

RESEARCH ARTICLE

Preoperative anemia, blood transfusion, and neutrophil-to-lymphocyte ratio in patients with stage i non-small cell lung cancer

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Perioperative and postoperative blood transfusions (BT), anemia and inflammation are associated with poor survivals in patients with non-small cell lung cancer (NSCLC). This study investigated the impact of perioperative BT on the survival of patients with NSCLC taking into account their preoperative inflammatory status and the presence of anemia. Demographic, perioperative, and survival data for 861 patients with stage I NSCLC was collected retrospectively. The primary endpoints of interest were recurrence-free (RFS) and overall survival (OS). Before and after propensity score matching, univariate and multivariable Cox proportional hazards models were used to evaluate the association between covariates and survival. A neutrophil-to-lymphocyte ratio (NLR) < 5 (hazard ratio [HR]: 0.58, 95% CI: 0.38-0.87; $p = 0.009$) and normal Hb concentration (HR: 0.72, 95% CI: 0.72; $p = 0.022$) were independently associated with longer RFS. The administration of blood perioperatively was associated with a trend towards worse RFS (HR: 0.69, 95% CI: 0.47-1.02; $p = 0.066$). The multivariate analysis also revealed that an NLR < 5 (HR: 0.48, 95% CI: 0.3-0.76; $p = 0.001$) and the absence of BT (HR: 0.63, 95% CI: 0.4-0.98; $p = 0.04$) were significantly associated with lower mortality risk. The propensity score matching analysis did not confirm the association between BT and poor RFS (HR: 0.63, 95% CI: 0.35-1.1; $p = 0.108$) and OS (HR: 0.52, 95% CI: 0.26-1.04; $p = 0.06$). Inflammation and anemia are common finding in patients with stage 1 NSCLC. After adjusting for these two important confounders, this study confirms that previous reports demonstrating an association between BT and poor survival after NSCLC surgery.

Keywords: Non-small cell; blood transfusions; anemia; cancer recurrence; survival

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Introduction

Approximately 2.8 million units of packed red blood cells (pRBCs) are transfused into patients undergoing surgery every year in the United States [1, 2]. Blood transfusions can expose patients to serious complications, including hemolytic reactions and transfusion-related immune suppression [3, 4], which may be one of the mechanisms underlying the association between blood transfusions and poor clinical outcomes [4]. Clinical studies have shown that in patients with gastrointestinal malignancies, perioperative allogeneic blood transfusion (ABT) is an independent risk factor for poor survival [5-7], and in-addition a recent meta-analysis demonstrated an association between ABT and shorter recurrence-free survival (RFS) and overall survival (OS) in lung cancer patients [8].

Other factors may influence the effect that ABT has on survival outcomes. In patients with non-small cell lung cancer, preoperative anemia is common and is an independent prognostic factor [9, 10]. In these patients, preoperative anemia can be multifactorial; however, low hemoglobin (Hb) concentrations, particularly in patients with more aggressive tumors, may also be related to complex interactions among the immune system, tumor microenvironment, and cancer cells [11, 12]. Moreover, the presence of both anemia and leukocytosis may be associated with worse prognosis than the presence of either condition alone is [10]. Preoperative inflammatory status is another important predictor of survival in patients with stage I NSCLC. In this patient population, those with a neutrophil-to-lymphocyte ratio (NLR) ≥ 5 have recurrence and mortality risks that are 2 times higher than those of patients with an NLR < 5 [13, 14].

To date no study has studied the clinical relevance of ABT, preoperative anemia and inflammatory status in patients with stage I NSCLC. We hypothesized that perioperative ABT, after adjustment for both preoperative anemia and inflammatory status, is an independent prognostic factor for poor survival in patients with stage I NSCLC. To test this hypothesis, we retrospectively assessed the effect that the combination of high NLR, preoperative anemia, and perioperative ABT has on the RFS and OS of these patients.

Materials and methods

Patients

This retrospective study was approved by The University of Texas MD Anderson Cancer Center's Institutional Review Board. Study data were collected and managed using REDCap electronic data capture tools hosted at MD

Anderson Cancer Center. We retrieved demographic, perioperative, and survival data for 861 patients with stage I NSCLC who underwent surgery with curative intent between January 1, 2004, and July 31, 2014, at The University of Texas MD Anderson Cancer Center. We excluded patients who had undergone palliative surgery or had secondary malignancies.

Preoperative Hb concentrations were obtained from routine blood tests. Preoperative anemia was defined according to World Health Organization (WHO) criteria (Hb concentration < 13 g/dL for men and < 12 g/dL for women) [15]. Perioperative ABT, given at the discretion of the treating physician, was defined as any pRBC unit given during surgery or within 30 days after surgery. The preoperative NLR was calculated by dividing the neutrophil count by the lymphocyte count. An NLR ≥ 5 and an NLR < 5 were used to define high preoperative inflammatory status and low preoperative inflammatory status, respectively [13].

Statistical analysis

The primary endpoint of interest was RFS, defined as the time from surgery to recurrence or death, whichever occurred first. OS was defined as the time from surgery to death from any cause. Descriptive statistics, including means, standard deviations, medians, and ranges, were used to describe continuous variables such as age, body mass index (BMI), and NLR. Frequency counts and percentages were used to describe categorical variables such as gender, American Society of Anesthesiologists (ASA) physical status, and perioperative ABT rate. The Fisher exact test or chi-square test was used to assess associations between 2 categorical variables. The Wilcoxon rank-sum test or Kruskal-Wallis test was used to assess differences in continuous variables between or among patient groups. The Kaplan-Meier method was used to estimate RFS and OS. The median RFS and OS durations and/or rates and 95% confidence intervals (CIs) for different patient groups were calculated. The log-rank test was used to evaluate the difference in RFS and OS durations and/or rates between patient groups. Univariate Cox proportional hazards models were fitted to evaluate the effects of continuous variables on RFS and OS durations and/or rates. Univariate analysis revealed to be significantly associated with RFS and OS durations were included in the multivariate analysis. Multivariable Cox proportional hazards models were used for the multivariate analysis.

To adjust for selection bias, we conducted a propensity score matching (PSM) analysis. The propensity score was the conditional probability of receiving a blood transfusion on a set of observed covariates. Age, BMI, ASA (1/2 vs. 3/4), tumor histology (no adenocarcinoma vs. adenocarcinoma), preoperative NLR, preoperative Hb, preoperative chemoradiation, and surgery type (thoracotomy vs.

Table 1. Patient and Tumor Characteristics of All Patients and According to Transfusion Status

Covariate	All patients, n = 861	Non-matched		p Value	Non-matched		Standardized Difference in % *
		No transfusion, n = 790	Transfusio n, n = 71		No transfusion, n = 62	Transfusio n, n = 62	
Mean age, years (SD)	65.29 (11.02)	65.08 (11.14)	67.64 (9.26)	0.103	66.97 (10.10)	67.33 (8.83)	3.79
Mean BMI (SD)	27.41 (5.55)	26.58 (5.48)	24.50 (5.95)	0.002	25.86 (4.03)	25.93 (6.14)	1.24
Gender							
Male	394 (45.76)	365 (46.2)	29 (40.8)	0.385	-	-	
Female	467 (54.25)	425 (53.8)	42 (59.2)	.	-	-	
ASA physical status							
1-2	96 (11.15)	92 (11.6)	4 (5.6)	0.166	3(42.9%)	4(57.1%)	6.94
3-4	765 (88.85)	698 (88.4)	67 (94.4)	.	59(50.4%)	58(49.6%)	
Mean NLR (SD)	2.67 (1.81)	2.58 (1.61)	3.65 (3.13)	0.002	3.07 (2.53)	3.08 (2.17)	0.42
NLR							
< 5	805 (93.5)	747 (94.6)	58 (81.7)	<0.0001	53(49.5%)	54(50.5%)	
≥ 5	56 (6.5)	43 (5.4)	13 (18.3)	.	9(52.9%)	8(47.1%)	
Mean Hb concentration, g/dL (SD)	13.43 (1.48)	13.51 (1.42)	12.51 (1.82)	<0.0001	12.79 (1.47)	12.84 (1.59)	3.15
Preoperative anemia							
Yes	188 (21.84)	159 (20.1)	29 (40.8)	0.0001	-	-	
No	673 (78.13)	631 (79.9)	42 (59.2)	.	-	-	
Histology adenocarcinoma							
No	360 (41.81)	322 (40.8)	38 (53.5)	0.036	35 (52.2%)	30 (52.6%)	9.64
Yes	501 (58.19)	468 (59.2)	33 (46.5)	.	27 (47.4%)	32 (47.8%)	
Neoadjuvant chemotherapy							
No	797 (92.57)	734 (92.9)	63 (88.7)	0.198	-	-	
Yes	64 (7.43)	56 (7.1)	8 (11.3)	.	-	-	
Neoadjuvant radiation							
No	857 (99.54)	787 (99.6)	70 (98.6)	0.291	-	-	
Yes	4 (0.46)	3 (0.4)	1 (1.4)	.	-	-	
Type of surgery							
Thoracotomy	557 (64.69)	510 (64.6)	47 (66.2)	0.781	43 (51.2%)	41 (48.8%)	6.85
Thoracoscopy	304 (35.31)	280 (35.4)	24 (33.8)	.	19 (47.5%)	21 (52.5%)	
Adjuvant chemotherapy							
No	89 (10.34)	708 (89.6)	64 (90.1)	0.890	-	-	
Yes	772 (89.66)	82 (10.4)	7 (9.9)	.	-	-	
Adjuvant radiation							
No	22 (2.56)	773 (97.8)	66 (93)	0.028	-	-	
Yes	839 (97.44)	17 (2.2)	5 (7)	.	-	-	
pRBC units							
0		790 (100)	0	0.0001	-	-	
1-3		0	56 (78.9)	.	-	-	
4-9		0	15 (21.1)	.	-	-	

Note: All data are no. of patients (%) unless otherwise specified. SD = standard deviation; BMI = body mass index; ASA = American Society of Anesthesiologists; NLR = neutrophil-to-lymphocyte ratio; Hb = hemoglobin; pRBC = packed red blood cells. *The standardized differences for all covariates were <=10.84% in the post-matching cohort, suggesting substantial reduction of bias between the two groups.

thoracoscopy) the covariates included in the multivariate logistic model to estimate the propensity scores. Some of these prognostic covariates were significantly imbalanced between the groups of patients who received or not BT (BMI (<=25 vs. >25; p-value=0.0029), tumor histology (no adenocarcinoma vs. adenocarcinoma; p-value=0.0368),

preop NLR (<5 vs. >=5; p-value= 0.0024), preoperative Hb (abnormal vs. normal; p-value<0.0001)).

Among the 861 patients, the propensity score was calculated. The *Greedy 5 → 1 digit match algorithm* was used to match the baseline covariates, so that the two groups

Table 2. Univariate Analysis of the Effects of Different Variables on 3- and 5-Year Recurrence-Free Survival (RFS) Rates

Variable	HR (95% CI)	3-Year RFS rate (95% CI)	5-Year RFS rate (95% CI)	p Value
All patients		0.76 (0.73-0.79)	0.67 (0.63-0.71)	
Age (continuous)	1.03 (1.01-1.04)			< 0.0001
Age				< 0.0001
< 65		0.83 (0.79-0.87)	0.76 (0.71-0.81)	
≥ 65		0.72 (0.67-0.76)	0.6 (0.55-0.66)	
BMI (continuous)	0.97 (0.94-0.99)			
BMI				0.012
≤ 25		0.71 (0.66-0.77)	0.6 (0.54-0.67)	
> 25		0.79 (0.76-0.83)	0.71 (0.66-0.75)	
Gender				0.001
Male		0.71 (0.67-0.76)	0.6 (0.54-0.66)	
Female		0.8 (0.77-0.84)	0.73 (0.69-0.78)	
ASA physical status				0.003
1-2		0.92 (0.86-0.98)	0.81 (0.72-0.92)	
3-4		0.74 (0.71-0.78)	0.65 (0.61-0.69)	
Adenocarcinoma				0.938
No		0.76 (0.71-0.81)	0.67 (0.62-0.73)	
Yes		0.76 (0.73-0.81)	0.67 (0.62-0.72)	
Type of surgery				0.396
Thoracotomy		0.76 (0.72-0.8)	0.65 (0.61-0.7)	
Thoracoscopy		0.77 (0.72-0.82)	0.71 (0.65-0.77)	
Neoadjuvant chemotherapy				0.043
No		0.77 (0.74-0.8)	0.68 (0.64-0.71)	
Yes		0.66 (0.55-0.8)	0.58 (0.46-0.73)	
Neoadjuvant radiation				0.405
No		0.76 (0.73-0.79)	0.67 (0.63-0.71)	
Yes		0.5 (0.19-1)	0.5 (0.19-1)	
Adjuvant chemotherapy				0.760
No		0.76 (0.73-0.8)	0.67 (0.63-0.71)	
Yes		0.76 (0.68-0.86)	0.66 (0.57-0.77)	
Adjuvant radiation				0.012
No		0.77 (0.74-0.8)	0.68 (0.64-0.71)	
Yes		0.57 (0.39-0.83)	0.39 (0.22-0.7)	
NLR (continuous)	1.13 (1.08-1.18)			<0.0001
NLR				0.0004
< 5		0.77 (0.74-0.81)	0.68 (0.65-0.72)	
≥ 5		0.58 (0.46-0.74)	0.44 (0.31-0.61)	
Preoperative anemia				0.0001
Yes		0.64 (0.57-0.72)	0.53 (0.46-0.62)	
No		0.8 (0.77-0.83)	0.71 (0.67-0.75)	
Hb (continuous)	0.89 (0.82-0.96)			0.006
Blood transfusion				0.0003
No		0.78 (0.74-0.81)	0.68 (0.65-0.72)	
Yes		0.62 (0.51-0.75)	0.5 (0.39-0.66)	

HR = hazard ratio; CI = confidence interval; BMI = body mass index; ASA = American Society of Anesthesiologists; NLR = neutrophil-to-lymphocyte ratio; Hb = hemoglobin.

(with perioperative BT or without perioperative BT) would have similar propensity scores. Sixty-two patients who received BT and with non-missing values for the covariates were matched with a 1:1 ratio to the non-transfused patients BT and with non-missing values for the covariates. Univariate and multivariate Cox proportional hazards models were fitted on the data after PSM to assess the association between BT and RFS or OS. *P* values < 0.05 were considered statistically significant.

All statistical analyses were performed using the statistical software programs SAS 9.3 (SAS, Cary, NC) and S-Plus 8.2 (TIBCO Software Inc., Palo Alto, CA).

Results

Patient characteristics

Table 3. Univariate Analysis of the Effects of Different Variables on 3- and 5-Year Overall Survival (OS) Rates

Variable	HR (95% CI)	3-Year OS rate (95% CI)	5-Year OS rate (95% CI)	p Value
All patients		0.87 (0.84-0.89)	0.78 (0.75-0.82)	
Age, years (continuous)	1.05 (1.03-1.06)			< 0.0001
Age, years				< 0.0001
< 65		0.93 (0.9-0.96)	0.88 (0.84-0.92)	
≥ 65		0.82 (0.78-0.86)	0.71 (0.67-0.76)	
BMI (continuous)	0.97 (0.95-1.00)			0.157
BMI				0.254
≤ 25		0.86 (0.82-0.9)	0.76 (0.71-0.82)	
> 25		0.87 (0.84-0.9)	0.8 (0.76-0.84)	
Gender				< 0.0001
Male		0.81 (0.77-0.85)	0.7 (0.65-0.75)	
Female		0.92 (0.89-0.94)	0.86 (0.82-0.9)	
ASA physical status				0.011
1-2		0.98 (0.95-1)	0.93 (0.86-0.99)	
3-4		0.85 (0.83-0.88)	0.77 (0.73-0.8)	
Adenocarcinoma				0.088
No		0.83 (0.78-0.87)	0.75 (0.7-0.81)	
Yes		0.89 (0.87-0.92)	0.81 (0.77-0.85)	
Type of surgery				0.180
Thoracotomy		0.86 (0.83-0.89)	0.77 (0.73-0.81)	
Thoracoscopy		0.89 (0.85-0.93)	0.83 (0.78-0.88)	
Neoadjuvant chemotherapy				0.112
No		0.87 (0.85-0.9)	0.79 (0.76-0.83)	
Yes		0.78 (0.69-0.9)	0.69 (0.58-0.83)	
Neoadjuvant radiation				0.824
No		0.87 (0.84-0.89)	0.78 (0.75-0.82)	
Yes		0.75 (0.43-1)	0.75 (0.43-1)	
Adjuvant chemotherapy				0.486
No		0.87 (0.84-0.89)	0.78 (0.75-0.82)	
Yes		0.87 (0.81-0.95)	0.81 (0.73-0.9)	
Adjuvant radiation				0.147
No		0.87 (0.84-0.89)	0.79 (0.76-0.82)	
Yes		0.77 (0.62-0.97)	0.58 (0.38-0.86)	
NLR (continuous)	1.16 (1.1-1.22)			< 0.0001
NLR				< 0.0001
< 5		0.88 (0.85-0.9)	0.8 (0.77-0.83)	
≥ 5		0.7 (0.58-0.84)	0.56 (0.43-0.72)	
Hb (continuous)	0.87 (0.79-0.96)			0.005
Preoperative anemia				0.0001
Yes		0.78 (0.72-0.85)	0.64 (0.57-0.73)	
No		0.89 (0.86-0.92)	0.82 (0.79-0.86)	
Blood transfusion				0.0001
No		0.88 (0.85-0.9)	0.8 (0.77-0.83)	
Yes		0.73 (0.63-0.84)	0.61 (0.49-0.75)	

HR = hazard ratio; CI = confidence interval; BMI = body mass index; ASA = American Society of Anesthesiologists; NLR = neutrophil-to-lymphocyte ratio; Hb: hemoglobin.

The 861 patients' clinical and tumor characteristics are given in Table 1. Overall, 56 patients (6.5%) had an NLR ≥ 5, 188 patients (21.84%) had preoperative anemia, and 71 patients (8.25%) received perioperative ABT. Of the patients who received ABT, more than three-fourths (78.87%; 56 patients) received 1–3 units of pRBCs. Compared with patients who did not receive perioperative ABT, those who did receive perioperative ABT were significantly more likely to have a BMI < 25 ($p = 0.002$), preoperative anemia ($p =$

0.0001), an NLR ≥ 5 ($p < 0.0001$), a histology other than adenocarcinoma ($p = 0.036$), and adjuvant radiation ($p = 0.028$). We found no statistically significant differences between the patients who did and those who did not receive ABT in terms of age, gender, ASA physical status, neoadjuvant chemotherapy, neoadjuvant radiation, or adjuvant chemotherapy.

RFS estimates

Table 4. Multivariate Analysis for Recurrence Free Survival (RFS) and Overall Survival (OS) Rates

Covariate	Recurrence Free Survival							Overall Survival								
	Before Matching			After Matching				Before Matching			After Matching					
	HR	95% CI	<i>p</i> value	HR	95% CI	<i>p</i> value	HR	95% CI	<i>p</i> value	HR	95% CI	<i>p</i> value				
Age, years (continuous)	1.02	1.01	1.04	1			1.04	1.02	1.06	<0.0001						
BMI (continuous)	0.96	0.94	0.99	0.015			0.97	0.94	1.00	0.138						
Gender (female vs. male)	0.71	0.55	0.91	0.008			0.57	0.42	0.77	0.0003						
ASA physical status (1-2 vs. 3-4)	0.58	0.35	0.95	0.138			0.63	0.35	1.15	0.138						
Adenocarcinoma (yes vs. no)							0.78	0.58	1.06	0.117						
Type of surgery (thoracoscopy vs. thoracotomy)							0.79	0.57	1.11	0.181						
NLR (< 5 vs. ≥ 5)	0.58	0.38	0.97	0.009			0.48	0.30	0.76	0.0017						
Preoperative anemia (no vs. yes)	0.72	0.54	0.95	0.053			0.72	0.52	1.00	0.053						
Blood transfusion (no vs. yes)	0.69	0.47	1.02	0.066	0.630	0.35	1.1	0.108	0.63	0.40	0.98	0.044	0.52	0.26	1.04	0.066

HR = hazard ratio; CI = confidence interval; BMI = body mass index; ASA = American Society of Anesthesiologists; NLR = neutrophil-to-lymphocyte ratio; Hb: hemoglobin.

The median follow-up time after surgery was 108.28 months. The results of the univariate analysis of the effects of different variables on 3- and 5-year RFS are given in Table 2. The 3- and 5-year RFS rates of the patients with an NLR ≥ 5 (58% and 44%, respectively) were significantly lower than those of the patients with an NLR < 5 (77% and 68%, respectively; $p = 0.0004$). The 3- and 5-year RFS rates of the patients with preoperative anemia (64% and 53%, respectively) were significantly lower than those of the patients without preoperative anemia (80% and 71%, respectively; $p = 0.0001$). The 3- and 5-year RFS rates of the patients who received ABT (62% and 50%, respectively) were significantly lower than those of the patients who did not receive ABT (78% and 68%, respectively; $p = 0.0003$). The number of pRBCs administered during and/or after surgery also had a negative impact on RFS rates. As expected patients who received > 4 units had the lowest 3- and 5- year RFS (Table 2). In addition, the 3- and 5- year RFS rates of patients age > 65 years, patients with a BMI < 25 , men, patients with an ASA physical status of 3-4, and patients who received adjuvant chemoradiation were significantly lower than those of patients age ≤ 65 years ($p < 0.0001$), patients with a BMI ≥ 25 ($p = 0.012$), women ($p = 0.001$), patients with an ASA physical status of 1-2 ($p = 0.003$), and patients who did not receive adjuvant chemoradiation ($p = 0.0027$), respectively.

The multivariate analysis confirmed that age ($p < 0.001$), BMI ($p = 0.015$), and gender ($p = 0.008$) were independent predictors of RFS (Table 4). Moreover, an NLR < 5 (hazard ratio [HR]: 0.58, 95% CI: 0.38-0.87; $p = 0.009$) and normal Hb concentration (HR: 0.72, 95% CI: 0.72; $p = 0.022$) were independently associated with longer RFS. Compared with patients who did receive ABT, patients who did not receive ABT showed a trend towards having better RFS (HR: 0.69, 95% CI: 0.47-1.02; $p = 0.066$). The univariate and multivariate model after PSM demonstrated that non-transfused patients had a lower risk of recurrence (HR: 0.64, 95% CI: 0.36-1.12; $p = 0.122$ and HR: 0.63, 95% CI: 0.35-1.1; $p = 0.108$, respectively), although it did not reach statistical significance.

OS estimates

The results of the univariate analysis of the effects of different variables on 3- and 5-year OS are given in Table 3. Patients age < 65 years, women, and patients with an ASA physical status of 1-2 had significantly better 3- and 5-year OS rates compared with patients age ≥ 65 years ($p < 0.0001$), men ($p < 0.0001$), and patients with an ASA physical status of 3-4 ($p = 0.019$), respectively. The 3- and 5-year OS rates of patients with preoperative anemia (78% and 64%, respectively) were significantly lower than those of patients without preoperative anemia (89% and 82%, respectively; p

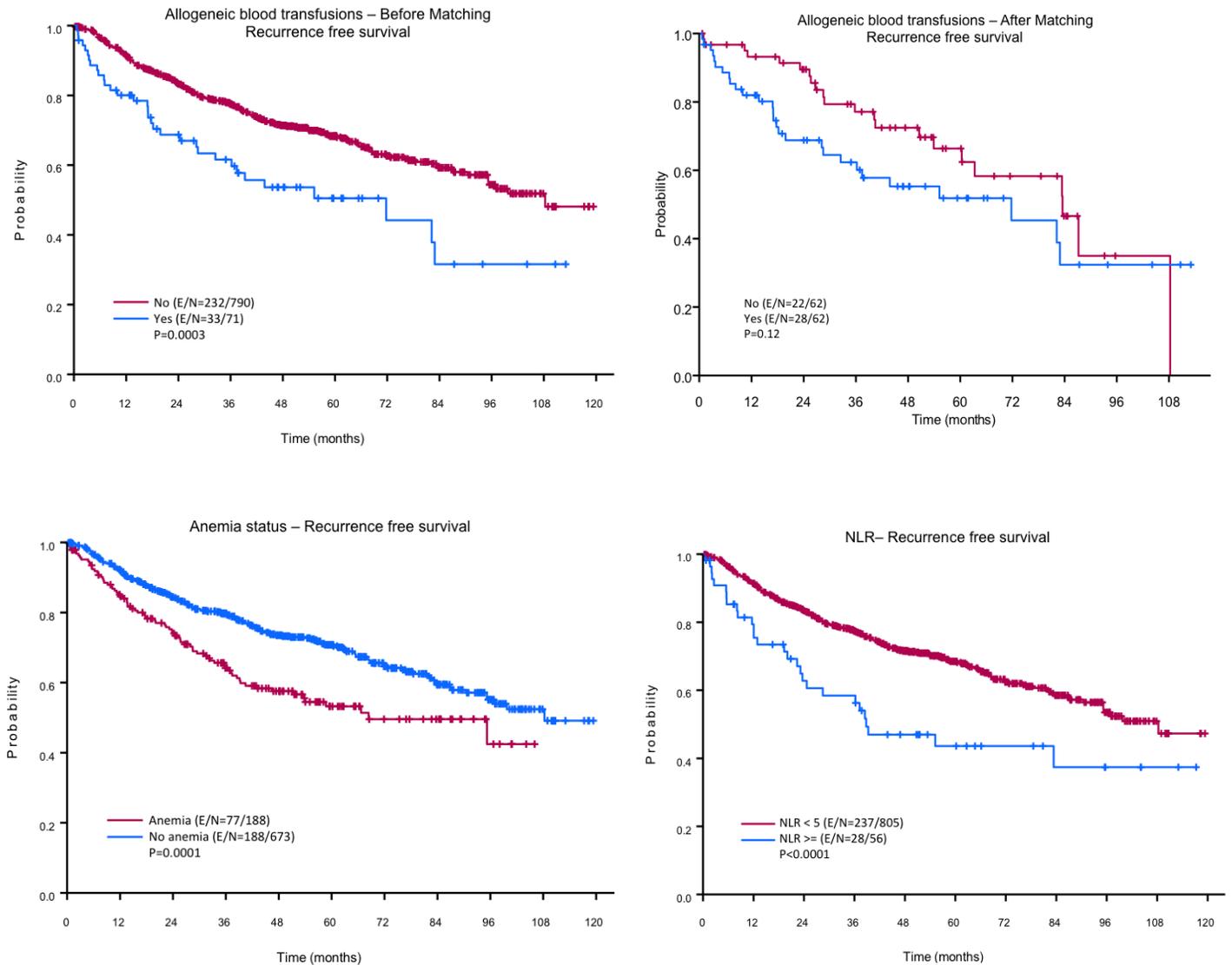


Figure 1. Kaplan-Meier curves for recurrence-free survival (RFS). Patients who did not receive allogeneic blood transfusion (ABT) had significantly better RFS than patients who did receive ABT (top panels). Patients who had preoperative anemia had significantly better RFS than patients who did have preoperative anemia (left bottom panel). The right bottom panel depicts the impact of preoperative neutrophil-to-lymphocyte ratio (NLR) on RFS.

= 0.0001), and the 3- and 5-year OS rates of patients with an $NLR \geq 5$ (70% and 56%, respectively) were significantly lower than those of patients with an $NLR < 5$ (88% and 80%, respectively; $p < 0.0001$). Patients who received ABT had 3- and 5-year OS rates (73% and 61%, respectively) that were significantly lower than those of patients who did not receive ABT (88% and 80%, respectively; $p = 0.0001$). The univariate analysis also demonstrated that the number of pRBCs transfused perioperatively also had a negative impact on OS rates. Thus, who received > 4 units had the lowest 3- and 5- year OS (Table 3).

The multivariate analysis revealed that a preoperative $NLR < 5$ (HR: 0.48, 95% CI: 0.3-0.76; $p = 0.001$) and avoidance of ABT (HR: 0.63, 95% CI: 0.4-0.98; $p = 0.04$) were associated with longer overall survival than an $NLR \geq 5$ (Table 4). Compared with patients without preoperative anemia, Patients with preoperative anemia showed a trend towards having worse OS (HR: 0.72, 95% CI: 0.52-1.00; $p = 0.053$). Gender and age were also independent predictors of OS. The univariate and multivariate model after PSM demonstrated that non-transfused patients had an important trend to have a lower risk of overall mortality (HR: 0.53, 95% CI: 0.27-1.06; $p = 0.07$ and HR: 0.52, 95% CI:

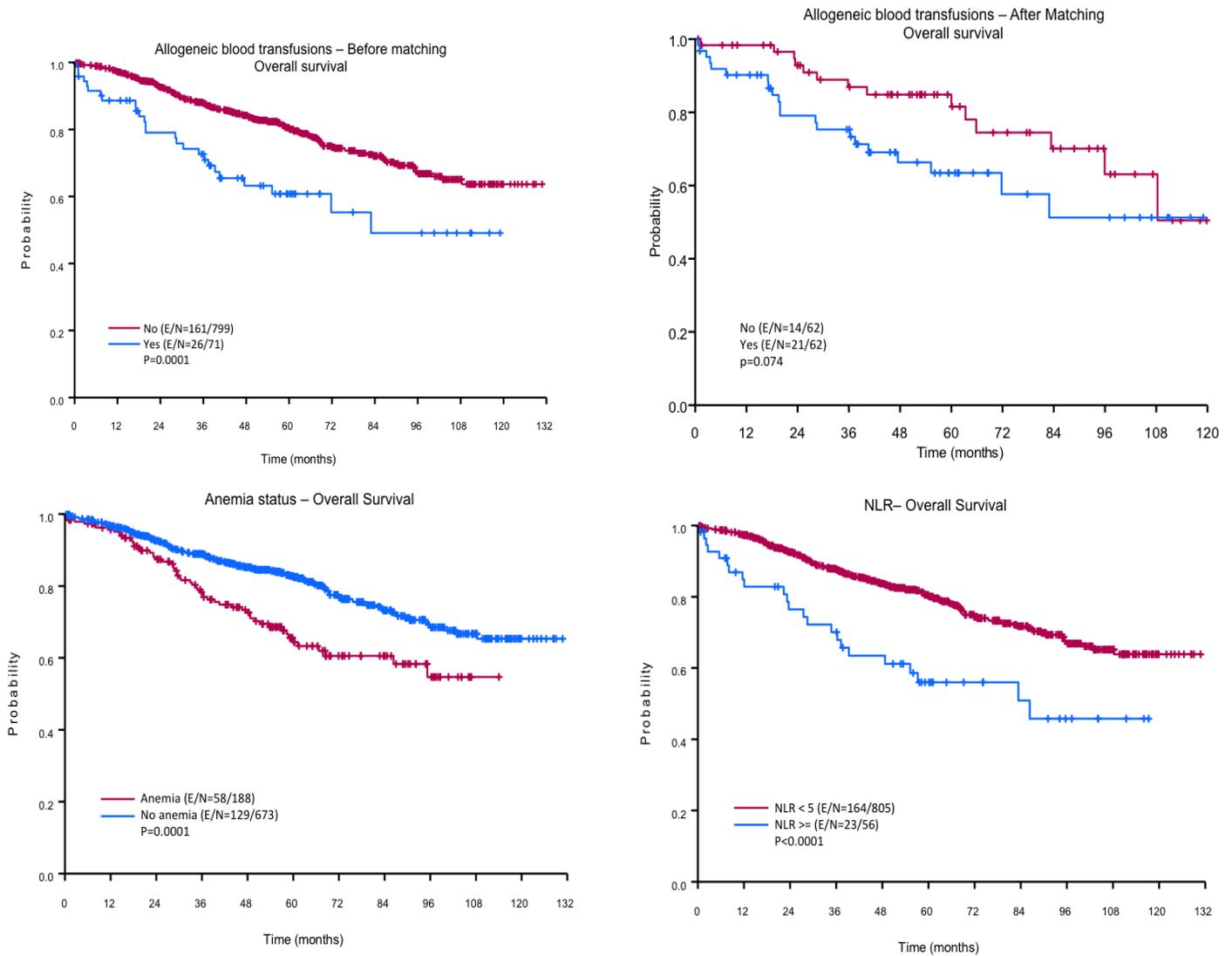


Figure 2. Kaplan-Meier curves for overall survival (OS). The top panels illustrate the effect of allogeneic blood transfusion (ABT) on OS. The left bottom panel shows the survival curves for patients who did and who did not have preoperative anemia. The right bottom panel depicts the impact of preoperative neutrophil-to-lymphocyte ratio (NLR) on OS.

0.26-1.04; $p = 0.06$, respectively) compared to patients who received pRBCs

Discussion

The ways in which ABT promotes tumor growth and cancer recurrence remain largely unclear; however, this association appears to be related to 2 mechanisms: One associated with the proposed immunosuppressive effect of blood transfusion (i.e., transfusion-related immune suppression) and the other associated with the direct action growth factors have on cancer cells [4, 16]. Our study

confirmed that, compared with NSCLC patients who do not receive perioperative ABT, those who do have an increased mortality risk and tend to have shorter RFS, although significant selection bias is a concern since the PSM analysis did not show such association. These results are in line with those of a recent meta-analysis demonstrating that patients with stage I NSCLC who receive ABT are 1.5 times as likely as patients who do not receive ABT to have cancer recurrence and 1.39 times as likely to die of their cancer [8]. However, the present study and the meta-analysis differ in several ways [17-22]. The meta-analysis included fewer patients, defined perioperative ABT as occurring within 7

days (rather than 30 days) following surgery, and did not report preoperative Hb concentrations. The patient population in the study by Peñalver *et al.*, which included more than 800 patients with stage I NSCLC, was much more similar to that of the present study. Peñalver *et al.* found no association between blood transfusion and OS; however, the authors did not consider preoperative anemia as a possible confounding variable. More importantly, the 5-year survival rate of the ABT patients in the present study (80%) was higher than that of those Peñalver *et al.* reported on (63%) [19].

Other groups have investigated the impact preoperative anemia has on lung cancer patients' postoperative oncological outcomes. In agreement with our study, Nosotti *et al.* and Ng *et al.* found that transfused patients had lower Hb concentrations than those not transfused [17, 18]. The preoperative Hb concentrations of the patients who did or did not receive transfusions in those studies were nearly identical to those we report here; however, Nosotti *et al.* did not include anemia as a variable in their analysis [17, 18]. Ng *et al.* also reported that anemia was associated with increased risks of cancer progression and cancer-related mortality, although the latter was not statistically significant [17]. In agreement with Tomita *et al.*, we found that the 5-year survival rate of patients with preoperative anemia was significantly lower than that of patients without preoperative anemia [9]. Several groups have offered hypotheses to explain how preoperative anemia reduces the survival of cancer patients; it has been suggested that the reduced partial pressure of oxygen at the tumor level triggers an exaggerated expression of hypoxia-inducible factor-1, which in turn triggers angiogenesis, epithelial-mesenchymal transformation, genetic mutations, and apoptosis resistance [23-26]. Although, our study did not address mechanisms by which anemia can be linked to cancer progression we accounted for an important mediator of preoperative anemia that is inflammation and for ABT that is a treatment of perioperative anemia.

The preoperative NLR is an independent predictor of survival in patients with a wide variety of cancers [14]. Previous studies from our group and others have highlighted the importance of the NLR as an independent predictor of survival in the overall NSCLC patient population [13, 27-29]. To date, only Sarraf *et al.* have assessed the impact of preoperative NLR on the survival of patients with stage I NSCLC [27]. The study, which included 83 patients and used an NLR cut-off value different from that used in the present study, revealed that patients with an NLR > 3.83 had poor survival, which is in agreement with our findings [27]. In our study, the multivariate analysis showed that the preoperative NLR, compared with anemia and postoperative NLR, had the

strongest predictive value for RFS and OS, indicating the importance of stratifying patients with stage I NSCLC according to their preoperative inflammatory status.

The present study has several potential limitations, including those inherent to all retrospective studies, such as the inability to account for unknown factors that may affect the studied outcomes. For example, our database does not include information on postoperative complications, which are known to negatively impact the mortality of patients who have undergone major thoracic surgery [30]. The present study's statistical analyses also included propensity-score matching that showed a 37% and 48% improvement in RFS and OS. Although this improvement in survivals did not reach statistical significance, it is important to remark that significant number of patients were lost after matching which limits the power of the study to detect significances [31].

In addition, because our study included patients who received both leukoreduced and non-leukoreduced pRBCs during the perioperative period, we could not estimate the impact of leukoreduction on survival. Several authors have questioned whether leukoreduced pRBCs affect cancer recurrence; for example, Ng *et al.* demonstrated that patients who received leukoreduced pRBCs had poorer outcomes than those who did not receive leukoreduced pRBCs [17]. Panagopoulos *et al.* suggested that pRBC transfusions, regardless of whether they are leukoreduced, explains the association between ABT and poor outcomes [32]. Another limitation is that we did not define the triggers of ABT, intraoperative blood loss or evaluated the impact of ABT timing (intraoperative or postoperative) on survival. Recent studies have suggested that intraoperative ABT has a more deleterious effect on survival than postoperative ABT does [33, 34]. Finally, including both patients with stage Ia and those with stage Ib disease in our analysis might be considered a confounder, as Ng *et al.* demonstrated that the survival rates of these 2 groups are different [17].

In conclusion, our study's findings suggest that preoperative anemia and an NLR ≥ 5 independent predictors of RFS and OS in patients with stage I NSCLC. Preoperative anemia as defined by the WHO criteria and an NLR ≥ 5 are potential biomarkers for identifying patients who have a high risk of recurrence. In the non-matched population of patients, allogeneic blood transfusion was an independent risk factor of reduced RFS and OS, but this association disappeared after propensity score matching.

Conflict of interests

The authors have declared that no conflict of interests exist.

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Author contributions

JPC was involved in design, or acquisition of data, or analysis and interpretation of data; writing of the manuscript and final approval. CG participate in acquisition of data, or analysis and interpretation of data; writing of the manuscript and final approval. RJM was involved in analysis and interpretation of data; writing of the manuscript and final approval, DR carried out analysis and interpretation of data; writing of the manuscript and final approval, JN was involved in design, analysis and interpretation of data; writing of the manuscript and final approval. Lei Feng contributed with the analysis and interpretation of data; writing of the manuscript and final approval. Andrea Rodriguez-Restrepo participated in acquisition of data, or analysis and interpretation of data; writing of the manuscript and final approval. Fernando Martinez was involved in analysis and interpretation of data; writing of the manuscript and final approval. Gabriel Mena participated in analysis and interpretation of data; writing of the manuscript and final approval. Vijaya Gottumukkala contributed in analysis and interpretation of data; writing of the manuscript and final approval.

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