

Neutrophils assist the metastasis of circulating tumor cells in pancreatic ductal adenocarcinoma

A new hypothesis and a new predictor for distant metastasis

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Abstract

During our research on circulating tumor cells (CTCs) derived from tumor-adjacent vessels in pancreatic ductal adenocarcinoma (PDAC), we found that CTCs are sometimes surrounded by white blood cells (WBCs) in blood. We hypothesize that such interaction between WBCs and CTCs in blood is a mechanism by which WBCs assist in the metastasis of CTCs. We present our laboratory finding, with our evaluation of the association between the neutrophil-to-lymphocyte ratio (NLR, the most investigated clinical parameter of WBCs) and distant metastasis after curative surgery in PDAC. The laboratory finding was presented through immunofluorescence. In the clinical segment, we performed a retrospective study on PDAC patients with distant metastasis after curative surgery who were referred to Peking University Third Hospital between 2005 and 2014. The data on the possible clinical factors were collected by a retrospective review of the patients' records. Immunofluorescence results showed that CTCs are surrounded by WBCs in tumor-adjacent vessels of PDAC patients. In the clinical segment, 112 (70%) of a total of 160 PDAC patients were found to have developed distant metastases after surgery; among the 112 patients, only 89 had entire data and were enrolled for further analysis (84.3% patients had liver metastasis). No significant association was found between the NLR and overall survival (hazard ratio [HR] = 1.027, 95% confidence interval [CI] 0.723–1.459, $P=0.88$); however, a significant relationship between the NLR and distant metastasis after curative surgery was found on the univariate (HR = 1.641, 95% CI 1.058–2.545, $P=0.027$) and multivariate analyses (HR = 2.15, 95% CI 1.279–3.615, $P=0.004$). Neutrophils might assist in distant metastasis through interaction with CTCs in blood. Moreover, NLR is an effective predictor for distant metastasis after curative surgery for PDAC.

Abbreviations: CTCs = circulating tumor cells, NLR = neutrophil-to-lymphocyte ratio, PC = pancreatic cancer, PDAC = pancreatic ductal adenocarcinoma, PLR = platelet-to-lymphocyte ratio, WBCs = white blood cells.

Keywords: circulating tumor cells, metastasis, neutrophils, pancreatic ductal adenocarcinoma

1. Introduction

Pancreatic cancer (PC) is one of the most aggressive malignancies, and it is a leading cause of cancer-related mortality.^[1,2]

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Pancreatic ductal adenocarcinoma (PDAC) is the most common histological type of PC. Most PC patients are diagnosed at an advanced stage, and above 38% of PDAC patients are found to have metastasis, particularly hepatic metastasis, and the 5-year survival rate of PC is 6%.^[1–3] According to the data of our hospital, approximately 70% of patients died of distant metastasis after curative surgery, and almost all of them died during the next 2 years. It is vital to establish additional prognostic factors to predict metastasis, which could be helpful to the development of therapeutic strategies and the selection of suitable treatment options for individual patients.

Recently, increasing evidence has focused on circulating tumor cells (CTCs), which indicate that CTCs might be responsible for metastasis.^[4–8] During our research on CTCs derived from tumor-adjacent vessels, we found that CTCs sometimes are surrounded by white blood cells (WBCs). We hypothesize that such interaction between WBCs and CTCs in blood is a mechanism by which WBCs assist in the metastasis of CTCs and one of the reasons why most pancreatic cancer metastasis occur in the liver. Neutrophil, one of the most important parts of WBCs, has been recognized as a key participator in metastasis, based on increasing evidence.^[9–12] The understanding of the mechanism of such participation of neutrophils is limited, and support from clinical evidence is lacking.

The neutrophil-to-lymphocyte ratio (NLR), one of the most used clinical parameters for the evolution of neutrophils, was considered a useful marker for the predictor of poor prognosis for pancreatic cancer.^[13–15] Whether NLR could predict the metastasis of PDAC remains unknown. We present our finding

and have performed a retrospective analysis of the predictor value of possible clinicopathological characteristics in the metastasis of PDAC after curative surgery, which included the NLR.

2. Methods

2.1. Ethical statement

The study was approved by the Clinical Ethics Committee of Peking University Third Hospital. The patients' data were analyzed anonymously because written consent was not obtained from all participants.

2.2. Study population and design

All participants were enrolled from Peking University Third Hospital (Beijing, China) from February 2005 to July 2014. The patients who underwent a curative resection and whose diagnoses were confirmed by pathological examination were included in this study. Seven PDAC patients who underwent neoadjuvant chemotherapy or chemoradiation therapy were excluded from the study. The NLR and platelet-to-lymphocyte ratio (PLR) of the 160 PDAC patients were calculated in the database. All the surgical specimens were evaluated pathologically to determine the extent of tumor differentiation, lymph node metastases, and surgical margins following surgery. The pathological PDAC stage was determined according to the American Joint Committee on Cancer (AJCC) 7th Edition.^[16] According to previous studies, the preoperative NLR was calculated as the neutrophil count divided by the lymphocyte count,^[17] and the preoperative PLR was calculated as the platelet count divided by the lymphocyte count.^[18]

For the first 3 years following surgery, the patients were followed up at intervals of at most 3 months. These follow-up visits consisted of a physical examination, laboratory examination including the measurement of tumor markers, and computed tomography (CT) or magnetic resonance imaging. In some cases, ultrasound or positron emission tomography-computed tomography (PET-CT) was also used. From the 3-year time point following surgery, the patients with no sign of metastasis or recurrence were monitored at 3- to 6-month intervals. The patients were followed up until death or December 31, 2015. The metastasis time was measured from the day of surgery to the date of the diagnosis of metastasis. The overall survival time was measured from the day of surgery to the date of death or the last follow-up.

2.3. Diagnosis of postoperative distant metastasis

During the postoperative follow-up, the patients underwent laboratory examinations, including Carbohydrate antigen 19-9, CT scanning, and chest x-ray. If a CT scan or x-ray showed a metastasis, with no definite evidence of metastasis from other cancers or recurrence elsewhere, we characterized the newly developed lesion by ultrasound or PET-CT. Needle biopsy for confirmation of metastasis could not routinely be performed for fear of soiling tumor cells via the needle tract.

2.4. Clinical laboratory tests

A biochemical blood examination, including a complete blood count, was generally performed 1 to 14 days before surgery. Serum was isolated for immediate detection or kept at -20°C for detection later. Surgical specimens were collected and evaluated

by hematoxylin and eosin (H&E) staining and immunohistochemistry (Abcam, ab41825 anticytokeratin 8, 18, and/or 19+ antibody).

2.5. Immunofluorescence cell staining

Portal venous blood (7.5 mL) was harvested in CellSave Preservative Tubes (Cat # 7900005, Janssen Diagnostics, LLC), containing Na₂EDTA and cell preservative for the epithelial cells. The buffy coat was isolated, and the epithelial derived CTCs were magnetically sorted by ferromagnetic labeling with a surface anti-Epithelial cell adhesion molecule antibody. The isolated cells were then stained for intracellular anti-cytokeratins 8, 18, and/or 19 Phycoerythrin antibody, Hoechst, and anti-CD45 Allophycocyanin antibody for leukocyte depletion.

2.6. Criteria for the determination of CTC

CTCs might lose some specific antigen because of epithelial-mesenchymal transition, such as EpCAM.^[19] Therefore, the determination of CTCs should be based on immunofluorescence and morphology. The following are the criteria for the determination of CTCs based on morphology:

Criterion I: nuclear atypia, irregular nuclear shape (including nodular and lobulated), rather than normal neutrophil including rod-shaped, leaf, a single core of horseshoe kidney, or ovoid;

Criterion II: a nuclear mass ratio greater than 0.8;

Criterion III: a cell diameter greater than 15 μm ;

Criterion IV: nuclear hyperchromatism and uneven coloring (because of cancer. The cells had increased chromatin and grain coarsening and were hyperchromatic);

Criterion V: the nuclear membrane became thickened, depressed or wrinkled, and the nuclear membrane presented a zigzag appearance;

Criterion VI: the nucleus chromatin moves to the edge or shows large nucleoli or abnormal fission.

Criteria

Criterion 1: cells stained with (Hoechst+, anti-CD45-) and in line with the above 4 criteria were determined to be CTCs; Criterion 2: cells stained with (Hoechst+, anti-CD45-) and meeting the sixth criterion and in line with the other 2 criteria were determined to be CTCs.

Criterion 3: epithelial cells with consistent morphology that are cytokeratin 8, 18, and/or 19+, Hoechst+ and CD45- are considered to be CTCs.

CTCs were later confirmed based on their Copy number variation and Single nucleotide variants/Insertion deletion profiles (data not shown).

2.7. Statistical analysis

The quantitative results are reported in the form of the means \pm standard deviation. The associations between the clinical and histopathological parameters with Overall survival and metastasis were analyzed using Kaplan-Meier curves and compared by the log-rank test. Univariate and multivariate Cox regression analyses were performed to determine the effects of possible prognostic factors on metastasis after curative surgery. Hazard ratios (HRs) estimated from the Cox analysis were shown as relative risks with corresponding 95% confidence intervals (CIs). All the analyses were conducted using SPSS 22.0 statistical software (SPSS, IL), and $P < 0.05$ was considered statistically significant.

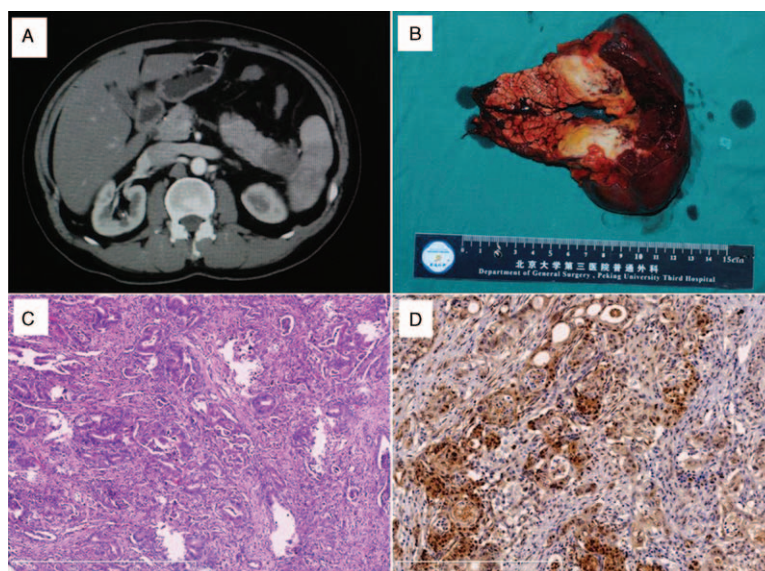


Figure 1. Kaplan–Meier curves for disease-free survival of patients with pancreatic ductal adenocarcinoma by a low versus high platelet–lymphocyte ratio. Neutrophil-to-lymphocyte ratio ≥ 2.5 is associated with poor survival ($P=0.023$).

3. Results

3.1. Patient characteristics

One hundred sixty patients who had undergone a primary attempt of a curative resection for PC were enrolled (R0=123 and R1=37), including 100 males and 60 females ranging in age from 23 to 86 years, with a mean age of 63.4 years. All the patient diagnoses were ultimately confirmed pathologically and clinically (Fig. 1). The clinical factors are summarized in Table 1.

Among the 160 patients, 112 (70%) patients were found to be developing distant metastases after surgery. However, among them, 23 patients were found to lack key clinical data for further analysis. We excluded those patients, and 89 patients remained for the comparison of the clinical variables in relationship to distant metastases after surgery. Among the 89 patients, 75 (84.3%) had liver metastasis, and 13 had other metastases including lung metastasis and bone metastasis. The time to the finding of metastasis after curative surgery and the overall survival time are 8.66 ± 6.15 and 12.75 ± 8.22 months, respectively. Among the 75 liver metastasis cases, 22 cases had additional metastasis or local recurrence. We summarized their characteristics in Table 2.

3.2. Comparison of the clinical variables in relationship to OS after pancreatic resection

In the univariate analysis, greater Carcino Embryonic Antigen (HR = 1.832, 95% CI 1.206–2.783, $P=0.007$), CA-125 (HR = 1.902, 95% CI 1.14–3.175, $P=0.014$), and AJCC-stage (HR = 1.952, 95% CI 1.356–2.810, $P<0.001$) were significant prognostic factors for OS (Table 1). The NLR and PLR were not significant predictors of OS ($P>0.05$ each; Table 1).

3.3. Comparison of the clinical variables in relationship to distant metastasis after pancreatic resection

In the univariate analysis, greater PLR (HR=1.897, 95% CI 1.182–3.047, $P=0.008$) and NLR (HR=1.641, 95% CI 1.058–2.545, $P=0.027$) were significant prognostic factors for

distant metastasis after surgery (Table 3, Figs. 2 and 3). In the multivariate analysis, only a greater NLR was a significant predictor of metastasis (HR=2.15, 95% CI 1.279–3.615, $P=0.004$; Table 3).

3.4. Patients' selection for immunofluorescence

Immunofluorescence cell staining was performed to detect the interaction between neutrophils and CTCs. The patients, who were confirmed as PDAC, were included in the detection of neutrophils and CTCs. A representative patient was found to have a tumor at the tail of the pancreas by CT (Fig. 1A). From the pathological sample removed during surgery, we could see it close to the spleen (Fig. 1B). All the patients were finally confirmed by H&E staining (Fig. 1C) and immunohistochemistry of cytokeratin 8, 18, and/or 19 (Fig. 1D). An intraoperative sample of 7.5 mL of blood from the splenic vein (tumor-adjacent vessel) was obtained before resection of the tumor.

3.5. Identification of neutrophils and CTCs and their interaction

The ideal determination of CTC is Criterion 3, which is an epithelial cell with consistent morphology showing cytokeratin 8, 18, and/or 19+ and Hoechst+ (Fig. 4A). However, tumor cells might lose some specific antigen because of the transformation of the epithelial mesenchyme, and the Criteria 1 and 2 also accepted for those were Hoechst+ (Fig. 4B) and CD45– (Fig. 4C). Hence, from Fig. 4D, there is a cell cluster and 2 single cells, from the comparison of Fig. 4E and F, and the CTCs were Hoechst+ and CD45–. There were CTCs surrounded by WBCs (the CTCs were marked by yellow arrows and the WBCs were marked by red arrows). Such interactions between the CTCs and WBCs are common in tumor-adjacent vessels of PDAC patients.

4. Discussion

Our data show that distant metastasis was the main cause (counted 70%) of death for PDAC patients after curative surgery,

Table 1**Patient characteristics and overall survival.**

Parameter	Mean	SD	Number	HR (95% CI)	P
Age, y	63.163	10.310			
<65			86		
≥65			74	1.049 (0.736–1.495)	0.792
Gender					
Male			100		
Female			60	0.788 (0.557–1.115)	0.178
Albumin, g/dL	40.625	5.564			
<3.5			18		
≥3.5			132	0.884 (0.506–1.545)	0.666
Missing			10		
CEA, ng/mL	5.082	7.293			
<5			106		
≥5			32	1.832 (1.206–2.783)	0.007
Missing			22		
CA 19-9, U/mL	429.920	691.324			
<39			25		
≥39			118	1.384 (0.863–2.221)	0.178
Missing			17		
CA 125, U/mL	21.876	26.837			
<35			120		
≥35			18	1.902 (1.14–3.175)	0.014
Missing			22		
Differentiation					
Well-to-moderate			76		
Others			75	1.122 (0.787–1.599)	0.524
Missing			9		
AJCC-stage (IA/IB/IIA/IIB/IIIA/IV)		(7/19/43/85/2/4)			
<IIB			69		
≥IIB			91	1.952 (1.356–2.810)	< 0.001
Missing					
R					
R0			123		
R1			37	1.316 (0.878–1.972)	0.184
Missing					
PLR	164.951	83.079			
<150			81		
≥150			73	1.030 (0.726–1.462)	0.867
Missing			6		
NLR	3.154	1.945			
<2.5			70		
≥2.5			84	1.027 (0.723–1.459)	0.88
Missing			6		

AJCC = American Joint Committee on Cancer, CI = confidence interval, HR = hazard ratios, NLR = neutrophil-to-lymphocyte ratio, PLR = platelet-to-lymphocyte ratio, SD = Standard deviation.

particularly liver metastasis (accounting for 84.3% of all distant metastasis cases), which has been seldomly reported. Therefore, a thorough understanding of distant metastasis after curative surgery is of great importance. CTCs have long been recognized as the seeds of metastasis in PC,^[4,5] and neutrophils have been indicated as mediators of metastasis only recently.^[9–11,20] Such a mediator role by neutrophils lacks clinical proof. We performed our retrospective investigation and found that there was no significant association between the NLR and overall survival (HR = 1.027, 95% CI 0.723–1.459, $P=0.88$), which is a different report from that of many other clinical studies.^[13–15] Such disunity might be because of the difference in patients. A significant relationship between the NLR and distant metastasis after curative surgery was found in both the univariate (HR = 1.641, 95% CI 1.058–2.545, $P=0.027$) and multivariate analyses (HR = 2.15, 95% CI 1.279–3.615, $P=0.004$). Therefore, the NLR might be an effective indicator for metastasis after

curative surgery in PDAC, which in turn might indicate that neutrophils assist in the process of metastasis.

Many mechanisms have recently been proposed concerning neutrophils assisting in metastasis.^[9–11] Wculek and Malanchi^[11] clarified the role of mature neutrophils as mediators of metastatic initiation, which could derive leukotrienes that aid the colonization of distant tissues by selectively expanding the subpool of cancer cells that retain high tumorigenic potential. Zhang et al's^[12] study suggests that the abundance of circulating tumor-associated neutrophils in advanced cancer patients contributes to circulating tumor cell survival and metastasis by suppressing peripheral leukocyte activation. During our research of CTCs isolated from a cancer-adjacent vein, we found that CTCs were surrounded by WBCs in some cases. As the major segment of WBCs, neutrophils have been demonstrated to have a crucial role in tumor metastatic progression; however, such contribution is multifaceted and contradictory. Neutrophils

Table 2
Patient characteristics of metastasis patients.

Characteristic	Mean	SD	Range
Age, y	63.4045	11.54706	23–86
Gender (male/female)	58/40		
Location of primary tumor (head/body–tail)	41/27		
AJCC-stage (I A/I B/II A/II B/III/IV)	(0/11/23/50/2/2)		
Location of metastasis (liver/liver and others/others)	53/22/13		
Platelet, ×103/mL	199.7303	61.0934	64–443
Lymphocyte, ×103/mL	1.4748	0.51908	0.45–2.7
Neutrophil, ×103/mL	3.909	1.75705	0.84–14.38
Albumin, mg/dL	40.5301	4.92224	31.4–61
PLR	153.6865	77.49639	52.5–402.73
PNR	3.0957	2.10437	0.61–11.53
CEA, ng/mL	4.5585	6.65587	0.58–45.97
CA 125, U/mL	21.1628	28.45597	3.12–244
CA 199, U/mL	532.4289	934.108	0.6–5381
Tumor size, cm	3.8341	1.72666	1.2–10.5

AJCC = American Joint Committee on Cancer, PLR = platelet-to-lymphocyte ratio, SD = Standard deviation.

promote tumor development by mediating the initial angiogenic switch and facilitating colonization of circulating tumor cells; in addition, neutrophils have cytotoxic and antimetastatic capabilities.^[21] Another study indicated that polymorphonuclear neutrophils (PMNs, the largest segment of neutrophils) could specifically recognize and kill cancerous cell; however, it also showed that PMNs exhibit poor cancer-killing activity in cancer patients compared to the cytolytic activity of average healthy donors.^[22] We hypothesize that neutrophils might try to kill CTCs by attaching and surrounding them; however, they do not have the capability of killing them in PDAC patients, and cluster

of CTCs formed surrounded by neutrophils. The surrounding WBCs might form a protective cover for CTCs from other killers in blood as well as serve as mediators between CTCs and the endothelium.^[20] By surrounding WBCs, neutrophils become metastasis assistants, and highly active CTCs might have an opportunity to seed in the liver or lung metastasize, which is different from the abovementioned mechanisms.^[9–11,21]

It has been reported that through interaction with neutrophils, CTCs could be brought to the endothelium, which is an essential step in hematogenous metastases.^[20] Studies have indicated that CTCs could activate and adhere to neutrophils directly through integrin.^[23,24] Integrin $\alpha V/\beta 3$, which is expressed by CTCs, could bind to surface receptors of neutrophils.^[25–28] In addition, research showed that its antibody

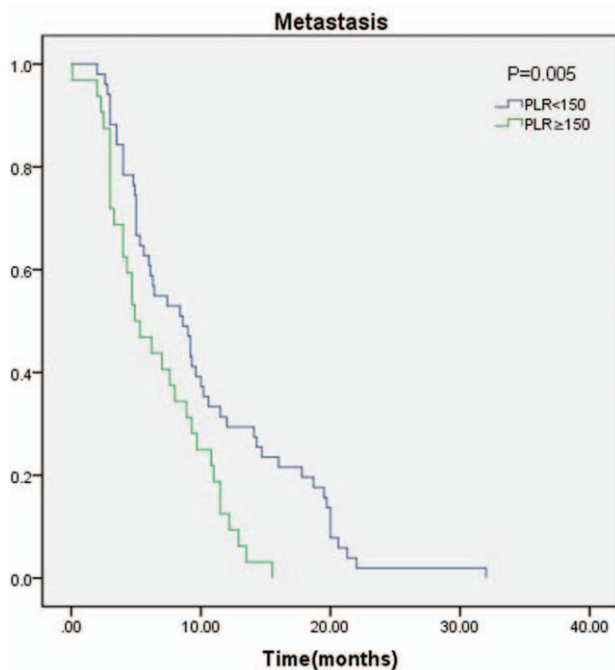


Figure 2. A representative patient was found to have a tumor at the tail of the pancreas by computed tomography (A). From the pathological sample removed during surgery, we could observe it close to the spleen (B). The pancreatic ductal adenocarcinoma diagnosis was confirmed by hematoxylin and eosin staining (C) and immunohistochemical staining of cytokeratin 8, 18, and/or 19 (D).

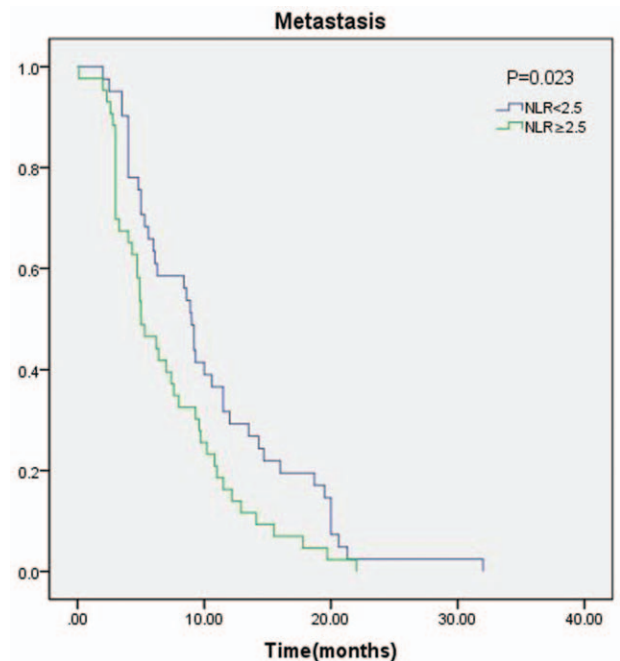


Figure 3. Kaplan–Meier curves for the overall survival of patients with pancreatic ductal adenocarcinoma by a low versus high platelet–lymphocyte ratio. Platelet-to-lymphocyte ratio ≥ 150 is associated with poor survival ($P=0.005$).

could reduce tumor cell adhesion in in vivo models of colon metastasis to the liver.^[29,30] In addition, neutrophils could enhance indirect binding through paracrine of interleukin-8 and matrix metalloproteinase.^[31,32] Neutrophils also play a key role in adhesion of CTCs to the endothelium. Neutrophils would be “primed” when exposed to the inflammatory cytokine IL-8, activating L-selectin, and N-cadherin and rolling along endothelial cells slowly and binding to inflamed endothelium.^[33–36] Primed neutrophils would also activate integrin $\alpha M/\beta$ (Macrophage-1 antigen) in response to IL-8.^[37–40] Rolling neutrophils with sufficient activation would bind to an endothelial cell if the endothelial cell is also sufficiently activated.^[41,42] Therefore, neutrophils might serve as mediators of the CTCs and endothelial cells, promoting tumor metastasis.

This study has 2 parts, the clinical part and the laboratory part. In the clinical part, our study is hospital-based in a single institution, not a population-based study. This design might have introduced a selection bias because of differential referral patterns. However, such a limitation was outweighed by the strength of the design. We do not conclude that we introduced an ascertaining bias for misdiagnosis because all the patients had pathologically and clinically confirmed PDAC as did the representative patient shown in Fig. 1. However, the loss of information on some clinical factors in a small number of the participants, as well as the small size of the number of patients who were found to have distant metastasis after surgery,

precluded us from estimating the magnitude of the PDAC risk for metastasis associated with these factors. Although our study has a relatively small sample size, the incidence of metastasis is high in PDAC after surgery, and given that this is a major focus of this study, the sample size is probably adequate.

In the laboratory segment, we found a very interesting phenomenon. During our research on CTCs derived from tumor-adjacent vessels (we first sampled blood from tumor-adjacent vessels), we found that CTCs were sometimes surrounded by WBCs. Although we failed to obtain a more representative picture of surrounded CTCs positive for cytokeratin 8, 18, and/or 19+, our result matched Criterion 1 and is convincing. Knowing whether the WBCs tried to help or kill the CTCs is difficult. Regardless of the action of the WBCs, such surrounding provides protection for CTCs from other killers in blood, if the WBCs did not have an effective attack on CTCs in vessels through such interaction. Many previous studies have shown that neutrophils serve as a mediator between CTCs and endothelial cells promoting tumor metastasis. Coupled with the clinical outcome above, we suggest that WBCs, predominantly the neutrophils, assist in distant metastasis by interacting with CTCs in blood. Such a hypothesis is immature and requires further demonstration.

The NLR is an effective predictor for distant metastasis after curative surgery for PDAC. One of the mechanisms by which neutrophils assist distant metastasis might be by surrounding

Table 3**Univariate and multivariate analysis of clinicopathologic variables in relation to metastasis after curative operation.**

Parameter	N	Univariate analysis		Multivariate analysis	
		HR (95% CI)	P	HR (95% CI)	P
Age, y			0.727		0.515
<65	46				
≥65	42	1.079 (0.703–1.657)		1.182 (0.714–1.955)	
Gender			0.311		0.163
Male	58				
Female	40	0.789 (0.5–1.248)		1.481 (0.853–2.57)	
Albumin, g/dL			0.337		0.917
<3.5	12				
≥3.5	76	0.719 (0.367–1.409)		1.055 (0.389–2.86)	
CEA, ng/mL			0.342		0.823
<5	67				
≥5	21	1.303 (0.755–2.249)		1.085 (0.532–2.214)	
CA 19-9, U/mL			0.653		0.741
<39	14				
≥39	74	1.154 (0.617–2.16)		1.131 (0.545–2.347)	
CA 125, U/mL			0.99		0.646
<35	74				
≥35	14	0.995 (0.477–2.078)		1.227 (0.513–2.933)	
Differentiation			0.309		0.428
Well-to-moderate	48				
Others	40	1.257 (0.809–1.955)		0.814 (0.489–1.354)	
AJCC-stage			0.614		0.693
<II B	34				
≥II B	54	1.119 (0.722–1.734)		0.896 (0.52–1.544)	
R			0.239		0.298
R0	74				
R1	14	1.43 (0.788–2.592)		0.691 (0.344–1.387)	
PLR			0.008		0.539
<150	53				
≥150	35	1.897 (1.182–3.047)		1.246 (0.617–2.519)	
NLR			0.027		0.004
<2.5	41				
≥2.5	47	1.641 (1.058–2.545)		2.15 (1.279–3.615)	

AJCC = American Joint Committee on Cancer, CI = confidence interval, HR = hazard ratios, NLR = neutrophil-to-lymphocyte ratio, PLR = platelet-to-lymphocyte ratio.

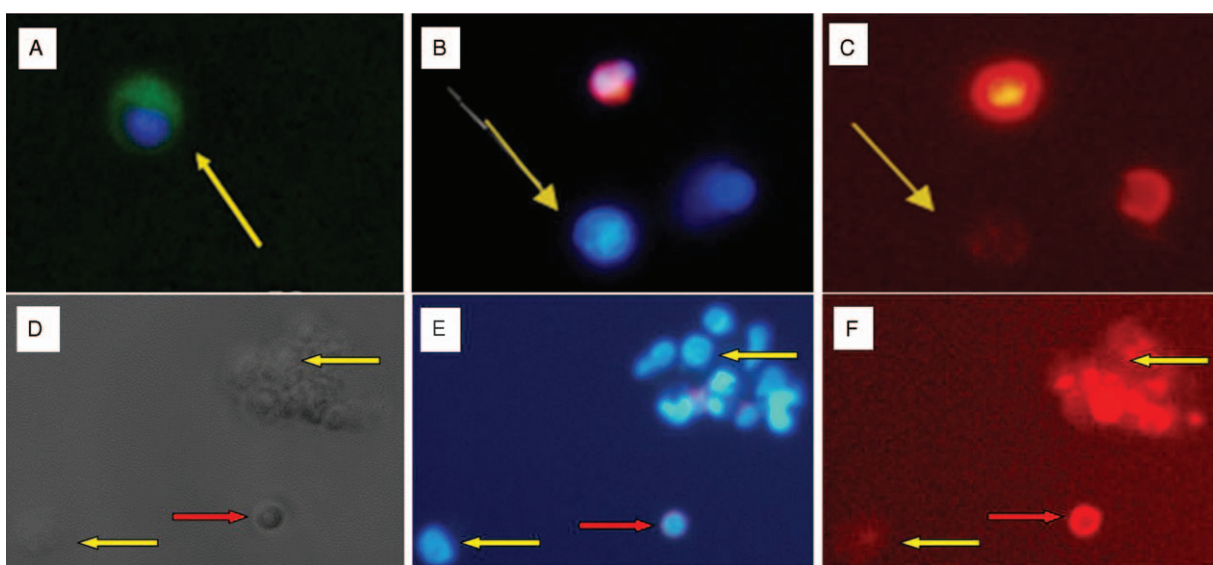


Figure 4. The ideal determination of circulating tumor cell is an epithelial cell with consistent morphology of cytokeratin 8, 18, and/or 19+ (green) and Hoechst+ (blue, A). However, some tumor cells might lose some specific antigen because of the process of epithelial–mesenchymal transition. Combined Hoechst+ (blue, B) and CD45– (red, C) plus the morphological characteristics also indicate the determination of CTCs. Among a cell cluster and 2 single cells (D), the CTCs that were Hoechst+ (E) and CD45– (F) were surrounded by white blood cells (WBCs) (the CTCs were marked by yellow arrows, the WBCs were marked by red arrows).

CTCs as protectors or mediators. The PDAC patients, who had a high NLR value before surgery, might be helped by planting a net screen in vessels or applying effective drugs postoperatively to reduce distant metastasis, which might be induced by the interaction between WBCs and CTCs.

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