Original article

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Quantitative assessment of liver function using hepatobiliary scintigraphy: the effect of microcirculatory alterations after portal vein embolization

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Objectives **Hepatobiliary scintigraphy using technetium-99m mebrofenin has been validated as a quantitative liver function test. Preoperative portal vein embolization (PVE) is performed in patients to increase future remnant liver function and volume. Changes in hepatic microcirculation after PVE remain largely unknown and may influence the uptake of mebrofenin. The aim was to evaluate microcirculatory changes after PVE to examine differences in perfusion that might influence the uptake of mebrofenin, and consequently, assessment of function.**

Patients and methods **Patients undergoing liver resection with or without preoperative PVE were included. Future remnant liver volume and function were measured before and after PVE. Hepatic microcirculation was measured in the embolized and the nonembolized lobes during resection. Microcirculatory flow index, perfused vessel density, sinusoidal diameter and red blood cell velocity were assessed.**

Results **A total of 16 patients, eight with preoperative PVE and eight control patients without PVE, were included. After PVE, both function and volume of the nonembolized lobe were significantly increased, and the functional increase exceeded the increase in volume. Perfused vessel density and sinusoidal diameter were significantly higher in the nonembolized liver lobe ($P < 0.002$ and <0.04). No significant differences between both lobes were found concerning microcirculatory flow index or red blood cell velocity.**

Conclusion **After PVE, the nonembolized lobe had a significantly higher (functional) microvascular density compared with the embolized lobe, without differences in microvascular flow. These findings indicate that the measured functional increase using hepatobiliary scintigraphy, which exceeded the volumetric increase, was not the consequence of an increase in hepatic perfusion, therefore, providing adequate representation of the liver function.** ***Nucl Med Commun*** **40:720–726**

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Background

Liver resection is in many cases the only curative treatment for liver tumours. Large resections are often needed to achieve tumour-free (R0) margins. In patients with insufficient future remnant liver (FRL) volume (FRLV) or function (FRLF), the risk of posthepatectomy liver failure is substantial and potentially lethal [1]. Therefore, preoperative assessment of FRL is crucial in patients undergoing major liver resection.

Preoperative assessment of FRL is usually performed using computed tomography (CT)-volumetry in which the volumetric share of the FRL is calculated as a percentage of the total volume [2]. More recently, technetium-99m (^{99m}Tc) mebrofenin hepatobiliary scintigraphy (HBS) has emerged as a quantitative liver function test that can be used to measure total and regional liver function [3]. This method relies on the hepatic uptake rate of mebrofenin as a reflection of liver function. Volumetric analysis of the FRL is used as an indirect measure of liver function in which the volume threshold for safe resection depends on the quality of liver parenchyma. However, liver function does not necessarily correlate with volume. Furthermore, the functional capacity may not be homogeneously distributed throughout the liver, especially in patients with impaired liver parenchyma [4]. Estimation of liver function on the basis of CT-volumetry alone can, therefore, be unreliable in patients with compromised liver parenchyma. Risk assessment based on HBS yields visual as well as quantitative information on liver function, using the same cutoff value regardless of parenchymal quality. HBS has been validated in a mixed series of patients undergoing liver resection to reduce the risk of posthepatectomy liver failure and related mortality [5].

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FRL function of less than 2.7%/min/m^2 is considered insufficient for safe liver resection or using CT-volumetry, a share of less than 25–40%, depending on the quality of liver parenchyma [4,6,7]. In these cases, preoperative portal vein embolization (PVE) can be used to increase FRL volume and function. Unilateral PVE induces atrophy of the embolized lobe and a compensatory hypertrophy of the nonembolized, contralateral lobe. Several studies have showed a more pronounced functional increase than a volumetric increase in the first weeks after PVE [8,9]. This can be attributed to an increase in function per volume unit of liver tissue. However, local physiological changes after PVE, particularly on the level of hepatic microcirculation, can also play a role and influence the uptake of mebrofenin. Microcirculation is essential for oxygen delivery to parenchymal cells and the complex functions of the liver tightly depend on the microcirculatory status of the liver. The hepatic sinusoids are key elements in the hepatic microcirculatory system through which supply of oxygen and nutrients (including mebrofenin) and the removal of metabolic products takes place. Two fundamental hemodynamic principles that determine this supply to tissue are microvascular distribution and diffusion. Distribution is quantified by flow (movement of blood cells), and diffusion is quantified by the density of the perfused microvessels. We previously showed hepatic microcirculatory changes after PVE that could interfere with the physiology of the uptake of mebrofenin at HBS [10].

The aim of this study was to evaluate hepatic microcirculatory changes after PVE to examine differences in perfusion that might influence the uptake of ^{99m}Tc-mebrofenin at HBS.

Patients and methods

Patients

A total of 16 patients who underwent major liver resection were included in this prospective, observational study. Eight patients who underwent preoperative PVE because of insufficient FRL were included along with eight control patients who did not require PVE. Patients with and without preoperative PVE were matched for sex, age and comorbidity. CT-volumetry and HBS were part of the preoperative workup of patients considered for major liver resection. These patients had been included in a previous study from our department [10].

During resection, the hepatic microcirculation was assessed using incident dark-field illumination (see below). The study was in compliance with ethical principles and appropriate regulatory requirements. The study has been approved by the Institutional Review Board of the Amsterdam UMC, the University of Amsterdam, under number W17_259. Informed consent was obtained from all individual participants included in the study.

Hepatobiliary scintigraphy and computed tomography-volumetry

Patients underwent HBS with ^{99m}Tc-mebrofenin and multiphase contrast-enhanced CT as part of the standard workup, as described previously [11]. Patients with preoperative PVE underwent CT and HBS before and ~3 weeks after the procedure.

Regions of interest were drawn on the geometric mean data sets around the liver, heart (serving as blood pool) and total field of view (Fig. 1). Total liver function is represented by the mebrofenin uptake rate, calculated according to Ekman *et al.* [12]. Subsequently, the nonembolized segments (FRL) were delineated on the SPECT data sets to calculate the functional share (the percentage of counts within the FRL; Fig. 2). The function of the FRL was calculated as the product of the functional share and total liver function, divided by BSA (m^2) to compensate for individual metabolic differences, and is represented as %/min/m^2.

For the calculation of the FRL volume, the liver was outlined on an axial scan in a semiautomated fashion using manual adjustment (Fig. 3). Total liver volume, tumour volume and FRLV were obtained to calculate the FRL volumetric share (FRLV%) as follows:

$$\frac{\text{FRLV}}{\text{Total liver volume} - \text{tumour volume}} \times 100\,\%\,.$$

Portal vein embolization

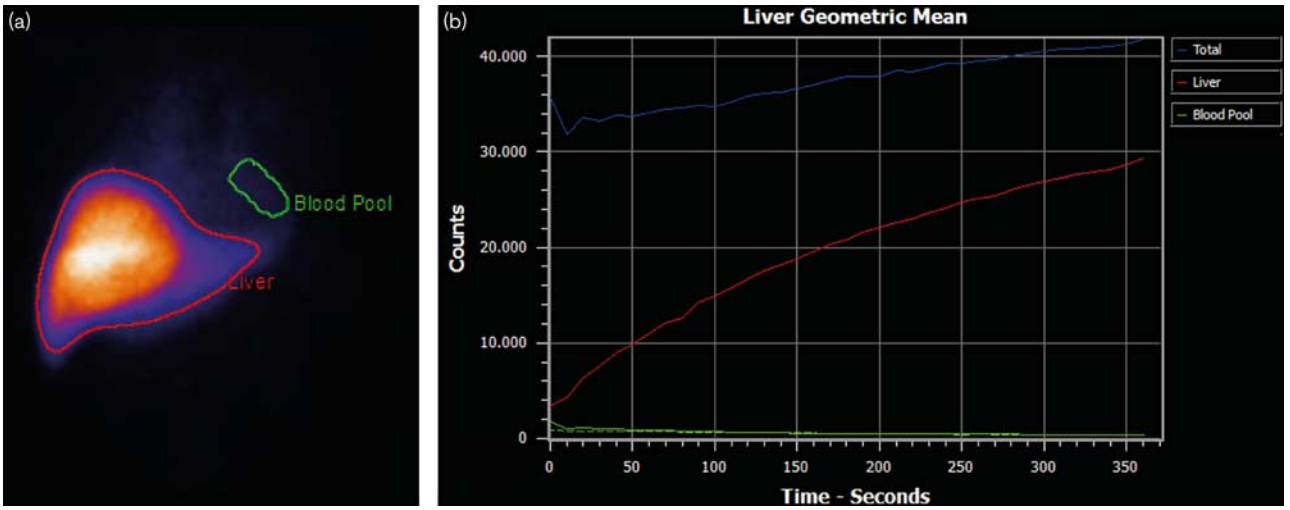
Patients with insufficient FRL function (< 2.7%/min/m^2) were considered for preoperative PVE. All patients underwent embolization of the right portal system by a percutaneous ipsilateral, transhepatic approach. The branches were embolized using polyvinyl alcohol particles (300–500 nm; Cook, Bloomington, Indiana, USA) and coils (Tornado Embolization Microcoil; Cook).

Microcirculation

Videos of the hepatic microcirculation were recorded intraoperatively using incident dark-field imaging using the Cytocam (Braedius Medical, Huizen, the Netherlands). This third-generation, handheld video-microscope emits illumination light at a wavelength of 548 nm with a pulse time of 2 ms. The light is absorbed by haemoglobin, rendering red blood cells visible as dark cells in the microcirculation [13,14]. Vessels that are not perfused will not be visible, whereas the tissue surrounding the vessels mostly reflects light and is seen as a white area.

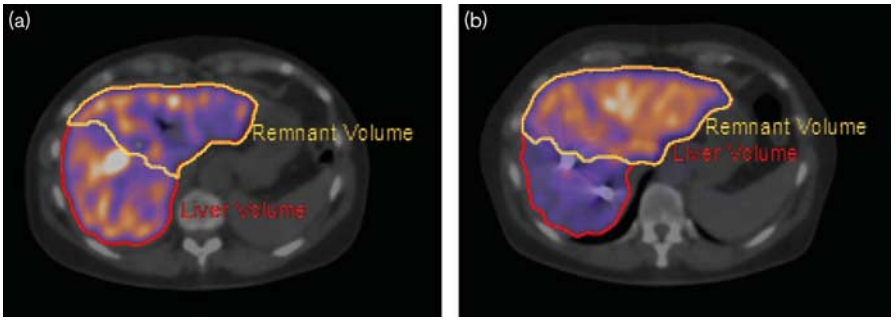
The hepatic microcirculation in the left and right lobes was measured in standardized regions (segments 3 and 6) corresponding with the embolized and nonembolized lobes, respectively, in patients having undergone PVE. The camera was perpendicularly placed on the hepatic surface exerting light pressure to prevent pressure-induced artefacts. Video clips were recorded with a

Fig. 1



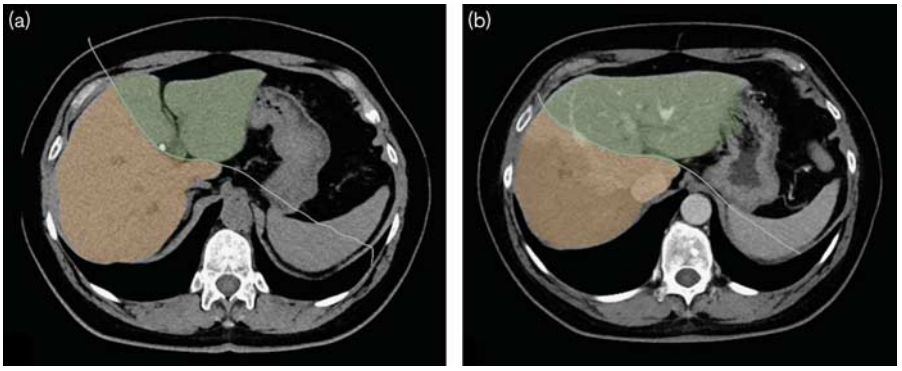
Hepatobiliary scintigraphy with regions of interest on summed dynamic images (a) and corresponding time–activity curves (b).

Fig. 2



Single-photon emission computed tomography delineation before (a) and 3 weeks after portal vein embolization (b).

Fig. 3



Computed tomography-volumetry before (a) and 3 weeks after portal vein embolization (b).

duration of 4 s at 25 frames/s and saved to the hard drive of the Cytocam computer.

Each video clip was assessed for adequate quality using the Microcirculation Image Quality Score [15]. Microcirculatory parameters were obtained using Automated Vascular Analysis Software v3.2 (Microvision Medical, Amsterdam, the Netherlands) [16].

Flow characteristics were quantified using the microcirculatory flow index (MFI) [17–19]. This semiquantitative score is based on the predominant type of flow patterns (absent = 0, intermittent = 1, sluggish = 2 or normal = 3). Each image was divided into four quadrants, and each quadrant was scored individually. The value of the four quadrants was averaged.

Diffusion was characterized by the perfused vessel density (PVD; mm/mm^2), which is the total density of vessels with MFI values of 2 and 3 [17,18]. This is used as a measure of functional capillary density. The red blood cell velocity (RBCv; μm/s) was quantified using the space-time diagram, and sinusoidal diameters (SinD; μm) were measured as the average diameter of four sinusoidal vessels [19].

Statistical analysis

Continuous variables were expressed as mean and SD. Noncontinuous variables were expressed in absolute number and percentage. Differences in continuous variables collected at two time points in one group were analysed with paired *t*-test, and variables collected at one time point in two groups were analysed with an unpaired *t*-test. A two-sided *P* value less than 0.05 was considered significant. Statistical analysis was performed using SPSS (SPSS 24.0; IBM, Chicago, Illinois, USA).

Results

Patient characteristics

A total of 16 patients, eight with preoperative PVE and eight control patients, were included. In the PVE group, the mean ± SD time to surgery after PVE was 45 ± 20 days. Patient characteristics are presented in Table 1.

Table 1 Patient characteristics

	PVE ($n = 8$)	No-PVE ($n = 8$)	*P* value
Sex (male)	4	5	1.00
Age (years)	65.5 ± 6.7	60.1 ± 12.9	0.31
BMI (kg/m^2)	25.6 ± 3.3	23.7 ± 6.4	0.46
BSA (m^2)	1.9 ± 0.1	1.8 ± 0.3	0.32
Comorbidities			
COPD	0	0	–
Hypertension	2	1	–
Diabetes	2	1	–
Smoking	0	1	–
Pathology			
Cholangiocarcinoma	4	7	–
Colorectal liver metastasis	2	0	–
Hepatocellular carcinoma	2	0	–
Liver adenoma	0	1	–

COPD, chronic obstructive pulmonary disease; PVE, portal vein embolization.

Liver volume and function

The FRLF was measured in seven of the eight included patients, whereas the FRLV% was measured in all of the eight patients with PVE. Both the function and the volume of the nonembolized lobe were significantly increased after a mean ± SD time of 23 ± 3 days after PVE. FRLF increased from 1.9 ± 0.9 to 3.9 ± 0.8%/min/m^2, $P < 0.002$, and the FRLV% increased from 27.8 ± 6.1 to 38.3 ± 8.5%, $P < 0.001$ (Fig. 4). The increase in liver function was 142.0 ± 124.2%, whereas the increase in FRL volume was 40.7 ± 26.5%.

Microcirculatory parameters

Microcirculatory parameters are presented in Table 2. PVD was significantly higher in the nonembolized (hypertrophic) liver lobe as compared with the embolized (atrophic) liver lobe after PVE (40.4 ± 8.9 vs. 26.7 ± 4.7 mm/mm^2, $P < 0.002$). This difference was not observed in the control group (29.5 ± 5.7 vs. 28.5 ± 7.4 mm/mm^2, $P = 0.759$; Fig. 5).

No significant differences between the left and right lobes were found concerning MFI or RBCv in both groups at 45 ± 20 days after PVE.

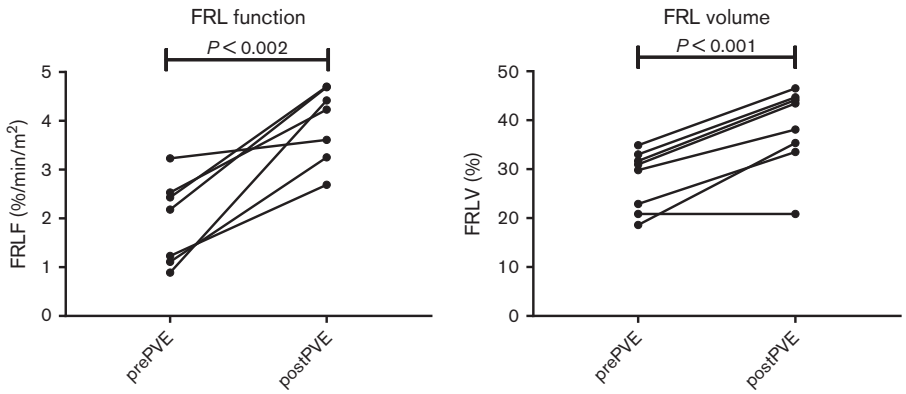
SinD was considerably higher in the nonembolized lobes compared with the embolized lobes (9.3 ± 1.8 vs. 6.4 ± 0.9 μm, $P < 0.04$). There were no differences in the SinD between the left and right lobes of the patients without PVE (7.44 ± 1.35 vs. 7.1 ± 0.56 μm, $P = 0.487$).

Discussion

In this study, we analysed the effect of microcirculatory alterations in the liver after PVE on the uptake of ^{99m}Tc-mebrofenin during HBS performed to assess the functional increase of the nonembolized FRL. Our main findings are that after PVE, there is a higher functional capillary density without changes in microvascular flow in the nonembolized lobe in comparison with the embolized lobe. This is accompanied by a significant increase in both function and volume of the FRL, indicating that the measured increase in the mebrofenin uptake rate results from an increase in functional hepatocytes rather than from increased hepatic perfusion [20].

Liver function assessed by HBS relies on the hepatic uptake rate of mebrofenin. After intravenous injection, mebrofenin is distributed in the blood pool and is conveyed to the liver through the hepatic artery where it reaches the sinusoids. There, mebrofenin comes into contact with the hepatocyte membrane where the uptake is mainly facilitated by organic anion transporting proteins B1 and B3 [21]. Mebrofenin undergoes no hepatic biotransformation and is consequently excreted in the bile ducts, mediated by the conjugate export pump multidrug

Fig. 4



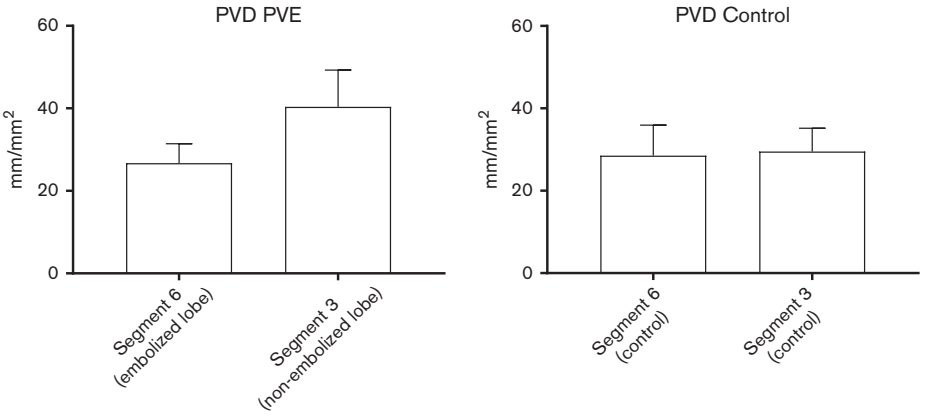
Future remnant liver function (%/min/m^2) and volume share (%) before and after portal vein embolization. FRL, future remnant liver; FRLF, future remnant liver function; FRLV, future remnant liver volume; PVE, portal vein embolization.

Table 2 Microcirculatory parameters

	PVE group			No PVE (control) group		
	Segment 3 (non-embolized lobe)	Segment 6 (embolized lobe)	*P* value	Segment 3 (control)	Segment 6 (control)	*P* value
PVD	40.4 ± 8.9	26.7 ± 4.7	< 0.002	29.5 ± 5.7	28.5 ± 7.4	0.759
MFI	3	3	1	3	3	1
RBCv	455.5 ± 54.0	510.8 ± 133.1	0.294	492.4 ± 52.3	491.3 ± 111.4	0.979
SinD	9.3 ± 1.8	6.4 ± 0.9	< 0.002	7.4 ± 1.4	7.1 ± 0.6	0.524

MFI, microcirculatory flow index; PVD, perfused vessel density (mm/mm^2); RBCv, red blood cell velocity (mm/s); SinD, sinusoidal diameter (μm).

Fig. 5



Perfused vessel density (mm/mm^2). PVD, perfused vessel density; PVE, portal vein embolization.

resistance protein 2 [21,22]. The amount of mebrofenin injected is of tracer concentration; therefore, saturation of the uptake receptors is unlikely where first-order kinetics apply [12,22]. In case of an increase in hepatic blood flow, the maximum concentration of mebrofenin in the sinusoidal spaces is reached faster. Considering the fact that the measurements of the uptake rate are performed 150–350 s after injection, the measurements will not be affected by the first pass effect. This has been showed in a cohort of patients who had undergone liver resection in which a strong correlation was found between the preoperatively determined FRLF and the actual function measured 1 day postoperatively, even though the blood flow of the remnant liver had doubled compared with the preoperative

situation [11]. However, the effect of the changes of the microcirculation on the uptake of the tracer remains largely unknown.

Animal studies investigating hepatic microcirculation have shown that after an initial period of lower flow in the embolized lobe, the flow normalizes within the next days [23]. The hepatic microcirculation derives its dual blood supply from the portal vein (70–80%) and hepatic artery (20–30%) [24]. In response to portal flow differences, the hepatic artery shows a compensatory, increased flow. This is achieved by the hepatic arterial buffer response, which aims to restore overall liver blood flow [25]. This is in line with the clinical findings of the present study in which after a period of 45 ± 20 days, no microvascular flow differences were found between both lobes in the PVE and non-PVE group. This indicates that the liver aims to keep the blood flow at a stable level, which is crucial to the many essential functions of the liver [23].

The increased PVD in the nonembolized lobe confirms an increase in the (functional) vascular density which can be explained by the formation of new blood vessels from pre-existing vessels during the regeneration phase accompanying hepatocellular proliferation [26]. This possible neoangiogenesis occurs in response to the increased oxygen demand during hepatocellular proliferation. It takes place under stable flow parameters and RBCv as an intrinsic regulatory mechanism to maintain adequate oxygen diffusion. Therefore, the increase in the mebrofenin uptake rate is most likely attributed to hepatocellular proliferation.

The SinD influences the local intrahepatic distribution of flow. In our cohort, we found an increased SinD in the nonembolized lobe compared with the embolized lobe. This is not in line with earlier findings of Gock *et al.* [27] reporting a decrease in SinD found 3 days after PVL in rats. This decrease, along with the constant flow and RBCv leads to an increase in shear stress, which is a trigger for hepatic regeneration. A possible explanation for the discrepancy with our findings is that our microcirculatory assessment took place 3 weeks after PVE when most of the hepatic regeneration had already been achieved.

This study confirms earlier findings that the increase in liver function is more prominent than the increase in the volume of the nonembolized lobe [8], suggesting that regeneration after PVE is mainly the result of hepatocellular proliferation (hyperplasia) rather than increased cell size (hypertrophy) [28]. Our findings indicate that the functional capacity per mass of tissue of the hypertrophied liver parenchyma is greater than before PVE. This has been showed in earlier studies after partial hepatectomy [29]. The mechanisms of functional recovery may be independent of those controlling volumetric regeneration. Therefore, assessment based on liver function, rather than volume alone, is more valuable in the selection of patients undergoing major liver surgery.

This study has several limitations; first, most patients who were included had parenchymal liver damage, which can influence hepatic microvascular architecture. The two patients with colorectal liver metastasis underwent neoadjuvant chemotherapy. The hepatic microcirculation can be affected by liver parenchymal changes like steatosis and steatohepatitis associated with chemotherapy [30,31]. However, these changes would be more generalized in the liver, whereas in both patients, a higher PVD in the nonembolized lobe was found. Hepatocellular carcinoma is in most cases accompanied by chronic parenchymal damage. Upon analysis of the resection specimen of these two patients, one patient had cirrhosis in background liver parenchyma, whereas the other only had periportal fibrosis without bridging. Despite this difference, a higher PVD was found in both patients in the nonembolized lobe. Finally, patients with cholangiocarcinoma present with cholestasis owing to posthepatic bile duct obstruction. Cholestasis with hyperbilirubinemia is known to be hepatotoxic and impairs liver regeneration [32]. To overcome this problem, all patients underwent biliary drainage before PVE and surgery. Even though not all pathologies were present in both groups, considering the consistent findings in microvascular changes, these changes are more likely to result from PVE, rather than because of pre-existing parenchymal damage owing to the underlying liver disease. Another limitation includes the small sample size.

Conclusion

On microcirculatory assessment of the liver after PVE, the nonembolized lobe had a significantly higher (functional) microvascular density, however, without differences in microvascular flow. These findings indicate that the measured increase in mebrofenin uptake rate after PVE was not caused by increased local perfusion but resulted from an increase in functional hepatocyte mass, therefore providing adequate representation of the liver function.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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