

Elevated platelet distribution width predicts poor prognosis in hilar cholangiocarcinoma

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Abstract

Although the platelet distribution width (PDW) has been reported as a reliable predictor of prognosis in several types of cancer, to our knowledge the prognostic value of PDW in hilar cholangiocarcinoma (HC) has not been studied. The aim of the study was to investigate the prognostic value of PDW in HC patients. A retrospective analysis of 292 consecutively recruited HC patients undergoing radical resection with at least a 5-year follow-up. The optimal cutoff value of PDW was determined by receiver operating characteristic (ROC) curve. Survival analysis by the Kaplan–Meier method and the difference between the clinico-pathologic variables and survival was evaluated by log-rank analysis. Multivariate analysis identified independent prognostic risk factors of overall survival (OS). ROC curve analysis suggested that the optimal cutoff value for the PDW was 16.55. There were significant associations of high PDW with high white blood cell ($P < .001$) and high neutril-to-lymph ratio ($P < .001$). In a multivariate analysis, the PDW was an independent prognostic factor for overall survival (HR=2.521, 95% CI 1.832–3.470, $P < .001$). In conclusions, our findings indicate that PDW may have clinical significance in predicting OS after surgery in HC patients.

Abbreviations: EMT = epithelial-to-mesenchymal transition, ENBD = endoscopic retrograde cholangiopancreatography, G-CSF = granulocytes colony stimulating factor, HC = hilar cholangiocarcinoma, IL-6 = interleukin-6, M-CSF = macrophage colony stimulating factor, OS = overall survival, PDW = platelet distribution width, PTCD = percutaneous transhepatic cholangiodrainage, ROC = receiver operating characteristic.

Keywords: hilar cholangiocarcinoma, platelet distribution width, prognosis, systemic inflammatory response

1. Introduction

Hilar cholangiocarcinoma (HC) is a neoplasm arising from the biliary epithelium at the common hepatic duct bifurcation, and may extend to intrahepatic biliary tree and liver.^[1,2] Primary sclerosing cholangitis, hepatolithiasis, biliary parasitic disease, hepatitis, choledochal cysts, and thorotrast exposure have been identified as risk factors associated with the development of HC.^[3,4] Despite advances in surgical techniques and instruments, the prognosis of HC patients remains extremely poor, with a 5-year OS rate of 10% to 44%.^[5–7]

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Platelets play a significant role in cancer progression and metastasis.^[8] There is evidence to suggest that activated platelets mediate tumor cell growth, angiogenesis, and invasion.^[9] Elevated platelets are related with a decreased overall survival (OS) and poorer prognosis in many types of cancer, including gastric cancer, colorectal cancer, pancreatic cancer, endometrial cancer, and ovarian cancer.^[10–14] However, platelet count is determined by the balance between the rate of production and consumption of platelets. A normal platelet count could conceal the presence of highly hypercoagulable and pro-inflammatory cancer phenotypes in the presence of efficient compensatory mechanisms.^[15] Instead, since platelet size reflects platelet activity, which can be assessed by platelet distribution width (PDW).^[16]

The purpose of the present study was to investigate the prognostic influence of the preoperative PDW on the OS in HC patients.

2. Materials and methods

2.1. Patients selection

A total of 252 consecutive patients who underwent radical resection for a pathological diagnosis of HC at the West China hospital between January 2005 and February 2012, were retrospectively enrolled and reviewed. The inclusion criteria were as follows:

- (1) HC was confirmed by pathology examination;
- (2) patients underwent radical resection (R0 resection).

Exclusion criteria included: patients with gallbladder or intrahepatic cholangiocarcinoma extending to the hilum, recurrent or metastatic tumor, non-radical resection (R1 and R2

resection), hematological disorders, autoimmune diseases, systemic inflammatory diseases, coronary artery disease, hypertension, diabetes mellitus, thyroid disease, renal disease, hepatic disorder and other cancer, and medical treatment with anticoagulant, statins, and acetylic salicylic acid. Written informed consents were obtained from all patients. This study was approved by the Institutional Review Board of West China Hospital of SiChuan University. All studies were conducted according to guidelines (Declaration of Helsinki) for biomedical research.

2.2. Preoperative workup

Preoperative assessment consisted of medical history, physical examination, laboratory tests and radiography. All patients were evaluated by contrast-enhanced ultrasound or contrast-enhanced computed tomography or magnetic resonance cholangiography along with magnetic resonance cholangiopancreatography to determine the location and extent of the tumor. Biliary drainage, including endoscopic retrograde cholangiopancreatography (ENBD) and percutaneous transhepatic cholangiodrainage (PTCD), was applied in obstructive jaundice patients with $>85 \mu\text{mol/L}$ total bilirubin. Preoperative portal vein embolization (PVE) was performed in patients with a future remnant liver (FRL) volume $<40\%$.

2.3. Surgical characteristics of the patients

To achieve negative resection margins, segmental bile duct resection and hepatic resection with extrahepatic bile duct resection were adopted. In addition, standard regional lymph node dissection was performed. The surgery was abandoned if metastases to the distant lymph nodes were diagnosed during surgery. The locations of regional lymph nodes are defined as follows: along the common bile duct, cystic duct, portal vein and proper hepatic artery. Vascular resection and reconstruction was only performed when vessels could not be detached from the tumor.

2.4. Pathological examination

The pathological evidence of cancer was determined by paraffin sections. All included HC were histopathologically confirmed by experienced pathologist. An R0 resection was defined as the presence of a macroscopically and microscopically tumor-free resection margin. An R1 resection was defined as microscopic evidence of tumor tissue at the resection margin. An R2 resection was defined as macroscopic evidence of tumor tissue at the resection margin.

2.5. Follow up

All enrolled patients had follow-up every 3 months in the first year and every 6 months subsequently until at least 5 years after the surgery. OS was defined as the interval from the date of surgery to death or last follow-up. The tumor markers (serum levels of carbohydrate antigen 19–9 and carcinoembryonic antigen), liver functions and ultrasonography were conducted. If there was a suspicion of recurrence, contrast-enhanced computed tomography or magnetic resonance imaging was further performed. Tumor recurrence was diagnosed based on the combined findings of typical radiological appearance, carbohy-

drate antigen 19–9 and clinical presentation, the date of the first suspicious radiological finding was recorded as the date of initial disease recurrence.

2.6. Statistical analysis

Patient data were retrospectively collected and statistical analyses were performed using SPSS version 20.0 (SPSS Inc. Chicago, IL). The quantitative variables are expressed as mean (SD) if they presented a normal distribution or otherwise as median and range. Qualitative variables are presented in absolute numbers and percentages. Normally distributed continuous data were compared by means of the Student *t* test and skewed-distributed by the Mann–Whitney *U* test and ordinal data were compared in a χ^2 test or Fisher exact test. The optimal cutoff value of PDW was determined by receiver operating characteristic (ROC) curve. Survival was described using the Kaplan–Meier method and differences between subgroups were reviewed with the log-rank test. The multivariate analysis for prognostic factors used a Cox proportional hazards model to analyze variables with $P < .1$ in the univariate analyses. Two-sided *P* values $< .05$ were considered to be statistically significant.

3. Results

The characteristics of the patients are outlined in Table 1. The 252 enrolled patients, including 143 men and 109 women with a median age of 60 years (20–78 years), underwent radical resection for hilar cholangiocarcinoma. Pre-operative biliary drainage was performed in 172 (81.9%) of the 210 obstructive jaundice patients, 114 patients underwent PTCD and 58 patients underwent ENBD. Preoperative portal vein embolization was performed in 11 patients.

According to the Bismuth–Corlette classification system, 24 patients (9.5%) were staged as type I, 35 (13.9%) patients as type II, 96 (38.1%) patients as type III and 97 (38.5%) patients as type IV. The radical surgery procedures included extrahepatic bile duct resection ($n=17$, 6.7%), extrahepatic bile duct resection combined with hepatectomy ($n=235$, 93.3%; 126 left hemihepatectomy, 4 left trisegmentectomy, 35 mesohepatectomy, 58 right hemihepatectomy, 8 right trisegmentectomy, 5 caudate lobectomy). Caudate lobectomy was conventionally performed, except for 17 type I patients with sufficient negative margins. The average operative time was 389.5 ± 125.2 minutes and the median blood loss volume was 500 ml (100–3000 ml). A total of 109 patients underwent intraoperative transfusions.

ROC curve analysis suggested that the optimal cutoff value for the PDW was 16.55 (Fig. 1). It indicated that PDW predicts HC prognosis with a sensitivity of 53.6% and a specificity of 79.1% (AUC=0.705, 95% CI: 0.627–0.784, $P < .001$). Of the total of 252 patients, 131 patients (52.0%) were detected with PDW of less than 16.55, while there were 121 patients (48.0%) whose PDW was greater than 16.55.

The relationships between PDW and clinical characteristics were shown in Tables 1 and 2. Our study indicated that PDW was associated with N stage ($P < .001$), AJCC stage ($P < .001$) and MPV ($P < .001$). While there were no significant differences in gender, age, Bismuth–Corlette classification, differentiation, T stage, perineural invasion, portal vein invasion, hepatic artery invasion, tumor size, transfusion, ALB, HGB, WBC, PLT, blood

Table 1
Baseline characteristics of patients with hilar cholangiocarcinoma according to PDW levels.

Variables	Total	PDW < 16.55	PDW > 16.55	P value
Gender				.866
Male	143	75	68	
Female	109	56	53	
Age				.631
<65	199	105	94	
>65	53	26	27	
Bismuth-Corlette classification				.504
I	24	15	9	
II	35	18	17	
III	96	45	51	
IV	97	53	44	
Differentiation				.797
Well/moderate	187	107	80	
poor	65	36	29	
T stage				.167
T1	6	5	1	
T2	182	98	84	
T3	55	23	32	
T4	9	5	4	
N stage				<.001
N0	155	97	58	
N1	97	34	63	
AJCC stage				<.001
I, II	108	75	33	
III, IV	144	56	88	
Perineural invasion				.393
Present	120	59	61	
Absent	132	72	60	
Portal vein invasion				.117
Present	52	22	30	
Absent	200	109	91	
Hepatic artery invasion				.674
Present	17	8	9	
Absent	235	123	112	
Tumor size				.130
<30mm	168	93	75	
>30mm	84	38	46	
Transfusion				.866
Yes	109	56	53	
No	143	75	68	

AJCC=American Joint Committee on Cancer.

loss and operation time. Linear correlation analysis revealed that PDW > 16.55 is associated with higher WBC count, higher NLR (Fig. 2),

Table 2
Baseline characteristics of patients with hilar cholangiocarcinoma according to PDW levels.

Variables	PDW < 16.55	PDW > 16.55	P value
Age (year)	60 (20–78)	59 (20–76)	.585
ALB (g/L)	38.8±5.0	36.4±4.7	<.001
HGB (g/L)	131.0±14.6	121.5±12.3	<.001
WBC (×10 ⁹ /L)	5.6 (3.47–9.49)	6.16 (3.7–9.90)	.035
PLT (×10 ⁹ /L)	204.7±74.1	220.0±78.8	.086
MCV (%)	93.6 (67.4–107)	92.8 (64.5–104.7)	.039
Operation time (min)	392.2±119.3	389.5±128.6	.855

ALB=albumin, HGB=hemoglobin, MCV=mean corpuscular volume, PLT=platelet, WBC=white blood cell.

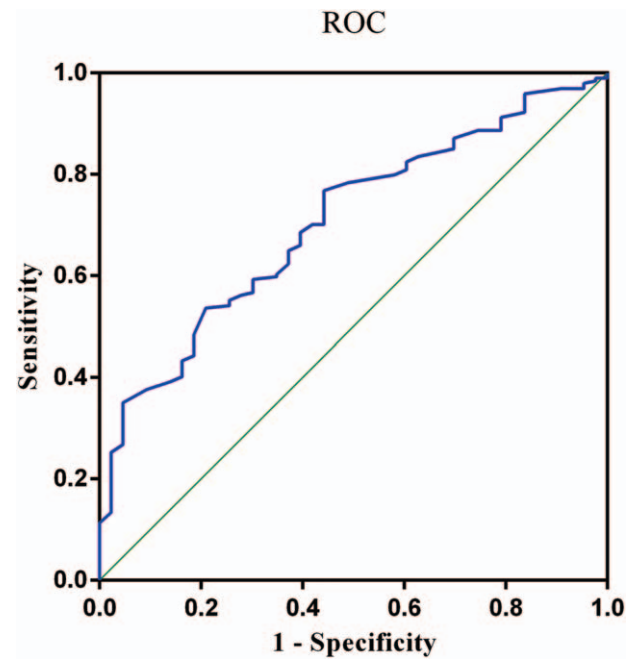


Figure 1. Optimized cutoff value was determined for red blood cell distribution width (PDW) using receiver operating characteristic curve (ROC) analysis.

In this cohort, the 5-year OS was 22.4%. Patients with PDW > 16.55 had a significantly worse 5-year OS than patients with PDW P < 16.55 (12.4% vs 30.3%, $P < .001$). The Kaplan–Meier OS curves showed a significant separation in the 2 subgroups (Fig. 3).

Univariate and multivariate analysis were performed and the results were presented in Table 3. Univariate analysis showed that PDW ($P < .001$), MPV ($P = .001$), differentiation ($P < .001$), T stage ($P < .001$), N stage ($P < .001$), AJCC stage ($P < .001$), portal vein invasion ($P = .001$) and hepatic artery invasion ($P = .037$) significantly influenced OS. All factors with $P < .05$ in the univariate analysis were included in the Cox regression model, in which PDW ($P < .001$), differentiation ($P = .001$), N stage ($P = .001$) and AJCC stage ($P = .028$) were independent prognostic risk factors for OS.

4. Discussion

The current study indicated that PDW is associated with patient’s survival and is an independent risk factor for prognosis in HC.

The PDW directly measures the variability in platelet size and is a marker of platelet activation.^[17] An elevated PDW in peripheral blood may be associated with atherosclerosis, coronary vascular disease, cerebrovascular disease and systemic inflammatory disease.^[18,19] Recent studies showed that PDW increased in patients with malignant tumors. However, the specific molecular mechanism to clarify the correlation between PDW and survival in HC has yet to be elucidated. The possible mechanisms might systemic inflammation and immune escape.

A growing body of evidence has suggested that systemic inflammatory response plays an important role in cancer progression. Tumor-related inflammatory microenvironment could facilitate tumor growth and metastasis by sustaining proliferation, inhibiting apoptosis, inducing epithelial-to-mesen-

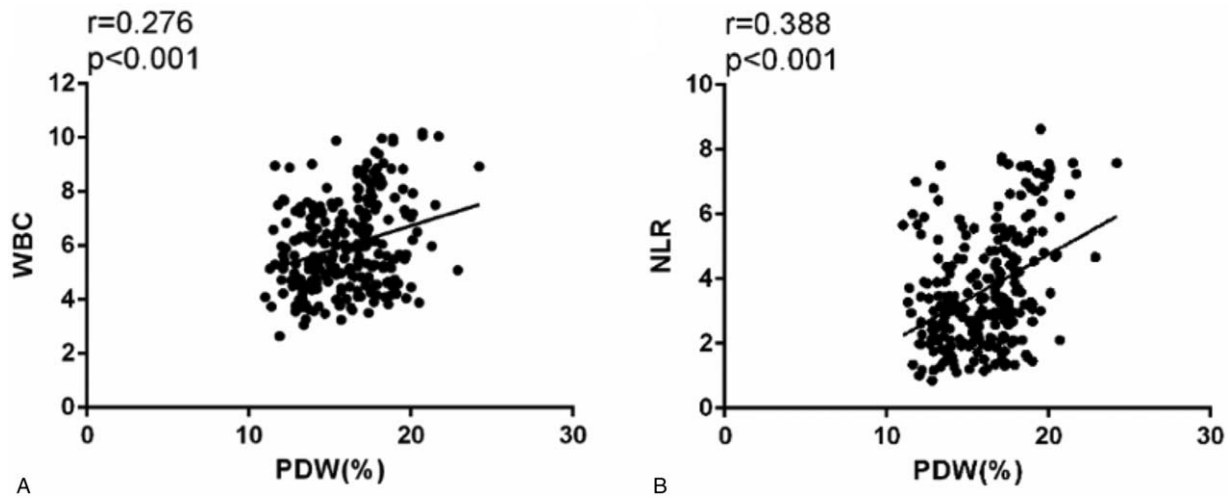


Figure 2. Correlation analysis of PDW with (A)WBC, (B) NLR.WBC = white blood cell count, NLR = neutrophil-to-lymphocyte ratio.

chymal transition, initiating angiogenesis, and suppressing host-anti-tumor immunity.^[20] As shown in Figure 2, higher WBC and higher NLR were associated with higher PDW. This result might support the idea that high levels of PDW reflect the chronic inflammation status of patients with HC.

Thrombocytosis is associated with reduced survival in patients with several types of malignancies, such as lung cancer, ovary cancer, endometrium cancer, rectum cancer, kidney cancer, stomach cancer, pancreas cancer and breast cancer.^[21] Fingas et al reported that the expression of platelet derived growth factor beta-receptor (PDGFR-β) was significantly increased in HC. PDGF-BB, via PDGFR-β promotes resistance against cytotoxicity by endogenous tumor necrosis factor related apoptosis inducing ligand (TRAIL).^[22]

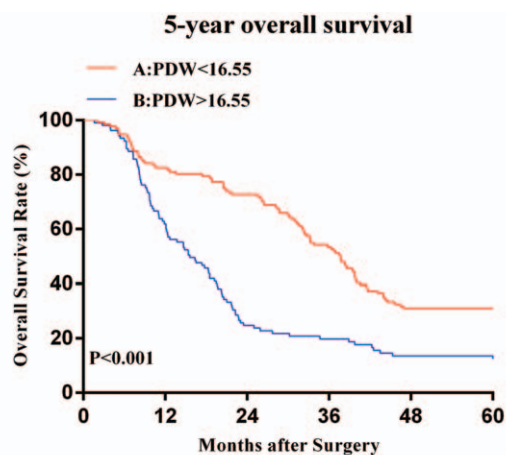
Furthermore, bone marrow cells (including megakaryocytes) malfunction may contribute to alter PDW. PDW is a measure of

platelet heterogeneity caused by heterogeneous demarcation of megakaryocytes. Recent reports demonstrated several cytokines, such as interleukin-6 (IL-6), granulocytes colony stimulating factor (G-CSF) and macrophage colony stimulating factor (M-CSF), regulate megakaryocytic maturation, platelet production and platelet size.^[23–25] IL-6 promotes tumor angiogenesis, metastasis and metabolism.^[26] Furthermore, the cytokines G-CSF and M-CSF that be secreted by tumor cells could stimulate megakaryopoiesis and subsequent thrombopoiesis in cancer.^[27] Another possible mechanism is that platelets promote the hypercoagulable state in cancer. Activated platelets create a procoagulant micro-environment that enables the tumor cells to cover themselves with platelets and evade the host immune system.^[28]

In this study, the cutoff value of PDW is 16.55. It is different from other malignant tumors including lung cancer, prostate cancer, esophageal cancer and hepatocellular carcinoma. We speculated that the prognosis-related PDW values may vary for different malignancies. However, we believe that the difference in laboratory measures and the number of patients included, may affect the cutoff value of PDW. We hope that, through multicenter cooperation, we can incorporate more patients, unify the PDW measurement standards, and achieve a more convincing cutoff value of PDW.

Some limitations of the study should also be taken into account when interpreting the results. First, our study was retrospective with inherent limitations in its design. Thus, some clinical bias was inevitable. We, also, did not collect relevant laboratory data regarding inflammatory factors (such as the ESR, CRP, and ILs). These indicators might greatly help further elucidation of the mechanism of PDW elevation in HC patients. Thirdly, PDW is essentially an inflammatory factor which is one of the cancer-causing factors, so the prognostic usefulness of PDW could be related to highly selected HC patients.

In conclusion, PDW is a simple, inexpensive, routinely measured and automatically reported blood test parameter, which reflects the degree of heterogeneity of platelet in peripheral blood. Our data revealed that PDW may have clinical significance in predicting OS after radical resection in HC patients. Further studies are warranted to clarify the specific role of PDW in HC



No. at risk	0	12m	24m	36m	48m	60m
A	132	109	96	69	40	40
B	105	65	26	19	13	13

Figure 3. Overall survival (OS) based on platelet distribution width (PDW) in hilar cholangiocarcinoma patients with radical resection: the 5-year OS rate in the PDW < 16.55 group were significantly higher than the PDW > 16.55 group.

Table 3**Univariate and multivariate analysis of overall survival in patients with hilar cholangiocarcinoma.**

Variable	5-year OS rate	Univariate analysis			Multivariate analysis		
		HR	95CI%	P value	HR	95CI%	P value
Gender		1.241	0.936–1.647	.134			
Male	17.6%						
Female	24.8%						
Age(years)		1.241	0.875–1.758	.225			
<65	21.9%						
>65	17.9%						
ALB(g/L)		0.719	0.524–0.986	.041			
<35	10.9%						
>35	24.5%						
PLT ($\times 10^9/L$)		1.251	0.838–1.868	.274			
≤ 300							
> 300							
PDW		3.122	2.325–4.193	<.001	2.521	1.832–3.470	<.001
<16.55	28.0%						
>16.55	11.9%						
MPV		1.686	1.233–2.307	.001	1.243	0.887–1.742	.206
≤ 13							
> 13							
Histologic grade		1.973	1.445–2.694	<.001	1.783	1.280–2.483	.001
Well/moderate	25.1%						
poor	9.4%						
Perineural invasion		1.261	0.951–1.672	.108			
Present	18.3%						
Absent	23.5%						
T stage		1.847	1.352–2.523	<.001	3.862	0.892–16.716	.071
T 1,2	23.9%						
T 3,4	12.5%						
N stage		2.488	1.854–3.339	<.001	3.127	1.543–6.334	.002
N0	30.0%						
N1	7.2%						
AJCC stage		2.687	1.988–3.632	<.001	1.424	1.198–1.909	.028
I, II	35.2%						
III, IV	15.5%						
Tumor size		1.152	0.858–1.548	.347			
≤ 30 mm	22.0%						
>30mm	19.0%						
Portal vein invasion		1.789	1.283–2.495	.001	1.331	0.427–4.146	.622
Present	11.5%						
Absent	23.5%						
Hepatic artery invasion		1.756	1.035–2.980	.037	0.931	0.325–2.662	.893
Present	5.9%						
Absent	21.7%						
Transfusion		1.016	0.763–1.351	.915			
Yes	21.1%						
No	20.3%						

ALB=albumin, MPV=mean platelet volume, PDW=platelet distribution width, PLT=platelet.

Author contributions

Li B analyzed the data and wrote the manuscript; Lu J and provided the collection of all the human materials; Peng DZ and Zhang XY helped with data analysis; You Z designed, organized, and supervised writing of the manuscript; all authors approved the final manuscript as submitted.

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