

# Low Arterial Oxygen Partial Pressure Induces Pulmonary Thrombocytopenia in Patients and a Mouse Model

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## Research article

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# Abstract

## Background

Recent basic studies demonstrate that the lung is a primary organ of platelet biogenesis. However, whether the pathophysiological state of the lung affect the platelets is little known. We aim to investigate the incidence of thrombocytopenia in patients with pulmonary infection (PIN) and risk factors associated with pulmonary thrombocytopenia.

## Methods

In total, 11941 patients with pulmonary infection (PIN) were enrolled, and patients with three other infectious diseases were collected as controls. The incidence of thrombocytopenia was compared, and the risk factors associated with thrombocytopenia in PIN patients were investigated by multivariate analysis. To explore the mechanism of thrombocytopenia, hypoxic model were constructed. Blood platelet counts from the angular vein (PLTs), left ventricle (PLT<sub>post</sub>) and right ventricle (PLT<sub>pre</sub>) were determined. Megakaryocyte staining with a CD41-specific antibody in the lungs was detected by flow cytometry and immunofluorescence.

## Results

The incidence of thrombocytopenia in PIN patients was higher than that in the other three types of infectious disease patients (9.8% vs 6.4%~5.0%,  $P < 0.001$ ). Relatively low arterial oxygen partial pressure (PaO<sub>2</sub>) was an important risk factor for thrombocytopenia (OR = 0.88;  $P < 0.001$ ). In a hypoxic mouse model, PLTs were decreased ( $518.38 \pm 127.92$  vs  $840.75 \pm 77.30$ ,  $P < 0.05$ ), which showed that the low PaO<sub>2</sub> could induce thrombocytopenia. The difference between the PLT<sub>post</sub> and PLT<sub>pre</sub> ( $\Delta$ PLT<sub>post-pre</sub>) represented the production of platelets in the lungs, which was significantly attenuated in hypoxic mice compared with normoxic mice ( $F = 25.47$ ,  $P < 0.05$ ). Additionally, CD41-positive megakaryocyte numbers in the lungs were decreased in hypoxic mice.

## Conclusion

PIN is a susceptibility factor for thrombocytopenia. Low PaO<sub>2</sub>-induced thrombocytopenia is associated with impaired platelet generation in the lungs.

## Background

Platelets are critical for hemostasis and thrombosis[1]. It is a classic view that megakaryocytes (MKs) produce platelets in the bone marrow. Interestingly, several studies have shown that a large mass of MKs exist in the lungs, which indicates that the lungs may be a specific organ for platelet biogenesis[2–4]. The latest discovery strongly indicated that a large number of MKs circulate through the lungs, where they dynamically release platelets, and an animal model showed that the lungs contributed approximately 50% of

total platelet production in mice[3]. It is often observed that thrombocytopenia appears in patients with pulmonary diseases, such as pneumonia, chronic obstructive pulmonary disease (COPD) or respiratory failure (RF), which leads to major bleeding events and death[4–7]. However, whether pathophysiological state of lung affects platelets is unclear from both clinical and basic research information. The present study, firstly, aims to explore whether the risk of thrombocytopenia varies among different organ infection, and we found that the incidence of thrombocytopenia in pulmonary infection (PIN) was the highest and low oxygen partial pressure ( $\text{PaO}_2$ ) is a key risk factor for thrombocytopenia in PIN. Secondly, mice hypoxia model were used to uncover the potential mechanism of thrombocytopenia associated with lung .

## Methods

### Subjects

Data were obtained from the large data center of the Second Affiliated Hospital of Nanchang University from January 1, 2014, to June 30, 2018. International classification of diseases code-10 (ICD-10) was applied to identify the diagnosis of PIN[8]. Additionally, patients with urinary tract infection (UTI), intestinal tract infection (ITI) or skin soft-tissue infection (SSI) were collected as controls to distinguish PIN-specific effects of infection. All subjects meeting any of the following criteria were excluded: 1) younger than 18 years old; 2) pregnant or lactating ; 3) presence of infection at two or more sites; 4) a total bilirubin, alanine aminotransferase or aspartate aminotransferase level 1.5 times higher than the normal value or a serum creatinine level 1.2 times higher than the normal value[9]; 5) presence of any disorder that could lead to thrombocytopenia, including hematopoietic disease, cancer, hepatitis, cirrhosis, hypersplenism, autoimmune disease, disseminated intravascular coagulation and hemorrhagic fever; and 6) concomitant intake of medication that could affect the platelet, including chemotherapeutic drugs, monoclonal antibodies, antiplatelet drugs, anticoagulant drugs, and some antibiotics (e.g. linezolid and vancomycin). The clinical pulmonary infection score (CPIS), which is used to assess the severity of PIN[10] and the Acute Physiology And Chronic Health Evaluation II (APACHE II) score, which is used to estimate the severity of RF[11], were calculated with data collected by a third person blinded to the experimental design to avoid bias.

Thrombocytopenia was defined as a platelet count lower than  $100 \times 10^9/\text{L}$ , which was defined according to the corresponding statement by the World Health Organization[12].All procedures were approved by the Medical Ethics Committee of the Second Affiliated Hospital of Nanchang University.

### Mouse models

Sixteen C57BL/6 male mice (20–28 g, age 10 weeks) were purchased from the Animal Center of Nanchang university. To verify whether the  $\text{PaO}_2$  is associated with thrombocytopenia, we generate a respiratory failure model in mouse and the protocol was well-accepted and utilized by other researchers [13]. The mice were randomly assigned into two groups. Hypoxic mice (N = 8) were housed in a hypobaric chamber with an 8%  $\text{O}_2$  concentration and 58%~66% humidity for 28 continuous days, while control mice (N = 8) were housed in normal air conditions. The mice were kept under conditions with controlled lighting (12 h per day) and temperature ( $21 \pm 2 \text{ }^\circ\text{C}$ ) and were free to standard laboratory food and water. Four mice were placed in one

cage. All procedures were approved by the Animal Care and Use Committee of the Second Affiliated Hospital of Nanchang University.

## Platelet counts and P-selectin assessment

Mice were anesthetized by inhalation of isoflurane (RWD, China) before blood collection. The platelet counts of blood samples collected from different sites including the angular vein (PLTs), right ventricle (PLT<sub>pre</sub>) and left ventricle (PLT<sub>post</sub>) were determined with an automatic blood cell analyzer. Plasma soluble P-selectin, a marker of platelet activation, was evaluated by enzyme-linked immunosorbent assay (Invitrogen, USA).

## Tissue processing

After 4 weeks of hypoxia exposure, the mice were deeply anesthetized with pentobarbital sodium salt (Sigma-Aldrich, Germany) at the dosage of 25 mg/kg by intraperitoneal injection. Then, eight mice of each group were decapitated to obtain lung, femur, spleen. Left lung, femur

## Flow cytometry

To obtain a single-cell suspension, 100 mg lung was digested with Liberase<sup>TM</sup> (Roche, Germany) at a concentration of 26 U/ml and 5% DNase I in a 37 °C warm bath for 30 min, followed by filtration through a 100- $\mu$ m sieve; and cell suspensions of bone marrow and spleen were performed as described previously [14, 15]. To assess the MK populations, cell suspensions treated with 1 ml RBC lysis buffer (Solarbio, China) were incubated with an anti-CD41 FITC-conjugated antibody (eBioscience, USA) or IgG FITC-conjugated isotype control antibody (eBioscience, USA) in the dark for 30 min and then evaluated by flow cytometry [16].

## Tissue immunofluorescence

Lung, femur and spleen of mice were rapidly removed after euthanasia and fixed with 4% paraformaldehyde for 24 hours. Sections (2-mm thick) were embedded in paraffin and incubated with an anti-CD41 primary antibody (Proteintech, USA) in PBS containing 1% BSA overnight at 4 °C [17]. All samples were treated with a secondary antibody conjugated to Alexa Fluor 488 or 568 (Invitrogen Corporation, Molecular Probes).

## Statistical analysis

Statistical analysis was performed by using SPSS 13.0 software (SPSS Inc. Chicago, Illinois, USA). Continuous variables are expressed as the mean  $\pm$  standard deviation ( $x \pm SD$ ), while categorical variables are presented as relative frequencies. Logistic regression models were used to identify clinical factors associated with thrombocytopenia. All covariates that reached statistical significance ( $P < 0.05$ ) in univariate analyses were selected for multivariate analysis. An independent  $t$  test was used for comparisons between two groups if the data conformed to a normal distribution. Otherwise, the Wilcoxon rank sum test was used. A paired  $t$  test was used to compare the PLT<sub>pre</sub> and the PLT<sub>post</sub>. To compare the difference in the  $\Delta$ PLT<sub>post-pre</sub> value between two groups, a covariance analysis model was used. A  $P$  value  $< 0.05$  was considered statistically significant.

## Results

# Patient cohort

A total of 11941(70.5%) patients with PIN were enrolled, while 3327(19.7%) patients with UTI, 1053 (6.2%) patients with ITI, and 602 (3.6%) patients with SSI were collected to be used as controls. The initial cohort included 16923 patients (Fig. 1), and the baseline characteristics are presented in Table 1. The incidence rates of thrombocytopenia were 9.8% (PIN), 6.4% (UTI), 5.0% (ITI) and 5.1% (SSI).

Table 1  
Baseline Characteristics of Patients with Infectious Diseases (N = 16923)

Variables	Thrombocytopenia (n = 1468)	Normal platelet (n = 15455)	p Value
<b>Demographic characteristics</b>			
Age, y	57.94 ± 23.62	58.57 ± 23.81	0.34
Sex, female, n(%)	876(59.7)	8771(56.8)	0.03*
<b>Infection location, n(%)</b>			< 0.001*
Lung	1171(79.8)	10770(69.7)	
Urinary tract	213(14.5)	3114(20.1)	
Intestinal tract	53(3.6)	1000(6.5)	
Skin soft-tissue	31(2.1)	571(3.7)	
<b>Biochemical indexes</b>			
WBC(*10 <sup>9</sup> /L)	9.92 ± 3.99	10.00 ± 4.09	0.54
HB(g/L)	128.46 ± 33.44	127.45 ± 33.42	0.27
PLT(*10 <sup>9</sup> /L)	70.33 ± 23.53	211.32 ± 80.26	< 0.001*
TBil(μmol/L)	19.94 ± 8.62	19.81 ± 8.53	0.58
AST(U/L)	36.07 ± 13.60	36.49 ± 13.66	0.26
ALT(U/L)	42.17 ± 18.80	41.84 ± 19.08	0.54
Creatinine (mmol/l)	93.86 ± 30.21	93.30 ± 30.18	0.49
CRP(mg/L)	50.48 ± 48.53	51.21 ± 49.21	0.67
PCT(ng/ml)	0.35 ± 0.21	0.25 ± 0.35	0.60
<b>Medical history, n(%)</b>			
Hypertension	453(30.9)	5512(35.7)	< 0.001*
Diabetes	220(15.0)	2613(16.9)	0.06
Date were presented as mean±(SD) or n(%).The physiological variables were collected within the first 24 hours of admission. *Significant differences (P< 0.05).			
Abbreviations: WBC, white blood cell; HB, hemoglobin; PLT, platelet; TBil, total bilirubin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; CRP, C-reactive protein; PCT, procalcitonin			

**Patients with PIN showed a higher incidence of thrombocytopenia than patients with other infectious**

# diseases

For all subjects with infectious disease, factors associated with thrombocytopenia by logistic analysis are shown in Table 2. By univariate analysis, sex, hypertension comorbidity and different infection sites were associated with thrombocytopenia. In multivariate analysis, after adjusting for the sex and hypertension comorbidity confounders, patients with other organ infections presented a lower incidence of thrombocytopenia than did PIN patients (UTI OR = 0.61, 95% CI: 0.52–0.71; ITI OR = 0.48, 95% CI: 0.33–0.69; SSI OR = 0.46, 95% CI: 0.35–0.61; all  $P < 0.001$ ), which indicates that patients with PIN are more likely to develop thrombocytopenia than patients with one of three other infections.

Table 2  
Comparison of the Incidence of Thrombocytopenia between Patients with Pulmonary Infection and Other Infections

Variables	Univariate Analysis			Multivariate Analysis		
	OR	95%CI	p Value	OR	95%CI	p Value
Age, y	0.99	0.99-1.00	0.33			
Sex, females	1.13	1.01–1.26	0.03*	1.09	0.98–1.22	0.14
<b>Infection Sites</b>						
Lung	1			1		
Urinary tract	0.63	0.54–0.73	< 0.001*	0.61	0.52–0.71	< 0.001*
Intestinal tract	0.49	0.37–0.65	< 0.001*	0.48	0.33–0.69	< 0.001*
Skin soft-tissue	0.50	0.35–0.72	< 0.001*	0.46	0.35–0.61	< 0.001*
<b>Biochemical indexes</b>						
WBC( $\times 10^9/L$ )	0.99	0.90–1.09	0.54			
HB(g/L)	1.00	0.99–1.03	0.27			
CRP(mg/L)	0.99	0.99–1.03	0.59			
PCT(ng/ml)	1.11	0.09–1.15	0.60			
<b>Medical history, n(%)</b>						
Hypertension	0.81	0.72–0.90	< 0.001*	0.74	0.66–0.84	< 0.001*
Diabetes	0.87	0.75–1.01	0.06			
Abbreviations: WBC, white blood cell; HB, hemoglobin; CRP, C-reactive protein; PCT, procalcitonin; OR odds ratio; CI, confidence interval; *Significant differences ( $P < 0.05$ ).						

PIN patients with RF comorbidity had a relatively high risk for thrombocytopenia

To explore the risk factors for thrombocytopenia in PIN patients, factors influencing thrombocytopenia were identified by logistic analysis (Table 3). The CPIS was evaluated to assess the severity of PIN[10]. Univariate analysis showed that the higher CPIS was (OR = 1.25, 95% CI: 1.18–1.32;  $P < 0.001$ ), the higher the risk of thrombocytopenia. RF (OR = 1.68, 95% CI: 1.35–2.09;  $P < 0.001$ ) and hypertension (OR = 0.73, 95% CI: 0.65–0.83;  $P < 0.001$ ) were associated with thrombocytopenia. Multivariate analysis showed that hypertension was a positive factor for thrombocytopenia (OR = 0.74, 95% CI: 0.65–0.85;  $P < 0.001$ ). RF (OR = 1.59, 95% CI: 1.27–1.98;  $P < 0.001$ ) and a high CPIS (OR = 1.24, 95% CI: 1.17–1.31;  $P < 0.001$ ) were risk factors for thrombocytopenia. Interestingly, patients with RF appeared more prone to thrombocytopenia than those without RF.

Table 3

Analysis on Risk Factors Associated with Thrombocytopenia in Patients with Pulmonary Infection (N = 11941)

Variables	Thrombocytopenia (n = 1171)	Normal platelet (n = 10770)	Univariate Analysis			Multivariate Analysis		
			OR	95%CI	p Value	OR	95%CI	p Value
Age, y(SD)	57.79 ± 24.06	58.76 ± 23.74	0.99	0.99- 1.00	0.19			
Sex, female, n(%)	454(38.8)	4356(40.4)	0.93	0.82- 1.06	0.27			
<b>Biochemical indexes</b>								
WBC(× 10 <sup>9</sup> /L)	9.90 ± 3.98	9.96 ± 4.08	0.99	0.98- 1.01	0.63			
HB(g/L)	126.75 ± 33.31	127.81 ± 33.49	0.98	0.98- 0.99	0.30			
CRP(mg/L)	49.54 ± 48.18	51.23 ± 49.29	0.99	0.98- 1.00	0.26			
PCT(ng/ml)	0.25 ± 0.15	0.31 ± 0.10	0.98	0.97- 1.99	0.87			
<b>CPIS scores</b>	7.25 ± 2.16	7.00 ± 2.99	1.25	1.18- 1.32	< 0.001*	1.24	1.17- 1.31	< 0.001*
<b>Medical history, n(%)</b>								
Hypertension	392(33.5)	4385(40.7)	0.73	0.65- 0.83	< 0.001*	0.74	0.65- 0.85	< 0.001*
Diabetes	177(15.1)	1798(16.7)	0.89	0.75- 1.05	0.17			
RF	101(8.6)	574(5.3)	1.68	1.35- 2.09	< 0.001*	1.59	1.27- 1.98	< 0.001*
COPD	78(6.7)	519(4.8)	1.24	0.91- 1.68	0.17			
Date were presented as mean±(SD) or n(%).								
Abbreviations: WBC, white blood cell; HB, hemoglobin; CRP, C-reactive protein; PCT, procalcitonin; CPIS, CLinical Lung Infection Score; RF, respiratory failure; COPD, chronic obstructive lung disease. OR odds ratio; CI, confidence interval; *Significant differences ( <i>P</i> < 0.05).								

## Low PaO<sub>2</sub> was a key risk factor for thrombocytopenia

To explore the key effective factor in PIN patients with RF, a subgroup analysis was conducted and is shown in Table 4. The APACHE II scoring system was adopted here to estimate the severity of RF [11]. Univariate analysis showed that the higher the APACHE II score was (OR = 1.09, 95% CI: 1.02–1.15;  $P = 0.007$ ), the higher the risk of thrombocytopenia. Relatively low PaO<sub>2</sub> (OR = 0.88, 95% CI: 0.85–0.91;  $P < 0.001$ ), hypertension (OR = 0.64, 95% CI: 0.41–0.99;  $P = 0.046$ ) and COPD (OR = 2.35, 95% CI: 1.22–4.53;  $P = 0.01$ ) were associated with thrombocytopenia. In multivariate analysis, both COPD (OR = 2.40, 95% CI: 1.22–4.76;  $P = 0.01$ ) and a high APACHE II score (OR = 1.06, 95% CI: 1.01–1.13;  $P = 0.03$ ) were risk factors for thrombocytopenia. It is important to emphasize that low PaO<sub>2</sub> was a potential risk factor for thrombocytopenia, which was supported by result of relatively high PaO<sub>2</sub> associated with a relatively low risk of thrombocytopenia (OR = 0.88, 95% CI: 0.85–0.92;  $P < 0.001$ ).

Table 4

Analysis on Risk Factors Associated with Thrombocytopenia in Pulmonary Infection Patients accompanying by Respiratory Failure (N = 675)

Variables	Thrombocytopenia (n = 101)	Normal platelet (n = 574)	Univariate Analysis			Multivariate Analysis		
			OR	95%CI	p Value	OR	95%CI	p Value
Age, y(SD)	58.92 ± 25.00	58.25 ± 23.45	1.00	0.99 - 1.01	0.79			
Sex, female, n (%)	34(33.7)	170(29.6)	1.21	0.77 - 1.89	0.41			
<b>Biochemical indexes</b>								
WBC(× 10 <sup>9</sup> /L)	9.48 ± 3.84	9.94 ± 4.01	0.97	0.92- 1.02	0.28			
HB(g/L)	126.39 ± 33.65	129.61 ± 33.32	0.09	0.99- 1.01	0.37			
CRP(mg/L)	45.39 ± 44.97	49.94 ± 48.43	0.99	0.99- 1.00	0.38			
PCT(ng/ml)	1.5 ± 1.2	1.6 ± 1.1	0.99	0.99- 1.01	0.56			
APACHEII scores	13.3 ± 3.9	12.3 ± 3.5	1.09	1.02- 1.15	0.007*	1.06	1.01- 1.13	0.03*
<b>ABGA</b>								
PH	7.39 ± 0.2	7.38 ± 0.2	1.08	0.30- 3.90	0.90			
PaO <sub>2</sub> (mmHg)	46.2 ± 7.5	52.8 ± 5.8	0.88	0.85- 0.91	< 0.001*	0.88	0.85- 0.92	< 0.001*
PaCO <sub>2</sub> (mmHg)	39.5 ± 16.1	38.7 ± 16.8	1.00	0.99- 1.02	0.64			
<b>Medical history, n (%)</b>								
Hypertension	34(33.7)	255(44.4)	0.64	0.41- 0.99	0.046*	0.65	0.41- 1.05	0.08

Date were presented as mean ± (SD) or n(%).

Abbreviations: WBC, white blood cell; HB, hemoglobin; CRP, C-reactive protein; PCT, procalcitonin; APACHE II scores, Acute Physiology and Chronic Health Evaluation; ABGA, arterial blood gas analysis; PaO<sub>2</sub> oxygen partial pressure; PaCO<sub>2</sub>, partial pressure of carbon dioxide; COPD, chronic obstructive lung disease. OR, odds ratio; CI, confidence interval. \*Significant differences ( $P < 0.05$ ).

Variables	Thrombocytopenia (n = 101)	Normal platelet (n = 574)	Univariate Analysis			Multivariate Analysis		
			OR	95%CI	p Value	OR	95%CI	p Value
Diabetes	16(15.8)	120(21.0)	0.71	0.40– 1.26	0.24			
COPD	34(33.7)	128(22.3)	2.35	1.22– 4.53	0.01*	2.40	1.22– 4.76	0.01*

Date were presented as mean± (SD) or n(%).

Abbreviations: WBC, white blood cell; HB, hemoglobin; CRP, C-reactive protein; PCT, procalcitonin; APACHE II scores, Acute Physiology and Chronic Health Evaluation; ABGA, arterial blood gas analysis; PaO<sub>2</sub> oxygen partial pressure; PaCO<sub>2</sub>, partial pressure of carbon dioxide ; COPD, chronic obstructive lung disease. OR, odds ratio; CI, confidence interval. \*Significant differences ( $P < 0.05$ ).

## Hypoxic mouse models with low PaO<sub>2</sub>

To verify whether low PaO<sub>2</sub> is a risk factor for thrombocytopenia, hypoxic mouse models were constructed[13] to analyze the blood gas content in left ventricular blood. The results showed that the PaO<sub>2</sub> (mmHg) was significantly decreased in blood from hypoxic mice (n = 8) compared with that from normoxic mice ( $59.63 \pm 6.39$  vs.  $76.63 \pm 9.58$ , respectively;  $P < 0.05$ ). For each Comparison, the sample size was eight.

## Reduction in PLTs independent of platelet activation after hypoxia

The count of PLTs ( $\times 10^9/L$ ) in angular vein blood was decreased in hypoxic mice compared with normoxic mice ( $518.38 \pm 127.92$  vs.  $840.75 \pm 77.30$ , respectively;  $P < 0.05$ ) and accompanied by increased hemoglobin (HGB) level (g/L) ( $196.0 \pm 10.56$  vs.  $140.0 \pm 5.78$ , respectively;  $P < 0.05$ ). There was no difference in the plasma soluble P-selectin concentration between these two groups ( $77.55 \pm 5.38$  vs.  $74.86 \pm 7.85$ , respectively;  $P > 0.05$ ). For each Comparison, the sample size was eight.

## MK numbers in the lungs decreased after hypoxia

The results of flow cytometry analysis showed lower proportions of CD41-positive cells (%) in the lungs ( $6.09 [5.66-6.71]$  vs.  $8.82 [8.26-10.27]$ , respectively;  $P < 0.05$ , Fig. 2A-B), bone marrow ( $2.11 \pm 1.12$  vs.  $5.03 \pm 1.72$ , respectively;  $P < 0.05$ , Fig. 2C-D) and spleen ( $0.39 [0.36-0.59]$  vs.  $0.74 [0.66-1.03]$ , respectively;  $P < 0.05$ , Fig. 2E-F) in hypoxic mice than in normoxic mice. Consistent with the flow cytometry results, the counts of CD41-positive cells calculated by fluorescence microscopy in the lungs ( $39.0 \pm 5.35$  vs.  $54.25 \pm 12.87$ , respectively;  $P < 0.05$ , Fig. 3A), bone marrow ( $10.00 \pm 2.78$  vs.  $24.88 \pm 3.68$ , respectively;  $P < 0.05$ , Fig. 3B) and spleen ( $2.75 \pm 1.04$  vs.  $8.75 \pm 5.29$ , respectively;  $P < 0.05$ , Fig. 3C) in hypoxic mice were lower than those in normoxic mice (Fig. 3D).

## Impaired platelet production was observed in hypoxic mouse lungs

To determine the number of platelets produced in the lungs, we investigated the  $PLT_{pre}$  and the  $PLT_{post}$ . The result showed that the  $PLT_{post}$  was higher than the  $PLT_{pre}$  in the normoxic group ( $713.63 \pm 124.15$  vs.  $543.75 \pm 121.17$ , respectively;  $P < 0.05$ ), which indicated that thrombopoiesis occur in the lungs. However, this phenomenon was not obvious in hypoxic mice ( $339.63 \pm 95.47$  vs.  $391.13 \pm 117.30$ , respectively;  $P > 0.05$ ). Namely, the  $\Delta PLT_{post-pre}$  value was less significant in the hypoxic group than in the normoxic group ( $F = 25.47$ ,  $P < 0.05$ ). For each Comparison, the sample size was eight.

## Discussion

There have been some cases of thrombocytopenia in PIN patients, but observational study of large samples is rare. We performed a retrospective study to observe the incidence of thrombocytopenia in patients with PIN, and patients with one of three other kinds of infections which was the most common in our hospital were chosen as controls. The results showed that the highest incidence of thrombocytopenia occurred in PIN patients among the four groups of infectious disease patients, which suggests that thrombocytopenia is likely associated with pulmonary infection. Subgroup analysis showed that PIN patients with RF had a higher risk of thrombocytopenia than those without RF. Furthermore, PIN with RF patients showing low  $PaO_2$  were more likely to have thrombocytopenia. These results indicated that low  $PaO_2$  might be a key risk factor for thrombocytopenia. In view of studies showing that the lungs can produce platelets[18, 19], it was reasonable to speculate that low  $PaO_2$  might induce thrombocytopenia by impairing platelet production in the lungs. To verify our hypothesis, we built a hypoxic mouse model, and the results showed that PLTs were decreased in hypoxic mice compared with normoxic mice, which demonstrated that low  $PaO_2$  indeed induced thrombocytopenia. In keeping with the fact that MKs circulate through the pulmonary capillaries where they release platelets[19], the  $PLT_{post}$  representing the postpulmonary(left ventricle) blood platelet was increased compared with the  $PLT_{pre}$  indicating the prepulmonary(right ventricle) blood platelet in normoxic mice. Hence, the  $\Delta PLT_{post-pre}$  index represented the generation of platelets in the lungs[20, 21]. Our results showed that  $\Delta PLT_{post-pre}$  was significantly attenuated in hypoxic mice compared with normoxic mice. The lower proportion of CD41-positive MKs indicated by histology and flow cytometry, and the decreased  $\Delta PLT_{post-pre}$  in hypoxic mice confirmed the speculation that low  $PaO_2$  could reduce MKs and impair the thrombocytopoiesis in the lungs.

Although infection is known to cause thrombocytopenia[22–24], cohort studies associated with different organ infections have not been reported. In the present study, the incidence of thrombocytopenia in PIN patients showed a significant increase, which suggested that the lungs could affect the physiological behavior of platelets in a particular way. Based on the conclusion that there were no associations between bacterial species and the incidence of thrombocytopenia in infectious diseases[23], we speculate that additional mechanisms might cause pulmonary thrombocytopenia.

Several previous studies described the link between low  $PaO_2$  and thrombocytopenia. A clinical observation showed that thrombocytopenia occurred in 31% of neonates with asphyxia versus 5% of matched controls without asphyxia[25]. Another study found that thrombocytopenia was a predictive factor for the progression of pneumonia to RF[26]. We confirmed in clinical cases that low  $PaO_2$  was a key risk factor for

thrombocytopenia through a relatively large sample of PIN patients for the first time. Severity of disease was associated with the incidence of thrombocytopenia [22, 24]. There is a relatively high incidence of thrombocytopenia ranging from 20–50% in critical patients[27]. Both the CPIS and the APACHE II score were positively associated with the risk of thrombocytopenia. It is worth mentioning that low PaO<sub>2</sub> is an independent risk factor for thrombocytopenia after adjusting for the APACHE II score, which makes the results more convincing.

The influence of hypoxia on bone marrow MKs is well described. Chronic hypoxia impair bone marrow MKs [28] and inhibit the differentiation of bone marrow MKs[29]. Or the erythroid system and the MK system share a common precursor in the bone marrow, and there is competition between erythroid and MK differentiation upon exposure to a hypoxic environment [30]. However, the effect of hypoxia on pulmonary thrombocytopoiesis has not been investigated as the lung is another important site of platelet biogenesis. We constructed hypoxic mouse models and found that low PaO<sub>2</sub> caused thrombocytopenia. P-selectin, an indicator of platelet activation, showed no significant difference between hypoxic and normoxic mice, which indicated that thrombocytopenia was not attributed to platelet activation. Researchers have paid close attention to the process of platelet generation in the lungs[3, 19]. There are abundant MKs in the pulmonary arterial blood but only a few MKs in the pulmonary venous blood under normal conditions[21], but thrombocytopenia occurs in patients with congenital heart diseases because a right-to-left shunt bypasses the lung where thrombocytopoiesis occurs [31]. A large number of MKs dynamically circulate through the lungs, where they release platelets [19]. Consistent with these findings, we observed a large number of CD41-positive MKs in the mouse lungs and found that the PLT<sub>post</sub> was higher than the PLT<sub>pre</sub>, indicating that the mouse lungs indeed were a site of platelet production. Interestingly, we found that hypoxia could reduce lung MKs and impair efficacy of thrombocytopoiesis in the lung.

It is important to note that there are several limitations to our study. First, A large-scale, multicenter, prospective investigation based on the relationship between thrombocytopenia and respiratory is needed to provide more convinced evidence. Second, the detailed molecular mechanisms underlying the process of platelet generation in the lungs and how low PaO<sub>2</sub> affects this process were not illuminated. In spite of these limitations, we believe that our results are the first to provide the correlation between lung diseases and thrombocytopenia with data from both clinical studies and mouse models, which would help to guide the management of patients with respiratory diseases, especially for those people with lung damage or chronic respiratory failure; but also optimize antiplatelet therapy in specific patients with respiratory comorbidities. We anticipate that future studies utilizing mouse models of chronic lung injury will focus on identification of mechanisms underlying pulmonary thrombocytopenia.

## Conclusions

We conclude that PIN relatively easily results in thrombocytopenia and thrombocytopenia induced by low PaO<sub>2</sub> might be associated with impaired thrombopoiesis in the lungs.

## Abbreviations

PIN: Pulmonary Infection; UTI:Urinary Tract Infection; MKs:Megakaryocytes; RF:Respiratory Failure; COPD:Chronic Obstructive Pulmonary Disease; PaO<sub>2</sub>:Oxygen Partial Pressure; ICD-10:International classification of diseases code-10; UTI:urinary tract infection; ITI:intestinal tract infection; CPIS:clinical pulmonary infection score; APACHE II:Acute Physiology And Chronic Health Evaluation II; PLTs:Blood platelets in angular vein; PLT<sub>post</sub>:Blood platelets in left ventricle; PLT<sub>pre</sub>:Blood platelets in right ventricle;  $\Delta$ PLT<sub>post-pre</sub>:The difference between the PLT<sub>post</sub> and the PLT<sub>pre</sub>.

## Declarations

**Acknowledgments:** Not applicable.

**Ethics approval and consent to participate:** The study procedures were approved by both the Medical Ethics Committee and the Animal Care and Use Committee of the Second Affiliated Hospital of Nanchang University. Details that might disclose the identity of the subjects under the study have been omitted.

**Authors' contributions:** Prof. KH conceived and designed the study. LW and NG completed the animal experiment. NG, QMX, ZX, JH and RW performed data collection and analysis. WWcalculated the CPIS and APACHE II scores. NG and LW wrote the paper. Prof. KH reviewed and edited manuscript. All authors have contributed to read and approved the manuscript.

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**Consent to publication:** Not applicable.

**Competing interests:** The authors declare that they have no competing interests.

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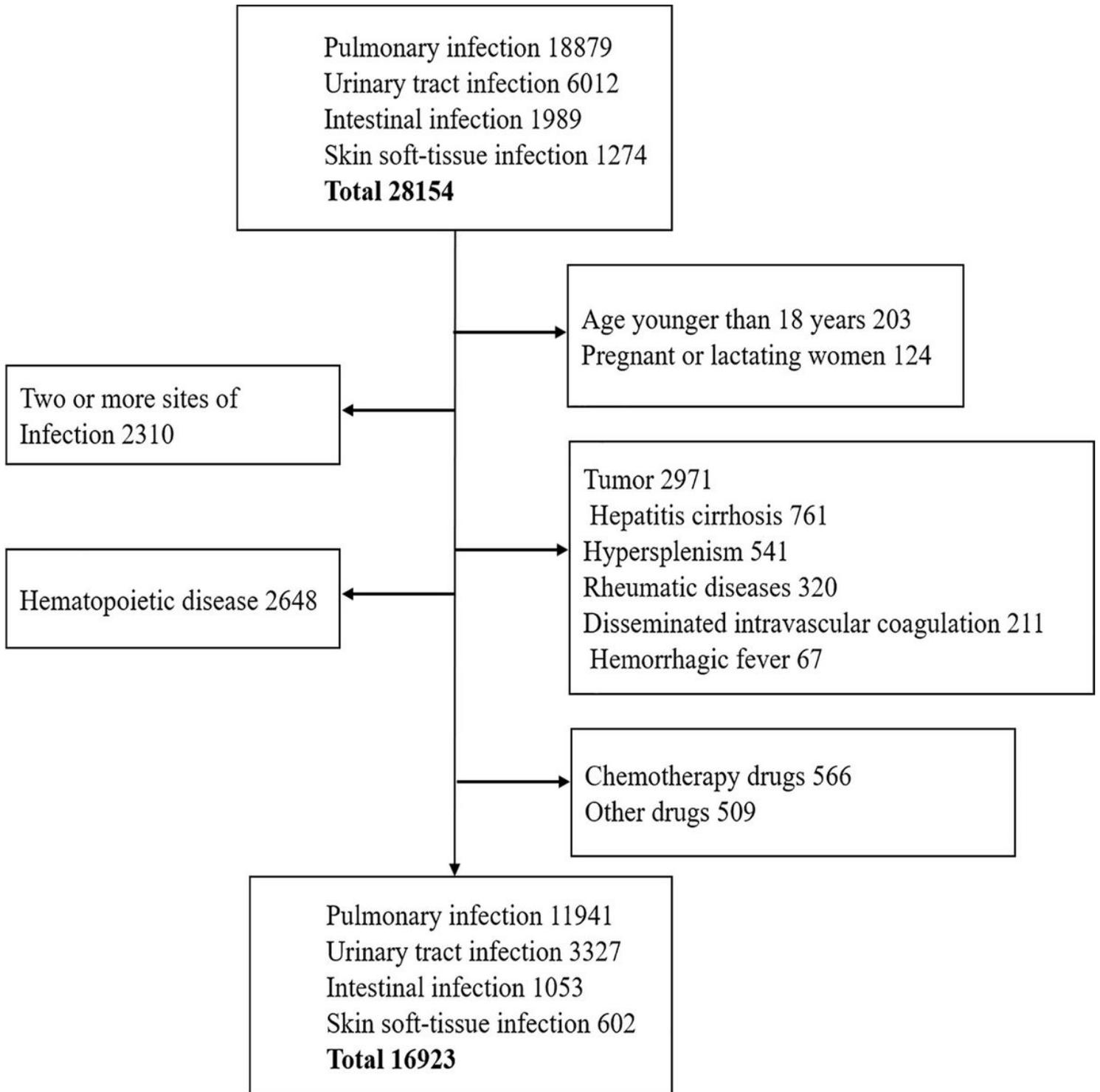
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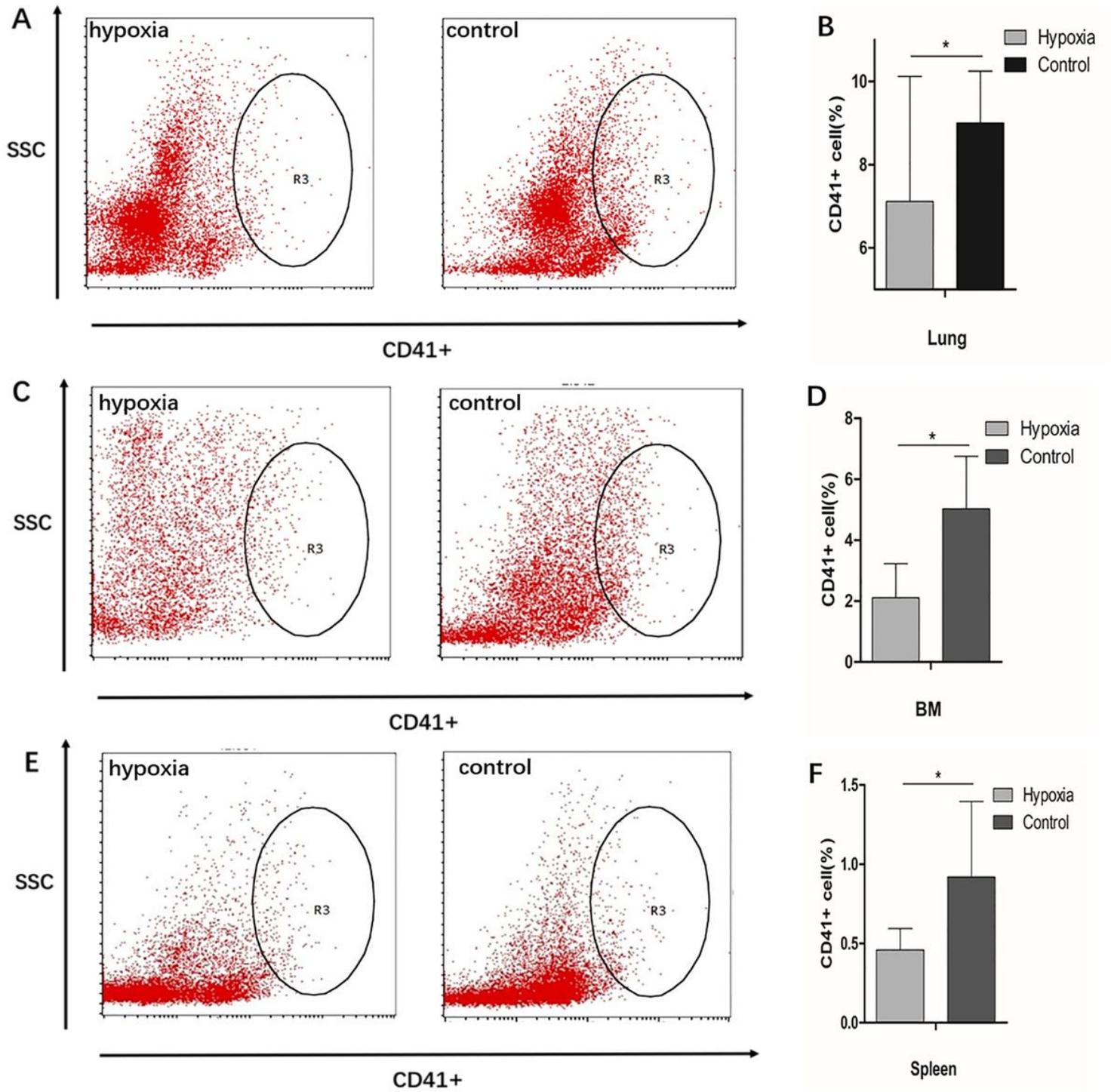
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## Figures



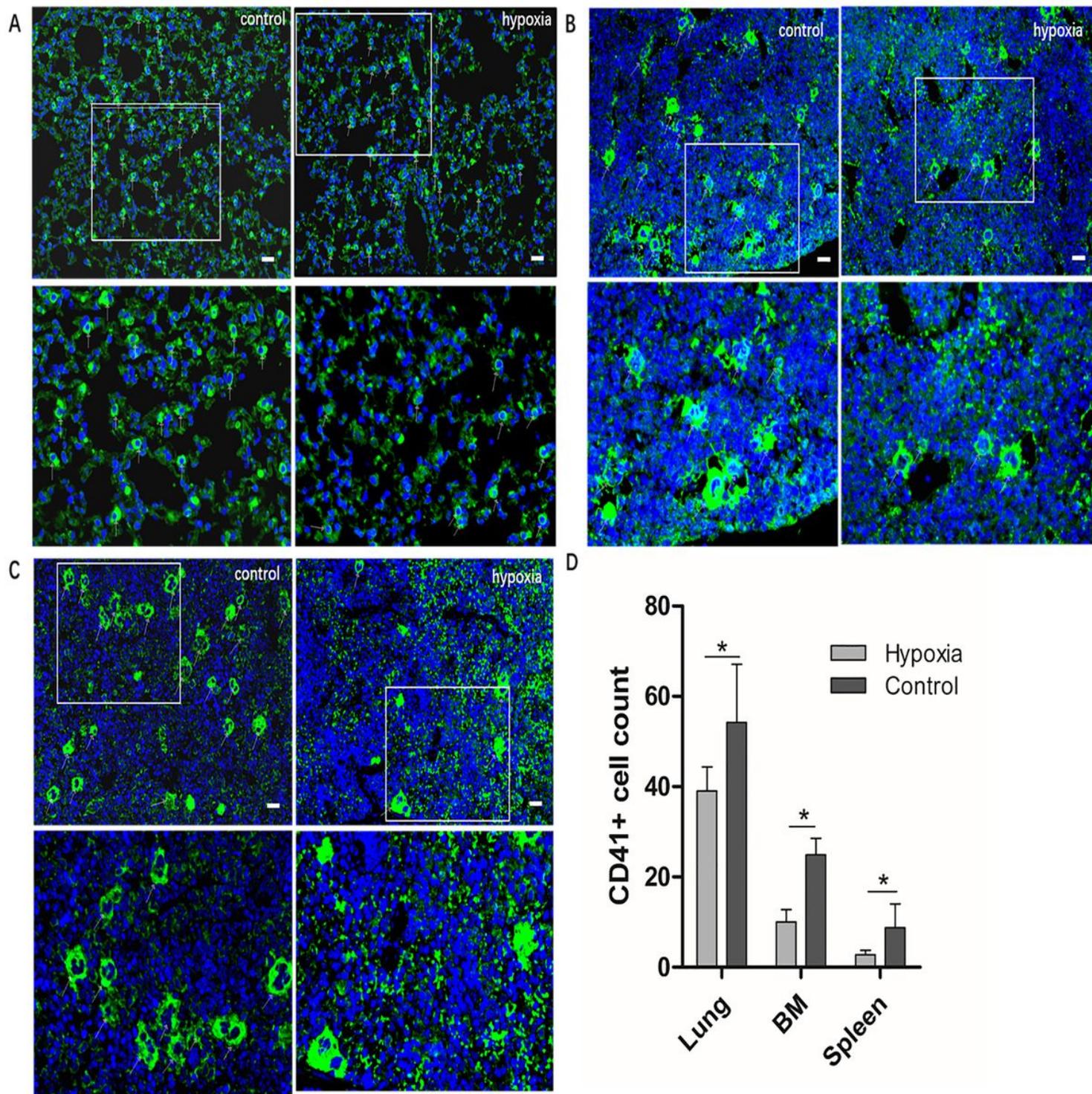
**Figure 1**

Flowchart showing the process of this study



**Figure 2**

Hypoxia reduced the megakaryocytes in tissues (flow cytometry). Gating strategy and analysis of megakaryocytes (CD41+) in lung (A-B), bone marrow (C-D) and spleen (E-F). (N=8). Values are mean  $\pm$  s.d. \*P<0.05.



**Figure 3**

Hypoxia reduced the megakaryocytes in tissues (immunofluorescence). Representative images of paraffin section from the lung(A), bone marrow (B), spleen (C). MKs (green, CD41) and nuclei (blue, DAPI) were showed. (N=8). Above, 200× amplified; below, 400× amplified. Relative quantitative analysis of megakaryocytes from tissue were showed (D). \*P<0.05.

## Supplementary Files

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- [supplement8.pdf](#)