histological confirmed liver fibrosis severity. These findings indicate that non-invasive diagnosis of/screening for severe FALD in patients with a Fontan physiology is ineffective to accurately assess liver fibrosis.

Screening of FALD

There is an unmet need to diagnose and assess liver fibrosis severity with non-invasive alternatives, as liver biopsy carries potential complications. Our data suggests that there is no role in clinical practice for ELF®, routine transient elastography or conventional MRI/CT to diagnose liver fibrosis in Fontan physiology, since it fails to correlate with histological fibrosis severity. Ultrasound abnormalities, such as heterogeneous aspect of parenchyma could be indicative for severe fibrosis, but absence of abnormalities does not rule out severe FALD. [39-41]This highlights that experiences from patients with other forms of liver disease (e.g. liver cirrhosis as a result of alcohol abuse, hepatitis B

and C) cannot be applied to the Fontan population, as interference of congestion remains an issue of concern in this unique physiology.[39-41]

Although we encountered no complications from the biopsy itself or from interruption of anticoagulant therapy, we acknowledge that liver biopsy is not an attractive tool to use for follow-up. Thus there remains an unmet need for alternatives to ease screening and follow-up in this population. MRI is subject to fast and major developments, and techniques such as T1-rho mapping, extracellular volume calculation,[42] susceptibility imaging,[43] and magnetic resonance elastography [14 44] are promising. We do stress that new techniques should be validated against histology before they are adopted in daily practice.

Pathophysiology

Although not fully elucidated, chronic passive hepatic congestion is thought to be the underlying mechanism that puts patients with a Fontan physiology at risk for complications of advanced liver disease.[45] In congestive hepatopathy, venous congestion elicits a sustained wound-healing response. Upon histological examination, this is characteristically depicted by sinusoidal dilatation, presumably resulting from venous pressure elevation. Consequently fibrosis arises, with also mainly a sinusoidal pattern.[46] Over time, injury precipitates formation of broad scars, bridging fibrosis and cirrhosis. Fibrosis is also generated by microthrombotic events in sinusoids and veins.[47] The ubiquitous presence of fibrosis in all biopsies examined in this study, underlines the profound impact of the Fontan physiology on the liver architecture, and contrasts with that in other aetiologies such as viral hepatitis, where only a minority of patients will ultimately develop liver fibrosis.[48] The specific histological pattern of sinusoidal fibrosis in combination with sinusoidal dilatation can help to distinguish FALD from other liver diseases, however correlation with clinical information should always be made to identify the definite cause of liver injury, as no pattern is unique in liver pathology.[49]

The spectrum of FALD

The findings from our study are consistent with the spectrum of FALD ranging from mild congestive hepatopathy to established cirrhosis. At 21 years after surgery, the majority of patients have developed advanced liver disease. Some 29% had histological evidence of liver cirrhosis and 50% had varices. These findings are in line with those from other Fontan cohorts, reinforcing the concept that liver fibrosis and portal hypertension are major extra-cardiac manifestations of Fontan physiology.[19, 21, 37, 38]

This reflects the ongoing continuum that starts postoperatively with hepatic vein congestion, which causes liver injury resulting in ongoing fibrogenesis.[5]

To address the true risk of advanced liver disease and its complications in patients with a Fontan

physiology we need longitudinal data that associate presence and severity of FALD to morbidity and mortality. [18, 50,66] In other chronic liver diseases, such as viral hepatitis and NAFLD, fibrosis stage determines portal hypertensive complications HCC development and ultimately prognosis.[6] If this is also the case in patients with a Fontan physiology needs to be further elucidated by prospective studies.

Portal hypertension

In our cohort, signs of portal hypertension were more frequently seen in patients with cirrhosis. Splenomegaly and portosystemic varices are uncommon in congestive hepatopathy, and are generally related to central venous pressure being transmitted through dilated hepatic sinusoids to the portal venous system. We did not perform measurements of the transhepatic pressure gradient, but in congestive hepatopathy and FALD values are usually normal.[45, 46] The presence of varices may be an indication of the transformation from congestion to fibrosis.[51] To what degree failure of Fontan physiology contributes to the onset of portal hypertension remains unknown.

Fontan nodules

Congestive hepatopathy is also associated with the onset of focal liver lesions. Arterialized large regenerative nodules or focal nodular hyperplasia (FNH) are often found in congestive hepatopathy.[52, 53] These lesions comprise of regions of parenchyma with compromised hepatic venous outflow and subsequently impaired portal inflow. Parenchymal perfusion thus becomes reliant on arterial flow, resulting in nodular regeneration of hepatocytes. It is generally assumed that FNH have no malignant potential.[54] On the other hand, cirrhosis is associated with an increased risk of hepatocellular carcinoma (HCC), and HCC has been described in young cirrhotic patients with a Fontan physiology.[55, 56] Hyperenhancing nodules were present in ~25% of patients from our cohort, independent of fibrosis stage, corroborating previous findings.[53, 57] Wash-out, in the setting of cirrhosis, is usually a specific feature of HCC, but in FALD may also be present in benign lesions.[53] Specific guidelines for screening of patients with a Fontan physiology for HCC are lacking,[58] but the value of AFP, liver-specific contrast agents, and additional ancillary features for discrimination between benign and potentially malignant Fontan nodules should be further explored.

Liver function & transplantation

Finally, liver function is an issue of concern. Liver function is defined as the ability to maintain bilirubin metabolism and intact coagulation, and not as the increase of transaminotransferases.[59] Liver fibrosis is an important prognostic determinant in patients with other chronic liver diseases as it may result in impaired liver function and increased mortality.[6] It is suggested that 5-year survival in cirrhotic patients with Fontan physiology was dismal which in turn was due to both cardiac and liver

related complications.[7] Clinical follow-up of patients with established liver disease is facilitated by the MELD (Model of End stage Liver Disease) score, prioritizing patients in need of liver transplantation.[60] Transplantation data are very limited and come from several small case series that describe combined heart and liver transplantation in patients with a failing Fontan physiology and liver cirrhosis.[61-63] These show good short term results, comparable to orthotopic heart transplant. Nonetheless, long term results are lacking. In adjusted form, MELD-XI probably predicts cardiac mortality or heart transplantation better than hepatological mortality, as similar outcomes are observed regardless of the presence of severe fibrosis.[64] In our cohort, MELD-XI scores were low.

Strengths & Limitations

The major strength of our study lies in the optimal identification of fibrosis in this population using a gold standard: histological confirmation. This allows us to interpret the observations as a result of either congestion, fibrosis or combined. The use of multiple invasive and non-invasive modalities for the same patients allows a fine comparison of the diagnostic value to detect congestion or fibrosis in relation to the gold standard. Previous studies investigating non-invasive alternatives to assess FALD lacked reference to the gold standard, hampering interpretation of results. Selection bias was limited since every consecutive patient was included and subjected to structural assessment. Liver fibrosis severity in our cohort is probably representative for the general population with Fontan physiology. Therefore we can answer to several important key knowledge gaps as identified by the stakeholders meeting of the American College of Cardiology in 2017.[65]

Our study is limited by the relatively small number of patients, but on the other hand our cohort underwent systematic and deep characterisation of the phenotype. The study design is cross-sectional, and exposure and outcome were simultaneously assessed. Without the presence of longitudinal data it is not possible to establish temporal relation between cause (Fontan physiology) and effect (liver fibrosis and its complications) or to assess the value of non-invasive diagnostics in monitoring of FALD. Longitudinal assessment of liver stiffness measurements has the potential to be helpful in monitoring patients with a Fontan physiology [44], but needs further confirmation. Future research should focus on assessing modalities for surveillance of these patients and on pathophysiology and risk factors for progression of FALD.[65]

Conclusion

In conclusion, we show that cirrhosis and signs of portal hypertension are present in substantial proportion of patients with a Fontan physiology, and that MRI, CT, transient elastography, and serum biomakers are unable to accurately assess severity of liver fibrosis in this cohort. This emphasizes the need for prospective longitudinal follow-up studies to further assess morbidity and mortality risk and to search for non-invasive alternatives to diagnose and monitor FALD.

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Figure Legends

Study flowchart: Legend: Flowchart of number of patients referred for hepatologic screening and actually screened by the several modalities incorporated in screening.

Figure 1A: Legend: Distribution of the assessed histological scores is displayed on the X-axis, with percentages of the scoring stages depicted on the Y-axis.

Figure 1B: Legend: Legend: Exemplary photographs of histological slides from patients with FALD, were taken with a camera attached to a microscope and processed in a digital image analysis programme. Panel 1: mild sinusoidal dilatatation (stage 1). Hematoxylin-Eosin stain, objective lens 10x. Panel 2: severe sinudoidal dilatation (stage 3). Hematoxylin-Eosin stain, objective lens 10x. Panel 3: mild sinusoidal fibrosis, with characteristic chicken-wire pattern of staining along the dilated sinudoids. Sirius Red stain, objective lens 20x. Panel 4: close-up of cirrhotic liver biopsy of patient with FALD, showing severe sinusoidal fibrosis and dilatation (stage 3) and gross architectural distortion (stage 4), with broad scarring and nodular regeneration. Sirius Red stain, objective lens 10x.

Figure 2: Legend: Data points in the graphs represent individual patient data, with the line representing the median value in patients with mild (n=16), compared to patients with severe fibrosis (n=22). Panel A represents ELF scores as measured in serum, Panel B liver stiffness (in kPa) as measured by Fibroscan®, and Panel C portal vein flow (in cm/s) as measured with Doppler flow on liver ultrasonography. Missing data: ELF score is missing in 3 patients with severe fibrosis.

Tables

Table 1. Patient characteristics	
Demographics	
Female gender	10/38 (26%)
Age (years)	27 ± 6.6
Body mass index (kg/m^2)	22.2 (20.3-24.6)
Congenital main diagnosis	
Atresia (pulmonary/tricuspidal)	18/38 (47%)
Double-inlet Left Ventricle	11/38 (29%)
Other*	9/38 (24%)
Type of Fontan procedure	
Atriopulmonary connection	14/38 (37%)
Lateral tunnel	7/38 (18%)
Extracardiac conduit	15/38 (40%)
Kawashima, secondary connection liver veins to pulmonary system	2/38 (5%)
Age at Fontan procedure (years)	5.0 (3.0-6.3)
Fontan duration (years)	21.4 ± 5.5
Medication	
Oral anticoagulants ɬ	26/38 (68%)
Hepatotoxic medication †	1/38 (3%)

Legend: Data are presented as mean (SD) or median (IQR) for continuous variables and as n, % for categorical variables. * Other cardiac main diagnosis include: hypoplastic left heart syndrome (n=4), double-inlet right ventricle (n=2), double-outlet right ventricle (n=2), left isomerism with univentricular heart (n=1). ɬ used anticoagulants are acenocoumarol (n=17), fenprocoumon (n=6), Direct Oral Anti-Coagulant (n=3)
† one patient used amiodaron and quinapril.

Table 2. Laboratory results of patients with mild vs severe fibrosis

	Mild fibrosis (n=16)†		**Severe fibrosis (n=22)†**		**p-value**
	Median (IQR)	n, % abnormal	Median (IQR)	n, % abnormal	
ALT (IU/L)	28 (24-33)	1/15 (7%)	28 (23-37)	3/22 (14%)	.915
AST (IU/L)	28 (23-33)	1/15 (7%)	28 (25-35)	5/22 (23%)	.551
ALP (IU/L)	70 (54-97)	2/15 (13%)	81 (70-96)	2/22 (9%)	.105
GGT (IU/L)	58 (46-104)	12/15 (80%)	62 (49-121)	19/22 (86%)	.636
Bilirubin (mmol/L)	13 (11-19)	3/15 (20%)	16 (11-22)	9/22 (41%)	.761
Albumin (g/L)	41 (40-43)	0/13	42 (40-44)	0/21	.529
MELD-XI score	9.44 (9.44-10.98)	1/12 (8%)	9.44 (9.44-11.08)	3/22 (14%)	.873

Legend: Data are presented as median with (IQR) and n,% of patients with an abnormal result (elevated or diminished, in comparison to the upper or lower limit of normal) on the corresponding test. * For APRI there is not a uniform cut-off available. P-values are shown for non-parametric analyses between median values of laboratory results in patients with mild and severe fibrosis. †Missing data: In one patient with mild fibrosis no blood results were present except for ELF. MELD-XI score could not be calculated, because of missing creatinin values in 4 patients with mild fibrosis. In 2 patients with mild fibrosis missed bilirubin and albumin. Albumin was missing in 1 and ELF in 3 patients with severe fibrosis. Total number of patients with available blood results are stated as denominator in the row showing n,% of abnormal test results.

Table 3. Parameters ultrasound and Doppler flow measurements

	Mild fibrosis (n=16)	**Severe fibrosis (n=22)**	**p-value**
Ultrasound parameters			
Enhanced pattern liver	3/16 (19%)	2/22 (9%)	.632
Heterogeneous aspect parenchyma	0/16	6/22 (27%)	.03
Irregular liver surface	5/16 (31%)	1/22 (5%)	.065
VCI compliance (<50%)	15/16 (94%)	18/20 (90%)	1.000
Spleen size (cm)	11.6 (10.3-13.0)	11.8 (10.5-13.0)	.895
Doppler Flow parameters			
Monophasic flow hepatic veins	2/16 (13)	3/22 (14%)	n.a.
Portal vein flow (cm/s)	16.5 (14.0-24.8)	16.3 (13.1-20.0)	.529
Hepatic artery flow max (cm/s)	69.4 (57.2-90.3)	65.8 (55.6-85.2)	.617
End diastolic hep artery flow (cm/s)	20.7 (14.4-33.0)	19.7 (16.8-23.8)	.660
Resistance Index	0.66 (0.58-0.84)	0.70 (0.64-0.79)	.800

Legend: Data are presented as median (IQR) for continuous variables and as n, % for categorical variables. Missing data: VCI compliance and resistance index could not be measured in 2 patients and end diastolic hepatic artery flow was missing in one patient.

Table 4. Radiological evaluation of fibrosis characteristics compared to histology

	Mild fibrosis (n=16)	**Severe fibrosis (n=22)**	**p-value**
*Periphery**			
None	0/11	1/19 (5%)	0.740
Mild	6/11 (55%)	10/19 (53%)	
Marked	5/11 (45%)	8/19 (42%)	
Nodularity			
None	3/16 (19%)	2/22 (9%)	0.684
Mild	6/16 (38%)	9/22 (41%)	
Marked	7/16 (44%)	11/22 (50%)	
*Reticulation**			
None	0/11	4/19 (21%)	0.179
Mild	7/11 (64%)	7/19 (37%)	
Marked	4/11 (36%)	8/19 (42%)	
Dysmorphy	16/16 (100%)	22/22 (100%)	-
Segment 1			
Atrophy	0/16	0/22	0.374
Normal	1/16 (6%)	4/22 (18%)	
Enlarged	15/16 (94%)	18/22 (82%)	
Segment 4			
Atrophy	4/16 (25%)	3/22 (14%)	0.534
Normal	9/16 (56%)	12/22 (55%)	
Enlarged	3/16 (19%)	7/22 (32%)	
ADC*	1.03 (0.96-1.30)	1.0 (0.96-1.04)	0.250
RER	0.45 (0.38-0.65)	0.51 (0.41-0.61)	.988

Legend: Data are presented as median (IQR) for continuous variables and as n, % for categorical variables.
*ADC, Periphery and reticulation characteristics can only be assessed on MRI, therefore not measured in 8 patients (5 mild, 3 severe fibrosis).

Table 5. Signs of portal hypertension in patients with mild and severe fibrosis

	Mild fibrosis (n=16)	Severe fibrosis (n=22)	p-value
Varices	7/16 (44%)	12/22 (54%)	0.511
Ascites	9/16 (56%)	13/22 (59%)	0.861
Splenomegaly	3/16 (19%)	4/22 (18%)	1.000
Combination of all 3*	2/16 (13%)	3/22 (14%)	n.a.
Platelets (10^09/L)	164 (137-186)	155 (136-191)	.963
Diminished platelet count (n,%)	4/15† (27%)	6/22 (27%)	1.000
Platelet/spleen ratio	1416 (1032-1763)	1297 (1123-1745)	1.000
VWF (%)	100 (100-100)	100 (100-105)	.417
VITRO score	63.4 (44.6-72.7)	64.9 (46.8-84.0)	.421

Legend: Data are presented as median (IQR) for continuous variables and as n, % for categorical variables. Missing data: In one patient no blood results were present, so platelet/spleen ratio and VITRO score could not be calculated.
*combination of varices, ascites and splenomegaly on advanced imaging. †platelet count was missing in one patient with mild fibrosis.

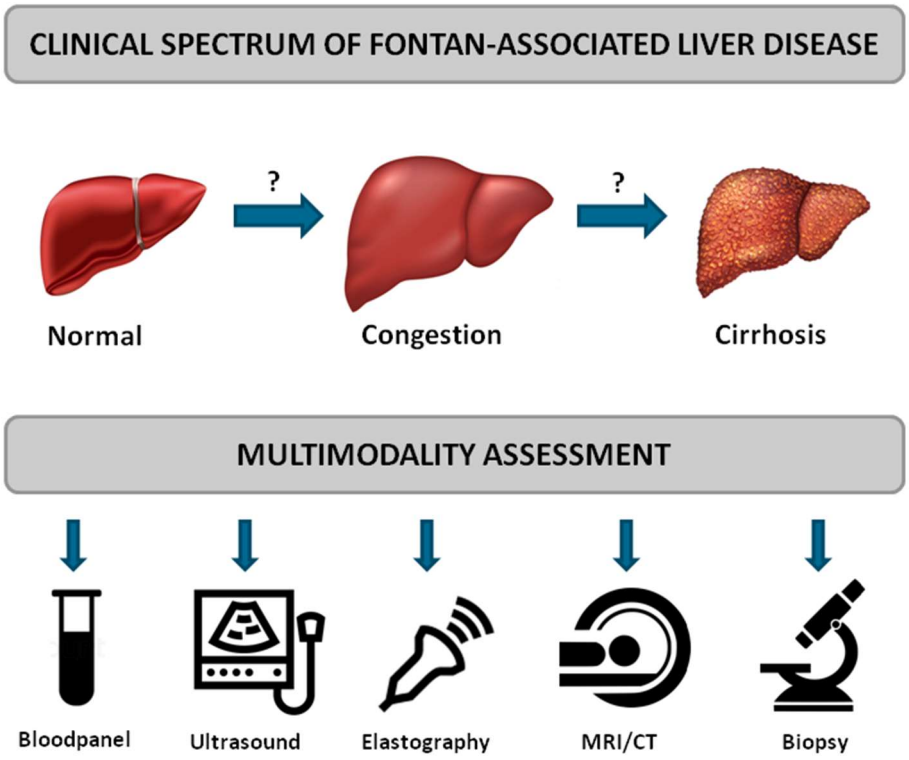
Table 6. Features of nodules > 10mm

25 Nodules in 9 patients	
Size (mm)	18.0 ±6.2
10-20 mm	19/25 (76%)
>20 mm	6/25 (24%)
Location	
Right hemi-liver	13/25 (52%)
Left hemi-liver	11/25 (44%)
Caudate lobe	1/25 (4%)
Signal intensity (T1-weighted)	
Hypo-intense	0
Iso-intense	20/25 (80%)
Hyperintense	5/25 (20%)
Signal intensity (T2-weighted)	
Hypo-intense	4/25 (16%)
Iso-intense	21/25 (84%)
Hyperintense	0
*Diffusion-weighted**	
Hyperintense	0
Arterial phase	
Hyper-intense	25/25 (100%)
Portal phase	
Hypo-intense	0
Iso-intense	3/25 (12%)
Hyperintense	22/25 (88%)
Delayed phase	
Hypo-intense	9/25 (36%)
Iso-intense	11/25 (44%)
Hyperintense	5/25 (20%)
Washout	
Hypo-intense on portal phase	0
Hypo-intense on delayed phase	9/25 (36%)
-Hyper>iso >hypo	-3/25 (12%)
-Hyper>hyper>hypo	-6/25 (24%)
No washout	13/25 (52%)
Hyperintense all phases	3/25 (12%)
Other	
Fat content	0
Capsule	5/25 (20%)
Central scar	4/25 (16%)

Legend: Data are presented as mean ± SD for continuous variables and as n, % for categorical variables. * Diffusion-weighted images only available on MRI, missing data on 5 nodules.

Figures:

Summarizing illustration:



Study flowchart

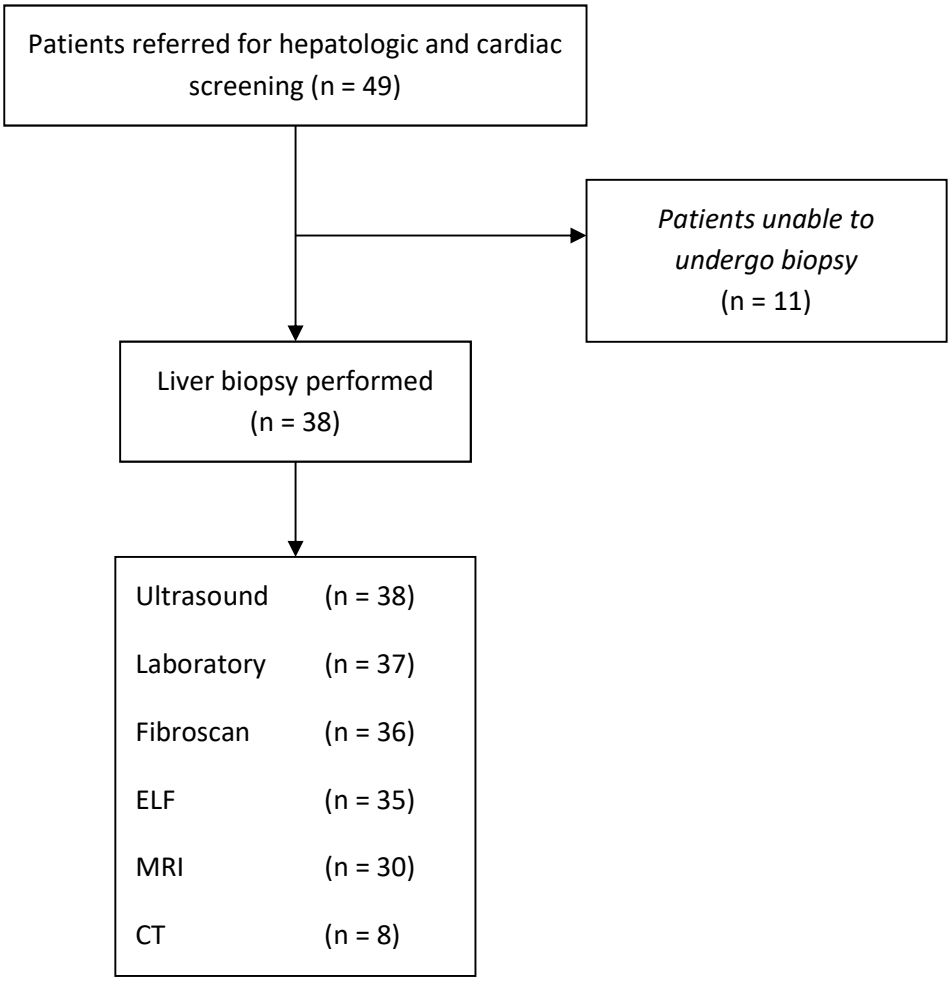


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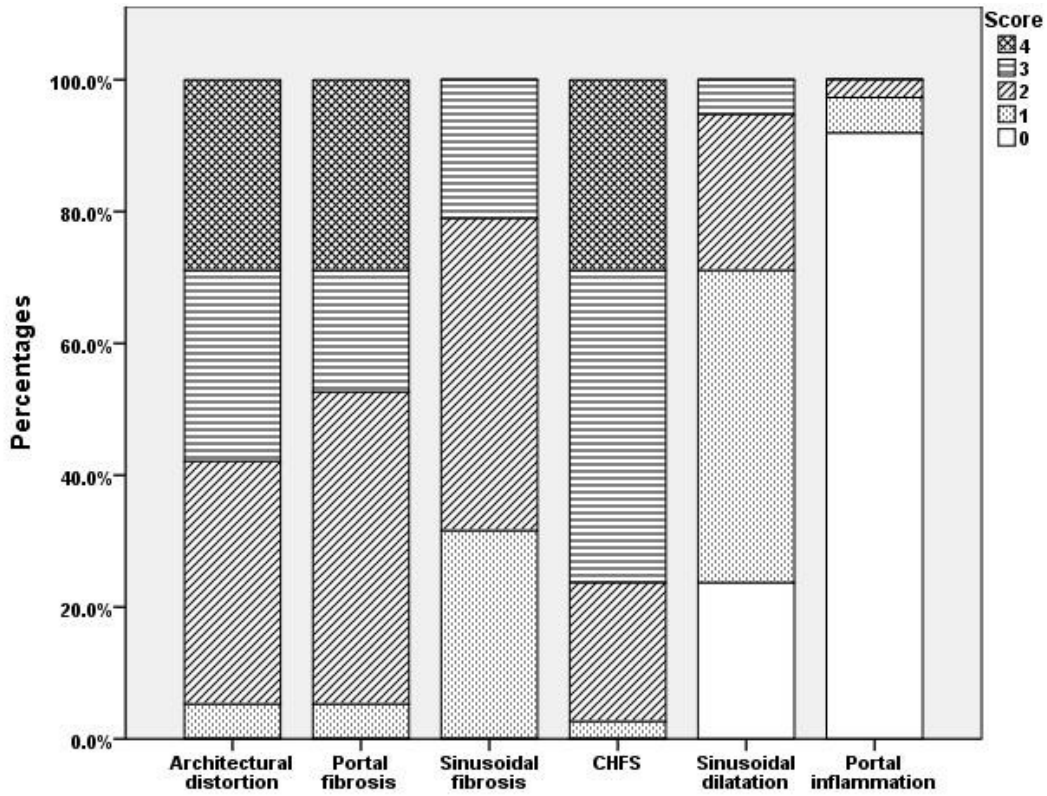
Figure 1. FALD histology

Panel A: Distribution of liver histology scores



Panel B: histological images of key pathological features of FALD

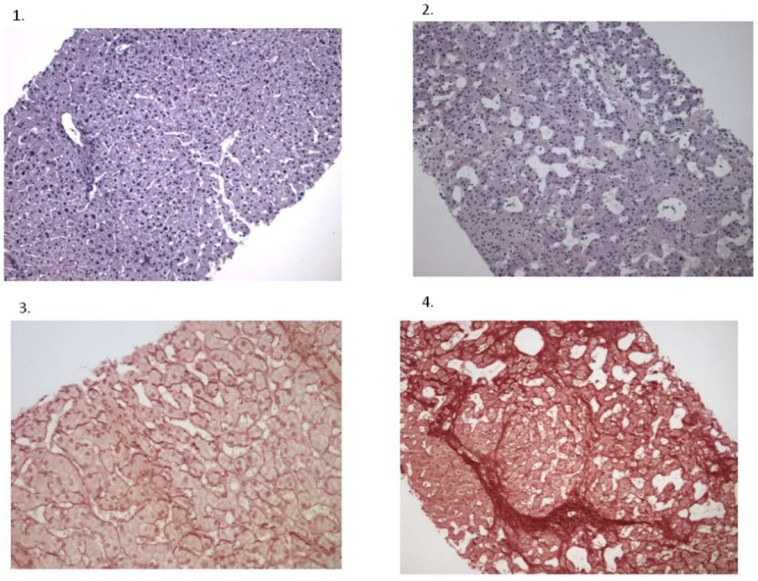
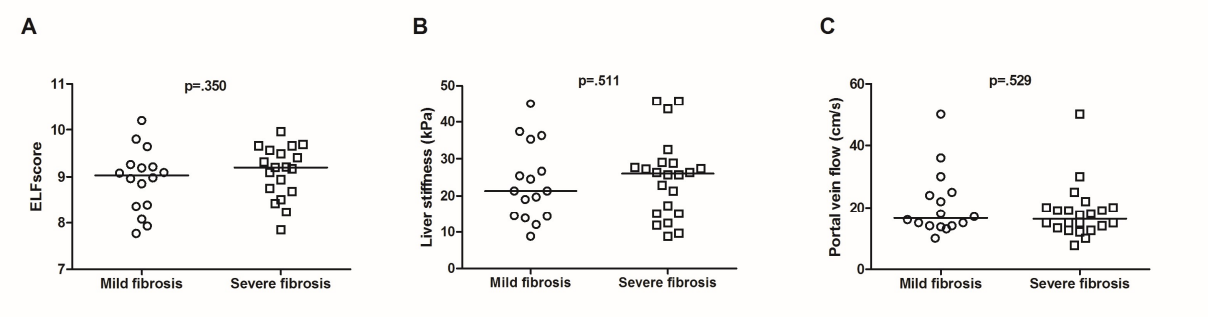


Figure 2. ELF, Liver stiffness and portal vein flow in mild vs severe fibrosis



Supplementary methods

Histology

Description histological fibrosis scores : First, the gross architectural distortion score (modified from METAVIR) was scored, ranging from 0 (no definite fibrosis); 1 (minimal fibrosis: no septa or rare thin septum); 2 (mild fibrosis: occasional thin septa); 3 (moderate fibrosis: moderate thin septa; up to incomplete cirrhosis) to 4(cirrhosis definite or probable).

Second, the Fontan Fibrosis score , a score consisting of 4 components (portal fibrosis, sinusoidal fibrosis, sinusoidal dilatation, and portal inflammation), was scored . Portal fibrosis was scored: 0 (no fibrosis); 1 (enlarged, fibrotic portal tracts); 2 (periportal, or portal-portal septa but intact architecture); 3 (fibrosis with distorted architecture, but no obvious cirrhosis); 4 (cirrhosis, probable or definite). Sinusoidal fibrosis was scored: 0 (no sinusoidal fibrosis); 1 (sinusoidal fibrosis in < 1/3 sinusoids); 2 (in 1/3-2/3 sinusoids); 3 (in > 2/3 sinusoids); 4 (cardiac cirrhosis: extensive central vein-central vein bridging). This score also includes sinusoidal dilatation (0-3): 0 (no dilatation); 1 (dilatation in <1/3 sinusoids); 2 (in 1/3-2/3 sinusoids); 3 (in > 2/3 sinusoids) and portal inflammation 0-3 scale (no/mild/moderate/marked inflammation in some or all portal tracts).

Lastly, the congestive heart failure fibrosis score (CHFS), was scored: 0 (no fibrosis); 1 (central zone fibrosis); 2A (central zone and mild portal fibrosis, with accentuation at central zone); 2B (at least moderate portal fibrosis and central zone fibrosis, with accentuation at portal zone); 3 (bridging fibrosis); 4 (cirrhosis).

Magnetic resonance imaging protocol

MR imaging was performed with a 3-T imager (Siemens Prisma/Skyra, the Netherlands) using a phased-array surface coil after 3 hours of fasting. Buscopan (1ml iv) was administered for anti-spasmolytic effect, previous to scanning.

The protocol included a T2-weighted single-shot sequence, a T2-weighted fast spin-echo sequence with spectral fat saturation, and a transverse breath-hold 3D T1-weighted fat-suppressed spoiled gradient-recalled echo sequence before and after dynamic injection of 0.1 mmol/kg of body weight (max 7.5 ml) of gadolinium chelates (Dotarem®; gadoterate meglumine) followed by a 20-mL saline solution flush at a rate of 2 mL/sec administered with a power injector. After the T2 haste (blanco) phases, the scanning of the arterial phase was initiated after bolus tracking (sufficient contrast uptake in a region of interest in the aorta). Arterial scanning phase lasts for 13 sec, followed after 30 seconds by the venous phase (also 13 sec). Delayed phase was fixed at 180 seconds after intravenous contrast injection. A free-breathing fat-suppressed single-shot echoplanar DW MR sequence was

performed before contrast injection with *b* values of 50, 500 and 800 sec/mm^2. Cardiac gating was not used. Sequence parameters are shown below.

Sequence parameters

Sequences	N slices	TR/TE (ms)	Slice thickness (mm)	Flip Angle (°)	N concatenations
T2-weighted haste	30	1400/87	5	160	3
T2-weighted haste with spectral fat saturation	35	1600/95	5	160	4
T1-weighted vibe	72	3.97/ 1.29 (TE1) 2.52 (TE2)	3	9	1
Diffusion-weighted	35	5900/52	5	-	1

Computed tomography protocol

Contrast-enhanced four phase CT was performed on 320-slides multidetector CT scanners (Toshiba Aquilion). As preparation patients fasted for 3 hours and drank 900 mL Telebrix Gastro 45 minutes and 450 mL water 30 minutes pre-scanning.

Unenhanced multidetector CT abdominopelvic images were initially obtained. Contrast-enhanced acquisitions were obtained following intravenous administration of Iomeprol contrast medium at 300 mg iodine per milliliter, calculated on total body weight (ranging 120-150 ml), through an 20-gauge catheter in a fixed time of 30 seconds (rate 4-5 mL/sec) by a power injector, followed by a 40-mL saline solution flush. The late arterial phases were acquired at an empirical fixed delay of 24 seconds after bolus tracking, and portal venous phases after another 20 seconds (around 60-70 sec). The late phases were fixed at 180 seconds after contrast administration had begun. See below for the used scanning and reconstruction parameters:

Scan parameters

	Unenhanced	Arterial	Venous	Late
Mode	Helical	Helical	Helical	Helical
Collimation	80 x 0.5	80 x 0.5	80 x 0.5	80 x 0.5
kV	Auto	Auto	Auto	Auto
Sure Exposure (mAs)	SD 17.5	SD 17.5	SD 17.5	SD 17.5
Rotation time	0.5	0.5	0.5	0.5
Pitch	Standard	Standard	Standard	Standard
Scan direction	Cranio-Caudal	Cranio-Caudal	Cranio-Caudal	Cranio-Caudal
API	Inspiration	Inspiration	Inspiration	Inspiration
Iterative	AIDR3D	AIDR3D	AIDR3D	AIDR3D

Reconstruction parameters

	Axial 1		Volume 1		Multiview 1	
Unenhanced	Body Axial	3 / 2.4	Body Volume	1 / 0.8		
Arterial	Body Axial	3 / 2.4	Body Volume	1 / 0.8		
Venous	Body Axial	3 / 2.4	Body Volume	1 / 0.8	MPR Coronal MPR Sagital	3 / 2 3 / 2
Late	Body Axial	3 / 2.4	Body Volume	1 / 0.8		

Calculation Relative Enhancement ratio

The relative enhancement ratio (RER) of liver pattern between pre-contrast and delayed phase was calculated, as a modification of the previously described method by Feier et al.[36] This ratio is based on signal intensity (SI) of the liver and the paraspinal muscles (to normalize the signal) on pre-contrast and delayed (post) phase T1-weighted imaging. The following equation was used:

$$\text{RER} = \frac{(\text{SI liver}_{\text{post}}/\ \text{SI muscle}_{\text{post}}) - (\text{SI liver}_{\text{pre}}/\ \text{muscle}_{\text{pre}})(\text{SI liver}_{\text{pre}}/\ \text{SI muscle}_{\text{pre}})}{}$$