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ORIGINAL ARTICLE

Thrombocytosis and immunohistochemical expression of connexin 43 at diagnosis predict survival in advanced non-small-cell lung cancer treated with cisplatin-based chemotherapy

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Abstract
Purpose Patients with advanced non-small-cell lung cancer (NSCLC) have poor survival, and platinum-based chemotherapy agents are the standard first-line chemotherapy agents for advanced NSCLC. This study aimed to identify predictive factors associated with the response to chemotherapy and survival in 258 patients with advanced NSCLC treated with platinum-based chemotherapy.
Methods Stage IIIA–IV NSCLC patients diagnosed in Kaifeng second people's hospital (Henan, China) between March 2002 and September 2011 were retrospectively reviewed. All of the patients had received platinum-based chemotherapy, and patients were followed up to date of death or last follow-up to obtain data of response to chemotherapy and survival. Potential prognostic factors such as gender, age, tumor size, tumor type, histologic stage, anemia, calcium levels, ECOG performance status (PS), thrombocytosis, TTF-1, p63, and connexin 43 were analyzed. Response to chemotherapy, overall survival (OS) and progression-free survival (PFS) were calculated by the Kaplan–Meier method and Cox regression model.
Results A univariate analysis indicated that thrombocytosis and connexin 43 were found to be significant prognostic factors ($p < 0.001$) and ECOG PS, Hb levels, and p63 presented a tendency toward association with survival. Kaplan–Meier survival showed that the mean OS and PFS in chemotherapy responders with connexin 43 $\geq+2$ were significantly longer than in chemotherapy responders with connexin 43 $\leq 1+$. In contrast, thrombocytosis was associated with increased mortality and resistance to chemotherapy in chemotherapy responders. In addition, all 21 patients of the 5-year OS were from chemotherapy responders with connexin 43 $\geq+2$ or non-thrombocytosis.
Conclusions Thrombocytosis and connexin 43 absence may be reliable surrogate markers for the prediction of chemotherapy response and prognosis for patients with advanced NSCLC, and assessment of these factors may identify a population of patients with advanced NSCLC that is likely to have a prolonged life expectancy.

Keywords Advanced non-small-cell lung cancer · Chemotherapy response · Survival · Thrombocytosis · Connexin 43

Introduction

Lung cancer remains the most common cause of cancer death worldwide, and non-small-cell lung cancer (NSCLC) constitutes about 85 % of all lung cancers [1]. Despite recent improvements in diagnostic technologies, approximately 50 % of patients diagnosed with NSCLC present advanced disease (stage III or IV), the absolute overall survival (OS) and the prognosis still remain poor because local and distant failures are common [2]. Although surgery can be curative at the early stages of NSCLC, the majority of patients with advanced stage NSCLC is not amenable to curative resection at diagnosis [3]. Undoubtedly, prediction

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of chemotherapy response and survival in the case of advanced NSCLC is instrumental in that it could shed light on the interpretation and design of future clinical trials. The current challenge is to define prognostic determinants of NSCLC and incorporate them into existing treatment regimens to improve therapeutic gain.

Many studies have reported predictive models for chemotherapy response and survival in advanced cancers, and the literature on prognostic factors (PFs) in lung cancer is exponentially increasing [4]. Up to now, most widely accepted prognostic determinants are disease stage and performance status [5]. Other PFs have been commonly reported, mainly gender, age, histology, hemoglobin level, lactate dehydrogenase level (LDH), lymphocyte count, interleukin 6 level, and tumor characteristics [6]. Obviously, the identification of adequate PFs in advanced stage NSCLC could delineate more homogeneous groups of patients with similar prognosis and give individual guidance for the clinicians in the decision-making process of choice of treatment options.

Platinum-based chemotherapy agents are the standard first-line chemotherapy agents for advanced NSCLC [7]. However, in clinical practice, the chemotherapy response varies wildly among individuals [8]. Although multiple studies examining factors individually may be helpful in predicting survival among patients with advanced lung cancer, results from different studies are inconsistent with one another, and currently proposed biomarkers still lack predictive power in order to be used for pre-therapeutic decision-making [9]. Therefore, the identification of predictive markers for chemotherapy response and survival is most clinically warranted to further improve the efficacy of chemotherapy. This study aimed to identify clinical and biological variables as outcome predictors for chemotherapy response and OS in patients with advanced NSCLC.

Materials and methods

Patients' selection

The investigation was a retrospective study. The study population consisted of 258 patients diagnosed with advanced NSCLC (stages IIIA–IV, not resectable) including 95 squamous cell carcinomas (SCC) and 163 adenocarcinomas (AC) at Kaifeng second people's hospital (Henan, China) from March 2002 to September 2011. All patients underwent chest and abdominal computed tomography (CT), brain magnetic resonance imaging (MRI), and bone scintigraphy at the first presentation for the evaluation of clinical staging. Diagnoses were confirmed by bronchoscopy or CT-guided lung biopsy; histologic diagnosis and grade of differentiation were assigned in accordance with the WHO criteria for lung tumors, and pathologic stage was based on the revised international system [10]. All patients were treated according to the standards applied to platinum-based chemotherapy, and none of them had malignant pleural effusion, severe acute or chronic inflammatory diseases, chronic liver diseases, and chronic renal failure. The last follow-up was performed on September 30, 2011.

Data collection

Information including age, gender, tumor size, performance status (using the Eastern Cooperative Oncology Group scale, ECOG PS), pathological stage, laboratory date (complete blood accounts, albumin, urine and kidney function) at diagnosis, date of death or last follow-up, and chemotherapy regimens was collected from the medical records. The paraffin-embedded tissue specimens from all of two hundred and fifty-eight patients were retrospectively investigated and were cut into 4-μm sections for immunohistochemical analysis (i.e., TTF-1, p63, connexin 43). Anemia was defined as hemoglobin of less than 11 g/dL; leukocytosis, white cell count over 10,000/μL; thrombocytosis, platelet count over 400,000/μL; hypoalbuminemia, serum albumin level of less than 3.5 g/dL; Hypercalcemia, serum calcium over 12 mg/dL; and chronic liver diseases, aspartate aminotransferase (AST) level over 37 U/L or alanine aminotransferase (ALT) level over 41 U/L. TTF-1 staining was assessed by the nuclear staining intensity relative to the staining intensity of type II pneumocytes as: 0, absent staining; 1+, weak staining; 2+, intermediate staining intensity; 3+, strong staining intensity. p63 expression was assessed by an staining intensity as follows: normal, absent or weak staining; overexpressed, intermediate or strong staining. Connexin 43 was assessed by the percentage of positive tumor cells as follows: 0, negative; 1+, <10 %; 2+, 10–50 %; 3+, >50 %. The primary data sources for abstraction were hand-written medical records, usually completed at the monthly patient visit.

Chemotherapy regimens and therapeutic effect evaluation

All the patients received cisplatin-based chemotherapy, including NP/NC regimens (vinorelbine plus carboplatin), GP/GC regimens (gemcitabine plus carboplatin), and TP/TC regimens (paclitaxol plus carboplatin). Dosage regimen: carboplatin AUC 4 g on day 1; vinorelbine 25 mg/m^2 on day 1 and day 8; gemcitabine 1 g/m^2 on day 1 and day 8; paclitaxol 175 mg/m^2 on day 1. All chemotherapeutic drugs were administered intravenously, and treatment cycles were repeated every 3 weeks for a maximum of 6 cycles. Patient responses to treatment were determined after four cycles by

the WHO criteria, which classify the response into four categories: complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD). CR was defined as complete disappearance of all measurable lesions. PR required at least 50 % reduction in measurable lesions. Patients with SD had less than a 50 % decrease or no more than a 25 % increase in the size of measurable lesions. PD was assigned to patients when measurable lesions increased by more than 25 % or new lesions appeared. For data analysis, CR and PR were combined as responders, and SD and PD were grouped as non-responders.

Follow-up

All patients were followed up by telephone, outpatient interviews, and letters every 6 weeks until death. Follow-up mainly included whether the patient died, with or without disease progression.

Statistical analysis

Overall survival was defined as the time from histologic diagnosis to the date of death whatever the cause, progression-free survival (PFS) was defined as the time from histologic diagnosis to progression disease. Survival curves for PFS and OS were constructed using the Kaplan–Meier method, and log-rank tests were carried out to evaluate differences between groups. Univariate logistic regression analyses followed by multiple logistic regression analyses were applied to evaluate the role of clinicopathological parameters, and Cox proportional hazards regression model was performed for univariate and multivariate analysis to produce hazard ratios (HRs) and 95 % confidence intervals (95 % CI). Factors included in the univariate analysis were gender, age, tumor size, tumor type, histologic stage, anemia, calcium levels, ECOG PS, thrombocytosis, TTF-1, p63, and connexin 43. All statistical tests were two-tailed, and $p < 0.05$ was considered significant. The SPSS software package, version 17.0 (SPSS Inc, Chicago, IL, USA) was used for statistical analysis.

Results

Patient clinical characteristics

The clinical and pathological characteristics of the series are shown in Table 1. The patients consisted of 195 males and 63 females, with a median age of 53 years (range, 31–75 years). The patients had a PS of 0–3. Regarding disease stage, 167 patients (64.7 %) had stage IIIA/IIIB disease and 91 (35.3 %) had stage IV disease. Histologically, 163 patients (63.2 %) had AC and 95 (36.8 %) had SCC. There were no significant differences between male and female patients in stage and histologic distribution. Median follow-up was 38 (3–86) months. Up to September 30, 2011, 252 (97.7 %) died of lung cancer, 3 (1.2 %) died of other causes, and 3 (1.2 %) were still alive. The 3-year OS rate was 31.4 % (81/258) and the 5-year OS rate was 8.1 % (21/258), with a median OS time of 20 months (range 6–95 months), and a median PFS time of 4 months (range 1–71 months) (Tables 2, 3).

Relationship between investigated factors and prognosis in patient

A univariate analysis indicated that thrombocytosis and connexin 43 were found to be significant prognostic factors ($p < 0.001$) and ECOG performance status, Hb levels, and p63 presented a tendency toward association with survival ($p < 0.20$), whereas gender, age, tumor size, tumor type, histologic stage, TTF-1, and calcium levels had no relationship with prognosis (Table 2). The statistically significant indicators in univariate analysis were introduced into the Cox proportional hazards model to perform multivariate analysis. In the multivariate analysis, the independent factors for a favorable prognosis were connexin 43 $\geq$+2, and the independent factors for poor prognosis were thrombocytosis. In subgroup analysis by histology, the associations between platelet count or connexin 43 and OS or PFS were statistically similar in overall advanced NSCLC (Table 3).

Impact of connexin 43 immunostaining on survival

As shown in Table 3, the 1-year PFS rate was 42.0 % (34/81) for connexin 43 $\geq$+2 patients, compared to 14.0 % (25/177) for connexin 43 $\leq$1+ patients ($p < 0.001$). The 3-year OS rate was 63.0 % (51/81) for connexin 43 $\geq$+2 patients, compared to 16.9 % (30/177) for connexin 43 $\leq$1+ patients ($p < 0.001$). All 21 patients of the 5-year OS were from connexin 43 $\geq$+2 patients, and 25.6 % (21/81) of the 5-year OS rate was significantly more than 8.1 % (21/258) in all of patients ($p < 0.001$). Kaplan–Meier survival showed that the mean OS and PFS in the patients with connexin 43 $\geq$+2 were significantly longer than that in the patients with connexin 43 $\leq$1+ (45.6 ± 2.2 and 14.2 ± 1.3 months vs 19.3 ± 0.8 and 4.3 ± 0.3 months) (Fig. 1a, b). We then analyzed the relationship between connexin 43 staining and clinicopathological factors. The rate of connexin 43 $\leq$1+ was 72.6 % (69/95) and 66.3 % (108/163) in squamous carcinoma and adenocarcinoma, respectively, and no significant difference in connexin 43 expression was observed between AC and SCC patients

Table 1 Clinical and pathologic characteristics of patients with ($n = 258$)

Characteristics	AC		SCC		p
	No.	(%)	No.	(%)	
Median age (years), (range)	49 (31–72)	63.2 (163/258)	56 (34–75)	36.8 (95/258)	0.175[a]
≥55	90	55.2 (90/163)	33	34.7 (33/95)	
<55	73	44.8 (73/163)	62	65.3 (62/95)	0.001[a]
Gender					
Male	119	73.0 (119/163)	76	80.0 (76/95)	
Female	44	27.0 (45/163)	19	20.0 (19/95)	0.207[a]
Tumor size					
≤3 cm	92	56.4 (92/163)	44	46.3 (44/95)	
>3 cm	71	45.6 (71/163)	51	54.7 (51/95)	0.116[a]
Stage of disease					
IIIA/IIIB	104	63.8 (104/163)	63	66.3 (63/95)	
IV	59	36.2 (59/163)	32	33.7 (32/95)	0.684[a]
PS					
≤1	80	49.1 (80/163)	43	45.3 (43/95)	
≥2	83	50.9 (83/163)	52	54.7 (52/95)	0.554[a]
TTF-1					
≤1+	129	79.1 (129/163)	19	20.0 (19/95)	
≥2+	34	20.9 (34/163)	76	80.0 (76/95)	<0.001[a]
p63					
Normal	29	17.8 (29/163)	22	23.2 (22/95)	
Overexpression	134	82.2 (134/163)	73	76.8 (73/95)	0.297[a]
Connexin 43					
≤1+	108	66.3 (108/163)	69	72.6 (69/95)	
≥2+	55	33.7 (18/163)	26	27.4 (26/95)	0.287[a]
PLT					
Thrombocytosis	99	60.7 (99/163)	52	54.7 (52/95)	
Non-thrombocytosis	64	39.38 (64/163)	43	45.3 (43/95)	0.345[a]
Hb					
Anemia	73	44.8 (73/163)	36	37.9 (36/95)	
Non-anemia	90	55.2 (90/163)	59	62.1 (59/95)	0.280[a]
Serum calcium					
Hypercalcemia	54	33.1 (54/163)	31	32.6 (31/95)	
Non-hypercalcemia	109	66.9 (109/163)	64	67.4 (64/95)	0.935[a]

AC adenocarcinoma, *SCC* squamous cell carcinomas, *PS* performance status (ECOG), *TTF-1* thyroid transcription factor 1, *PLT* platelet, *Hb* hemoglobin

[a] Pearson's Chi squared test

($p > 0.05$). There was no significant correlation between connexin 43 expression and gender or age ($p > 0.05$, data not shown). In order to clarify if the reduced connexin 43 expression was associated with patients' prognoses, we also employed the Cox's proportional hazard regression model, and the result showed that reduced connexin 43 expression (≤1+) was a hazard factor for OS and PFS of patients with advanced NSCLC (HR = 5.81 with 95 % CI 4.13–8.20 for OS and 3.27 with 95 % CI 2.38–4.46 for PFS, Fig. 1c, d).

Association of thrombocytosis with OS and PFS

The estimated survival distributions were calculated by the Kaplan–Meier method. As shown in Table 3, the median survival time was 36 months (range 13–95 months) for 107 patients with non-thrombocytosis, compared to 14 months (range 6–46 months) for 151 patients with thrombocytosis. The 3-year OS rate was 49.5 % ($n = 53$) for 107 patients with non-thrombocytosis, compared to 18.5 % ($n = 28$) for 151 patients with thrombocytosis. Figure 2 illustrates

Table 2 Univariate analysis of prognostic factors affecting OS and PFS in patients with advanced NSCLC

Variables	Cases	OS (months)				PFS (months)			
		Median	95 % CI	HR	*p*	Median	95 % CI	HR	*p*
Total	258	20	17.0–23.0			4	3.4–4.6		
Age									
≥55	123	20	15.9–24.1			4	3.0–5.0		
<55	135	21	16.7–25.3	1.097	0.727[b]	4	3.4–4.6	1.111	0.519[b]
Gender									
Male	195	19	14.6–23.4			4	3.2–4.8		
Female	63	22	19.2–24.8	1.267	0.822[b]	4	3.0–5.0	0.935	0.885[b]
Pathologic type									
SCC	95	17	12.2–21.8			4	2.9–5.1		
AC	163	21	18.0–24.0	0.924[a]	0.324[b]	4	3.3–4.7	1.580[a]	0.058[b]
Tumor size									
≤3 cm	136	19	15.2–22.8			4	3.1–4.9		
>3 cm	122	21	17.1–24.9	1.136[a]	0.747[b]	4	3.0–5.0	1.107[a]	0.586[b]
Stage of disease									
IIIA/IIIB	167	21	17.0–25.0			4	3.2–4.8		
IV	91	20	15.7–24.3	0.873[a]	0.206[b]	4	3.2–4.8	1.137[a]	0.493[b]
PS									
≤1	123	19	14.7–23.3			3	2.2–3.8		
≥2	135	21	17.0–25.0	0.856[a]	0.112[b]	4	3.0–5.0	0.917[a]	0.166[b]
TTF-1									
≤1+	148	22	18.5–25.5			4	3.0–5.0		
≥2+	110	16	12.4–19.6	0.880[a]	0.811[b]	3	2.1–3.9	0.959[a]	0.363[b]
p63									
Normal	102	16	12.5–19.5			3	2.0–4.0		
Overexpression	156	22	18.9–27.1	0.824[a]	0.107[b]	4	3.0–5.0	0.604[a]	0.127[b]
Connexin 43									
≤1+	177	15	14.0–16.0			3	2.7–3.3		
≥2+	81	43	38.2–47.8	0.172[a]	<0.001[b]	10	8.4–11.6	0.306[a]	<0.001[b]
PLT									
Thrombocytosis	151	14	13.1–14.9			2	1.7–2.3		
Non-thrombocytosis	107	36	30.5–41.5	0.241[a]	<0.001[b]	10	7.6–12.4	0.288[a]	<0.001[b]
Hb									
Anemia	109	19	15.9–22.1			3	1.9–4.1		
Non-anemia	149	22	18.0–26.0	0.931[a]	0.100[b]	4	3.3–4.7	0.939[a]	0.188[b]
Serum calcium									
Hypercalcemia	85	19	14.5–23.5			3	1.9–4.1		
Non-hypercalcemia	173	21	16.9–25.1	0.789[a]	0.216[b]	4	3.3–4.7	0.756[a]	0.188[b]
Chemotherapy									
Responders	85	43	40.7–45.3			12	11.4–12.5		
Non-responders	173	15	14.0–16.0	4.71[a]	<0.001[b]	3	2.8–3.2	4.00[a]	<0.001[b]

OS overall survival, *PFS* progression-free survival, *HR* hazard ratios, *95 % CI* 95 % confidence intervals

[a] HR adjusted for age, gender

[b] Log-rank (Mantel–Cox) test

patient OS and PFS according to platelet count. The thrombocytosis in patients with advanced NSCLC shows a prognostic significance for OS and PFS on Kaplan–Meier and log-rank test analyses (Fig. 2a, b, $p < 0.001$), and the 1-year PFS rate was 46.7 % (50/107) for patients with non-thrombocytosis and 6.0 % (9/151) for patients with

Table 3 Multivariate analysis using the Cox proportional hazards model for OS and PFS in patients with advanced NSCLC

Variables	OS					PFS			
	3 years	5 years	HR	95 % CI	*p*	1 years	HR	95 % CI	*p*
Total									
Thrombocytosis	18.5 % (28/151)	0 (0/151)				6.0 % (9/151)			
Non-thrombocytosis	49.5 % (53/107)	19.6 % (21/107)	0.241[a]	0.179–0.324	<0.001[b]	46.7 % (50/107)	0.288[a]	0.215–0.385	<0.001[b]
Connexin 43									
≤1+	16.9 % (30/177)	0 (0/177)				14.0 % (25/177)			
≥2+	63.0 % (51/81)	25.9 % (21/81)	0.172[a]	0.122–0.242	<0.001[b]	42.0 % (34/81)	0.306[a]	0.224–0.420	<0.001[b]
SCC									
Thrombocytosis	11.5 % (6/52)	0 (0/52)				3.8 % (2/52)			
Non-thrombocytosis	62.8 % (27/43)	27.9 % (12/43)	0.207[a]	0.123–0.347	<0.001[b]	60.5 % (26/43)	0.229[a]	0.135–0.389	<0.001[b]
Connexin 43									
≤1+	20 % (14/70)	0 (0/70)				17.1 % (12/70)			
≥2+	76 % (19/25)	48 % (12/25)	0.133[a]	0.063–0.281	<0.001[b]	64 % (16/25)	0.203[a]	0.103–0.401	<0.001[b]
AC									
Thrombocytosis	22.2 % (22/99)	0 (0/99)				7.1 % (7/99)			
Non-thrombocytosis	40.6 % (26/64)	14.1 % (9/64)	0.276[a]	0.190–0.399	<0.001[b]	37.5 % (24/64)	0.343[a]	0.240–0.491	<0.001[b]
Connexin 43									
≤1+	15.0 % (16/107)	0 (0/107)				12.1 % (13/107)			
≥2+	57.1 % (32/56)	16.1 % (9/56)	0.199[a]	0.135–0.294	<0.001[b]	32.1 % (18/56)	0.352[a]	0.245–0.506	<0.001[b]
Chemotherapy responders									
Thrombocytosis	18.5 % (28/151)	0 (0/151)				6.0 % (9/151)			
Non-thrombocytosis	49.5 % (53/107)	19.6 % (21/107)	0.236[a]	0.133–0.418	<0.001[b]	46.7 % (50/107)	0.539[a]	0.320–0.910	0.021[b]
Connexin 43									
≤1+	16.4 % (29/177)	0 (0/177)				14.0 % (25/177)			
≥2+	67.9 % (52/81)	25.9 % (21/81)	0.164[a]	0.091–0.294	<0.001[b]	42.0 % (34/81)	0.220[a]	0.128–0.378	<0.001[b]
Chemotherapy non-responders									
Thrombocytosis	0 (0/151)	0 (0/151)				0 (0/151)			
Non-thrombocytosis	0 (0/107)	0 (0/107)	0.118[a]	0.072–0.193	<0.001[b]	0 (0/107)	0.295[a]	0.199–0.437	<0.001[b]
Connexin 43									
≤1+	0 (0/177)	0 (0/177)				0 (0/177)			
≥2+	0 (0/81)	0 (0/81)	0.174[a]	0.104–0.290	<0.001[b]	0 (0/81)	0.393[a]	0.253–0.609	<0.001[b]

[a] HR adjusted for age, gender, stage, Hb, and PS

[b] Log-rank (Mantel–Cox) test

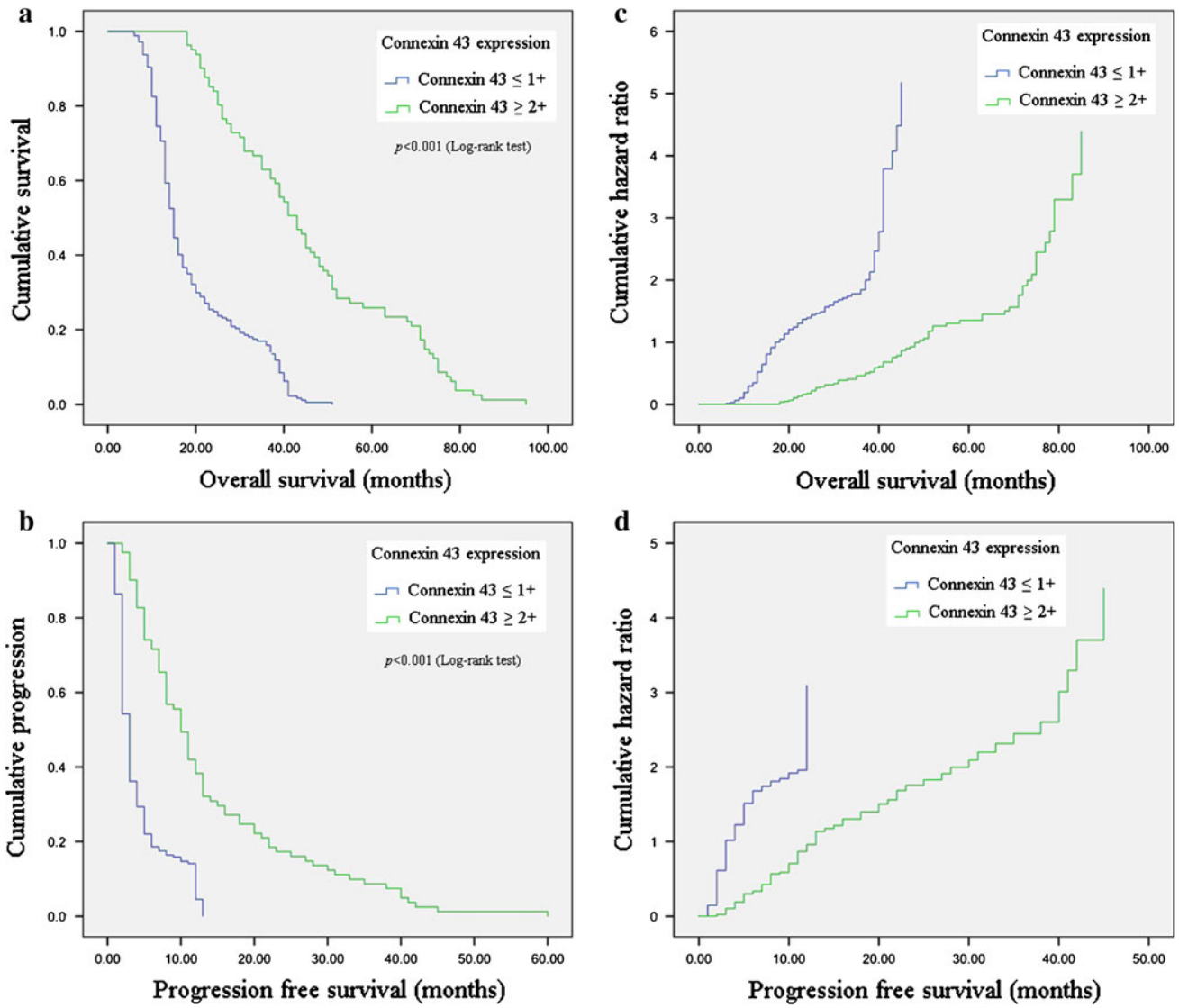

Fig. 1 Relationship between connexin 43 immunostaining and OS or PFS in patients with advanced NSCLC. Kaplan–Meier curve showed that reduced connexin 43 expression (≤1+) was a hazard factor for OS and PFS of patients with advanced NSCLC and the mean OS and PFS in the patients with connexin 43 ≥+2 were significantly longer than that in the patients with connexin 43 ≤1+

thrombocytosis ($p < 0.001$). In the Cox's proportional hazard regression models, it showed that thrombocytosis was associated with increased mortality in patients with advanced NSCLC at diagnosis (HR = 4.15 with 95 % CI 3.09–5.59 for OS and 3.47 with 95 % CI 2.60–4.65 for PFS, Fig. 2c, d). This association became stronger after additional adjustment for connexin 43, the mean OS and PFS in the patients with non-thrombocytosis and connexin 43 ≥+2 were significantly longer than that in the patients with thrombocytosis and connexin 43 ≤1+ (54.9 ± 3.1 and 19.3 ± 2.0 months vs 14.5 ± 0.7 and 2.8 ± 0.2 months), and the 5-year OS rate was 45.7 % (21/46) in patients with non-thrombocytosis and connexin 43 ≥+2 far more than 8.1 % in all of patients (Fig. 3a–d).

Chemotherapy response

The median number of chemotherapy cycles was 3 (range from 2 to 6 cycles). As shown in Table 2, of all patients subject to received platinum-based chemotherapy, 32.9 % (85/258) had chemotherapy response (CR + PR) and 67.1 % (173/258) showed no chemotherapy response (SD + PD). The distribution of connexin 43 ≥+2 was significantly higher in chemotherapy responders than in chemotherapy non-responders [64.7 % (55/85) vs 15.0 % (26/173), $p < 0.001$]. In contrast, the thrombocytosis distribution was significantly lower in chemotherapy responders than in chemotherapy non-responders [21.2 % (27/85) vs 76.9 % (124/173), $p < 0.001$]. Logistic regression analysis

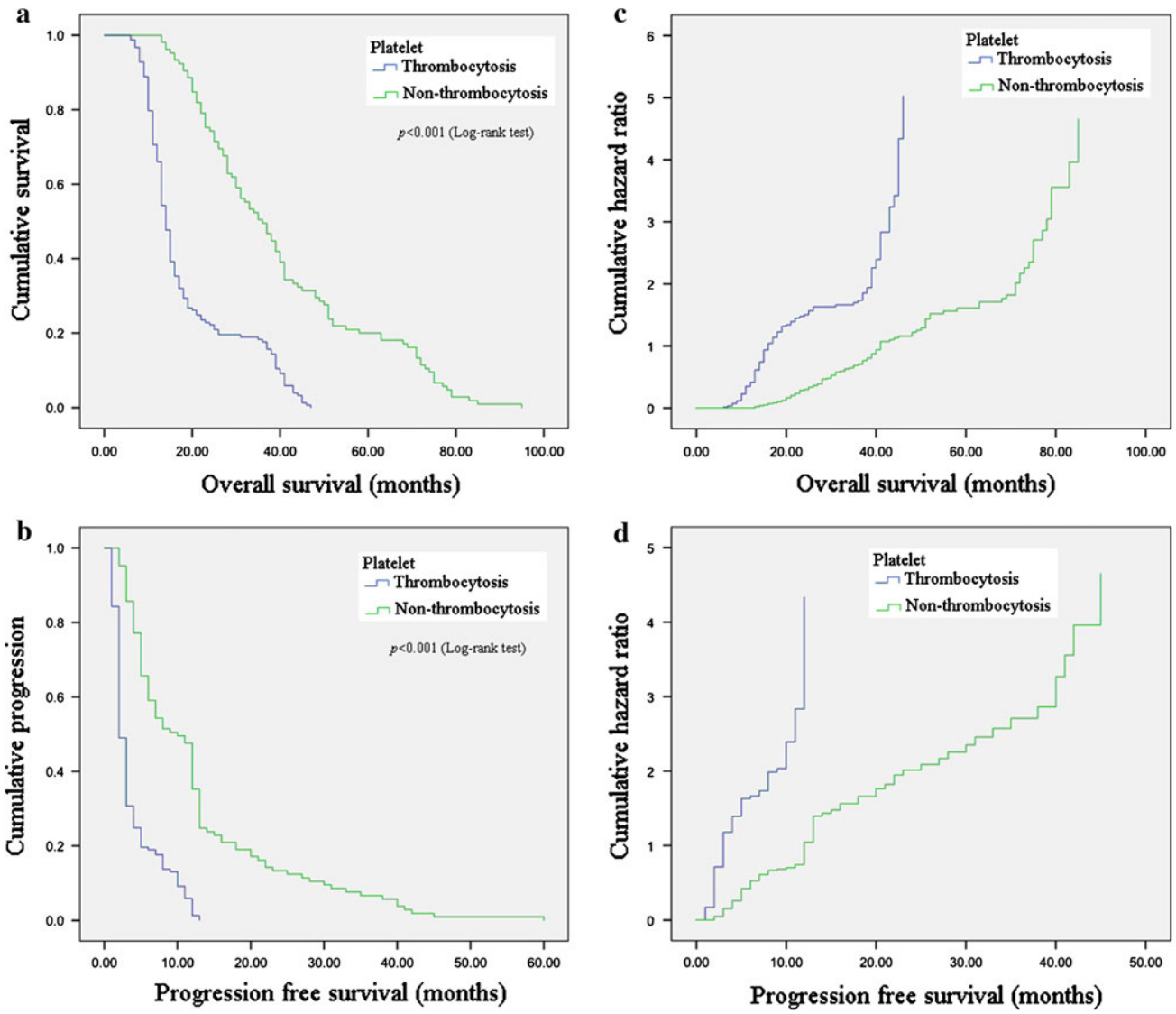

Fig. 2 Relationship between thrombocytosis and OS or PFS in patients with advanced NSCLC. Kaplan–Meier curve showed that thrombocytosis was associated with increased mortality in patients with advanced NSCLC at diagnosis and the mean OS and PFS in the patients with non-thrombocytosis were significantly longer than that in the patients with thrombocytosis

showed a significantly increased chance of being a chemotherapy non-responder for the distribution of thrombocytosis and connexin 43 $\leq$1+ compared with non-thrombocytosis and connexin 43 $\geq$+2 (HR = 2.20 with 95 % CI 1.73–2.79, $p < 0.001$) after adjustment with sex, age, tumor histology, and disease stage. The 3-year OS and 1-year PFS rate were significantly higher in chemotherapy responders with connexin 43 $\geq$+2 than in chemotherapy responders with connexin 43 $\leq$1+ (67.9 % and 42.0 % vs 16.4 % and 14.0 %, respectively), and HR for OS and PFS were 1.64 (95 % CI 1.30–2.07) and 1.81 (95 % CI 1.44–2.28), respectively. Similarly, the 3-year OS and 1-year PFS rate were also significantly higher in chemotherapy responders with non-thrombocytosis than in chemotherapy responders with thrombocytosis (49.5 % and 46.7 % vs 18.5 % and 6.0 %, respectively), and HR for OS and PFS were 1.48 (95 % CI 1.20–1.84) and 1.33 (95 % CI 1.07–1.64), respectively. All 21 patients of the 5-year OS were from chemotherapy responders with connexin 43 $\geq$+2 or non-thrombocytosis (Table 3; Fig. 4a–d).

Discussion

A recent consensus paper on medical treatment of advanced lung cancer reported several factors influencing clinical outcomes, some important factors which have been correlated with worse survival are high white blood cell counts, low Hb levels, ECOG PS > 0, body mass index (BMI) <18.5 kg/m^2, stage IV disease, quality of life, and

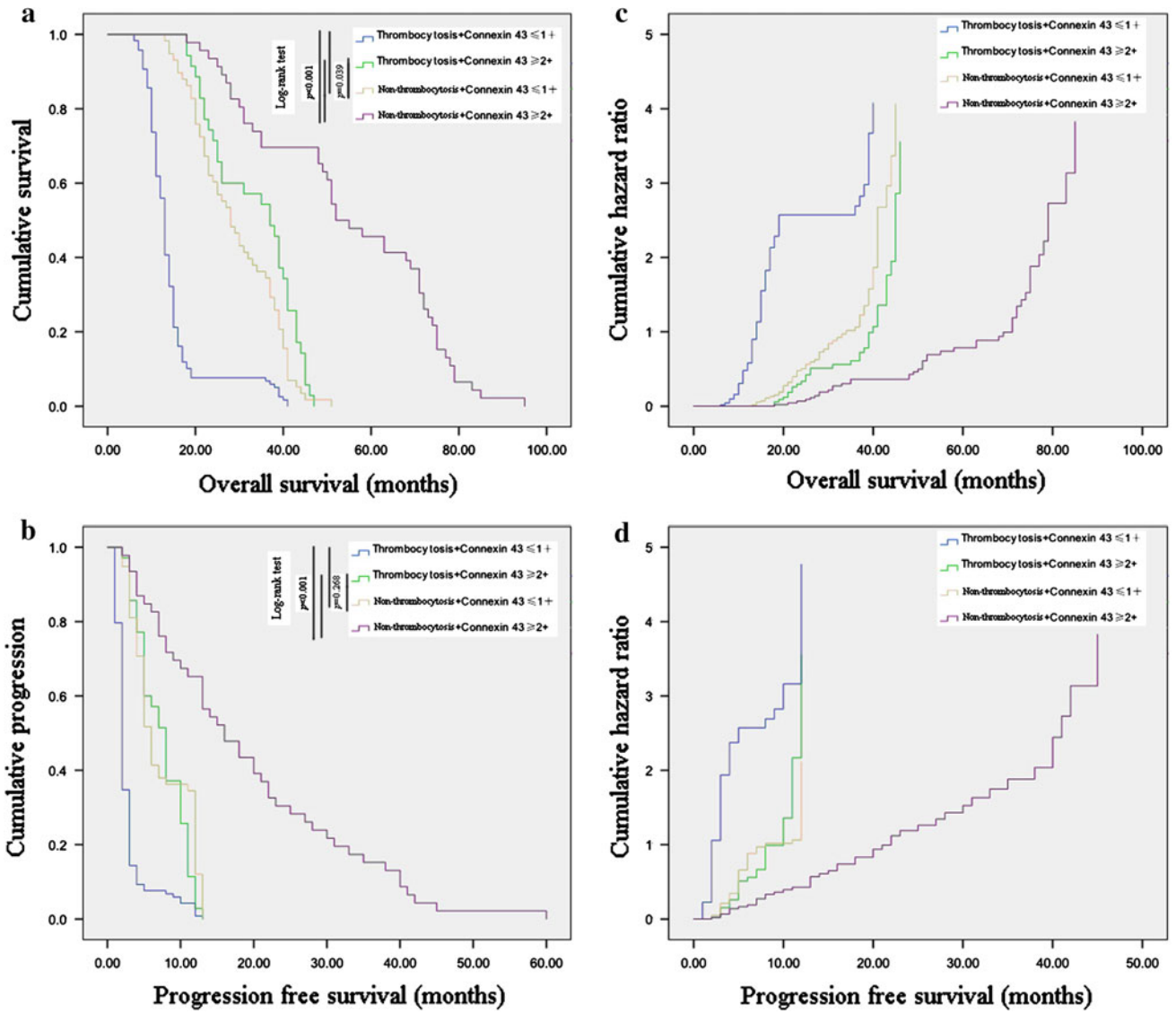

Fig. 3 Relationship between thrombocytosis and OS or PFS in patients with advanced NSCLC after additional adjustment for connexin 43. Kaplan–Meier curve showed that thrombocytosis and connexin 43 absence was associated with increased mortality in patients with advanced NSCLC at diagnosis and the mean OS and PFS in the patients with non-thrombocytosis and connexin 43 ≥+2 were significantly longer than that in the patients with thrombocytosis and connexin 43 ≤1+

comorbidity score [6]. In this retrospective study, we investigated factors related to chemotherapy response and survival of 258 patients with advanced NSCLC. As previously reported, we have confirmed that ECOG performance status, Hb levels, thrombocytosis, and connexin 43 were independent prognostic factors for advanced NSCLC treated with cisplatin-based chemotherapy. More interestingly, we found that thrombocytosis and connexin 43 absence (≤1+) at diagnosis were associated with poorer survival for advanced NSCLC treated with cisplatin-based chemotherapy, independent of the effects of other prognostic factors.

Connexins (Cxs) are a family of homologous proteins that serve as the building blocks of gap junctions (GJs). Among the 21 Cxs found in human, Cx43 is the most abundant gap junction protein and is believed to have a role in carcinogenesis [11]. Several studies showed that reduced Cx43 gap junction plaque expression differentiates carcinomas from benign disease [12] and the loss of connexin 43 (Cx43) expression in tumors is correlated with significantly shorter relapse-free and overall survival [13]. In contrast, expressing connexin 43 in cancer cells reduces their metastasis and reverses malignant phenotypes [14–17]. There was some evidence that connexin 43 is a potential prognostic biomarker for some tumors and can predict disease outcome [13, 18]. This study demonstrated that the distribution of connexin 43 ≥+2 was significantly higher in chemotherapy responders than in chemotherapy

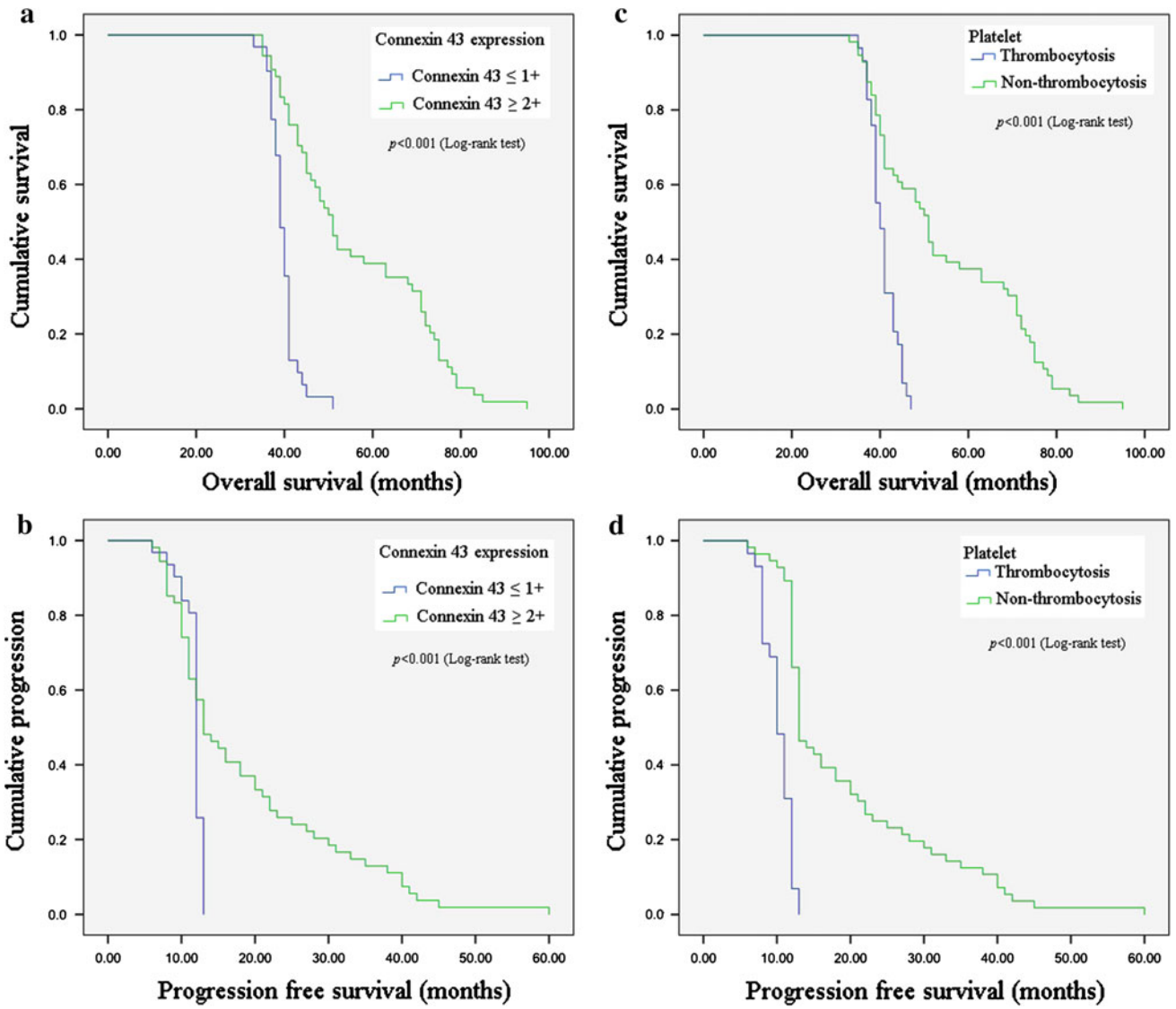

Fig. 4 Relationship between thrombocytosis or connexin 43 and OS or PFS in responders treated with cisplatin-based chemotherapy. Kaplan–Meier curve showed that thrombocytosis and connexin 43 absence were significantly correlated with response to cisplatin-based chemotherapy and the OS and PFS rate were significantly higher in chemotherapy responders with connexin 43 ≥+2 or non-thrombocytosis than in chemotherapy responders with connexin 43 ≤1+ or thrombocytosis

non-responders, and 3-year OS and 1-year PFS rate were significantly higher in chemotherapy responders with connexin 43 ≥+2 than in chemotherapy responders with connexin 43 ≤1+, which is in concordance with other studies [19].

Thrombocytosis had been found to be associated with tumor metastasis and poor prognosis in malignant tumors including lung cancer [20]. In clinic, nearly 40 % of persons incidentally found to have platelet counts exceeding 400,000 per cubic millimeter in the absence of iron deficiency and benign inflammatory conditions have an occult cancer [21], most commonly, gastrointestinal cancer, lung cancer, and colorectal or rectal cancer [22–25]. Previous studies have reported that thrombocytosis was significantly associated with advanced disease and shortened survival [26]. At present, the main focus is on genetic predictive markers while the prognostic value of thrombocytosis has been subjected to relatively limited attention. The experimental evidence suggests that platelets actively promote cancer progression through diverse mechanisms, including protection of cancer cells from immune surveillance, negotiation of cancer-cell arrest in the micro-vasculature, and stimulation of angiogenesis [27]. In our result, thrombocytosis distribution was significantly lower in chemotherapy responders than in chemotherapy non-responders, and 3-year OS and 1-year PFS rate were also significantly higher in chemotherapy responders with non-thrombocytosis than in chemotherapy responders with thrombocytosis.

In addition, thrombocytosis was found in 58.5 % (151/258) of all patients with advanced NSCLC and was related to the prognosis of advanced NSCLC patients treated with cisplatin-based chemotherapy, and this result indicates that thrombocytosis is more common among patients with advanced NSCLC.

Although connexin 43 absence and thrombocytosis in advanced NSCLC patients might be regarded as a paraneoplastic phenomenon, their overall role in cancer onset, progression, and metastasis is somewhat controversial [28, 29]. Our results showed that thrombocytosis and connexin 43 absence were significantly correlated with response to cisplatin-based chemotherapy and might be useful for predicting survival of advanced NSCLC patients, and this study findings may form a foundation for the growing corpus of knowledge explaining the outcome differences in the treatment of patients with advanced NSCLC, potentially helping to create more personalized counseling and treatment. The limitations are that this study was a single-center retrospective study on a relatively small scale, and thus, replication studies with large independent cohorts are warranted.

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Conflict of interest The authors declare that they have no conflict of interest.

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