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Fontan protein-losing enteropathy is associated with advanced liver disease and a proinflammatory intestinal and systemic state

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Abstract

Background and aims: Protein-losing enteropathy (PLE) after Fontan surgery carries significant morbimortality. Its pathophysiology and association with other Fontan complications are poorly understood. Our aims were to examine whether Fontan-PLE is associated with greater liver damage and to assess the presence of systemic and intestinal inflammation.

Methods: Fontan patients with PLE and Fontan controls without PLE matched for age and Fontan surgery procedure were included. Data were prospectively compiled on blood and stool tests, liver imaging, elastography, cardiac-MRI and cardiac catheterization.

Results: Twenty-nine Fontan patients were enrolled (14 with PLE and 15 controls without PLE). Patients with PLE had more advanced liver disease estimated by non-invasive methods: blunt liver margins on ultrasonography (71.4% vs 26.7%, $P = .027$), greater median liver stiffness (25.4 vs 14.5 kPa, $P = .003$) and higher FIB-4 ($P = .016$). Portal hypertension-related signs were more common in patients with PLE including ascites ($P = .035$), larger spleen size ($P = .005$), oesophageal varices/splanchnic collateral shunts ($P = .03$), higher liver stiffness-spleen size-to-platelet ratio risk score ($P < .001$) and lower platelet count ($P = .01$). Systemic proinflammatory cytokines (TNF-α, interleukin-6), biomarkers of intestinal permeability (intestinal fatty-acid binding protein) and faecal calprotectin concentrations were also significantly increased in Fontan-PLE ($P < .05$). Faecal calprotectin directly correlated with alpha-1 antitrypsin clearance and inversely with cardiac index, total serum proteins and body mass index.

Conclusion: Fontan-PLE is associated with advanced liver disease and increased markers of systemic inflammation and intestinal permeability. Faecal calprotectin is elevated and correlates with Fontan-PLE severity. Liver assessment is mandatory in all Fontan patients, and especially in those with PLE.

Abbreviations: ALT, Alanine aminotransferase; AP, Alkaline phosphatase; AST, Aspartate aminotransferase; BNP, Brain natriuretic peptide; CI, Confidence interval; CT, Computed tomography; FALD, Fontan-associated liver disease; GGT, Gamma-glutamyl transferase; I-FABP, Intestinal fatty acid-binding protein; IL-6, Interleukin-6; LSPS index, Liver stiffness-spleen size-to-platelet ratio risk index; MRI, Magnetic resonance imaging; NYHA, New York Heart Association; PLE, Protein-losing enteropathy; TNFα, Tumour necrosis factor-alpha.

Enrique Rodríguez de Santiago and Luis Téllez share first co-authorship.

KEYWORDS

calprotectin, Fontan, inflammation, liver disease, protein-losing enteropathy

1 | INTRODUCTION

Fontan surgery comprises several techniques that divert systemic venous return to the pulmonary arterial system, usually without an intervening ventricle. This form of surgery is the palliative procedure of choice for many patients with a single functional ventricle.[1] Fontan surgery survivors almost invariably experience long-term complications involving the heart, lungs, kidneys, brain, liver and gut.[2]

Protein-losing enteropathy (PLE) is a life-threatening disease in which proteins spill into the gut and affects 3%-18% of patients after a Fontan operation.[3,4] The pathogenic mechanism of Fontan-associated PLE differs, at least in part, from other forms of PLE, and this makes Fontan-PLE a unique clinical entity.[3] Fontan-PLE can be improved by bringing down central venous pressure via fenestration of the Fontan conduit or fully resolved by heart transplantation. However, an association between PLE and elevated systemic venous pressure is an inconstant finding, suggesting that other factors play a role in its pathogenesis.[4] Inflammation is thought to be a critical pathophysiological mechanism, a contention based on small reports suggesting that inflammatory markers may be increased in serum of patients with Fontan-PLE,[3] and the improvement in the course of the latter by systemic corticosteroids and budesonide.[5-10] Faecal calprotectin is a neutrophilic cytosolic protein that has proven to be a reliable non-invasive biomarker of gut inflammation.[11] It has been shown to be increased in short case series of Fontan patients,[12] but its potential role for the diagnosis and follow-up of this population and its relationship with other complications of Fontan surgery have not yet been addressed.

Fontan-associated liver disease (FALD) refers to a wide range of structural and functional alterations of the liver caused by haemodynamic changes associated with the Fontan circulation. Over the last decade, observational studies have revealed that portal hypertension-related complications may appear in the long term. We hypothesized that FALD and Fontan-associated PLE could be two sides of the same coin since haemodynamic derangement is common in both entities and mucosal inflammation and intestinal barrier disruption may promote liver disease.[13] To the best of our knowledge, this is the first study designed to explore this issue. The aims of our study were thus to investigate in patients with Fontan circulation and PLE (a) the presence of intestinal and systemic inflammation, and (b) the relationship between PLE and the severity of cardiac and liver damage.

Key points

- Fontan surgery is a palliative intervention for complex congenital heart malformations in which there is only one functional ventricle. This procedure prolongs survival and improves quality of life, but long-term complications such as advanced liver disease and Fontan-associated protein-losing enteropathy (PLE) may develop.
- We found that advanced chronic liver disease is more common in Fontan-associated PLE than non-PLE Fontan patients. Faecal calprotectin, proinflammatory and intestinal permeability biomarkers were also increased in Fontan-PLE patients.
- Liver assessment and close surveillance are mandatory in all Fontan patients, and especially in those with PLE.

2 | MATERIAL AND METHODS

2.1 | Study design

This was an observational, single-centre case-control study based on data from a prospective database. Cases were all patients diagnosed with Fontan-associated PLE. PLE was defined as a 24 hours alpha-1-antitrypsin faecal clearance value above 27 mL/day.[3] Inflammatory bowel disease, coeliac disease and gastrointestinal infection were exclusion criteria. Controls were matched at a 1:1 ratio by age (±2 years) and Fontan surgery procedure (atriopulmonary vs total cavopulmonary), since both these factors may influence FALD outcome.[14,15] The inclusion period was December 2015-March 2019. The study protocol adhered to the principles of the Declaration of Helsinki and was approved by the Ethics Committees for Clinical Research of our institution on December 3, 2015 (code: 384/14, HRC-FONLIVER). Written informed consent for inclusion in this study was obtained in all cases.

2.2 | Study population and procedures

At our institution, from the year 2015, all patients who have undergone Fontan surgery are routinely monitored through an established followed up protocol and the resultant data entered in a prospective database. This protocol has been described in detail elsewhere.[14] In brief, patients are initially assessed through a structured medical interview, physical examination, blood and faeces tests, abdominal Doppler-ultrasonography, liver elastography (Fibroscan®, Probe M/XL Echosens®), abdominal angio-magnetic resonance imaging (MRI) or computed tomography (CT) scan when MRI is contraindicated, and echocardiography. A cardiac-MRI and cardiac haemodynamic study are also performed when clinically indicated.

For liver elastography and abdominal ultrasonography the patient is requested to fast for at least 8 hours. Abdominal Doppler-ultrasonography is performed by a gastroenterologist with

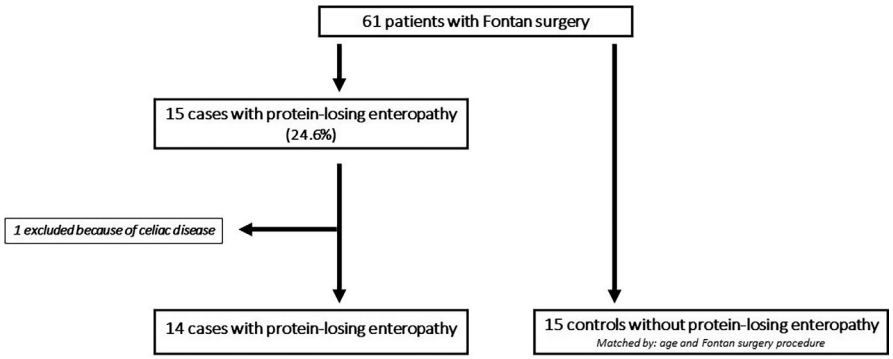

FIGURE 1 Study flowchart

expertise in abdominal imaging (>15 000 abdominal procedures). Liver elastography is performed by a trained nurse (>10 000 procedures) blinded to the patient's clinical history. In all cases, at least 10 measures are obtained. Only results with more than 10 valid measurements, a procedural success rate >60% and an interquartile range <30 are included in the database. The result is drawn from an average value expressed in kilopascals (kPa). Abdominal MRI and CT scans are assessed by two independent radiologists with expertise in vascular liver diseases. Echocardiography, cardiac-MRI and haemodynamic studies are performed and interpreted by experienced paediatric cardiologists and radiologists trained in congenital heart disease. Faeces tests including alpha-1-antitrypsin and faecal calprotectin (normal range <50 μg/g) are carried out in the first visit. Stool cultures are also requested when chronic diarrhoea is present. All tests are initially performed within a period of 6 months. Data from the initial assessment were used for this study.

The data compiled for each participant were: demographic data (age, sex), clinical data (New York Heart Association functional scale [NYHA], height [m], weight [kg], alcohol abuse defined as >20 g/day in women and >30 g/day in men, blood pressure [mm Hg], Fontan procedure [atriopulmonary or total cavopulmonary], treatment and clinical signs and symptoms), systemic oxygen saturation and laboratory data (glucose, creatinine, total bilirubin, alanine and aspartate aminotransferases [ALT, AST], gamma-glutamyl transferase and alkaline phosphatase [GGT, AP], C-reactive protein, brain natriuretic peptide [BNP], serology [HAV, HBV, HCV and HIV], immunoglobulins [IgG, IgA and IgM], albumin, total proteins, platelet count and international normalized ratio). Tumour necrosis factor-alpha (TNFα), interleukin-6 (IL-6) and intestinal fatty acid-binding protein (I-FABP) were measured in peripheral blood according to the enzyme-linked immunosorbent assay manufacturer´s instruction (Elabscience® ELISA kit). Coeliac disease was ruled out through IgG and IgA antitransglutaminase antibodies. In patients with a high suspicion of coeliac disease an upper endoscopy and a histological and flow cytometric analysis of the duodenal mucosa were performed. We also calculated the FIB-4 index for each participant (FIB-4 = age [year] × AST [IU/L]/(platelets [10^9/L] × (ALT [IU/L]). Despite its limitations, this index has been described as a good predictor of liver fibrosis in several forms of liver disease.[16] Blunt liver margins and ascites were determined as binary categories in the abdominal ultrasonography as indirect markers of liver disease. Gastroesophageal varices or intraabdominal splanchnic collaterals observed on MRI/CT, spleen long axis (mm) and the liver stiffness-spleen size-to-platelet ratio risk score (LSPS index = liver stiffness × spleen diameter/platelet count)[16,17] were recorded as surrogate non-invasive markers of portal hypertension. Ventricular ejection fraction obtained in the cardiac MRI, cardiac index (Fick equation) and pulmonary artery and systemic venous medium pressures obtained in the haemodynamic study were selected to assess cardiac function.

2.3 | Statistical analysis

Median and range were calculated for continuous variables as most variables were non-normally distributed. Normality was tested through distributional graphs and the Shapiro-Wilk test. Frequency counts and percentages were used for categorical data. 95% confidence intervals (CI) for proportions were calculated based on the Wilson method. Continuous data were compared using the Mann-Whitney U test. Chi-squared and Fisher's exact tests were used for categorical data. Correlation between continuous variables was assessed through Spearman's rank coefficient. A type I error associated with multiple comparisons was controlled by adjusting *p*-values of liver assessment using Sidak's method and by minimising the number of variables selected for the analysis. Significance was set at a *p*-value less than .05. All statistical tests were two-tailed and performed at the promoting institution (Hospital Universitario Ramón y Cajal, Madrid) using STATA version 14.1 (StataCorp).

3 | RESULTS

From a database of 61 patients with Fontan surgery, we identified 15 patients with Fontan-associated PLE (prevalence 15/61, 24.6%, 95% confidence interval: 15.5%-36.7%) and 15 controls without PLE matched for age and Fontan procedure. One female patient with PLE was subsequently diagnosed with coeliac disease and excluded, leaving 14 patients (10 males, 71.4%) in the case group (Figure 1).

The baseline characteristics of the cases and controls are outlined in Table 1. No clinically or statistically relevant differences between the groups were detected in age, sex, time since surgery

TABLE 1 Baseline characteristics

Variable	Patients with PLE	Patients without PLE	*P* values
Number of patients	14	15	
Age years	28.1 (13-38)	28.2 (13-39)	1
Male Sex	71.4% (10/14)	53.3% (8/15)	.31
Treatment			
Antiaggregant/NSAID	57.1% (8/14)	73.3% (11/15)	.45
Anticoagulant	35.7% (5/14)	26.7% (4/15)	.7
Oral budesonide	21.4% (3/14)	0%	.1
Beta-blocker	50% (7/14)	26.7% (4/15)	.20
Diuretics	50% (7/14)	6.7% (1/15)	**.01**
Amiodarone	7.1% (1/14)	0%	.48
Sildenafil	14.3% (2/14)	0%	.22
Angiotensin-converting enzyme inhibitors	7.1% (1/14)	13.3% (2/15)	1
Dyhidropyridin Calcium channel blockers	0%	6.7% (1/15)	1
Endothelin receptor antagonist	7.1% (1/14)	0%	1
Prior fenestration	14.3% (2/14)	6.7% (1/15)	.59
Alcohol abuse	0%	6.7% (1/15)	1
Main congenital heart defect			1
Tricuspid atresia	35.7% (5/14)	33.3% (5/15)	
Double inlet left ventricle	35.7% (5/14)	26.7% (4/15)	
Pulmonary atresia	21.4% (3/14)	26.7% (4/15)	
Other	7.1% (1/14)	13.3% (3/15)	
Serologyat this level			.48
HCV	7.1% (1/14)	0%	
HBV/HIV	0%	0%	
Fontan surgery			.87
Atriopulmonary	42.9% (6/14)	40% (6/15)	
Total cavopulmonary	57.1% (8/14)	60% (9/15)	
Time since Fontan (years)	17.9	18.8	1
Body mass index (kg/m^2)	21.4	24.3	.24
NYHA			.2
I	50% (7/14)	60% (9/15)	
II	28.6% (4/14)	40% (6/15)	
III	21.4% (3/14)	0	
Systemic O_2 saturation	93%	96%	**.025**
Systolic pressure (mm Hg)	110 (90-134)	119 (96-135)	.09
Diastolic pressure (mm Hg)	72 (60-87)	75 (63-89)	.8
Creatinine (mg/dL)	0.78 (0.42-1.15)	0.76 (0.59-0.92)	.84
Total proteins (g/dL)	4.9 (3.2-6.8)	7.4 (5.9-8.3)	**<.001**
Albumin (g/dL)	2.6 (1.2-4.6)	4.2 (3.3-5)	**<.001**
Immunoglobulins (mg/dL)			
IgG	489 (93-1160)	1160 (871-1710)	**.007**
IgA	83 (30-333)	173 (56-425)	**.008**
IgM	74.8 (20-172)	92 (43-312)	**.03**
C-reactive Protein	3.2 (0.2-4.5)	3.4 (0.5-7.1)	.26
BNP	40.9 (10-336)	26 (10-201)	.94

(Continues)

TABLE 1 (Continued)

Variable	Patients with PLE	Patients without PLE	*P* values
EF MRI	58.5% (48.5-74%)	63.5% (57-78%)	**.04**
Haemodynamic			
Cardiac index (L/min/m^2)	2.54 (1.4-5.1)	4.25 (2.4-6.1)	**.016**
Systemic venous pressure (mm Hg)	16.6 (10-22)	15.1 (8-20)	.62
Pulmonary artery medium pressure (mm Hg)	15.7 (7-28)	13.9 (8-20)	.49
Alpha-1-antitrypsin faecal clearance (mL/day)	84 (27-784)	5.8 (3.1-13)	**<.001**
Clinical signs and symptoms			
Asymptomatic	21.4% (3/14)	80% (12/15)	**.002**
Cyanosis	57.1% (8/14)	0	**<.001**
Diarrhoea	42.9% (6/14)	13.3% (2/15)	.11
Peripheral oedema	42.9% (6/14)	0	**.006**
Ascites grade II-III	28.6% (4/14)	6.6% (1/15)	.17
Abdominal pain	14.3% (2/14)	6.6% (1/15)	.60
Frequent abdominal discomfort/bloating	14.3% (2/14)	6.6% (1/15)	.60
Spider angioma	14.3% (2/14)	0	.22
Palmar erythema	14.3% (2/14)	6.6% (1/15)	.60
Jaundice	7.1% (1/14)	0	.48
Hepatic Encephalopathy	7.1% (1/14)	0	.48

Note: Values are expressed as medians (range).

Numbers in bold indicate significance.

Abbreviations: BNP, brain natriuretic peptide; EF, ejection fraction; MRI, magnetic resonance; NSAID, nonsteroidal anti-inflammatory drugs; NYHA, New York Heart Association.

or main confounders of faecal calprotectin (NSAID/antiaggregant therapy).

The most common signs and symptoms in the PLE group were cyanosis (57.1%) and diarrhoea (42.9%). Peripheral oedema and cyanosis were significantly more frequent in the PLE-group, consistent with the findings of lower oxygen saturation, hypoproteinaemia and lower cardiac index (Table 1). One patient in the PLE-group presented hepatic encephalopathy and spontaneous bacterial peritonitis. Three patients received oral budesonide; in two of them, the treatment was started after faecal calprotectin measurements. Additional treatments are detailed in Table 1.

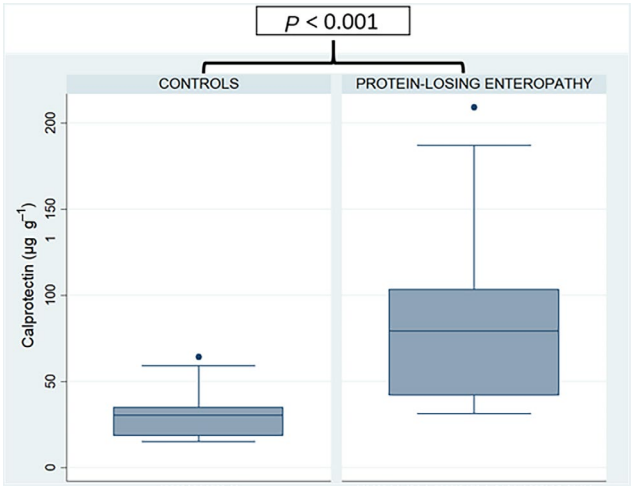

FIGURE 2 Increased faecal calprotectin in patients with Fontan-associated protein-losing enteropathy compared to Fontan patients without protein-losing enteropathy

3.1 | Intestinal and systemic inflammation in patients with PLE

Faecal calprotectin was significantly increased in the Fontan-PLE group (80 vs 30 µg/g, *P* < .001) (Figure 2). Faecal calprotectin was above the normal range (50 µg/g) in nine patients (64.3%) with PLE and in two without PLE (59 and 64 µg/g, 13.3%) (*P* = .006). Three patients with Fontan-PLE (21.4%) and elevated faecal calprotectin were asymptomatic. Faecal calprotectin correlated with markers of PLE severity as shown by a direct correlation with alpha-1-antitrypsin clearance (rho = 0.6, *P* = .03), and an inverse correlation with cardiac index (rho = −0.65, *P* = .04), body mass index (rho = −0.5, *P* = .04) and total proteins (rho = −0.68, *P* = .03).

Serum I-FABP, measured as a marker of intestinal barrier disruption, was significantly increased in the Fontan-PLE group (9 vs 5 ng/mL, *P* = .019). Systemic inflammation, as addressed by the serum levels of the proinflammatory cytokines TNF-α and IL-6, was greater in patients with PLE (TNF-α, 99 vs 7 pg/mL, *P* = .019; IL-6, 42 vs 26 pg/mL, *P* = .035) (Figure 3).

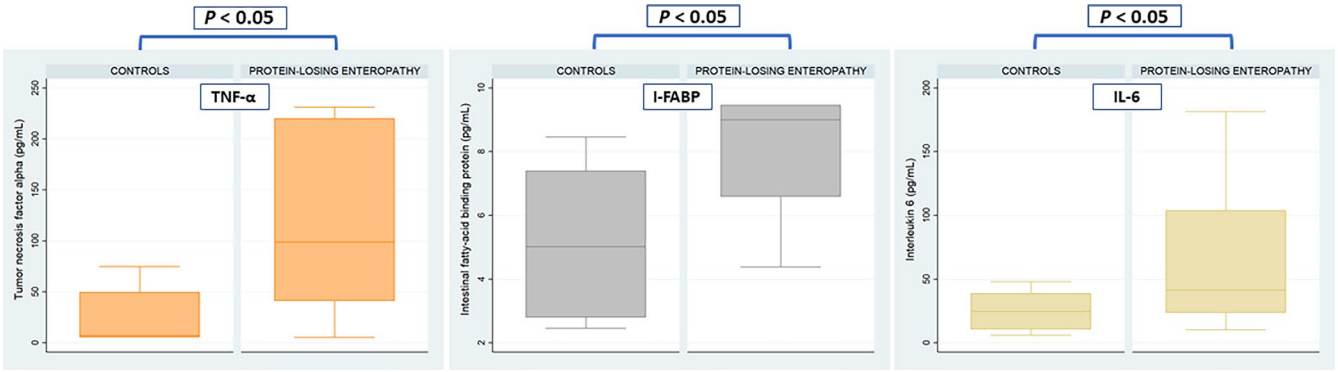

FIGURE 3 Increased biomarkers of systemic inflammation and intestinal permeability in patients with Fontan-associated protein-losing enteropathy compared to Fontan patients without protein-losing enteropathy. Abbreviations: I-FABP, Intestinal fatty acid-binding protein; IL-6, Interleukin 6; TNF-α, Tumour necrosis factor-alpha

3.2 | Greater severity of cardiac impairment in patients with protein-losing enteropathy

The most common Fontan surgery in both groups was the total cavopulmonary variant. Controls without PLE (NYHA I-II: 100%) had better functional class than patients with PLE (NYHA I-II: 78.6%; NYHA III: 21.4%), although this difference was not statistically significant. No differences were detected in blood pressure, creatinine and BNP serum levels (Table 1).

Participants with PLE had a lower cardiac index in the hemodynamic study (2.54 vs 4.25 L/min/m^2, P = .016), lower ventricular ejection fraction on cardiac MRI (58.5% vs 63.5%, P = .04) and lower systemic oxygen saturation (93% vs 96%, P = .025). Systemic venous pressure and pulmonary artery medium pressure were similar in both groups (Table 1).

3.3 | Greater liver damage in patients with protein-losing enteropathy

Both groups were similar regarding known risk factors for chronic liver disease (HCV, HBV, alcohol abuse and overweight). One case of chronic HCV infection was diagnosed in the PLE group (Table 1). No differences were observed in liver function test results (bilirubin, AST, ALT, GGT and AP) (Table 2).

Non-invasive methods were indicative of more advanced FALD in PLE patients (Table 2). Platelet counts were lower in the Fontan-associated PLE group (117 000 vs 153 000/mm^3, P = .01), whereas median liver stiffness assessed by Fibroscan® was significantly higher in the PLE group (25.4 vs 14.3 kPa; P < .001). Abdominal US revealed that the presence of blunt liver margins, ascites and a larger spleen size was significantly more common in Fontan-PLE. In CT/MRI, oesophageal varices and intrabdominal splanchnic collaterals were also more often seen in the PLE group. Finally, LSPS and FIB-4 indexes values were higher and indicative of more advanced liver disease in the presence of PLE (Table 2). No cases of hepatocarcinoma were diagnosed in either subgroup.

TABLE 2 Liver parameters in Fontan patients with and without protein-losing enteropathy (PLE)

Variable	Patients with PLE	Patients without PLE	*P* values
Number of patients	14	15	
Bilirubin (mg/dL)	1.4 (0.3-3.6)	1.3 (0.5-3.6)	.91
AST (IU/L)	25 (15-55)	26 (18-37)	.84
ALT (IU/L)	24 (14-98)	21 (11-61)	.74
Alkaline phosphatase (IU/L)	72 (41-172)	79 (47-643)	.52
GGT (IU/L)	95.5 (17-358)	68 (16-279)	.38
Platelets*103/ mm3	117 (71-259)	153 (97-298)	**.01**
Liver stiffness (kPa)	25.4 (14.1-66.4)	14.5 (11.1-26.3)	**.003**
LSPS index	2.2 (0.8-9.6)	0.38 (0.1-1.6)	**<.001**
FIB-4	1.4 (0.3-2.8)	0.9 (0.3-1.8)	**.016**
Ascites in US	42.9% (6/14)	6.7% (1/15)	**.035**
Blunt liver margins	71.4% (10/14)	26.7% (4/15)	**.027**
Spleen long axis (cm)	13.6 (10.4-18.6)	11 (9.1-15)	**.005**
Oesophageal varices or intrabdominal splanchnic collaterals in CT/MRI	64.3% (9/14)	33.3% (5/15)	**.03**

Note: Numbers in bold indicate significance.

Abbreviations: ALT, alanine transaminase; AST, aspartate aminotransferase; CT, computerized tomography; FIB-4, fibrosis index based on four factors; GGT, gamma-glutamyl transferase; LSPS, liver stiffness-spleen size-to-platelet ratio risk score; MRI, magnetic resonance imaging; US, Abdominal ultrasound.

4 | DISCUSSION

This case-control study explores the relationship between PLE, intestinal and systemic inflammation, liver damage and cardiac haemodynamics in patients with Fontan circulation. Our results indicate that Fontan patients with PLE show more severe liver damage and cardiac impairment, along with greater systemic inflammation,

intestinal inflammation and intestinal permeability than those without PLE.

Intestinal and systemic inflammation in patients with Fontan-PLE were remarkable findings of our study. Intestinal inflammation was assessed by faecal calprotectin. Values of faecal calprotectin were greater in patients with PLE than in those without, and paralleled the severity of PLE and cardiac derangement. These observations indicate that intestinal inflammation contributes to intestinal barrier damage and hyperpermeability in patients with PLE, as has been shown in experimental models of PLE and cirrhosis.[18] The benefits of budesonide and systemic corticosteroids in the course of Fontan-associated PLE provides further evidence of the pathogenetic contribution of inflammation to the intestinal damage produced in these patients.[5,6] In turn, the inverse correlation observed between faecal calprotectin and cardiac index suggests that inflammation could result from venous congestion and intestinal hypoxia. Intestinal barrier damage in our patients was further shown by the greater levels detected in patients with PLE of I-FABP, an intracellular protein mainly expressed in enterocytes that has proven to be a sensitive biomarker of intestinal barrier integrity.[19] It is conceivable that the derangement of the intestinal barrier in patients with Fontan surgery leads not only to PLE, but also to increased passage of bacterial products to the systemic circulation which results in activation of the proinflammatory immune system. The latter is supported by the more severe systemic inflammation reflected by greater serum proinflammatory cytokines in patients with PLE. As in other forms of chronic liver disease, systemic inflammation itself can worsen liver damage in patients with Fontan circulation.[20] Taken together, these findings endorse a pathogenetic link among intestinal barrier damage, intestinal and systemic inflammation and the severity of liver damage in patients with Fontan-PLE, as the disruption of the gut-liver axis is known to be implicated in the development and progression of chronic liver disease.[13]

In our study, we assessed both faecal alpha-1-antitrypsin clearance and faecal calprotectin. We suggest that in the clinical work-up of Fontan surgery patients, faecal calprotectin could provide complementary information to faecal alpha-1-antitrypsin. Measurement of faecal calprotectin has numerous advantages such as not requiring concomitant blood tests, the need for only a single small amount of stool, good reproducibility, universal availability and the stability of the protein in stool samples at room temperature for up to 3-7 days.[11,17] Noticeably, faecal calprotectin was elevated in three of our asymptomatic patients with Fontan-PLE. The question arises as to whether this marker may be useful for the early diagnosis of Fontan-PLE and monitoring of its treatment. Moreover, we hypothesise that faecal calprotectin may serve to identify patients with greater intestinal inflammation, who are most likely to benefit from budenoside.

Liver disease is almost a constant in patients with Fontan circulation, compromising survival when portal hypertension-related complications and hepatocellular carcinoma appear.[14] This is the first study to establish a relationship between liver disease and PLE in patients who have had Fontan surgery. In other studies on this topic, this relationship has not been observed,[6] probably because of a lack of data from a complete liver assessment (ie liver imaging, elastography and serologic indices) and because the study was not specifically designed to address this question. Our data indicate that the severity of liver disease is greater in patients who develop PLE after Fontan surgery as shown by their greater liver stiffness, higher FIB-4 and LSPS indices and more frequent signs of portal hypertension. Because of the constant presence of hepatic congestion, it could be argued that transient elastography will overestimate liver stiffness in Fontan patients.[16,21,22] However, the similar systemic venous pressure and BNP recorded in our patients with and without PLE suggests a major contribution of liver fibrosis to increased liver stiffness, as suggested by previous studies.[22] Nonetheless, it should be remarked that elastography results must be interpreted with caution in the Fontan population as, contrary to other forms of chronic liver disease, no validated cut-offs of severe liver fibrosis are yet available.

Another important finding of our study was a greater impairment of cardiac function paralleling liver disease severity in the Fontan patients with PLE, as shown by a lower cardiac index and ejection fraction. The lower systemic oxygen saturation in the Fontan-PLE patients, which could be explained by the lower cardiac index and development of venous collaterals to the left atrium, suggests that tissue hypoxia could be a pathogenetic factor shared by liver disease and PLE. It is important to remark that, contrary to portal hypertension of other aetiologies, in which there is an increased cardiac index and a hyperdynamic circulatory state, the circulation in Fontan patients is hypodynamic with a cardiac index unable to fulfil the greater demands of an expanded splanchnic vascular bed, probably contributing to liver hypoxia. Indeed, it has been suggested that low cardiac index and reduced mesenteric flow may further aggravate FALD and PLE.[23,24]

The strength of our study lies in its prospective nature. All participants were subjected to a complete assessment of liver and bowel disease using a standardized protocol so that we could exclude other causes of liver or bowel damage. Its main limitations are its reduced sample size and the observational design, which make us acknowledge the exploratory nature of our study. It could also be argued that we did not perform an endoscopic evaluation of our population to rule out inflammatory bowel disease as a cause of faecal calprotectin elevation. However, considering its estimated prevalence of 10-200 cases per 100,000 persons, the likelihood of this bias seems low (<1 case expected in our study).[25] The lack of liver biopsy could also be considered a limitation. Nonetheless, this procedure is potentially risky, especially for patients on antithrombotics with high systemic venous pressures. Additionally, since there is no specific treatment available, from our perspective, liver biopsy is only justified in candidates for double transplantation (hepatic and cardiac) and in those in whom another aetiology of liver disease is suspected. Besides, we consider that the high agreement across all non-invasive methods for liver assessment

was robust enough to support the relationship proposed between FALD and Fontan-PLE.

In conclusion, our findings indicate that severe intestinal damage, PLE and advanced liver fibrosis frequently coexist in Fontan surgery patients. This relationship justifies a complete assessment of both intestinal and liver function when either PLE or signs of liver disease develop in patients with Fontan circulation. Our study also provides additional evidence of the presence of systemic and intestinal inflammation in Fontan-associated PLE, and suggests faecal calprotectin could be a useful biomarker for the diagnosis and follow-up of these patients. The risk of life-threatening complications beyond the purely cardiopulmonary sphere in patients with Fontan surgery supports their management by a multidisciplinary team.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest.

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