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Histological differences between patients with porto-sinusoidal vascular disorder and fibrosis regression of chronic hepatitis B: an analysis based on machine learning algorithms

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Research Article

Keywords: Porto-sinusoidal vascular disorder, Fibrosis regression, Chronic hepatitis B, Second-harmonic generation, Machine learning, Hepatic vasculature

Posted Date: November 19th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11055162/v1

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Additional Declarations: No competing interests reported.

Abstract

Background

Porto-sinusoidal vascular disorder (PSVD) alters portal hemodynamics, leading to portal hypertension without obvious fibrosis. Incomplete septal fibrosis (ISF) is recognized as one of the pathological hallmarks of PSVD; however, the differences in ISF between patients with PSVD and those with fibrosis regression remain controversial. This study aimed to analyze the vascular and fibrotic characteristics of liver biopsies between two cohorts using machine learning algorithms.

Methods

41 patients with PSVD and 40 with chronic hepatitis B who achieved fibrosis regression after 48 weeks of entecavir treatment were enrolled. The fibrosis stage was assessed using the Ishak system. Images of unstained liver biopsy slides were obtained using second-harmonic generation/two-photon excited fluorescence. Machine learning algorithms were adopted for the analysis: arterial density (AD), portal vein density (PVD), central vein density (CVD), collagen proportionate area (CPA), and different areas of CPA on the portal area, zones 1/2/3, and ISF.

Results

In contrast to fibrosis regression, patients with PSVD had significantly higher AD (17.98 ± 10.38/mm² vs 8.59 ± 8.28/mm², $P < 0.05$) and CVD (6.93 ± 4.93/mm² vs. 3.30 ± 2.40/mm², $P < 0.05$), while PVD (19.18 ± 13.37/mm² vs. 33.05 ± 22.61/mm², $P < 0.05$) was significantly lower. PSVD had more collagen fibers and longer and wider collagen distribution in the portal area ($P < 0.05$). In contrast, in the ISF, patients with PSVD had fewer collagen fibers and shorter collagen distribution ($P < 0.05$).

Conclusion

This study not only revealed the pathological differences in vascular and fibrotic changes between patients with PSVD and fibrosis regression in chronic hepatitis B, but also clarified the commonalities of ISF.

Introduction

Porto-sinusoidal vascular disorder (PSVD) is a relatively new medical concept that mainly encompasses diseases characterized by portal vein abnormalities and their associated changes[1, 2]. Patients with PSVD are usually asymptomatic until portal hypertension and its related complications occur.

However, the disease concept of PSVD remains controversial. Unlike hepatic pathologists in Europe, pathologists in the United States and the Asia-Pacific region do not consider it a newly described specific disease[3–5]. They argue that the concept centered on obliterative portal venopathy (OPV) was already described and widely accepted for more than 50 years, and there is no need to propose a new concept, which would be misleading. The controversy between the two camps mainly focuses on the vascular changes of the disease.

In liver fibrosis, vascular changes and collagen deposition are often mutually [6–8].Although the liver fibrosis in patients with PSVD is often mild, incomplete septal fibrosis (ISF) is also recognized as one of the characteristic histological manifestations of PSVD in the guidelines of the European Association for the Study of the Liver Previously[2], ISF was generally considered one of the histological manifestations of liver fibrosis[9, 10]..To our knowledge, there is currently no study focusing on the differences of ISF between PSVD and liver fibrosis regression, such as collagen type and morphology. The present study therefore compared vascular and fibrotic features between patients with PSVD and those with fibrosis regression of CHB, using second-harmonic generation/two-photon excited fluorescence (SHG/TPEF) imaging combined with quantitative machine-learning algorithms (qVessel and qFibrosis), with the goal of refining the diagnostic strategy for PSVD.

Methods

Clinical cohorts

This study included 41 patients with PSVD and 40 previously untreated patients with chronic hepatitis B (CHB) who achieved liver fibrosis regression after 48 weeks of entecavir therapy. All patients were recruited from the Shuguang Hospital and the Affiliated Hospital of Hubei University of Chinese Medicine. All clinical and pathological data were anonymized and the study was approved by the Ethics Review Committee of the relevant institution.

Pathological evaluation

The Ishak fibrosis staging system (F0-F6) was used to assess the liver fibrosis stage, which ranged from F1 to F4 in this cohort (Table 1). The diagnoses were made collectively by a pathological team consisting of three senior hepatic pathologists (Professor Qi Miao, Professor Zunguo Du, and Professor Jinsheng Zhang).

The diagnosis of PSVD was made according to the relevant guidelines[2]. The definition of fibrosis regression in chronic hepatitis B patients was as follows: the fibrosis stage after-treatment was decreased by ≥ 1 compared with that pre-treatment.

The sample exclusion criteria were as follows: a. samples shorter than 1.2cm; b. samples with inconsistent evaluation; c. samples without ISF; d. fragmented samples.

Image Acquisition

Acquisition of images from unstained formalin-fixed paraffin-embedded (FFPE) tissue slides was performed using the Genesis® imaging system (a fully automated, stain-free multiphoton fluorescence imaging microscope; HistoIndex Pte. Ltd., Singapore) equipped with a 20× objective (numerical aperture 0.75). Collagen fibers were visualized via second harmonic generation (SHG) signals and cellular structures via two-photon excited fluorescence (TPEF) signals. Specifically, a 780 nm two-photon laser (laser power ~ 15–20 mW at the sample) was used to irradiate the samples, with SHG signals detected at 390 nm and TPEF signals at 550 nm. For each whole-liver specimen, tile-based imaging was performed at 20× magnification; each tile had a resolution of 512 × 512 pixels and corresponded to an actual tissue area of 200 × 200 μm². Adjacent tiles were acquired to ensure complete coverage of the entire tissue section on each slide.

Vessel density quantification

A machine learning (ML)-based algorithm, called qVessel was developed to detect and quantify arteries and veins from the SHG/TPEF images of liver tissue[11]. The vessel density in liver tissue was quantitatively analyzed using arterial density (AD), portal vein density (PVD) and central vein density (CVD). AD represents the number of arteries per mm², PVD the number of portal veins per mm², and CVD the number of central veins per mm² (Supplemental Table 1).

Fibrosis quantification

The collagen proportionate area (CPA) in liver tissue was quantitatively analyzed using the qFibrosis algorithm[12], with evaluation focused on the following regions: overall, representing the total fibrosis area percentage within the liver tissue; portal tract, defined as the fibrosis area surrounding the portal vein, hepatic artery, and bile duct; Zone 1, corresponding to the liver parenchyma adjacent to the portal tract; Zone 2, the intermediate region between Zone 1 and Zone 3; Zone 3, the liver parenchyma surrounding the central vein; central vein, comprising the central vein and its immediate surrounding area; and ISF, characterized with incomplete, slender, and blind-ended fibrosis septa[13]. (Supplemental Table 1)

Statistical analysis

The pre-specified primary outcomes were the three vessel density parameters (AD, PVD and CVD), and the secondary outcomes were the seven regional CPA parameters (overall, portal tract, Zone 1, Zone 2, Zone 3, central vein and ISF). Measurement data are presented as mean ± standard deviation. Comparisons between groups were performed using the chi-squared test or Fisher's exact test for categorical variables, Student's t-test for normally distributed continuous variables, and the Mann–Whitney U test otherwise. For the seven regional CPA parameters, the Benjamini–Hochberg procedure was applied to control the false discovery rate at 5%. All statistical analyses were performed using SPSS

22.0, with the significance level set at P < 0.05 (two-sided). In the figures, *P < 0.05, **P < 0.01 and ***P < 0.001. The study is reported in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement.

Results

Patients’ cohort

A total of 41 patients with PSVD and 40 with fibrosis regression who underwent liver biopsies within clinical care were included in this analysis. The characteristics of the study cohort are presented in Table 1.

Table 1
Clinical characteristics of patients with porto-sinusoidal vascular disorder (PSVD) and of patients with fibrosis regression of chronic hepatitis B

Gender (N)		**PSVD (n = 41)**	**Fibrosis regression (n = 40)**	***P value***
		M (26)/F (15)	**M (31)/F (9)**	**0.165**
Age (Year)		45.58 ± 11.11	40.18 ± 8.11	0.014
Stage	**F1 (N)**	12	0	< 0.001
	F2 (N)	24	0	
	F3 (N)	5	16	
	F4 (N)	0	24	
Etiology	**CHB**	20	40	/
	NAFLD	1	/	
	DILI	5	/	
	Others	15	/	
PLT (10^9/L)		128.30 ± 79.53	140.28 ± 49.18	0.418
ALT (U/L)		35.78 ± 28.50	26.17 ± 14.05	0.059
AST (U/L)		37.26 ± 27.99	26.42 ± 8.61	0.216
ALB (g/L)		41.54 ± 4.74	48.16 ± 4.40	< 0.001
TBil (μmol/L)		18.61 ± 7.11	13.97 ± 5.78	0.002
Ascites		7	0	/
Gastroesophageal varices		18	0	/
Bleeding		4	0	/
Child-Pugh	**A**	39	40	/
	B	2	0	
	C	0	0	

Fibrosis stage was assessed with the Ishak scoring system (F0–F6); the stages observed in this cohort ranged from F1 to F4. Continuous variables are presented as mean ± standard deviation (SD) and categorical variables as the number of patients. Between-group comparisons were performed using the chi-squared test or Fisher's exact test for categorical variables and Student's t-test or the Mann–Whitney U test for continuous variables, as appropriate. $P < 0.05$ was considered statistically significant, and P values below the precision threshold are displayed as “<0.001”. Abbreviations: ALB, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CHB, chronic hepatitis B; DILI, drug-induced liver

injury; NAFLD, non-alcoholic fatty liver disease; PLT, platelet count; PSVD, porto-sinusoidal vascular disorder; SD, standard deviation; TBil, total bilirubin; "/", not applicable or not determined.

Differences of vessel densities between PSVD and fibrosis regression

As shown in Fig. 1, the AD, PVD, and CVD in patients with PSVD were 17.98 ± 10.38/mm², 19.18 ± 13.37/mm², and 6.93 ± 4.93/mm², respectively; while those in patients with fibrosis regression of CHB were 8.59 ± 8.28/mm², 33.05 ± 22.61/mm², and 3.30 ± 2.40/mm², respectively. Compared to patients with fibrosis regression of CHB, those with PSVD had higher AD and CVD but lower PVD.

Differences of CPA distributions between PSVD and fibrosis regression

As shown in Fig. 2, in the CPA of the portal area, patients with PSVD had a higher count of total collagen fibers, short collagen fibers, a longer total collagen length, and a wider total collagen width compared with those with fibrosis regression of CHB ($P < 0.05$). In the CPA of the ISF, patients with PSVD had fewer distributed collagen fibers, long collagen fibers, and thin collagen fibers, and a shorter total collagen length than those with fibrosis regression ($P < 0.05$). The differences in CPA parameters across all evaluated regions are summarized in Fig. 3, which is consistent with the above: the parameters of the portal tract and portal region were higher, whereas those of the ISF were lower, in patients with PSVD than in patients with fibrosis regression. Representative SHG/TPEF images showing how vessels and collagen were identified in the two groups are presented in Fig. 4.

Discussion

The occurrence of PSVD is associated with a variety of factors, including chronic hepatitis, immune disorders, and thrombosis[1,14,15], which may lead to liver insufficiency, cirrhosis and other serious complications. The diagnosis of PSVD faces numerous challenges, especially in the differences of ISF in PSVD and fibrosis regression, which have not yet been clarified.

Here, ultra-high-resolution laser scanning microscopy (SHG/TPEF) combined with quantitative analysis methods ([11] and [12]) was employed to provide accurate data support for comparing differences in vascular changes and collagen compositions. In compensated advanced chronic liver disease (cACLD), angiogenesis represents an adaptive response to chronic inflammation and [6,16], driven by mediators such as released platelet-derived growth factor (PDGF) and vascular endothelial growth factor [17–19] However, in chronic hepatitis B, increasing portal vein density (PVD) is more prominent than arterial density [11] likely because long-standing portal inflammation drives portal vein [20] By contrast, increasing AD is more pronounced in PSVD, which may reflect its unique histological changes: obliterative portal venopathy (OPV) on one hand and generally mild inflammation on the other. Because OPV reduces portal venous flow, hepatic arterial proliferation becomes the dominant angiogenic response. Similarly, central vein density (CVD) was higher in PSVD than in fibrosis regression. Because inflammation is

milder in PSVD, hepatic lobular architecture is better preserved, so the central vein compartment is comparatively less affected. Previous reports have shown that loss of lobular integrity in CHB reflects long-term [21] Collectively, the discrepancies in vessel density reflect distinct microenvironments in PSVD and CHB-related fibrosis regression.

The differences in CPA between PSVD and fibrosis regression are mainly reflected in the portal tracts and ISF, which relate to the different histological states of the two diseases. During fibrosis progression, the number of collagen fibers increases from few to many and their morphology changes from short to long. Whereas, during fibrosis regression, the collagen fibers become thinner. Therefore, in patients with fibrosis regression, the CPA of the portal tract is characterized by a lower count and a smaller size. However, ISF is considered evidence that the previously wide and thick collagen has not yet been degraded[9]. ISF in patients with fibrosis regression therefore contains more thick and long collagen fibers.

This study has several limitations. First, the sample size was modest (41 PSVD and 40 fibrosis regression cases), which limits statistical power for subgroup analyses. Second, the PSVD cohort was etiologically heterogeneous, including chronic hepatitis B (20/41), NAFLD (1/41), DILI (5/41) and other causes (15/41); although this reflects real-world clinical practice, residual confounding from underlying disease activity cannot be fully excluded. Third, fibrosis stage distribution was imbalanced between groups (PSVD: predominantly F1–F2; FR: predominantly F3–F4), which is intrinsic to the design—FR requires a baseline F ≥ 3—but may affect direct comparability of vessel density. Fourth, the cross-sectional design does not allow causal inference about whether the observed vascular changes precede or follow fibrotic remodelling. Finally, mechanistic validation (e.g., sinusoidal endothelial markers, VEGF/PDGF expression) was not performed. Larger prospective cohorts with etiology stratification, paired pre/post-treatment biopsies and mechanistic assays are needed to confirm and extend these findings.

In summary, this study demonstrated clear histological differences between PSVD and fibrosis regression in CHB, characterized by higher arterial and central vein densities but lower portal vein density in PSVD, together with more pronounced portal-tract collagen deposition but reduced incomplete septal fibrosis in PSVD. Together, these features—the paradoxical increase in arterial density with decreased portal vein density, and the divergent collagen patterns in the portal tract versus ISF—may serve as a useful histological signature to support the differential diagnosis of PSVD when both entities show ISF. Future studies with larger, etiology-stratified cohorts and mechanistic validation are warranted.

Declarations

Funding

This study was funded by the National Natural Science Foundation of China (82274305) and National Science and Technology Major Project (2018ZX10302204). Open foundation of Center of Traditional Chinese Medicine Modernization for Liver Diseases, Hubei University of Chinese Medicine

(100604060125). Histoindex Pte. and Hangzhou Choutu Co. supported this study by providing logistic support and analysis of liver slides.

Conflict of interest

The authors declare the following competing interests: Y.Y.R. is an employee of Hangzhou Choutu Tech. Co., Ltd. and D.C.S.T. is an employee of Histoindex Pte. Ltd. Both companies provided logistic support and assisted with the analysis of liver slides, but had no role in the study design, data collection, analysis, interpretation or the decision to submit the work for publication. All other authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contributions

Author contributions according to the CRediT taxonomy: Conceptualization: C.-H.L. and Q.C.F. Methodology: Z.X.L., M.L., J.Z.W. and Y.Y.R. Software: Y.Y.R. Validation: Z.X.L. and M.L. Formal analysis: Z.X.L. and M.L. Investigation: Z.X.L., M.L. and J.Z.W. Resources: D.C.S.T. and C.-H.L. Data curation: Z.X.L. Writing - original draft: Z.X.L., M.L. and J.Z.W. Writing - review & editing: all authors. Visualization: Z.X.L. and Y.Y.R. Supervision: D.C.S.T. and C.-H.L. Project administration: C.-H.L. and Q.C.F. Funding acquisition: C.-H.L. and D.C.S.T. Z.X.L., M.L. and J.Z.W. contributed equally to this study and share first authorship. All authors have read and approved the final manuscript.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional review board and with the 1964 Helsinki Declaration and its later amendments. The study was approved by the Ethics Review Committee of Hubei Provincial Hospital of Traditional Chinese Medicine (approval No. 2015-445-73-01).

Written informed consent was obtained from all individual participants included in the study. For participants whose biopsy archives were used retrospectively, the requirement for additional consent was waived by the Ethics Review Committee of Hubei Provincial Hospital of Traditional Chinese Medicine because all data were anonymized.

Patient consent statement

Data availability

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request, subject to approval by the institutional ethics committee and the data-sharing agreements of the participating centers.

Declaration of generative AI in scientific writing

During the preparation of this work, the authors used Doubao (an AI language model) to improve the readability and language of selected paragraphs. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication. The AI tool was not used to generate, analyze or interpret the data, nor to draw scientific conclusions, nor to produce any of the images, statistical results or reference citations reported in this manuscript.

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Figures

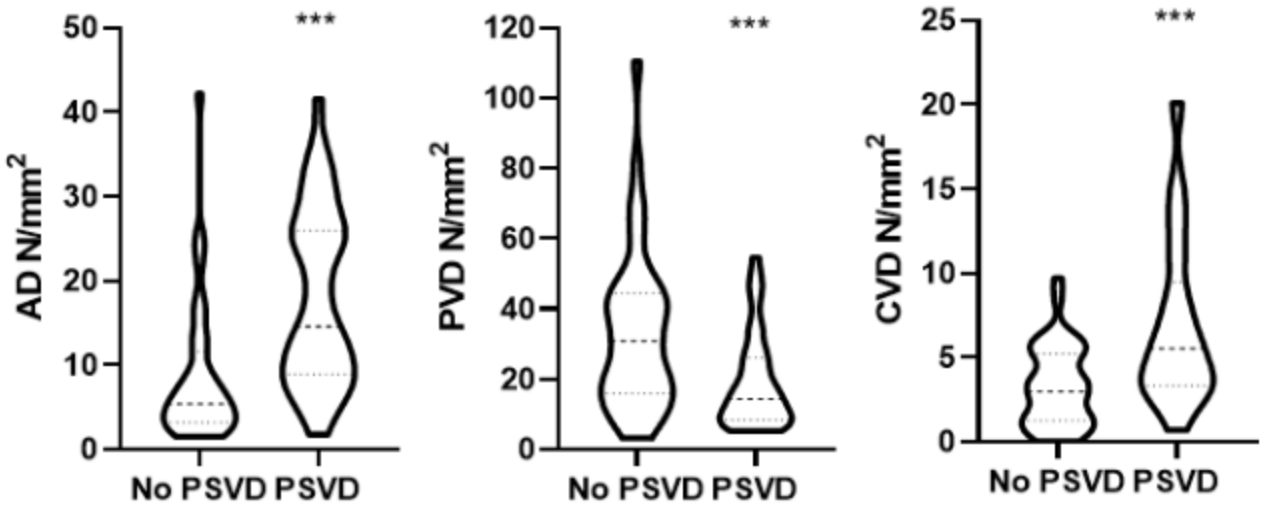


Figure 1

*Comparison of vessel densities between patients with porto-sinusoidal vascular disorder (PSVD) and patients with fibrosis regression of chronic hepatitis B (CHB). Violin plots show arterial density (AD), portal vein density (PVD) and central vein density (CVD) in patients with fibrosis regression (labeled "No PSVD" in the figure; n = 40) and patients with PSVD (n = 41). Within each violin, the dashed horizontal line indicates the median and the dotted horizontal lines indicate the 25th and 75th percentiles. Between-group comparisons were performed using the Mann-Whitney U test. ***P < 0.001. Abbreviations: AD, arterial density; CHB, chronic hepatitis B; CVD, central vein density; PSVD, porto-sinusoidal vascular disorder; PVD, portal vein density.*

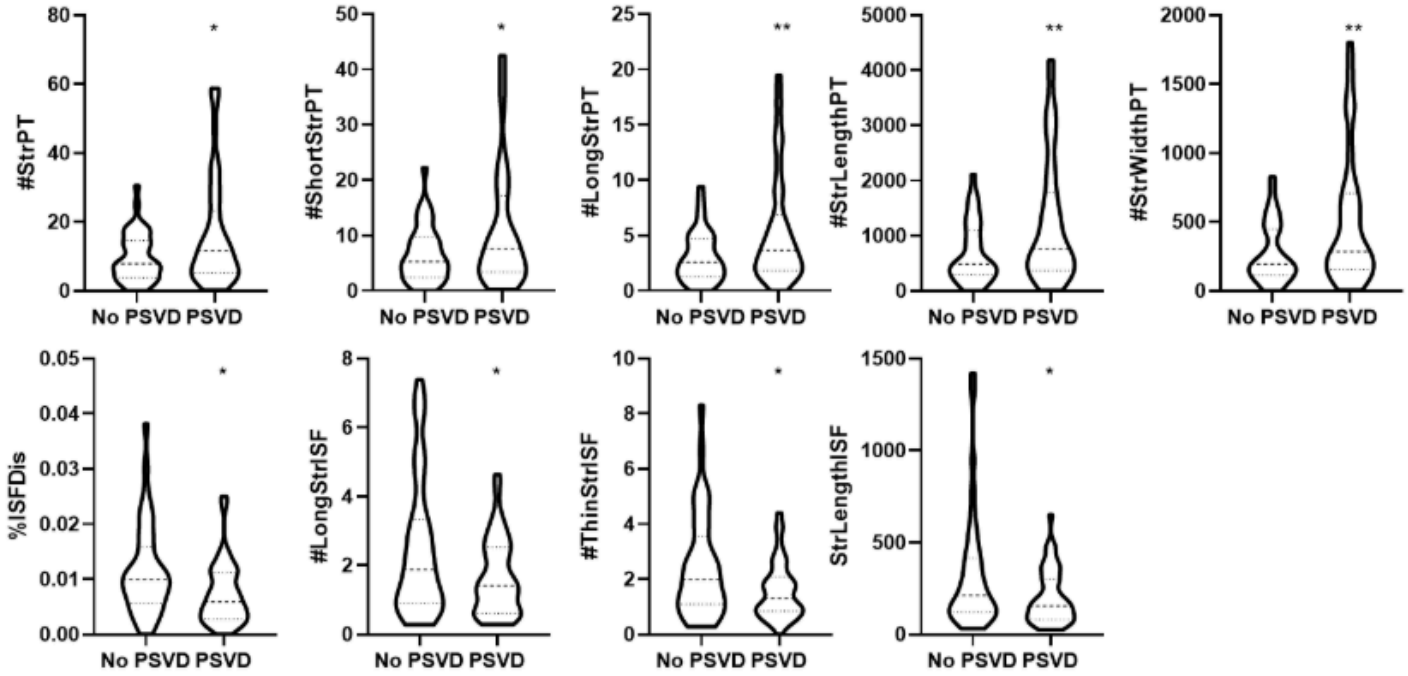


Figure 2

*Comparison of collagen proportionate area (CPA) parameters in the portal tract and in incomplete septal fibrosis (ISF) between patients with fibrosis regression of chronic hepatitis B and patients with porto-sinusoidal vascular disorder (PSVD). Upper row: parameters measured in the portal tract (PT), including total number of collagen fibers (#StrPT), number of short collagen fibers (#ShortStrPT), number of long collagen fibers (#LongStrPT), total collagen length (StrLengthPT) and total collagen width (StrWidthPT). Lower row: parameters measured in the ISF, including distribution of collagen fibers (%ISFDis), number of long collagen fibers (#LongStrISF), number of thin collagen fibers (#ThinStrISF) and total collagen length (StrLengthISF). Violin plots show the distribution in patients with fibrosis regression (labeled "No PSVD" in the figure; n = 40) and patients with PSVD (n = 41). Within each violin, the dashed horizontal line indicates the median and the dotted horizontal lines indicate the 25th and 75th percentiles. Between-group comparisons were performed using the Mann-Whitney U test. *P < 0.05, **P < 0.01. Abbreviations: CPA, collagen proportionate area; ISF, incomplete septal fibrosis; PSVD, porto-sinusoidal vascular disorder; PT, portal tract.*

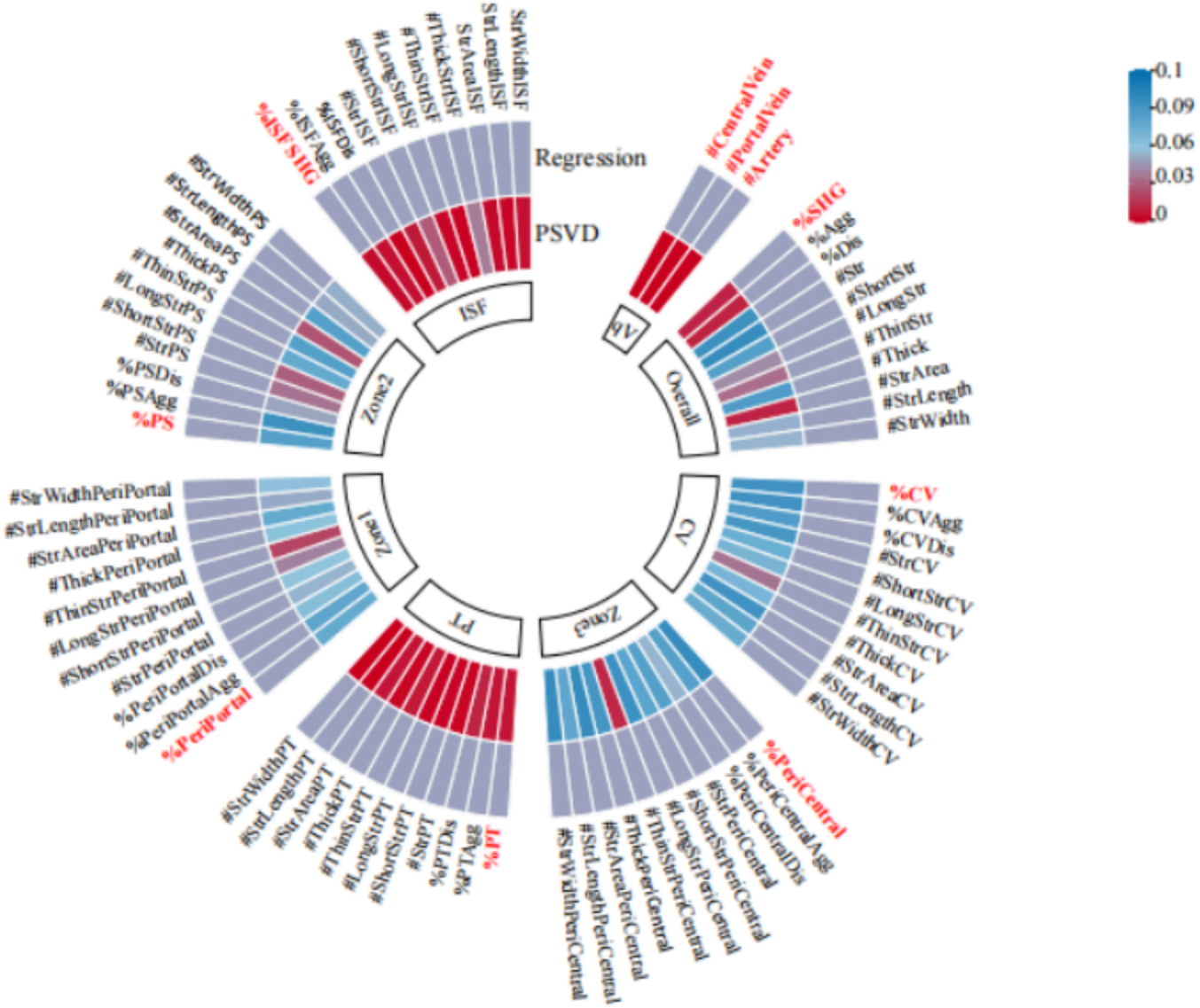


Figure 3

Circular heatmap comparing the collagen proportionate area (CPA) and vessel parameters between patients with fibrosis regression of chronic hepatitis B and patients with porto-sinusoidal vascular disorder (PSVD). Each sector of the ring corresponds to one anatomical region in which the parameters were measured: overall liver tissue (Overall); central vein (CV) and the pericentral region (PeriCentral); the hepatic parenchymal zones (Zone 1, Zone 2, Zone 3); the portal tract (PT) and the periportal region (PeriPortal); the perisinusoidal region (PS); incomplete septal fibrosis (ISF); and the vessel counts (NV). Within each sector, every segment represents one parameter measured in that region (for the complete list of parameters, see Supplemental Table 1); the inner track shows the value in patients with fibrosis regression (labeled "Regression" in the figure) and the outer track shows the value in patients with PSVD. Segment color and segment length are scaled to the same value for each parameter, according to the color key at the upper right (red, 0; blue, 0.1). Abbreviations: CPA, collagen proportionate area; CV, central

vein; ISF, incomplete septal fibrosis; NV, vessel counts; PS, perisinusoidal; PSVD, porto-sinusoidal vascular disorder; PT, portal tract.

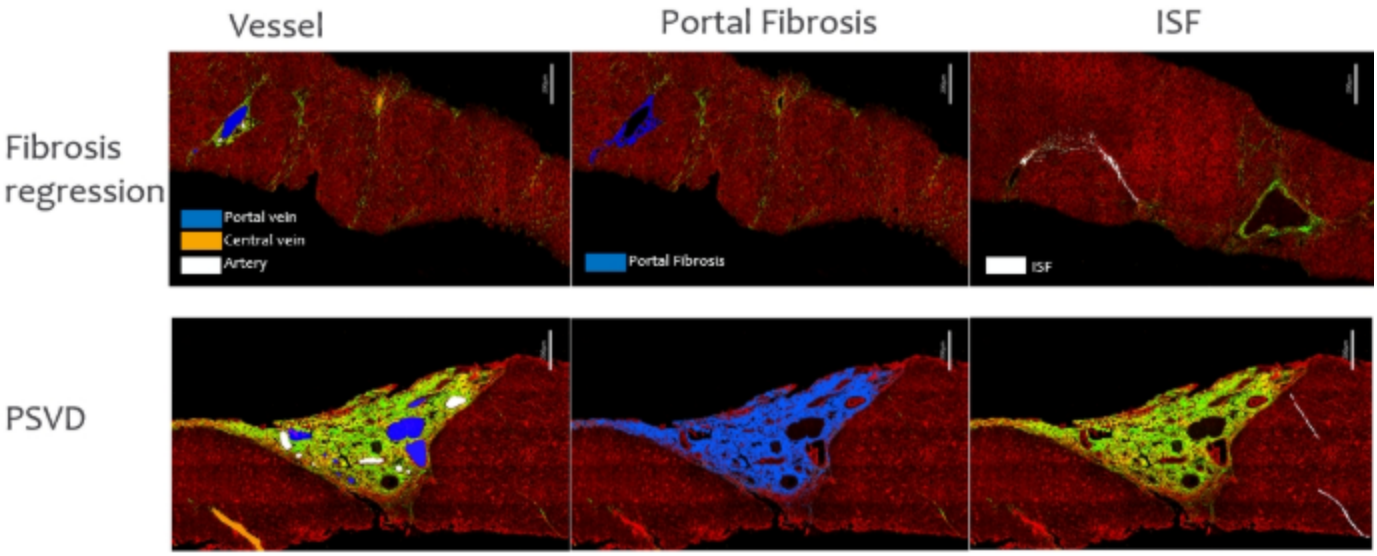


Figure 4

Representative second-harmonic generation/two-photon excited fluorescence (SHG/TPEF) images showing the automated identification of vessels and collagen in liver biopsies from a patient with fibrosis regression (upper row) and a patient with porto-sinusoidal vascular disorder (PSVD, lower row). Collagen is visualized in red (SHG) and cellular structures in green (TPEF). Columns show the segmentation of vessels (portal vein, blue; central vein, orange; artery, white), portal fibrosis (blue) and incomplete septal fibrosis (ISF, white). Scale bars are shown in the upper right corner of each panel (100 µm). Images were acquired with a Genesis® multiphoton imaging system (Histoindex Pte. Ltd., Singapore) at 20× magnification, with a numerical aperture of 0.75 and a laser power of 15-20 mW. Abbreviations: ISF, incomplete septal fibrosis; PSVD, porto-sinusoidal vascular disorder; SHG, second-harmonic generation; TPEF, two-photon excited fluorescence.