Original Article
The independent association of platelet parameters with overall survival in pancreatic adenocarcinoma receiving intensity-modulated radiation therapy

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Abstract: Background: Platelets are involved in multiple links of the process of thrombus formation and development. The aim of this study is to investigate the impact of pretreatment platelet parameters, such as plateletcrit (PCT), platelet mean distribution width (MDW), and mean platelet volume (MPV) in a cohort of patients with locally advanced pancreatic adenocarcinoma treated with a combination of chemotherapy and radiation therapy. Methods: A retrospective analysis was conducted of 163 locally advanced unresectable consecutive pancreatic adenocarcinoma patients who received chemoradiotherapy in Zhejiang cancer hospital from January 2009 to December 2011. The effects of platelet parameters on overall survival (OS) were assessed using Kaplan-Meier analysis. Independent prognostic factors were identified in the multivariate Cox analysis. Results: The median of the PC, PCT, MDW, MPV and CRP were 175×10^9, 20.0%, 14.0%, 10.8 fl and 7.0 mg/L, respectively. MDW was positively correlated with PC (r=0.156, P=0.047) and CRP (r=0.591, P<0.001). The median survival time of high MDW group was significantly worse than that of low MDW group (14.0 m Vs 11.0 m, P=0.008). Patients with high PCT were found to have shorter overall survival time (15.0 m Vs 11.0 m, P=0.018). Multivariate analysis indicated that MDW and N stage were two independent prognostic factors for overall survival (P<0.05). Patients with higher MDW had a 1.48-fold increased risk of death compared to those with low MDW. Conclusions: MDW is an independent negative prognostic factor for overall survival in pancreatic adenocarcinoma patients.

Keywords: Pancreatic adenocarcinoma, platelet, platelet parameters, prognosis

Introduction

The prognosis of cancer patients is not only determined by tumor characteristics, patient-related factors are also important factors. Cancer-associated thrombosis is a key determinant of disease progression and survival in most cancers [1]. Platelets are the smallest circulating blood cells and their major function is to stop bleeding by clumping and clogging blood vessel injuries. Platelets are involved in multiple links of the process of thrombus formation and development. In fact, increased circulating platelet counts have been reported with unfavorable prognosis in pancreatic cancer [2-5]. Several evidences support a role for platelets in contributing to systematic inflammation and promoting thrombosis [6, 7]. The changes in platelet parameters were reported to reflect the platelet activation [8]. It has been indicated that platelet count (PC) and its parameters including plateletcrit (PCT), platelet mean distribution width (MDW), and mean platelet volume (MPV) might be useful together with other acute phase reactants to define inflammation activation. Recently, Ma et al. [9] showed that the combination of thrombocytosis and maximal aggregation rate are independent negative prognostic factors for overall survival in epithelial ovarian cancer patients, but there is no study regarding these markers in pancreatic cancer.

In our previous study [10], the rate of thrombocytosis was 13.1% in pancreatic adenocarcinoma and a significant association can be found between thrombocytosis and the poor survival of patients with pancreatic adenocarcinoma as evidenced by a multivariate analysis. Therefore, this study was undertaken to identify the poten-

tial prognostic significance of pretreatment indices including plateletcrit (PCT), platelet mean distribution width (MDW), and mean platelet volume (MPV) in a cohort of patients with locally advanced pancreatic adenocarcinoma treated with a combination of chemotherapy and radiation therapy.

Patients and methods

Patient's selection

A retrospective analysis was conducted of 168 locally advanced unresectable consecutive pancreatic adenocarcinoma patients who received chemoradiotherapy in Zhejiang cancer hospital from January 2009 to December 2011. All patients were newly confirmed to have pancreatic adenocarcinoma and had not received treatment previously. Patients with other malignancies were excluded from this study. Those patients with concomitant disease or conditions suspected of influencing platelet parameters, such as hematological disease, coronary artery disease, end-stage renal disease, heart failure, cerebrovascular disease, peripheral arterial disease, were excluded. Finally, a total of 163 patients were enrolled in current study. Medical records were reviewed, and each patient's gender, age, tumor location, clinical TNM stage, treatment response, and overall survival after treatment were obtained. The extent of the disease was determined by TNM staging according to the new IASLC staging system.

Complete blood count (CBC) test results were obtained within 1 week prior to treatment. Our study was approved by the institutional review board of the hospital. All patients provided informed consent before treatment.

Treatment schedule

All patients were treated with intensity modulated radiation therapy (IMRT). Patients were immobilized in a supine position, arms overhead, with thermoplastic cast. Patients underwent CT-simulation on CT scanner (GE, Lightspeed, USA), using 5 mm slices with contrast enhancement. Radiation plans for IMRT were generated using Pinnacle Version 8.0. All patients underwent external beam radiation therapy with 6 MV X-rays. The area of solid macroscopic tumors in pancreas, the surrounding tissue infiltrated, and the regional lymph node

metastases were defined as the gross tumor volume (GTV). The GTV plus a margin of at least 5 mm, including any areas of microscopic spread and the regional lymph nodes (peripancreatic, celiac, superior mesenteric, portal hepatic, retroperitoneal), was defined as the clinical target volume (CTV). The plan target volume (PTV) was defined as the CTV plus 0.5-1.0 cm to account for the daily setup variation and respiratory movement. A fractional daily dose of 1.8 Gy (Gray) (5 days per week, over 5 weeks) at an isocenter was prescribed. The median delivered dose of IMRT was 50 Gy (range: 44.0-55.8 Gy). The dose to the adjacent normal structures was constrained as follows: The liver dose was limited to V_5<75%, V_{20}<50%, V_{30}<30%, and a mean dose less than 28 Gy. The kidney dose was limited to V_{12}<50% and $V_{22.5}$<30%. Spinal cord maximum dose was held to 45 Gy. The CTV encompassed at least 95% isodose line. The dose volume histogram (DVH) was obtained for CTV, PTV, spinal cord, liver, and kidney. The small bowel contour was confined to the small bowel loops within 3 cm of the PTV and was limited to maximal dose <54 Gy.

Concurrent chemoradiotherapy, with or without adjuvant chemotherapy, was used in 101 patients. The chemotherapy regimens consisted of S1 at 50 mg/m^2 twice daily from days 1 to 14 or gemcitabine at 1,000 mg/m^2 on day 1 and day 8 of a 21 days cycle. The CR (complete response), PR (partial response), SD (stable disease), and PD (progressive disease) were assessed at an interval of at least 4 weeks to confirm the objective response. All patients received standardized follow-up, occurring at 3 months interval for 2 years, 6 months interval the 3rd year, and yearly thereafter. Evaluations comprised a physical examination, pancreatic CT, complete blood count, liver and kidney function tests, abdominal ultrasound, chest radiography, and pelvic CT.

Statistical analysis

Overall survival was calculated from the initial event (radiotherapy) to the death or censoring. The cutoff values of PCT, MDW, and MPV were determined using receiver operating characteristic (ROC) curve analysis, and the dependent variable was the OS for 2 years. The optimal cutoff levels for PCT, MDW, and MPV were established at 18.00%, 14.15%, and 12.00 fl, respectively, and these cutoff values were used to categorize high and low PCT, MDW, and MPV

Table 1. Baseline patient characteristics (n=163) and univariate analysis of prognostic factors of OS by the Kaplan-Meier method in pancreatic adenocarcinoma

Characteristics	Total (n, %)	Median OS time (months)	2 year OS rate (%)	P
Gender				
Female	60 (36.8)	14.0	9.6	0.887
Male	103 (63.2)	12.0	14.7	
Age (year)				
<60	74 (45.4)	14.0	15.9	0.297
≥60	89 (54.6)	12.0	9.6	
Tumor location				
Head	77 (47.2)	12.0	8.5	0.397
Body and tail	86 (52.8)	13.0	15.5	
BMI (kg/m^2)				
<20	70 (42.9)	13.0	15.3	0.756
≥20	93 (57.1)	13.0	17.3	
Chemotherapy				
No	62 (38.)	10.0	5.7	0.049
Yes	101 (62.0)	14.0	16.4	
T stage				
T_{1-2}	45 (27.6)	13.0	12.1	0.882
T_{3-4}	118 (72.4)	12.0	16.1	
N stage				
N_0	77 (47.2)	14.0	13.7	0.045
N_1	86 (52.8)	11.0	11.6	
CRP (mg/L)				
<3	39 (23.9)	14.0	26.3	0.161
≥3	124 (76.1)	12.0	13.5	
Platelet count				
<300×10^9	142 (87.1)	14.0	13.7	0.015
≥300×10^9	21 (12.9)	9.0	5.7	
MPV (fl)				
<12.00	128 (78.5)	13.0	14.3	0.381
≥12.00	35 (21.5)	11.0	7.3	
PCT (%)				
<18.00	100 (61.3)	15.0	18.5	0.018
≥18.00	63 (38.7)	11.0	13.5	
MDW (%)				
<14.15	85 (52.1)	14.0	20.5	0.008
≥14.15	78 (47.9)	11.0	5.4	

Abbreviations: OS, overall survival; HR, hazard ratio; BMI, body mass index; CRP, C-reactive protein; MPV, mean platelet volume; PCT, plateletcrit; PDW, platelet distribution width; MDW, platelet distribution width.

groups. Chi square test or Fisher exact test was used for comparisons of categorical variables. The correlation between PC, PCT, MDW, MPV and C-reactive protein (CRP) was determined through linear correlation analysis. Survival curves were estimated by the univariate Kaplan-Meier method. The log-rank test was applied to check the significant difference in the curves among groups. Furthermore, we used the Cox proportional hazards model with the backward selection method for multivariate analysis. All statistical calculations were performed with SPSS 13.0 software for Windows (Chicago, IL, USA). Two-sided *P*-values of <0.05 were considered to be statistically significant.

Results

Characteristics of patients

Preoperative PC, PCT, MDW, and MPV were confirmed in 163 pancreatic adenocarcinoma patients. Patient characteristics are shown in **Table 1**. The study population had a median age of 61 years (range: 34-83 years). Tumor invasion depths of T_1, T_2, T_3, and T_4 were observed in 17 (10.4%), 28 (17.2%), 75 (46.0%), and 43 (26.4%) of the patients, respectively. On the basis of image parameters, tumor was found with regional lymph node metastases in 86 (52.8%) patients. The overall follow-up durations ranged from 4 to 48 months (median, 12.0 months). 137 of 163 (84.0%) patients died of disease progression.

Relationship between PC, PCT, MDW, MPV and CRP

The median of the PC, PCT, MDW, MPV and CRP were 175×10^9, 20.0%, 14.0%, 10.8 fl and 7.0 mg/L, respectively. MDW was positively correlated with PC (r=0.156, P=0.047, **Figure 1A**) and CRP (r=0.591, P<0.001, **Figure 1B**). However, no significant correlation was observed between PCT, PC, MPV and CRP (P>0.05).

Survival analysis

The 1 year, 2 year and 3 year cumulative overall survival rate were 50.2%, 16.3%, 4.7%. We performed univariate analysis for platelets parameters and other clinicopathological variables
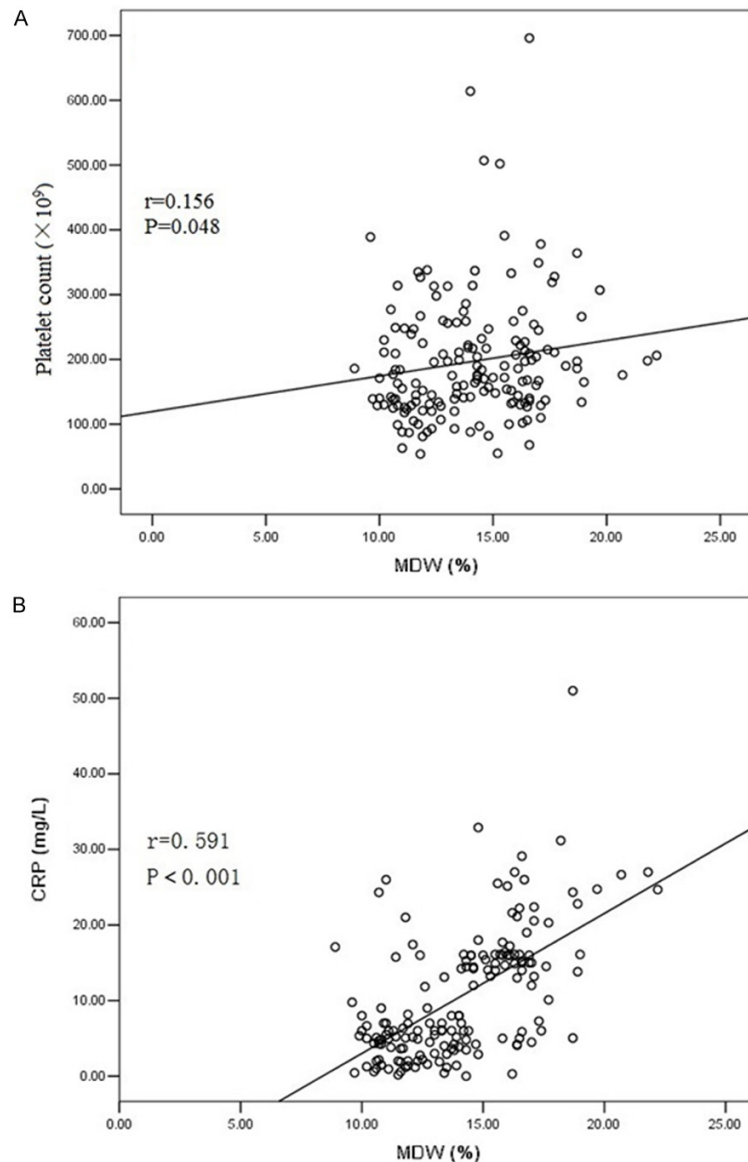
Figure 1. A. Spearman's correlation revealed that MDW was positively correlated with PC (r=0.156, P=0.047). B. Spearman's correlation revealed that MDW was positively correlated with CRP (r=0.591, P<0.001).

significantly correlated with overall survival, with low MDW patients with longer overall survival time (**Figure 2B**).

We then performed multivariate analysis for these factors whose presence significantly affected prognosis. The Cox proportional hazards regression indicated that MDW and N stage were two independent prognostic factors for overall survival (P<0.05, **Table 2**). Patients with high MDW had a 1.48-fold increased risk of death compared to those with low MDW. The 95% confidence interval (CI) was 1.05-2.09. However, PC and PCT were not independent prognostic factors adjusted for other prognostic factors (P> 0.05).
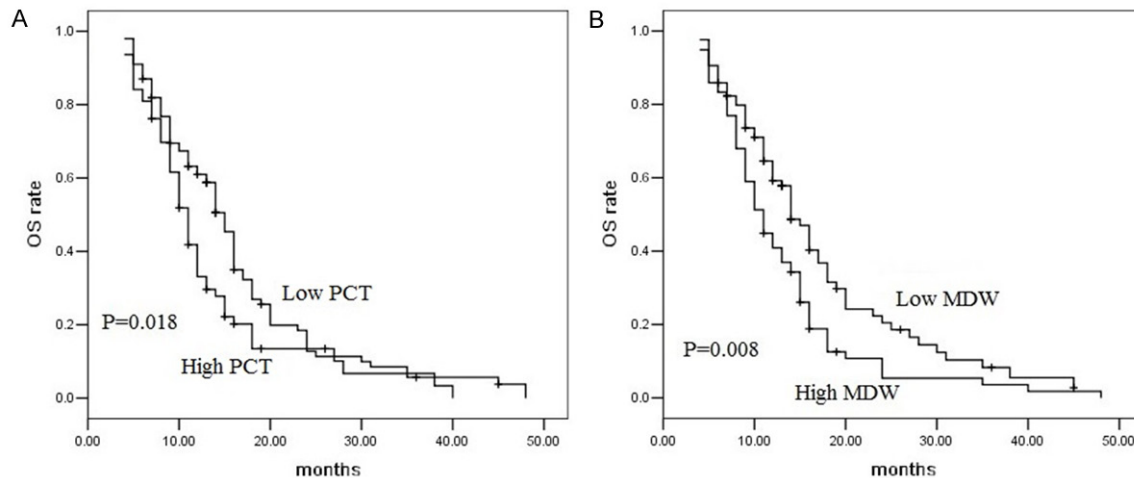
Discussion

To the best of our knowledge, our study is the first study to analyze the relationship between platelet parameters (MPV, PCT and MDW) and prognosis in pancreatic adenocarcinoma. In the present study, we found a significant association between thrombocytosis, high PCT or high MDW and poor overall survival in locally advanced pancreatic adenocarcinoma treated with radiation therapy or chemoradiotherapy. Furthermore, according to the multivariate analysis, MDW is one of the independent prognostic factors.

Platelets activation plays a significant role in the process of thrombus formation and development. The alteration in platelet parameters could reflect the platelet activation. MPV represents the average volume of a single platelet and is an important biological variable and that larger platelets have higher thrombotic potential. MPV is a simple marker showing platelet

(including gender, age, tumor location, BMI, T stage, lymph node metastasis, and chemotherapy) to find useful prognostic factors for pancreatic adenocarcinoma (**Table 1**). The results were shown in **Table 1**. Chemotherapy, N stage, PC, PCT and MDW were five significant prognostic factors for overall survival (Chemotherapy: P=0.049; N stage: P=0.045; PC: P=0.015; PCT: P=0.018; MDW: P=0.008). The pretreatment PCT was correlated with overall survival, with high PCT patients with shorter overall survival time (**Figure 2A**). The pretreatment MDW was

Figure 2. A. Kaplan-Meier overall survival curves of the patients with high pretreatment PCT (n=63) and low PCT (n=100). All patients underwent radiotherapy (P=0.018, log-rank test). B. Kaplan-Meier overall survival curves of the patients with high pretreatment MDW (n=78) and low PCT (n=85). All patients underwent radiotherapy (P=0.008, log-rank test).

Table 2. Multivariable analysis on overall survival

Variables	Hazard ratio	95% CI	P
Chemotherapy			
Yes Vs No	0.73	0.51-1.05	0.091
N stage			
N_0 Vs N_1	0.70	0.49-0.99	0.043
T stage			
T_{3-4} Vs T_{1-2}	0.92	0.63-1.35	0.666
Platelet count			
$\geq 300 \times 10^9$ Vs $<300 \times 10^9$	1.48	0.90-2.44	0.124
PCT (%)			
≥ 18.00 Vs <18.00	1.32	0.92-1.92	0.131
MDW (%)			
≥ 14.15 Vs <14.15	1.48	1.05-2.09	0.027

Abbreviations: CI, confidence interval; PCT, plateletcrit; MDW, platelet distribution width.

function and activation that can be influenced by inflammation. Increased MPV levels were reported in myocardial infarction, acute ischemic stroke and Crohn's disease [11]. Furthermore, MPV is a useful inflammatory marker to differentiate cancer patients from healthy controls [12, 13]. The increase in MPV levels is considered to be related with early diagnosis of gastric cancer but not with TNM stage [12]. However, no significant correlation was observed between MPV and TNM stage. There is only one study investigate the association between MPV and pancreatic cancer. In

2011, Karaman et al. [14] evaluate the MPV both in pancreatic adenocarcinomas and pancreatic neuroendocrine tumors. There was not a significant difference among the preoperative MPV between resectable pancreatic adenocarcinomas and unresectable pancreatic adenocarcinomas. In our current study, the preoperative MPV did not related with prognosis in pancreatic adenocarcinoma. This is consistent with previous findings [12, 14].

MDW is another platelet parameter which can indicate variation in platelet size and differentially diagnoses thrombocytosis. Elevated MDW is an indication for the anisocytosis of platelets. MDW is an often forgotten platelet indice and clinicians pay less attention than PC and MPV. Recent studies show that MDW is a more specific marker of platelet activation and is associated with vascular damage than PC and MPV [15]. Jindal et al. [16] also reported that higher value of MDW is a special characteristic of diabetics with microvascular complications. In present study, MDW and PC are both related with overall survival. One possible reason is that tumor cell have been not only shown to have the ability of aggregating platelets, but also it can change the platelet shape and function to more aggressive tumor biology. Chronic inflammation has been proposed as an independent

risk factor for the development of pancreatic cancer in a number of important studies [17, 18]. CRP is one of the major proteins helpful in determination of activity of chronic inflammation [19]. It is also a diagnostic factor and independent prognostic factor in pancreatic adenocarcinoma [20]. In this study, PC and MDW were positively related with CRP. Moreover, MDW was significantly higher in patients with thrombocytosis than those without thrombocytosis. Spearman's correlation also revealed that MDW was positively related with PC. Higher level of PC and MDW reflected enhancement activity of bone marrow hematopoietic function. Based on these findings, we speculated that during the process of development of cancer, hematopoietic function of bone marrow could be enhanced by inflammation. Consistent with our study findings, Alexandrakis MG et al. [21] reported that proinflammatory cytokines, such as interleukin-1 beta (IL-1 beta) and interleukin-6 (IL-6), play a significant role in the reactive thrombocytosis. Obesity is another factor which will play important role during pancreatic cancer development. In a multicenter retrospective study, Kasenda B et al. [22] found that increasing BMI was significantly associated with worse survival prognosis (HR 1.22, 95% CI 1.04-1.41, P=0.012). Accumulative evidence shows that obesity is associated with oxidative stress and chronic inflammation [23]. However, it seems there was no relationship between MDW and BMI. Furthermore, BMI was not associated with overall survival. One possible reason is our sample is relatively small. Therefore, further prospective study is needed to confirm the relationship between MDW and BMI.

PCT is a marker of total platelet mass, which also related with platelet activation. PCT and MPV usually changed in the same direction. PCT could be used as a marker in the discrimination of autoimmune gastritis and functional dyspepsia patients [24]. In our study, increased PCT is related with worse prognosis in univariate analysis. The CR (complete response), PR (partial response), SD (stable disease) and PD (progressive disease) were assessed at an interval of at least 4 weeks after treatment to confirm the objective response. CR and PR were more common in the low PCT group (PCT <18.00%). This might explain the lower mortality in this group compared with the high PCT group (PCT ≥18.00%).

In conclusion, the present study suggests that high MDW and PCT are associated with worse prognosis in pancreatic adenocarcinoma. MDW is a new novel independent prognostic biomarker for overall survival in pancreatic adenocarcinoma.

Disclosure of conflict of interest

None.

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