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Coronary flow reserve in patients with primary biliary cholangitis

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ABSTRACT

Background: It is still not clear whether primary biliary cholangitis (PBC) is associated with abnormalities of the cardiovascular system. We aimed to assess the relationship between PBC and coronary flow reserve (CFR).

Methods: Our inclusion criterion was a diagnosis of PBC with no clinical evidence of heart disease or metabolic syndrome. Coronary flow velocity in the left anterior descending coronary artery was measured using transthoracic Doppler echocardiography at rest (DFVr), and during adenosine infusion (DFVh). The corrected CFR (cCFR) was defined as the ratio of DFVh to DFVr corrected for cardiac workload (cDFVr). Microvascular resistance was also assessed in baseline (BMR) and hyperemic conditions (HMR).

Results: 37 PBC patients and 37 sex- and age-matched controls were considered. The cCFR was significantly lower in PBC patients (2.8 ± 0.7 vs. 3.7 ± 0.7, $p < 0.0001$), and abnormal (≤ 2.5) in 13 (35%) of them, but in none of the controls ($p < 0.0001$). The cDFVr was higher in patients with abnormal cCFR (29.0 ± 6.0 vs. 20.4 ± 4.5 cm/sec, $p < 0.0001$). The CFR and cCFR did not correlate with any characteristics of PBC, comorbidities or Framingham risk scores. The BMR and HMR correlated with time since PBC diagnosis and duration of symptoms.

Conclusion: The CFR is reduced in PBC, apparently due to mechanisms correlating with the time since diagnosis. In particular, the higher cDFVr with a lower basal resistance in patients with cCFR ≤ 2.5 suggests a compensatory mechanism against any cardiomyocyte bioenergetics impairment.

1. Introduction

Primary biliary cholangitis (PBC) is a chronic cholestatic autoimmune liver disease primarily affecting middle-aged women [1]. When diagnosed at an early stage, and in patients responding to ursodeoxycholic acid therapy, PBC has a long natural history [2–5]. It is nonetheless associated with a significantly higher all-cause mortality rate than in the general population, with a significant proportion of this mortality having causes unrelated to the liver [6]. This excess mortality does not seem to be related to malignancies [6,7], but may be attributable to cardiac causes. This hypothesis is supported by data describing an excess cardiac mortality in PBC patients with severe fatigue [8], a symptom associated with autonomic nervous system (ANS) dysfunction [9]. Hypercholesterolemia has been investigated in the past as a possible risk factor for atherosclerotic cardiovascular disease in patients with PBC, since it occurs in up to 75% of them [10,11]. Four epidemiological studies [11–14] explored this issue, but none confirmed a definitely higher risk of cardiovascular mortality associated with PBC. A nationwide follow-up study conducted in Sweden on patients hospitalized for immune-mediated diseases without previous or concomitant coronary heart disease (CHD) found that patients with PBC had a standardized incidence ratio for subsequent CHD of 1.72. This figure is comparable with the case of other immune-mediated diseases, such as psoriasis, Sjögren's syndrome, rheumatic polymyalgia, and Hashimoto's thyroiditis [15].

Coronary microvascular dysfunction is defined as the presence of functional and structural abnormalities of the coronary microcirculation capable of impairing myocardial perfusion and causing ischemia [16]. The condition is associated with major cardiac events [17], and it can be detected in patients with chronic inflammatory disorders, such as systemic lupus erythematosus, psoriasis, or sys-

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temic sclerosis, in the absence of significant coronary artery disease or cardiovascular risk factors [18–21]. Coronary microvascular dysfunction can be caused by various pathogenic mechanisms that alter coronary vasomotor function and increase oxidative stress. In systemic lupus erythematosus it has been associated with an impaired motor function and higher than normal total antioxidant levels in the serum [22–24].

In a healthy coronary circulation, the epicardial conduit arteries account for no more than 10% of coronary resistance, while the coronary arterioles maintain a high level of resistance, and constitute the main site of myocardial flow regulation. These resistance vessels are responsible, in the healthy vasculature, for keeping coronary flow stable across a wide range of physiological perfusion pressures – a process called coronary autoregulation – and for adapting myocardial flow to myocardial demand by means of metabolic vasodilation [25]. The maximal diastolic coronary flow velocity (DFV) is impaired in the setting of microvascular disease, which limits the vasodilatory capacity of the coronary microcirculation. The coronary flow reserve (CFR) is the ratio of coronary flow (velocity) during maximal vasodilation to coronary flow (velocity) in resting conditions. This widely-studied and well-validated, flow-based physiological parameter has been used successfully in a broad array of invasive and non-invasive diagnostic modalities [26]. Flow velocity variations are proportional to the total blood flow if the vessel's lumen remains constant — a reasonable assumption if drugs such as dipyridamole or adenosine are administered, which induce an endothelial-independent coronary microvascular dilation. Since the CFR is the ratio between the coronary flow velocities with maximal vasodilation (during adenosine infusion) and in baseline conditions, an increase in the latter due to hemodynamic conditions such as high blood pressure or high heart rate would lead to the CFR being underestimated, erroneously suggesting a coronary microvascular dysfunction. That is why using the corrected CFR (the ratio between the coronary flow velocities during adenosine infusion and in baseline conditions, adjusted for the product of blood pressure and heart rate) can improve the quality of the ratio's interpretation in patients with coronary microvascular dysfunction [27].

Measuring the CFR with transthoracic Doppler echocardiography (TDE) is a simple and useful way to assess coronary microvascular function in cardiac and systemic diseases. The CFR is impaired in various immune-mediated and inflammatory diseases [18–22], and may ameliorate after therapy [28].

The important question of whether PBC is associated with abnormalities of the cardiovascular system, and the possible clinical implications of the latter, remains unanswered. Given the long natural history of PBC nowadays, in patients who respond to therapy, any such related cardiovascular abnormalities could have a more significant impact on their quality of life and survival than in the past. Hence the aim of the present study was to investigate coronary microvascular function in patients with PBC, using CFR by TDE, and drawing a comparison with a group of controls.

2. Patients and methods

2.1. Study population

We prospectively enrolled 37 consecutive PBC patients (4 males and 33 females, with a median age of 58 [49–66] years) being followed up at Padua University's Center for Cholestatic and Rare Hepatic Diseases, with at least 1 year of follow-up since their PBC was diagnosed. After giving their written informed consent, the patients were included in a case-control study (observational design), that was approved by the local ethics committee. The non-randomized control group consisted of 37 volunteers recruited from members of staff matched with the patients for age and sex. The controls did not undergo any cardiovascular conditioning program. None of the PBC patients or controls had coronary heart disease. All the controls were asymptomatic and had no history of cardiac disorders. For all study participants (patients and controls), the following were reasons for exclusion: cerebral vascular disease, carotid artery bruit, peripheral bruit or abnormal pulse, history of angina or myocardial infarction, presence of metabolic syndrome (MS), as defined by the American Heart Association [29] (apart from cases with a single component of MS in stable conditions on therapy for at least 1 year), alcohol intake >14 units a week, history of allergic reactions to drugs or food. Other exclusion criteria for the PBC patients were: a history or clinical evidence of concomitant malignant liver diseases, or cirrhosis with portal hypertension. The cases with single components of MS were comparable between patients and controls. All participants had a normal ECG at rest and during adenosine-induced hyperemia. Patients and controls came from the same geographical area. The absence of coronary heart disease was judged from clinical history, physical examination, and ECG. PBC was diagnosed according to the EASL criteria [30].

2.2. PBC patients' clinical characteristics

After being diagnosed with PBC, all patients were treated with UDCA at a stable dose of 15 mg/kg/day, and they were routinely followed up with clinical and biochemical assessments every 6 months. The following data were obtained for each patient: response to UDCA according to the Barcelona criteria [3], age at PBC diagnosis, interval between PBC diagnosis, symptom onset and CFR assessment, symptoms of liver disease (fatigue, pruritus, and sleep disorders), comorbidities and their duration, and concomitant therapies. Patients also underwent a complete physical and biochemical assessment at the time of their CFR assessment, as well as upper abdominal ultrasound and transient elastography to test for cirrhosis, and their Framingham risk [31] and Mayo prognostic risk scores were calculated [32].

2.3. CFR assessment

TDE was performed with a commercially-available ultrasound system (Vivid 7, GE Medical System, Inc., Hortem, Norway). Coronary images were obtained in the distal part of the left anterior descending artery (LAD) with a 7-MHz transducer. Coronary blood flow was obtained by color Doppler flow-mapping guidance, and the sample volume was positioned within the color signal in the LAD by pulse-wave Doppler. After obtaining baseline recordings of the coronary diastolic flow velocity at rest (DFVr), adenosine was infused intravenously (140 mg/kg-1/min) for 3 min to establish the hyperemic Doppler flow velocity (DFVh). The CFR was estimated as the ratio of the DFVh to the DFVr.

The impact of myocardial workload on CFR was ascertained by correcting the DFVr for the double product (DPr), defined as the product of heart rate and systolic blood pressure at rest [33]. The corrected diastolic flow velocity at rest (cDFVr) was obtained using the following formula:

$$cDFVr = (DFVr/DPr) * 10.000$$

The corrected CFR (cCFR) was calculated as the ratio of the DFVh to the cDFVr. A cCFR ≤ 2.5 was considered abnormal, and the patient population was dichotomized according to this cutoff. To describe the increase in Doppler flow velocity after adenosine infusion, the difference between the DFVr and the DFVh was calculated both as an absolute value (ΔDFV), and as a percentage (ΔDFV%). Microvascular resistance (mmHg.s/cm) was calculated as the ratio between mean blood pressure (mean pressure = [2 × diastolic + systolic]/3)

and the average DFVr and DFVh (BMR and HMR, respectively) [34]. The corrected BMR (cBMR), i.e. the ratio between mean blood pressure at rest and cDFVr, was calculated as well. The cCFR and DFVh were integrated by creating 4 subgroups based on whether patients had a concordant or discordant impairment of these coronary flow indices. A cCFR ≤ 2.5 and a DFVh ≤ 65 cm/sec were considered impaired.

2.4. Statistical analysis

Continuous variables are reported as mean ± standard deviation (SD), or median and interquartile range if not normally distributed. Patients and controls were compared using Student's unpaired t-test for normally-distributed data, or Wilcoxon's signed rank sum test if data were highly skewed. Matched pairs were compared with Student's paired t-test for normally-distributed data, or Wilcoxon's signed rank sum test if data were highly skewed. Categorical variables are reported as percentages and were compared with the χ^2-test or Fisher's exact test, as appropriate. The distribution of the data was analyzed with a 1-sample Shapiro-Wilk test. An unadjusted linear regression analysis was performed between the CFR, BMR, cBMR, HMR, and risk factors or clinical conditions. Statistical significance was assumed if the null hypothesis could be rejected at p = .05. Data were analyzed with the SPSS software, version 24.0 (Chicago, SPSS, Inc. Chicago, IL).

3. Results

3.1. Clinical characteristics of PBC patients

The 37 PBC patients (33 females) had a median 7 years of follow-up. All patients had received UDCA since being diagnosed with PBC, and 32 were responders according to the Barcelona criteria. At the time of their CFR assessment, alkaline phosphatase was below 1.5 (the upper the limit of normal) in 28 patients (76%). Five patients (14%) were cirrhotic. None had portal hypertension.

Seventeen patients (46%) had a single component of metabolic syndrome (arterial hypertension in 5, type II diabetes in 2, hypercholesterolemia in 6, hypertriglyceridemia in 1, and 3 patients were overweight [BMI 25–29.9]). All patients had been taking a stable dose of appropriate therapy for at least 1 year before entering the study, and patients with hypercholesterolemia were treated with statins. At the time of their CFR assessment, their altered parameter had normalized, except for the 3 overweight patients, who were on a low-calorie diet. Despite the presence of cardiovascular comorbidities, the PBC population had a low Framingham risk score of 1 (1–3).

Twenty-six patients (70%) also had one or more concomitant extrahepatic autoimmune or chronic inflammatory diseases diagnosed a median 10 (4–16) years prior to their CFR assessment. In detail, Sjögren's syndrome was identified in 21 patients, Raynaud's phenomenon in 12, Hashimoto's thyroiditis in 6, psoriasis in 3, rheumatoid arthritis in 2, celiac disease in 1, autoimmune thrombocytopenia in 1, dermatomyositis in 1, polymyositis in 2, and Horton's arteritis in 1.

At the time of their CFR assessment, 17 patients complained of fatigue, and 7 reported pruritus and sleep disturbances. The patients' clinical and biochemical characteristics at the time of their CFR assessment are listed in Table 1.

3.2. CFR assessment in patients and controls

Overall, the cCFR was significantly lower in PBC patients than in controls (2.8 ± 0.7 vs. 3.7 ± 0.7, p < 0.0001) (Fig. 1). None of the patients showed any significant left ventricular wall motion abnormality during adenosine infusion. The echocardiographic features, including left ventricular mass, were comparable in PBC patients and controls, and none of the study participants had left ventricular hypertrophy. All PBC patients had normal left ventricular dimensions and ejection fractions. Table 2 shows the hemodynamic parameters in the PBC patients and controls. Both the DFVr and the DFVh were significantly lower in the PBC patients than in the controls (p < 0.0001). The cDFVr was lower in the patients too (p = .006), and the ΔDFV was significantly lower in the PBC patients than in the controls (p < 0.0001). As expected, the PBC patients' hemodynamic parameters differed significantly before and after adenosine infusion, showing a normal response to adenosine infusion in both groups (Table 3).

Table 1
Clinical and biochemical features of 37 PBC patients at the time of CFR assessment.

Characteristics	PBC patients (n = 37)
Age at CFR assessment (years)	58 (49–66)
BMI (Kg/m^2)	23 (21–25)
Total bilirubin (mg/dL)	0.6 (0.4–0.9)
Alkaline phosphatase (x ULN)	0.9 (0.7–1.6)
GGT (x ULN)	1.4 (0.7–2.6)
AST (x ULN)	0.7 (0.6–1.2)
ALT (x ULN)	0.7 (0.5–1.1)
Total cholesterol (mg/dL)	214 (185–234)
HDL cholesterol (mg/dL)	64 (54–71)
Triglycerides (mg/dL)	89 (68–109)
Serum glucose (mg/dL)	88 (80–97)
Hemoglobin (mg/dL)	13.4 (12.8–14.6)
Liver stiffness (kPa)	7.1 (5.6–8.8)
Mayo risk score	4.1 (3.5–4.5)
TSH within the range	36 (97)
Vitamin D deficiency or insufficiency	14 (45)
Increased PTH	9 (43)

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CFR, coronary flow reserve; GGT, gamma-glutamyltransferase; TSH, thyroid stimulating hormone; PTH, parathyroid hormone; ULN, upper limit of normal. Continuous variables are expressed as median (interquartile range). Qualitative variables are expressed as n (%).

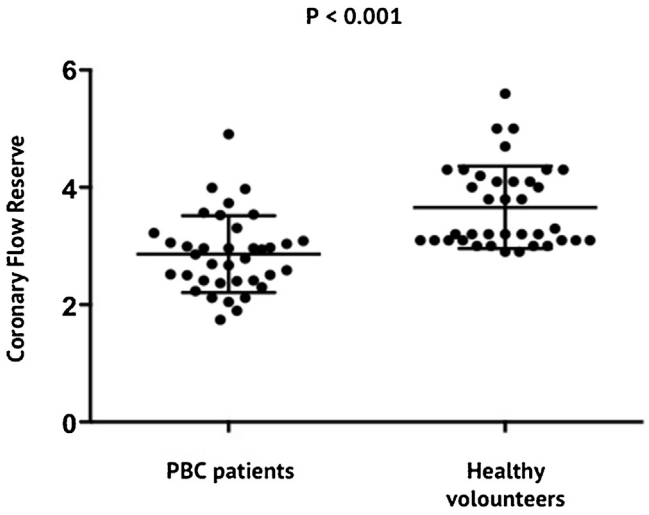

Fig. 1. Corrected coronary flow reserve (cCFR) in PBC patients and healthy controls.

3.3. Hemodynamic parameters based on cCFR

Stratifying the PBC population according to the 2.5 cutoff, the cCFR was normal in 24 patients, and abnormal in 13. The hemodynamic parameters of the two subgroups are presented in Table 4. The corrected DFVr was significantly higher in PBC patients with a lower CFR (p < 0.0001), indicating a higher baseline flow velocity after correcting for the impact of cardiac workload; and, in keeping

Table 2
Hemodynamic parameters in PBC patients and controls.

	Controls (N = 37)	PBC patients(N = 37)	p	CI 95%
DFVr (cm/sec)	29.5 ± 6.3	21.4 ± 4.2	<0.0001	5.10–11.1
cDFVr (cm/sec)	30.4 ± 8.2	23.2 ± 6.4	0.006	2.2–12.2
DFVh (cm/sec)	104.6 ± 18.2	64.4 ± 10.6	<0.0001	32–48.3
ΔDFV (cm/sec)	75 ± 14.1	40.8 ± 8.1	<0.0001	27.9–40.4
ΔDFV (%)	260 ± 52	187 ± 64	<0.0001	37.9–107.8
CFR	3.7 ± 0.7	3.0 ± 0.3	<0.0001	0.31–0.8
cCFR	3.54 ± 0.5	2.8 ± 0.6	0.001	0.28–1.04
BMR (mm Hg.s/cm)	3.9 ± 0.9	5.1 ± 0.1	0.001	−1.79 to −0.55
cBMR (mm Hg.s/cm)	3.2 ± 0.2	4.9 ± 0.2	<0.0001	−2.45 to −0.94
HMR (mm Hg.s/cm)	1.08 ± 0.1	1.5 ± 0.2	<0.0001	−0.65 to −0.23

Abbreviations: BMR, microvascular resistance at rest; cBMR, corrected microvascular resistance at rest; cCFR, corrected coronary flow reserve; CFR, coronary flow reserve; cDFVr, corrected diastolic flow velocity at rest; DFVr, diastolic flow velocity at rest; DFVh, diastolic flow velocity during adenosine infusion; ΔDFV, difference between DFVh and cDFVr; ΔDFV%, percentage of difference between DFVh and cDFVr; HMR, microvascular resistance during hyperemia.

Table 3
Hemodynamic parameters in controls and PBC patients before and after adenosine infusion.

	Controls (n = 37)		p	PBC patients (n = 37)		p
	Before adenosine	After adenosine		Before adenosine	After adenosine	
HR(bpm)	77 ± 12	89 ± 13	<0.001	78 ± 14	87 ± 14	<0.001
SBP (mmHg)	126 ± 17	105 ± 14	<0.001	122 ± 15	110 ± 14	<0.001
DBP (mmHg)	75 ± 6	65 ± 4	0.003	73 ± 8	66 ± 7	0.004
DFV (cm/sec)	29 ± 6	104 ± 18	<0.0001	21* ± 4	64* ± 11	<0.001

Abbreviations: DBP, diastolic blood pressure; DFV, diastolic flow velocity; HR, heart rate; SBP, systolic blood pressure. Hemodynamic parameters are expressed as mean ± standard deviation.
* P < 0.0001 vs. controls.

Table 4
Hemodynamic parameters in PBC patients according CFR value and controls.

	Controls (N = 37)	cCFR > 2.5 (N = 24)	cCFR ≤ 2.5 (N = 13)	p*	CI 95%
DFVr (cm/sec)	29.5 ± 6.3	20.6 ± 3.7[a]	22.2 ± 4.8	0.1	−0.57 to 5.25
cDFVr (cm/sec)	30.4 ± 8.2	20.4 ± 4.5[a]	29.0 ± 6.0	<0.0001	−12.3 to −5.04
DFVh (cm/sec)	104.6 ± 18.2	63.5 ± 9.7[a]	66.0 ± 12.5	0.5	−9.9 to 5.07
ΔDFV (cm/sec)	75 ± 14.1	43.2 ± 7.1[a]	36.5 ± 8.4	0.01	1.4–12.0
ΔDFV (%)	260 ± 52	221 ± 54[b]	126 ± 25	<0.0001	62.3–127.1
CFR	3.7 ± 0.7	3.1 ± 0.3[c]	2.9 ± 0.3	0.04	−0.01 to 0.44
cCFR	3.54 ± 0.5	3.2 ± 0.5	2.3 ± 0.3	<0.0001	0.63–1.28
BMR (mm Hg.s/cm)	3.9 ± 0.9	5.4 ± 0.3[a]	4.6 ± 0.2	0.04	0.02–1.53
cBMR (mm Hg.s/cm)	3.2 ± 0.2	5.6 ± 0.3[a]	3.7 ± 0.3	<0.0001	0.98–2.78
HMR (mm Hg.s/cm)	1.08 ± 0.1	1.5 ± 0.3[a]	1.4 ± 0.2	0.2	−0.63 to 0.34

Abbreviations: as in Table 1.
[a] p < 0.0001 versus controls.
[b] p = 0.02 versus controls.
[c] p = 0.001 versus controls.
* P value for comparison between PBC patients with cCFR > 2.5 and patients with cCFR ≤ 2.5.

with this, their cBMR was lower (p < 0.0001). Table 4 shows further microvascular perfusion indexes in the PBC patients with a normal cCFR and in controls. Intriguingly, the CFR, DFVr and cDFVr were lower in patients with a cCFR>2.5 than in controls (p < 0.0001 for all parameters), and the BMR, cBMR and HMR were significantly higher in patients with a cCFR > 2.5 than in controls (p < 0.0001 for all parameters) (Table 4).

3.4. Integrated assessment of coronary flow reserve and hyperemic flow

Table 5 shows the hemodynamic parameters for the patient population, divided into 4 subgroups by the concordance or discordance of their cCFR or DFVh impairment. A cCFR ≤ 2.5 and DFVh ≤ 65 cm/sec (the latter representing the median value for the whole patient population) were considered impaired. Although the CFR and cCFR were comparable, the basal and hyperemic microvascular resistance values (BMR, cBMR and HMR) were lower in group 2 than in group 1. In contrast, the cCFR was higher in group 3 than in group 2, while group 3 had a lower cDFVr and a higher basal and hyperemic microvascular resistance (cBMR, BMR and HMR). In addition, despite the comparable CFR and cCFR, the basal and hyperemic microvascular resistance values (BMR, cBMR and HMR) were higher in group 3 than in group 4.

3.5. Correlations between hemodynamic parameters and risk factors or clinical conditions

The CFR and cCFR did not correlate with any of the following variables: age at PBC diagnosis or age at CFR assessment; time since diagnosis; presence and number of symptoms of PBC; presence, number and duration of other extrahepatic autoimmune, inflammatory diseases or cardiovascular comorbidities; biochemical features at the time of CFR assessment (total bilirubin, transaminases, alkaline phosphatase, GGT, immunoglobulin M level, total and HDL cholesterol, triglycerides, glycemia, albumin, INR, hemoglobin, vitamin D, TSH and PTH levels); Framingham risk score or Mayo risk score. On the other hand, both the BMR and the HMR correlated with time since diagnosis (p = .03 and p = .002, respectively), symptom duration (p = 0.04 and p = 0.02,

Table 5
Hemodynamic parameters in four PBC patients groups.

	Impaired cCFR		Preserved cCFR		p value			
	Impaired DFVh (group 1)	Preserved DFVh (group 2)	Impaired DFVh (group 3)	Preserved DFVh (group 4)	1 vs. 2	1 vs. 3	3 vs. 4	2 vs. 3
DFVr (cm/sec)	19.0 ± 3.8	25.3 ± 3.7	18.0 ± 2.3	23.6 ± 2.6	0.01	0.5	<0.0001	<0.0001
cDFVr (cm/sec)	24.3 ± 5.8	32.3 ± 3.5	17.8 ± 3.0	23.2 ± 4.2	0.01	0.007	0.003	<0.0001
DFVh (cm/sec)	53.4 ± 7.2	73.8 ± 7.3	56.1 ± 5.6	72.2 ± 5.0	0.002	0.4	<0.0001	<0.0001
ΔDFV (cm/sec)	29 ± 5.4	41.1 ± 6.1	38.2 ± 4.9	49.1 ± 4.0	0.006	0.01	<0.0001	0.3
ΔDFV (%)	124 ± 33	126 ± 20	220 ± 49	221 ± 62	0.9	<0.0001	0.9	<0.0001
CFR	2.84 ± 0.2	2.93 ± 0.2	3.15 ± 0.3	3.08 ± 0.4	0.3	0.1	0.7	0.3
cCFR	2.24 ± 0.3	2.23 ± 0.1	3.20 ± 0.4	3.20 ± 0.6	0.7	<0.0001	0.9	<0.0001
BMR (mm Hg.s/cm)	5.4 ± 0.5	4.1 ± 0.7	6.1 ± 0.7	4.5 ± 0.7	0.02	0.2	<0.0001	<0.0001
cBMR (mm Hg.s/cm)	4.3 ± 0.5	3.2 ± 0.6	6.2 ± 0.3	4.8 ± 0.5	0.04	0.01	0.006	<0.0001
HMR (mm Hg.s/cm)	1.69 ± 0.2	1.27 ± 0.1	1.76 ± 0.2	1.35 ± 0.2	0.01	0.5	<0.0001	<0.0001

Abbreviations: as in Table 2.

respectively), and Framingham risk score ($p = 0.008$ and $p = 0.01$, respectively). Finally, the HMR correlated directly with the duration of autoimmune/inflammatory comorbidities ($p = 0.02$). We also noted that the times elapsing since diagnosis, and since symptom onset were significantly longer in group 3 than in group 1 (data not shown).

4. Discussion

This study showed that patients with PBC but without metabolic syndrome or clinical evidence of coronary artery disease have a lower CFR than controls. This difference became more significant after correcting for cardiac workload, suggesting that the patients' reduced CFR was not due to any differences in basal heart rate or blood pressure between patients and controls.

These novel findings suggest that patients with PBC might have an early impairment of coronary flow reserve, and this could mean a greater risk of cardiovascular events over time, even though our PBC cohort's Framingham risk scores indicated a low risk profile for cardiac events. A previous study conducted at our center found significantly more cardiovascular events in patients with PBC and MS as opposed to PBC alone (30% vs. 5%, $p < 0.0001$), and age at the time of PBC diagnosis was the only predictor of mortality in the PBC patients with MS [35].

The second important finding of the present study lies in that, on stratifying PBC patients by a cCFR ≤ 2.5 or >2.5, those with a lower cCFR (one in three of our patients) had an increased coronary flow in basal conditions, even after adjusting for cardiac workload. There were no differences between these two groups in terms of the variables characterizing liver disease, the presence of extrahepatic autoimmune diseases, or cardiovascular risk factors.

Previous studies exploring the mechanisms of fatigue in PBC found severe fatigue associated with an ANS dysfunction [9], and a higher prevalence of sudden cardiac death [8]. ANS dysfunction was correlated with severity of PBC symptoms [36,37], and the early onset of ANS dysfunction in patients with severe fatigue suggested that the heart may be a target of accelerated aging processes in PBC patients [38]. Impaired cardiomyocyte bioenergetics were also documented in PBC, so the ANS dysfunction was interpreted as a compensatory mechanism against an altered myocardial function [39]. In line with these studies, we suggest that the increase in coronary flow in basal conditions could likewise be a compensatory mechanism of the coronary microcirculation in response to impaired cardiomyocyte bioenergetics in a subgroup of PBC patients.

Another relevant finding of our study stems from the comparison between PBC patients with a normal cCFR (cCFR > 2.5) and controls: the former had significantly lower CFR and cCFR than the latter, even though the ratios were within the limits of normality. This intriguing finding is probably due to PBC patients having lower diastolic flow velocities and a higher microvascular flow resistance in both basal and hyperemic conditions than controls, even after adjusting for cardiac workload. In other words, even PBC patients with a normal cCFR may have some degree of microvascular dysfunction, and cCFR alone cannot identify all patients with this condition.

The group of PBC patients with a cCFR > 2.5, and lower basal and hyperemic diastolic flow velocities than in controls, shared some features with patients with arterial hypertension and NAFLD [40], who had a lower CFR than hypertensive patients without NAFLD due to lower hyperemic flow values, suggesting a defective vasodilatory response. In patients with NAFLD and arterial hypertension, moreover, severity of liver fibrosis was an independent predictor of CFR impairment [40]. Similarly, the 5 patients in our study with cirrhosis all had a cCFR >2.5 and lower basal and hyperemic flow velocities.

Given these results, and to better characterize our patients, we divided them into 4 groups by integrating their cCFR with their hyperemic diastolic flow velocities. Group 1 (impaired cCFR and DFVh) had a higher hyperemic microvascular resistance than group 4 (normal cCFR and DFVh). The coronary microcirculation of patients in group 1 was unable to vasodilate, meaning their cardiovascular risk is generally higher than normal, as is the likelihood of epicardial multivessel disease [41]. Angiography might therefore be considered in such cases to identify any epicardial coronary artery disease or coronary microvascular disease. In contrast, group 4 had the lowest cardiovascular risk and are unlikely to have flow-limiting epicardial coronary disease. Patients in group 2 (impaired cCFR and preserved DFVh), with high basal diastolic flow velocities and a low basal resistance, also carry an increased cardiovascular risk according to the literature [42]. Patients in group 3 (preserved cCFR but impaired DFVh) had a higher basal and hyperemic microvascular resistance and lower diastolic flow velocities than in group 4, but their cCFR was normal because it identifies the ratio between two velocities. Their increased microvascular resistance suggests a process of coronary microvascular structural remodeling, and these patients' microcirculation is not normal despite their normal cCFR. We also documented a specific relationship between the time elapsing since PBC was diagnosed or symptoms developed and microvascular resistances. In particular, the interval was longer in group 3 than in group 1, suggesting that the two groups may represent different stages of the same coronary microvascular disease. In keeping with this hypothesis, we found direct relationships between basal microvascular resistance and time since diagnosis, and between hyperemic microvascular resistance and time since diagnosis.

The main limitation of our study lies in that none of the patients underwent coronary angiography because they were all asymp-

tomatic for heart disease, and their CFR (before correcting for double product) was always higher than 2. We consequently cannot exclude the possibility of a reduced CFR being secondary to epicardial stenosis. The likelihood of epicardial coronary disease was low, however, given the low Framingham risk scores.

5. Conclusions

In conclusion, our preliminary study demonstrated that PBC patients have a lower CFR than healthy controls. This could be due to different mechanisms: an impaired CFR and a high resting coronary flow associated with a low microvascular resistance in some patients; or a concordantly impaired CFR and hyperemic coronary flow in others. In a third group, we also found signs suggestive of coronary microvascular structural remodeling associated with a preserved CFR and a high resting and hyperemic resistance. These different patterns seem to relate to the time elapsing since PBC was diagnosed.

Further studies are needed in this field to investigate whether cardiomyocyte bioenergetics are impaired in PBC, to explore the pathogenic mechanisms behind cholestatic damage to coronary microvascular function, and to assess the clinical impact of these findings.

Conflict of interest

None declared.

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