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Original Article

The Imbalance of Homeostasis in Neutrophil Extracellular Traps is Associated with Portal Vein Thrombosis in Patients with Decompensated Cirrhosis

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Abstract

Background and Aims: Portal vein thrombosis (PVT) is a challenging complication in liver cirrhosis, with no currently available sensitive diagnostic markers. This study aimed to investigate the potential of neutrophil extracellular traps (NETs) and Deoxyribonuclease (DNase) as diagnostic indicators for PVT in chronic hepatitis B (CHB)-related decompensated cirrhosis. ***Methods:*** We analyzed 145 CHB-related decompensated cirrhosis patients from the Ditan study and 33 from the Changgung validation study, categorizing them based on PVT occurrence. Plasma samples were assessed for NET markers, including cell-free DNA (cfDNA) and histone-DNA complexes, along with DNase activity. ***Results:*** PVT patients exhibited elevated levels of cfDNA and histone-DNA complexes, and reduced DNase activity. This pattern persisted regardless of hepatocellular carcinoma (HCC) status. Histone-DNA levels, DNase activity, and hemoglobin were identified as independent risk factors for PVT. Receiver operating characteristic curve analysis revealed that high histone-DNA levels may serve as a potential diagnostic marker for PVT, with an area under the curve of 0.8628 in the Ditan study and 0.7521 in the Changgung study. When combined with cfDNA and DNase activity, the area under the curve improved to 0.8774 in the Ditan study and 0.7975 in the Changgung study. ***Conclusions:*** Imbalances in NET homeostasis are associated with PVT in CHB-related decompensated cirrhosis, including cases involving HCC. Histone-DNA complexes, a significant risk factor for PVT, show potential as a diagnostic marker for PVT in decompensated cirrhosis, particularly in HBV-related HCC.

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Introduction

Portal vein thrombosis (PVT) is a prevalent and severe complication associated with liver cirrhosis, potentially leading to portal vein obstruction, increased portal vein pressure, mesenteric ischemia, intestinal necrosis, and ultimately contributing to a poor prognosis in cirrhosis patients. The incidence of PVT is notably high, affecting up to 26% of cirrhosis patients and a staggering 40% of those with hepatocellular carcinoma (HCC), including cases with tumor-related thrombosis.[1–3] While the majority of patients are asymptomatic at the time of diagnosis,[4] the condition can be life-threatening if not promptly and accurately identified. Currently, ultrasound is the primary non-invasive diagnostic method for PVT, yet its sensitivity and accuracy remain suboptimal.[5]

Recent research has highlighted the critical regulatory role of neutrophil extracellular traps (NETs) in inflammation and coagulation disorders. NETs, which consist of extracellular chromatin decorated with proteins derived from neutrophil granules, are implicated in conditions such as acute coronary syndrome, ischemic stroke, and thrombosis.[6] Circulating NET levels serve not only as biomarkers for assessing disease severity but also as potential therapeutic targets.[7] The role of NETs in PVT development is multifaceted, including their contribution to thrombus formation, the inflammatory environment, and HCC metastasis. However, the relationship

Keywords: Portal vein thrombosis; Neutrophil extracellular trap; Deoxyribonuclease; Chronic hepatitis B; Decompensated cirrhosis; Hepatocellular carcinoma.

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between NETs and PVT in cirrhosis patients remains controversial. Some studies have reported elevated plasma markers of NET formation in patients with cirrhosis or HCC, correlating with the severity of liver dysfunction,[8,9] suggesting a predictive role for NETs in PVT. However, a prospective cohort study indicated that NET levels may not predict PVT development in cirrhosis patients.[10] These inconsistent conclusions may stem from differences in the Child-Pugh grade of liver cirrhosis among the study populations. Notably, the latter study focused on patients with Child-Pugh grades A and B, with 72% having Child-Pugh grade A. Therefore, this study will account for the Child-Pugh grade of cirrhosis when examining the association between NETs and PVT in patients with chronic hepatitis B (CHB)-related decompensated cirrhosis.

The balance of NET content results from a dynamic equilibrium between NET formation and nuclease-mediated degradation.[11] Triggers such as pro-inflammatory cytokines and microbial components can induce NET formation, while the primary clearance mechanism relies on nuclease degradation, primarily through Deoxyribonuclease (DNase).[12] DNase, a type of nuclease, is essential for cleaving extracellular DNA and maintaining low NET concentrations during physiological processes.[13] Reduced DNase activity has been identified as a novel biomarker and pathogenic factor in conditions like systemic lupus erythematosus and cystic fibrosis.[14,15]

Despite the established importance of NETs in PVT development, limited focus has been placed on NET clearance and the maintenance of homeostasis. This study aims to investigate the relationship between NET homeostasis and PVT occurrence by assessing NET content and DNase activity in the plasma of patients with CHB-related decompensated cirrhosis. Furthermore, we aim to explore the potential of NETs and DNase activity as diagnostic indicators for PVT in these patients, regardless of concurrent HCC, using the Ditan study as a foundation and the Changgung study for external validation.

Methods

Study population

Demographic data and clinical laboratory test results for patients with CHB-related decompensated cirrhosis were obtained from electronic medical records at Beijing Ditan Hospital, Capital Medical University, between May 2017 and June 2018. The study was approved by the Ethics Review Committee of Beijing Ditan Hospital, Capital Medical University (NO. DTEC-KT2023-005-01). Inclusion criteria included individuals aged 18 years or older with CHB on antiviral therapy who met the clinical, biochemical, hematological, radiological, or histological diagnostic criteria for decompensated cirrhosis and provided informed consent. Exclusion criteria included incomplete data and the presence of neoplasms other than HCC. Of the patients assessed at Ditan Hospital, 145 met the inclusion criteria and were enrolled, while 16 were excluded—three due to incomplete data and 13 due to the presence of other neoplasms. The flowchart is shown in Figure 1A. Participants were categorized into PVT and non-PVT groups based on the presence of PVT, which was diagnosed using color Doppler ultrasound, with contrast-enhanced computed tomography used in ambiguous cases.[16] HCC diagnosis was confirmed by integrating histological features from liver biopsies with imaging studies, verified by specialists.

A validation study was conducted using patients with decompensated cirrhosis from Beijing Tsinghua Changgung Hospital between September 2022 and September 2023 and was approved by its Ethics Review Committee (22444-4-02). Thirty-three patients met the inclusion criteria for this study, with the flowchart shown in Figure 1B.

Laboratory examinations

Participants fasted for 12 h prior to blood sampling. Blood samples were centrifuged at 4°C at 3,000 rpm for 10 m to isolate plasma, which was then stored at −80°C. Routine biochemical tests were performed following standard protocols, including complete blood count, hepatitis B virus (HBV) DNA, alpha-fetoprotein, hepatic and renal function tests, ion concentration, and coagulation function. HBV DNA was quantified using a real-time PCR assay, with a lower limit of quantification of 20 IU/mL.

Assessment of cell-free DNA (cfDNA)

The Quant-iT™ PicoGreen® dsDNA assay kit (Thermo Fisher Scientific, USA) was utilized to quantify cfDNA levels in plasma according to the manufacturer's instructions.

Assessment of histone-DNA

The Cell Death Detection ELISA Kit (Roche, Switzerland) was employed to measure histone-DNA levels in plasma according to the manufacturer's instructions.

Assessment of DNase activity

The DNase Activity Fluorometric Assay Kit (Beyotime, China) was applied to assess DNase activity in plasma, following the provided directions.

Statistical analysis

Quantitative variables are presented as mean ± standard deviation, with differences evaluated using Student's t-test. Categorical variables are expressed as frequencies and percentages, with differences assessed using the Chi-square test or Fisher's exact test. Non-normally distributed variables are shown as a median and interquartile range, with significance tested using the Mann-Whitney test. Binary logistic regression was used to identify independent risk factors associated with PVT, with variables significant at $p < 0.05$ included in multivariate logistic regression by forward stepwise selection. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to measure the strength of associations. Cutoff points for test variables were determined using receiver operating characteristic (ROC) curves, and correlations were assessed using Spearman's correlation. Statistical significance was set at $p < 0.05$. All analyses were conducted using SPSS 26.0 (IBM, USA) and GraphPad Prism version 9.0.0 (GraphPad Software, USA).

Results

Clinical characteristics of patients in the Ditan study

The Ditan study enrolled 145 patients with CHB-related decompensated cirrhosis from Beijing Ditan Hospital, 39 of whom had PVT and 106 did not. The incidence of PVT varied across Child-Pugh grades A to C, with 15.4%, 64.1%, and 20.5%, respectively. A significant difference was observed in grades A (42.5% vs. 15.4%, $p = 0.003$) and B (38.7% vs. 64.1%, $p = 0.004$). HCC co-occurred in 54 (37.2%) patients, including 19 with PVT. No significant difference in HCC incidence was observed between the PVT and non-PVT groups (33.0% vs. 48.7%, $p = 0.083$). Demographic and

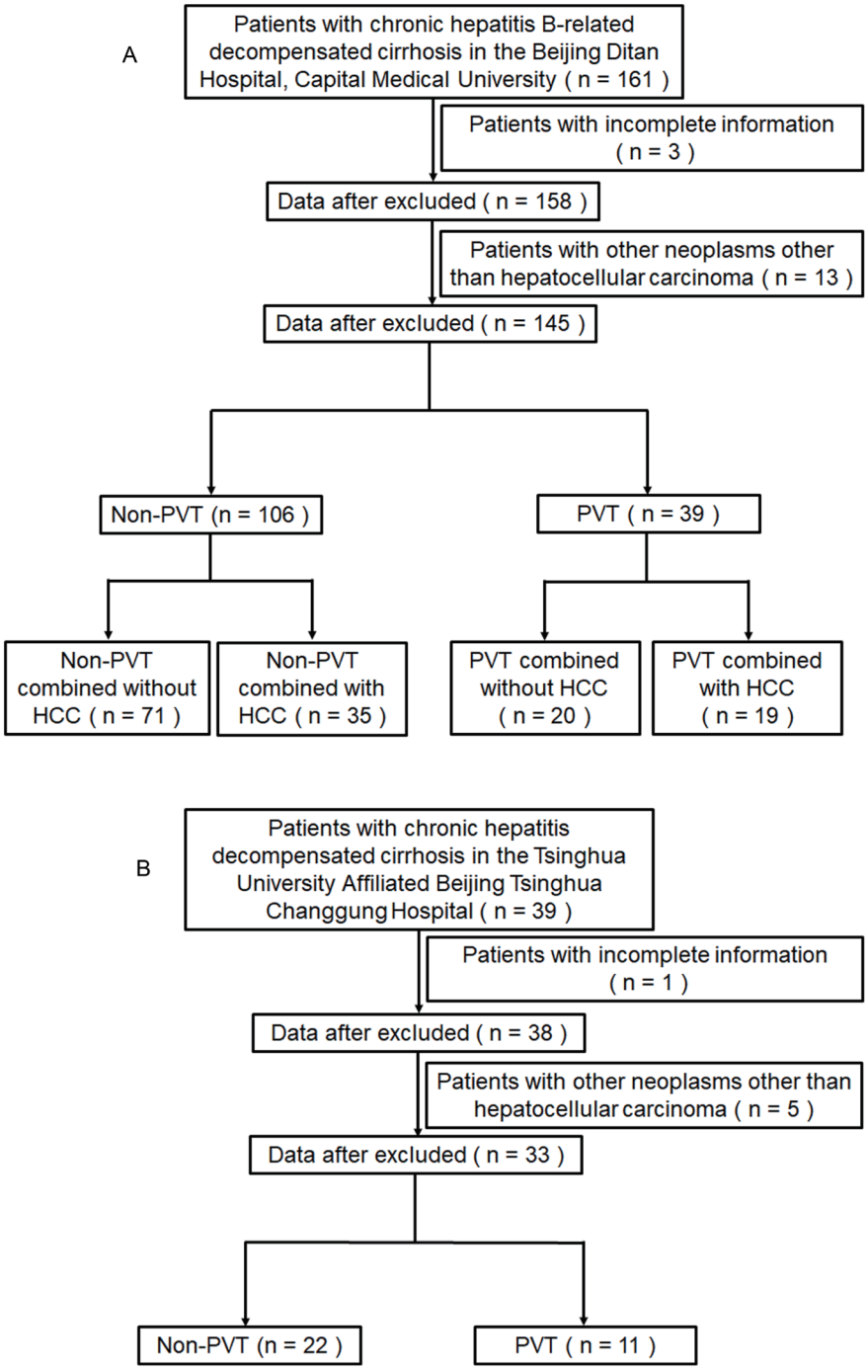

Fig. 1. Flowchart of the Ditan study (A) and Changgung study (B). PVT, portal vein thrombosis; HCC, hepatocellular carcinoma.

clinical characteristics are detailed in Supplementary Table 1. Notably, Child-Pugh scores were significantly higher in PVT patients (p = 0.006). Laboratory indices are presented in Table 1.

NET homeostasis association with PVT in CHB-related decompensated cirrhosis

We analyzed the NET formation and degradation in the plasma of patients from the Ditan study, with NET contents indicated by cfDNA and histone-DNA complexes, and degradation ability by DNase activity. PVT patients exhibited significantly higher NET levels (cfDNA and histone-DNA complexes) and lower DNase activity compared to non-PVT patients (Fig. 2A–C). Specifically, cfDNA, histone-DNA, and DNase activity levels in PVT patients were 1.215-fold, 2.970-fold, and 0.865-fold those of non-PVT patients, respectively.

Further analysis revealed a positive correlation between histone-DNA and liver enzymes aspartate aminotransferase

Table 1. Laboratory indexes of patients with CHB-related decompensated cirrhosis in the Ditan study

Variables	Non-PVT (n = 106)	PVT (n = 39)	*p*-value
Routine blood test			
WBC (10^9/L)	3.8 [2.6–5.2]	3.5 [2.1–4.4]	0.105
RBC (10^{12}/L)	4.0 ± 0.8	3.6 ± 0.7	**0.021**
HGB (g/L)	130.0 [109.3–142.1]	113.0 [93.0–129.0]	**0.001**
PLT (10^9/L)	78.0 [51.0–129.2]	90.1 [42.0–122.0]	0.729
Liver function			
ALT (U/L)	23.6 [17.6–39.9]	26.3 [21.3–37.1]	0.392
AST (U/L)	28.7 [22.6–51.6]	38.7 [26.7–70.7]	0.058
ALP (U/L)	86.3 [67.8–125.7]	89.9 [72.0–165.8]	0.577
ALB (g/L)	36.3 ± 6.4	34.1 ± 5.6	0.066
TBil (μmol/L)	18.9 [11.9–37.5]	26.6 [16.8–41.2]	**0.048**
γ-GT (U/L)	27.7 [17.7–76.4]	42.4 [18.18–129.6]	0.144
CHE (U/L)	4,742 [3,193–6,280]	3473 [2,167–5,179]	**0.005**
GLU (mmol/L)	5.4 [4.9–6.6]	5.4 [4.9–6.2]	0.971
Coagulation			
PT (s)	13.5 [12.1–15.5]	14.6 [12.9–17.5]	**0.036**
PTA (%)	76 ± 21	69 ± 17	0.071
INR	1.2 [1.1–1.4]	1.3 [1.2–1.5]	**0.025**
APTT (s)	32.7 [30.3–36.6]	32.3 [29.0–36.4]	0.401
TT (s)	17.6 [16.5–18.9]	18.6 [16.9–20.3]	0.050
FIB (g/L)	217 ± 63	209 ± 88	0.268
Renal function			
UREA (mmol/L)	5.2 [4.2–6.2]	5.7 [4.4–6.9]	0.138
CREA (μmol/L)	68.0 [59.6–77.1]	69.4 [57.4–79.0]	0.751
URCA (mmol/L)	293 [248–359]	302 [260–346]	0.840
Electrolytes			
K (mmol/L)	3.8 ± 0.4	3.8 ± 0.5	0.603
Na (mmol/L)	141.6 [139.7–143.2]	141.4 [138.0–143.4]	0.568
Cl (mmol/L)	106.1 [104.4–107.9]	106.1 [101.6–108.1]	0.984
Others			
AFP (ng/mL)	3.9 [2.0–11.6]	3.4 [1.7–178.1]	0.878
HBV-DNA (IU/mL)	20 [0–74]	20 [0–162]	0.910

Data are presented as mean ± standard deviation or as median [interquartile range] for continuous variables. The differences between the two groups were tested by one-way t-tests (for normally distributed continuous variables) or the Mann-Whitney nonparametric test (for non-normally distributed variables). A two-sided α of less than 0.05 was considered statistically significant. *p*-values < 0.05 are shown in bold. WBC, white blood cell; RBC, red blood cell; HGB, hemoglobin; PLT, platelet; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; ALB, albumin; TBil, total bilirubin; γ-GT, γ-glutamyl transpeptidase; CHE, cholinesterase; GLU, glucose; PT, prothrombin time; PTA, prothrombin activity; INR, international normalized ratio; APTT, activated partial thromboplastin time; TT, thrombin time; FIB, fibrinogen; UREA, urea; CREA, creatinine; URCA, uric acid; K, potassium; Na, sodium; Cl, chloride; AFP, alpha-fetoprotein; HBV-DNA, hepatitis B virus-deoxyribonucleic acid.

and alanine aminotransferase, while DNase activity negatively correlated with histone-DNA and albumin. Notably, cfDNA showed the highest correlation with total bilirubin (r = 0.84, $p < 0.0001$) and the lowest correlation with cholinesterase (CHE) (r = −0.66, $p < 0.0001$; Fig. 2D).

When stratified by Child-Pugh grade, cfDNA levels increased with the severity of cirrhosis, while histone-DNA levels and DNase activity remained relatively stable (Fig. 3A–C). Within each sub-group, PVT patients consistently displayed higher NET levels and lower DNase activity (Fig. 3A–C), indicating a close association between NET dynamics and PVT across all cirrhosis stages.

NET homeostasis association with PVT in HCC patients

To determine if HCC influences the NET-PVT relationship, patients were divided into non-HCC and HCC sub-groups. Clinical characteristics and laboratory indices were comparable

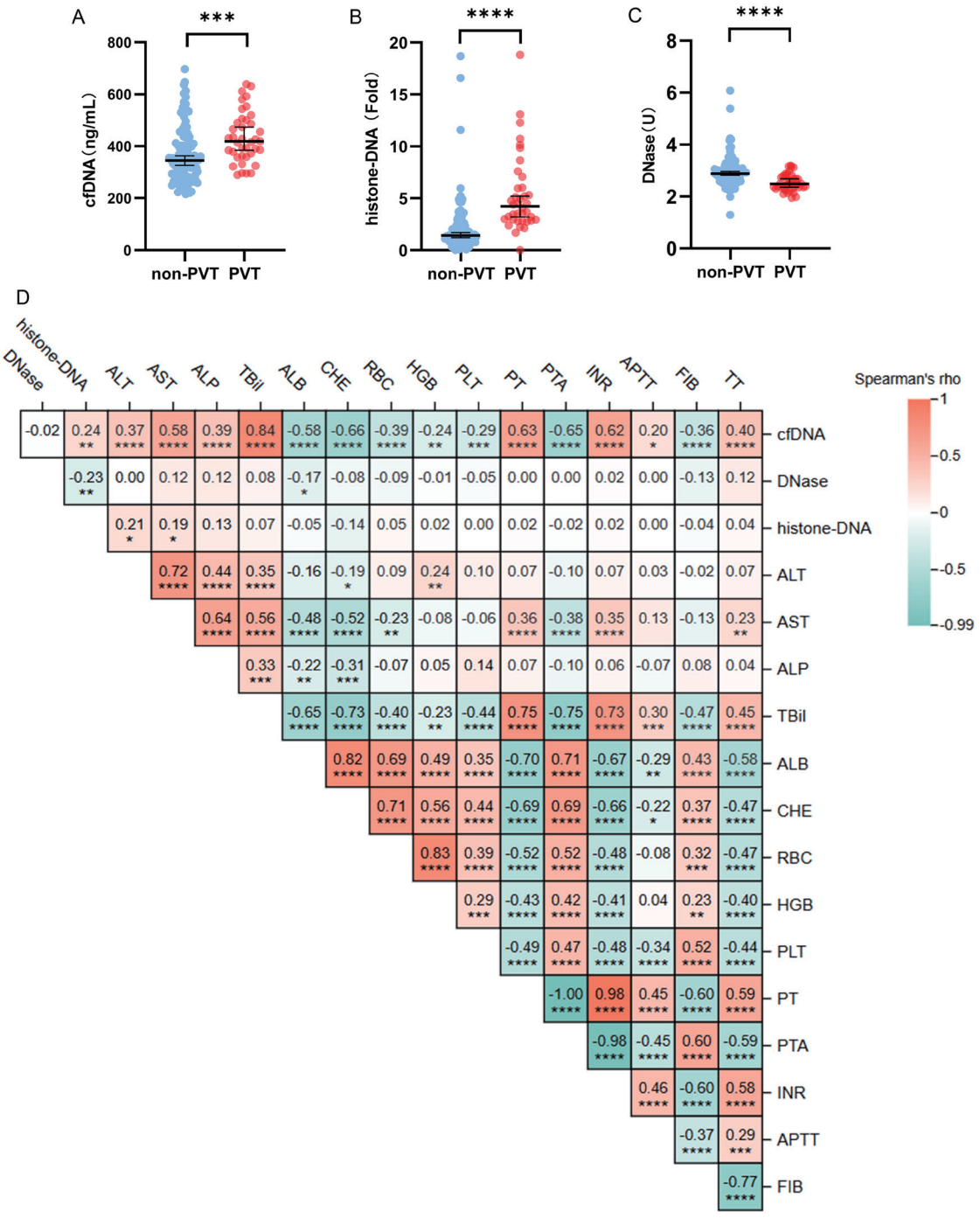

Fig. 2. Association of NET formation and degradation with PVT in patients with CHB-related decompensated cirrhosis in the Ditan study. Plasma levels of cfDNA (A), histone-DNA (B), and DNase activity (C) in patients with PVT vs. without PVT. Non-PVT, n = 106; PVT, n = 39. Data are presented as median (95% CI). *p*-values were obtained using the Mann–Whitney test. (D) Correlation analysis between cfDNA, histone-DNA, DNase, and laboratory parameters. Spearman correlation analysis was used. The numbers in the grid indicate R values. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. The graph was drawn on https://www.chiplot.online/. PVT, portal vein thrombosis; cfDNA, cell-free DNA; DNase, deoxyribonuclease; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TBil, total bilirubin; ALB, albumin; CHE, cholinesterase; RBC, red blood cell; HGB, hemoglobin; PLT, platelet; PT, prothrombin time; PTA, prothrombin activity; INR, international normalized ratio; APTT, activated partial thromboplastin time; FIB, fibrinogen.

between PVT and non-PVT patients in both sub-groups (Supplementary Tables 2 and 3). No significant difference in NET levels or DNase activity was observed between PVT patients with and without HCC (Fig. 4A–C). However, PVT patients in both sub-groups consistently had higher cfDNA and histone-DNA levels and lower DNase activity (Fig. 4A–C), suggesting a robust association between NET imbalance and PVT occurrence, independent of HCC status.

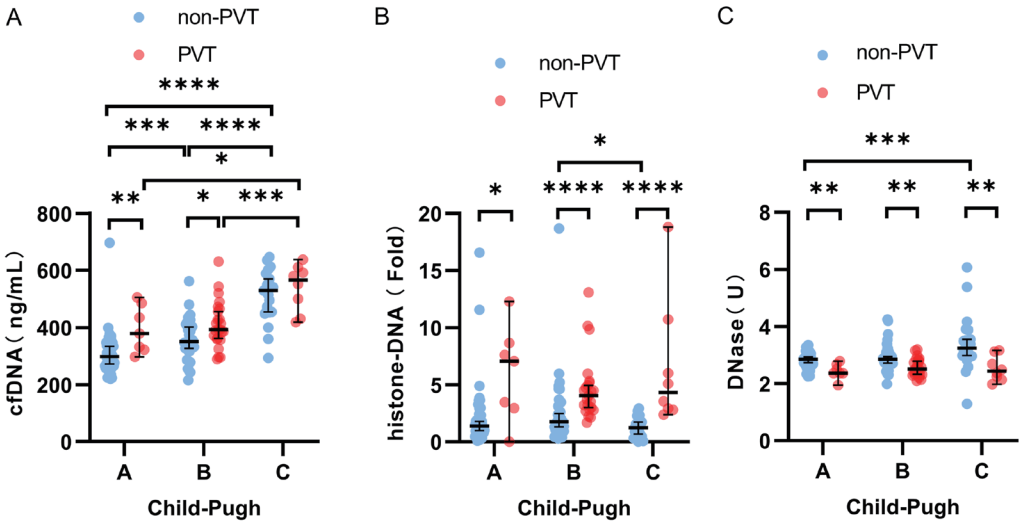

Fig. 3. Plasma levels of cfDNA, histone-DNA, and DNase activity in patients with different Child-Pugh grades in the Ditan study. Plasma levels of cfDNA (A), histone-DNA (B), and DNase activity (C). Child-Pugh A: non-PVT, n = 48; PVT, n = 7; Child-Pugh B: non-PVT, n = 37; PVT, n = 24; Child-Pugh C: non-PVT, n = 21; PVT, n = 8. Data are presented as median (95% CI). *p*-values were obtained using the Mann–Whitney test. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. PVT, portal vein thrombosis; cfDNA, cell-free DNA; DNase, deoxyribonuclease.

Risk factors for PVT

Univariate and multivariate logistic regressions identified risk factors for PVT occurrence (Table 2). In univariate analysis, seven variables, including albumin, CHE, red blood cell count, hemoglobin, cfDNA, histone-DNA, and DNase activity, significantly differentiated PVT from non-PVT patients. Multivariate analysis identified histone-DNA (OR, 1.351 [95% CI, 1.139–1.603]; $p = 0.001$), DNase activity (OR, 0.095 [95% CI, 0.024–0.386]; $p = 0.001$), and hemoglobin (OR, 0.965 [95% CI, 0.947–0.984]; $p < 0.001$) as independent risk factors for PVT.

Diagnostic values of NETs and DNase activity in PVT

ROC curve analysis was performed to assess the diagnostic potential of NET markers for PVT in CHB-related decompensated cirrhosis patients. The area under the ROC curve (AUC) values for cfDNA, histone-DNA, and DNase activity were 0.7068, 0.8628, and 0.7744, respectively (Fig. 5A and Supplementary Fig. 1). The combination of the three markers yielded the highest AUC value of 0.8774 for PVT diagnosis (Fig. 5B), indicating superior diagnostic efficiency.

Diagnostic efficacy was further evaluated in non-HCC and HCC sub-groups. In the non-HCC sub-group, the AUC for histone-DNA was 0.8617, and the combination of NET markers resulted in an AUC of 0.8829 for PVT diagnosis (Fig. 5C and D). In the HCC sub-group, the AUC for histone-DNA was 0.8451, with no significant increase upon combining the three markers (0.8632; Fig. 5E and F). NETs demonstrated high diagnostic efficiency for PVT occurrence in CHB-related decompensated cirrhosis patients, regardless of HCC presence.

Association of cfDNA and histone-DNA levels with PVT in the Changgung validation study

The Ditan study findings were validated in an external cohort of 33 patients with decompensated cirrhosis from Beijing Changgung Hospital, including 11 patients with PVT and 22 without. The incidence of PVT was 33.3%, with varying

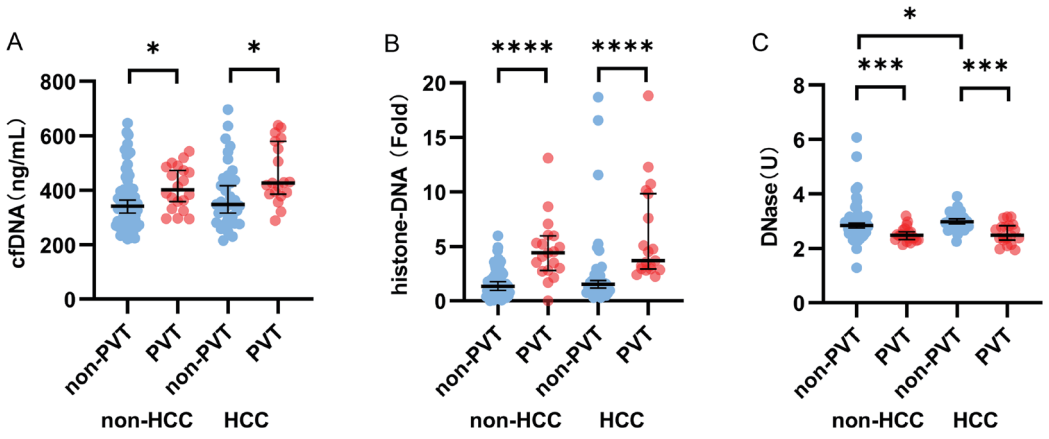

Fig. 4. Plasma levels of cfDNA, histone-DNA, and DNase activity in patients with or without HCC in the Ditan study. Plasma levels of cfDNA (A), histone-DNA (B), and DNase activity (C). Non-HCC: non-PVT, n = 71; PVT, n = 20; HCC: non-PVT, n = 35; PVT, n = 19. Data are presented as median (95% CI). *p*-values were obtained using the Mann–Whitney test. HCC, hepatocellular carcinoma; cfDNA, cell-free DNA; DNase, deoxyribonuclease; PVT, portal vein thrombosis.

Table 2. Univariate and multivariate analyses of PVT risk factors in the Ditan study

Variables	Univariate			Multivariate		
	***p*-value**	**OR**	**95%CI**	***p*-value**	**OR**	**95%CI**
ALT (U/L)	0.448	1.002	0.997–1.007	–	–	–
AST (U/L)	0.190	1.004	0.998–1.009	–	–	–
ALP (U/L)	0.366	1.002	0.998–1.006	–	–	–
TBil (μmol/L)	0.610	1.002	0.996–1.007	–	–	–
ALB (g/L)	0.069	0.946	0.891–1.004	–	–	–
γ-GT (U/L)	0.110	1.003	0.999–1.006	–	–	–
CHE (U/L)	0.006	1.000	1.000–1.000	–	–	–
GLU (mmol/L)	0.538	1.031	0.935–1.138	–	–	–
K (mmol/L)	0.600	0.789	0.326–1.912	–	–	–
Na (mmol/L)	0.367	0.961	0.882–1.048	–	–	–
Cl (mmol/L)	0.346	0.963	0.891–1.041	–	–	–
UREA (mmol/L)	0.113	1.067	0.985–1.157	–	–	–
CREA (μmol/L)	0.421	1.003	0.995–1.011	–	–	–
URCA (μmol/L)	0.877	1.000	0.997–1.004	–	–	–
WBC (10^9/L)	0.125	0.849	0.689–1.046	–	–	–
RBC (10^{12}/L)	0.023	0.585	0.368–0.930	–	–	–
HGB (g/L)	0.001	0.976	0.961–0.990	0.000	0.965	0.947–0.984
PLT (10^9/L)	0.930	1.000	0.994–1.005	–	–	–
PT (s)	0.663	1.016	0.947–1.090	–	–	–
PTA (%)	0.073	0.983	0.965–1.002	–	–	–
INR	0.608	1.223	0.567–2.635	–	–	–
APTT (s)	0.737	0.988	0.923–1.058	–	–	–
FIB (mg/dL)	0.575	0.998	0.993–1.004	–	–	–
TT (s)	0.336	1.082	0.921–1.272	–	–	–
cfDNA (ng/mL)	0.001	1.006	1.002–1.009	–	–	–
DNase (U)	0.000	0.073	0.021–0.250	0.001	0.095	0.024–0.386
histone-DNA (Fold)	0.000	1.404	1.186–1.662	0.001	1.351	1.139–1.603

Predictors were estimated using regression modeling of sub-distribution functions in competing risk scenarios. WBC, white blood cell; RBC, red blood cell; HGB, hemoglobin; PLT, platelet; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; ALB, albumin; TBil, total bilirubin; γ-GT, γ-glutamyl transpeptidase; CHE, cholinesterase; GLU, glucose; PT, prothrombin time; PTA, prothrombin activity; INR, international normalized ratio; APTT, activated partial thromboplastin time; TT, thrombin time; FIB, fibrinogen; UREA, urea; CREA, creatinine; URCA, uric acid; K, potassium; Na, sodium; Cl, chloride; AFP, alpha-fetoprotein.

rates across Child-Pugh grades. Clinical characteristics and laboratory indices are detailed in Supplementary Tables 4 and 5. PVT patients exhibited significantly higher levels of cfDNA (1.492-fold; $p < 0.05$) and histone-DNA (1.626-fold; $p < 0.05$), with no notable difference in DNase activity (Fig. 6A–C). Correlations between cfDNA and total bilirubin, and between cfDNA and CHE, were consistent with the Ditan study results (Fig. 6D).

In the Changgung study, ROC curve analysis confirmed the utility of NET markers in differentiating PVT in patients with decompensated cirrhosis. The AUC for histone-DNA was 0.7521, and combining the three markers (cfDNA, histone-DNA, and DNase activity) increased the AUC to 0.7975 for PVT diagnosis, demonstrating diagnostic significance (Fig. 6E and F). These results corroborate the Ditan study findings, indicating an association between elevated NET formation and PVT occurrence in decompensated cirrhosis patients.

Discussion

Our study presents novel findings demonstrating that NET homeostasis, characterized by cfDNA and histone-DNA, along with DNase activity, is significantly associated with PVT in patients with CHB-related decompensated cirrhosis. A key observation is that these markers are effective in diagnosing PVT, regardless of the presence of HCC. The combined assessment of these markers yielded a high AUC value, particularly in the non-HCC sub-group, underscoring their potential as diagnostic tools.

Patients with liver cirrhosis and PVT have lower survival rates compared to those without PVT.[17] Therefore, there is an urgent need for effective biomarkers to predict the development of PVT. Additionally, PVT should be considered a key marker of decompensated cirrhosis, irrespective of clinical events such as ascites, encephalopathy, or variceal bleeding. Including PVT in the definition of decompensated cirrhosis

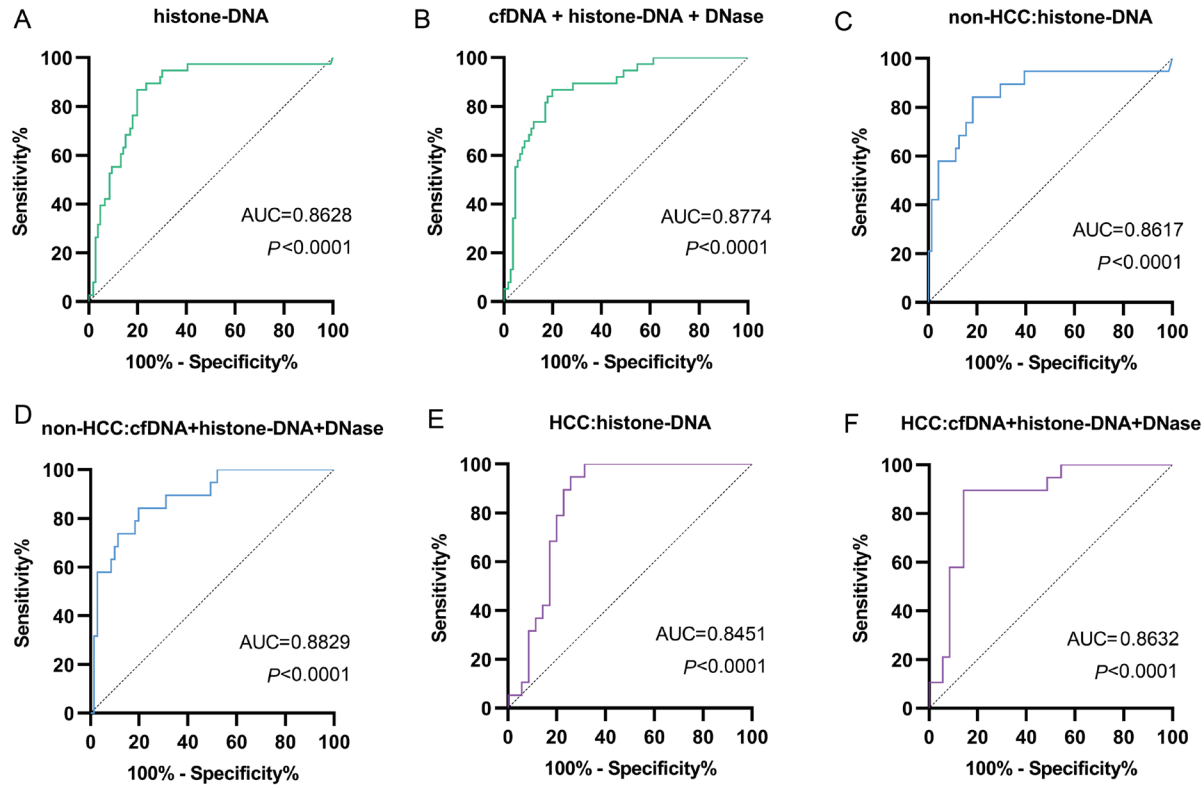

Fig. 5. ROC curves of plasma histone-DNA and the combination of three indicators for the diagnosis of PVT in the Ditan study. (A) and (B) Diagnostic ROC curves in patients with CHB-related decompensated cirrhosis (n = 145). (C) and (D) Diagnostic ROC curves in patients without HCC (n = 91). (E) and (F) Diagnostic ROC curves in patients with HCC (n = 54). Logistic regression analysis was used to establish the diagnostic model. Based on this model, prediction values were calculated, followed by ROC curve analysis. cfDNA, cell-free DNA; DNase, deoxyribonuclease; HCC, hepatocellular carcinoma; ROC, receiver operating characteristic; PVT, portal vein thrombosis; AUC, area under the curve.

would remind clinicians and researchers of the crucial role PVT plays in the natural history of the disease. It is essential to establish new systems for the prevention and treatment of PVT in cirrhosis.[18] HCC can tilt the hemostatic balance in cirrhosis toward a hypercoagulable state through various interconnected mechanisms. Currently, there are no specific guidelines for thrombosis prophylaxis in this unique population, and prospective studies are urgently needed to assess which patients have the highest pre-thrombotic characteristics and would benefit from early thrombo-prevention.[19]

The incidence of PVT in our study (26.9%) aligns with previous reports and was notably higher among patients with HCC.[20–23] Complications of cirrhosis, such as varices and ascites, were more prevalent in PVT patients, and the incidence of PVT varied significantly across Child-Pugh grades, highlighting the need for early and accurate PVT detection. The association between PVT and the severity of liver disease is further supported by the lower survival rates observed in patients with PVT, emphasizing the urgent need for effective biomarkers for early detection.

NETs play multifaceted roles in the pathogenesis of PVT and the progression of HCC. NETs contribute to thrombosis, shape the inflammatory microenvironment, and facilitate HCC metastasis, emerging as novel inducers of PVT.[24] While previous research has examined NETs in PVT within the context of cirrhosis or HCC, our study uniquely investigates NETs in PVT patients with CHB-related decompensated cirrhosis, irrespective of HCC co-occurrence. This approach reveals that HCC-induced NETs are pivotal in cancer-host interactions, promoting metastasis and presenting as potential immune-based therapeutic targets to curb HCC progression.[25] By inducing a tumor inflammatory response, NETs may also accelerate HCC metastasis.[26] Our findings suggest that therapeutic strategies targeting NETs could be more effective than those directly targeting neutrophils.

Our study reinforces the established link between PVT and coagulation disorders, confirming the role of D-dimer, P-selectin, and platelet count as key risk factors in cirrhotic patients.[27–30] Specifically, the platelet count is a strong predictor of PVT, especially in patients with cirrhotic portal hypertension following devascularization.[31] Comprehensive analyses have uncovered further predictors of PVT in cirrhosis, including advanced Child-Pugh scores, elevated end-stage liver disease scores, reduced portal flow, ascites, and the use of non-selective beta-blockers.[32] The association between platelet fibrin clot strength, platelet activation, and PVT in decompensated cirrhosis underscores the complexity of thrombotic complications in these patients.[33] While the platelet ratio has been considered a promising biomarker for thrombotic risk in decompensated cirrhosis,[20] our findings highlight the novel role of NETs in immune modulation related to PVT development. This insight suggests that NETs could provide a unique perspective in understanding and potentially managing PVT.

The Changgung study validated our findings, demonstrating elevated NET markers in PVT patients compared to non-PVT patients. However, it differed from the Ditan study by showing no significant difference in DNase activity between PVT and non-PVT patients, possibly due to differences in patient etiologies and sample sizes. These discrepancies underscore the need for further research with diverse etiologies

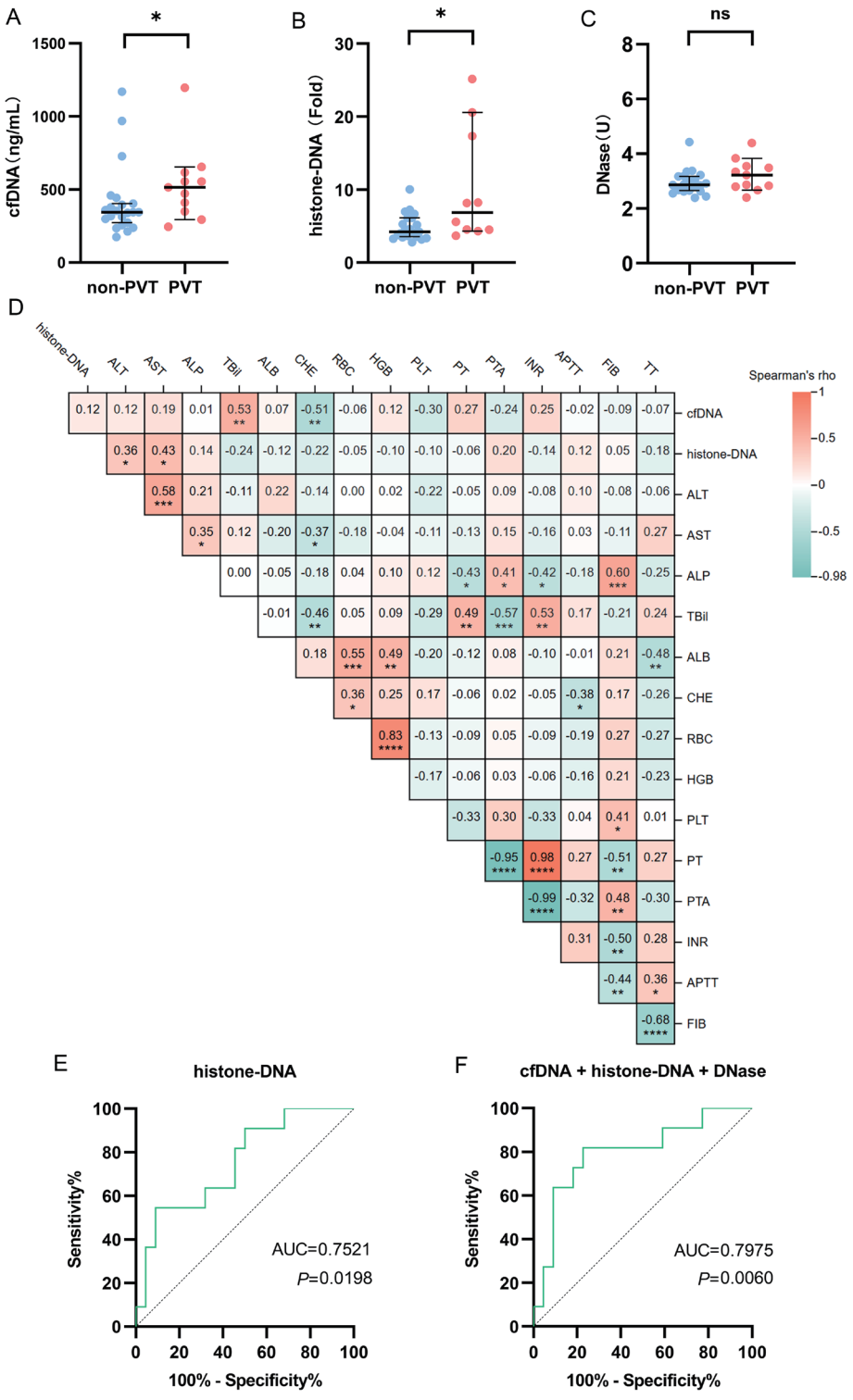

Fig. 6. NET formation is associated with PVT in patients with decompensated cirrhosis in the Changgung study. Plasma levels of cfDNA (A), histone-DNA (B), and DNase activity (C) in patients with PVT vs. without PVT. Non-PVT, n = 22; PVT, n = 11. Data are presented as median (95% CI). *p*-values were obtained using the Mann–Whitney test. (D) Correlation analysis between cfDNA, histone-DNA, and laboratory parameters. Spearman correlation analysis was used. The numbers in the grid indicate R values. **p* < 0.05, ***p* < 0.01, ****p* < 0.001, *****p* < 0.0001. The graph was drawn on https://www.chiplot.online/. (E) and (F) Diagnostic ROC curves in patients with decompensated cirrhosis (n = 33). Logistic regression analysis was used to establish the diagnostic model. Based on this model, prediction values were calculated, followed by ROC curve analysis. PVT, portal vein thrombosis; cfDNA, cell-free DNA; DNase, deoxyribonuclease; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; TBil, total bilirubin; ALB, albumin; CHE, cholinesterase; RBC, red blood cell; HGB, hemoglobin; PLT, platelet; PT, prothrombin time; PTA, prothrombin activity; INR, international normalized ratio; APTT, activated partial thromboplastin time; FIB, fibrinogen; ROC, receiver operating characteristic.

and larger cohorts to confirm the relationship between PVT and NETs.

It is important to acknowledge the limitations of our study. As an observational clinical study, we cannot establish causality, and interventional trials are required to validate our findings. Additionally, more research is needed to explore the potential of NETs as predictive markers and therapeutic targets for PVT.

Conclusions

In summary, our study demonstrates that imbalances in NET homeostasis, characterized by elevated cfDNA and histone-DNA levels alongside diminished DNase activity, are significantly associated with PVT in patients with CHB-related decompensated cirrhosis, including those with HCC. Notably, histone-DNA is an independent risk factor for PVT and shows potential as a valuable diagnostic marker for PVT in the context of decompensated cirrhosis, irrespective of HBV-related HCC.

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Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Conceptualization (YC, YH, LZ), methodology (MH, YL, YZ, SD), investigation (MH, YL, YY, XY, HX), writing – original draft (MH, YL, YC, LZ), funding acquisition (YC, LZ). All authors read and approved the final version and publication of the manuscript.

Ethical statement

This study was conducted in accordance with the Declaration of Helsinki principles and Good Clinical Practice Guidelines and was approved by the Ethics Review Committee of Beijing Ditan Hospital, Capital Medical University (NO. DTEC-KT2023-005-01) and the Ethics Review Committee of Beijing Tsinghua Changgung Hospital (22444-4-02). Written informed consent or waiver of informed consent was obtained from the patients.

Data sharing statement

The data supporting this study are available from the corresponding author, Liuluan Zhu, upon reasonable request.

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