Platelet aggregates in lung capillaries in severely decompensated pulmonary hypertension

Camille Miard,[1] Vincent Thomas de Montpreville,[2] Jean-François Bernaudin,[2,3,4] Julien Adam,[2] Chakib Djediat,[5] Francois Stephan [1,6]

[1]Cardiothoracic Intensive Care Unit, Hospital Marie Lannelongue, Le Plessis-Robinson, France
[2]Pathology Department, Hospital Marie Lannelongue, Le Plessis-Robinson, France
[3]INSERM 1272 Hypoxia & Lung SMBH 93000, Bobigny, France
[4]Faculty of Medicine, Sorbonne Université 75013, Paris, France
[5]Department of electronic microscopy, Museum National d'Histoire Naturelle, Paris, France
[6]Paris Saclay University, School of Medicine, 94270, Le Kremlin-Bicêtre, France

Correspondence to
Professor Francois Stephan; f-stephan@hotmail.fr
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ABSTRACT

The mechanism of thrombocytopenia during acute pulmonary hypertension (PH) decompensation may be partly due to platelet aggregation in the lung. Platelet aggregates in explanted lung from 16 lung transplant patients during acute PH decompensation with and without thrombocytopenia were identified by immunohistochemistry. Scanning electron microscopy (SEM) was performed. 7 explant lung controls without PH and thrombocytopenia were also examined. Compared with controls, the median number of platelet aggregates was higher in patients with acute PH decompensation with thrombocytopenia (19.4 [IQR 3.4–38.3] vs 147.5 [IQR 26.5–203.2]). SEM showed capillaries filled with platelet aggregates. Our study suggests that platelets may aggregate in the lungs during acute PH decompensation.

INTRODUCTION

Thrombocytopenia is reported to occur in about 20% of patients with pulmonary hypertension (PH).[1] The severity of PH may result in a more pronounced platelet nadir.[2] Given the multiple confounding factors, the mechanisms causing thrombocytopenia in PH remain largely enigmatic.[1] One report suggested that thrombocytopenia in PH may not stem from increased platelet consumption.[3] Acute PH decompensation results in pulmonary blood-flow disturbances, endothelial dysfunction and inflammation,[3–5] all of which may promote platelet aggregation and sequestration.[3 4]

We hypothesised that thrombocytopenia in patients with decompensated PH may be at least partially due to platelet aggregation in the lungs.

PATIENTS AND METHODS

This retrospective study approved by the French Ethical Committee for Research in Anaesthesia and Critical Care (IRB 00010254-2023-069) was designed to compare blood platelet counts and intracapillary platelet abundance in explanted lung tissue after lung transplantation.

We identified 105 patients with PH who underwent high-priority lung transplantation between July 2012 and July 2022. The inclusion criteria were acute PH decompensation defined by low peripheral perfusion pressure and the occurrence of organ failure requiring catecholamine treatment on intensive care unit admission before lung transplantation,[6] absence of chronic thromboembolic PH, availability of blood platelet counts at the time of PH decompensation and availability of paraffin-embedded tissue samples from the removed lungs. The 16 patients who met these criteria were divided into two groups depending on blood platelet count: thrombocytopenic (<150 G/L)[1 2] (group1, n=8) and normal platelet count (≥150 G/L) (group 2, n=8). We also selected seven transplant patients without PH and thrombocytopenia (controls, n=7) (table 1).

Platelet aggregates and megakaryocytes in lung-tissue sections were identified by immunohistochemistry (IHC) and counted. The markers used for IHC were CD41 (CD41 clone EP178 diluted at 1:100; Vitro Master Diagnostica) and CD61 (CD61 clone EP65 diluted at 1:100; Bio SB) anti-human rabbit monoclonal antibodies[7] (figure 1A,B). Scanning electronic microscopy (SEM) on paraffin-embedded tissue sections (see figure 1 legend) was done on samples from a patient with severe thrombocytopenia (figure 1C) and a control.

The Kolmogorov–Smirnov test was applied to assess normality of data distribution. Quantitative variables were described as mean±SD if normally distributed and as median (IQR) if otherwise. Baseline categorical variables were described as n (%). Fisher's exact test was applied to compare proportions and rates. Comparisons of the three groups relied on analysis of variance followed by Scheffe's F test for post-hoc comparisons of quantitative variables. *P* values of ≤0.05 were considered significant.

Because the number of platelet aggregates and megakaryocytes was right-skewed, we performed logarithmic transformation and analysed the IHC results on the transformed values. The results are expressed as antilogs, ie the geometric mean with its 95% 95% CI.[8]

RESULTS

Platelet aggregates and megakaryocytes were identified as CD41+or CD61+ brown immunolabelled spots or as characteristic cells, respectively, as shown in (figure 1A,B). In addition, intracapillary features evocative for platelet aggregates were observed by SEM (figure 1C).[9]

As shown in table 2, compared with the controls, the number of CD41+platelet aggregates was significantly higher in group 1 (p=0.018) and non-significantly higher in group 2 (p=0.08). The higher number of intravascular CD41+megakaryocytes in group 1 was nearly significant versus controls (p=0.059).

Table 1 Patient characteristics and biological variables

Variables	Group 1 (platelet count<150 G/L) (n=8)	Group 2 (platelet count≥150 G/L) (n=8)	Controls (n=7)	ANOVA or Fisher's exact test
General characteristics				
Age, y, mean±SD	49.5±11.2	36.6±16.0	59.0±6.7	0.007*
Males, n, (%)	1 (12.5)	4 (50.0)	3 (43.0)	0.31
Weight, kg, mean±SD	60.9±16.0	69.5±14.2	69.7±10.2	0.37
Height, cm, mean±SD	161.5±7.0	168.1±14.7	170.1±5.8	0.24
Simplified acute physiologic score II, point, mean±SD	28±9	31±12	–	
Classification of pulmonary hypertension,† n (%)				–
Pulmonary arterial hypertension (PAH)				
Idiopathic	3 (37.5)	1 (12.5)	–	
Pulmonary veno-occlusive disease‡	2 (25.0)	1 (12.5)	–	
Heritable	1 (12.5)	3 (37.5)	–	
Drug-induced	1 (12.5)	–	–	
Pulmonary hypertension due to lung disease				
Interstitial lung disease	1 (12.5)	3 (37.5)	–	
Chronic lung diseases	–	–		
Idiopathic pulmonary fibrosis			3 (43)	
Obstructive lung disease			4 (57)	
Treatment of pulmonary hypertension, n (%)				
Phosphodiesterase-5 inhibitors	6 (75.0)	5 (62.5)	–	
Endothelin receptor antagonist	6 (75.0)	6 (75.0)	–	
Parenteral prostacyclin therapy	5 (62.5)	5 (62.5)	–	
Epoprostenol§	4 (50.0)	2 (25.0		
Treprostinil¶	1 (12.5)	3 (37.5)		
Soluble guanylate cyclase stimulator	0	1 (12.5)	–	
Anticoagulants, n (%)				
Oral anticoagulants	1 (12.5)	0	–	
Unfractionated heparin	2 (25.0)	4 (50)	–	
Low-molecular-weight heparin	5 (62.5)	4 (50)	–	
Identified triggering factor, n (%)**	4 (50)	2 (25)		
Biochemical variables				
Creatinine at steady-state, µmol/L, mean±SD	73±19[1]	63±9[2]	54±14[3]	0.06
Creatinine during decompensated pulmonary hypertension, µmol/L, mean±SD	111±30	90±30	54±13	0.002††
Aspartate aminotransferase, UI/mL, mean±SD	46±27	218±538	26±8	0.44
Alanine aminotransferase, UI/mL, mean±SD	27±11	347±905	29±8	0.41
Bilirubin, µmol/L, mean±SD	27±16	19±8	6±2	0.004‡‡
Pro-brain natriuretic peptide, pg/mL, mean±SD	4659±4108	2925±2524	65±36	0.034§§
Haemodynamic data				
Pulmonary artery pressure, mmHg, mean±SD	52±16	74±22	19±6	<0.0001¶¶
Right atrial pressure, mmHg, mean±SD	14.9±4.5	13.2±4.3	–	0.49***
Dobutamine, n (%)	8 (100)	5 (62.5)	–	–
Norepinephrine, n (%)	6 (75)	3 (37.5)	–	–
Pretransplantation veno-arterial ECMO, n (%)	3 (37.5)	1 (12.5)	–	–
Pretransplantation veno-venous ECMO, n (%)	0	1 (12.5)	–	–
Haematological variables				
White blood cells, G/L, mean±SD	6.3±2.2	8.4±4.1	10.9±6.9	0.58
Haemoglobin, g/dL, mean±SD†††	12.2±3.2	13.4±1.7	13.1±2.5	0.58
Platelet count at steady-state, G/L, mean±SD	180±35	233±38	255±57	0.013‡‡‡

Continued

Table 1 Continued

Variables	Group 1 (platelet count<150 G/L) (n=8)	Group 2 (platelet count≥150 G/L) (n=8)	Controls (n=7)	ANOVA or Fisher's exact test
Platelet count and characteristics during decompensated pulmonary hypertension and before ECMO placement if relevant, G/L, mean±SD§§§	138±8	207±34	252±47	<0.0001¶¶¶
Mean platelet volume, fL, mean±SD	10.1±1.4	10.0±1.2	10.5±0.6	0.78
Platelet distribution width, fL mean±SD	15.4±2.7	11.0±2.8	12.1±1.5	0.05****
Mean Outcome				
Post-transplant death in the ICU, n (%)	4 (50)	2 (25)	0	0.13

- Creatinine at steady state vs creatinine during decompensated pulmonary hypertension (paired-samples t-test procedure): 1P=0.003, 2P=0.001, 3P=0.93.
- For the ANOVA or X^2 test, P values ≤0.05 were considered statistically significant. Group 1: pulmonary hypertension and thrombocytopenia. Group 2: Pulmonary hypertension and normal platelet count.
*Group 2 vs. controls, P=0.007
†Pulmonary arterial hypertension plexiform lesions were found in native lungs from five patients with severe thrombocytopenia, three patients with moderate thrombocytopenia, and none of the controls (P=0.04).
‡A specific association with platelet aggregates was not observed.
§Epoprostenol was prescribed in five patients in group 1 (mean dose=37.7±12.4 ng/kg/min) and two patients in group 2 (mean dose=22.5±2.1 ng/kg/min).
¶Treprostinil was prescribed in one patient in group 1 (dose=31 ng/kg/min) and three patients in group 2 (mean dose=34.0±12.8 ng/kg/min).
**Triggering factor: group 1= infection (n=2), haemorrhage (n=1), effort (n=1); group 2=thrombosis of Potts shunt (n=1), haemorrhage (n=1). In other cases, acute PH decompensation was a manifestation of disease worsening.[6]
††Group 1 vs. controls, P=0.002; Group 2 vs. controls, P=0.04.
‡‡Group 1 vs. controls, P=0.004.
§§Group 1 vs. controls, P=0.036.
¶¶Group 1 vs. controls, P=0.016; Group 2 vs. controls, P<0.0001.
***By Student's t-test.
†††There was no evidence of a microangiopathic picture on blood smears (absence of schizocytes) and haemolysis index was zero.
‡‡‡Group 1 vs. controls, P=0.016.
§§§A median of 25.5 day [IQR 10.0-72.0] elapsed between the steady-state platelet count and that taken on the day of pulmonary hypertension decompensation.
¶¶¶Group 1 vs. group 2, P=0.002; Group 1 vs. controls, P<0.0001; Group 2 vs. controls, P=0.005.
****Group 1 vs. group 2, P=0.06.
ECMO, extracorporeal membrane oxygenation; ICU, intensive care unit.

We found significant correlations linking the numbers of CD41+and CD61+ platelet aggregates (r=0.79 [0.54–0.91], p<0.0001) in all patients studied; that is, the numbers of CD41+and CD61+ megakaryocytes (r=0.67 [0.34–0.85], p=0.0009), CD41+platelet aggregates and CD41+megakaryocytes (r=0.64 [0.30–0.83], p=0.001) and CD61+platelet aggregates and CD61+megakaryocytes (r=0.85 [0.67–0.94], p<0.0001).

The blood platelet count correlated significantly with the numbers of CD41+platelet aggregates in the lung (figure 1D) and megakaryocytes. Similar correlations were found for CD61+platelet aggregates and megakaryocytes.

The platelet counts normalised (>150 G/L) at a median of 1 [IQR 1–5] day after transplantation, with a mean platelet count of 177±29 G/L.

DISCUSSION

These results strongly suggest that platelets aggregate in the lungs in patients with thrombocytopenia and acute decompensated PH. The number of lung platelet aggregates and megakaryocytes correlated with the severity of thrombocytopenia.

Normally, most platelets travel unimpeded through the alveolar capillaries.[4] The vascular, endothelial and immune abnormalities associated with PH[1 3–5] may contribute to platelets trapping within lung capillaries. The abnormal pulmonary blood flow in acute decompensated PH increases the pressure within microvessels.[5] The resulting hydrodynamic changes, notably increased shear stress, may promote the formation of platelet aggregates, which may obstruct the blood flow. This possibility is consistent with our finding that capillaries containing platelet aggregates were dilated (figure 1A,C). A higher platelet distribution width with a normal mean platelet volume may indicate platelet activation and selective platelet destruction with a compensatory response.[10] Thus, a vicious circle of PH-related flow disturbances, platelet aggregation, lumen obstruction and further flow disturbances may be created.[4 5] Vascular thrombosis is a common finding in advanced PH,[1 3] and the more severe the PH, the more platelet aggregates can be observed.[3]

Intracapillary megakaryocyte numbers were also increased in patients with thrombocytopenia. The lung is a site of active megakaryopoiesis,[11] and the occurrence of thrombocytopenia may trigger a compensatory pulmonary response.[11] However, the regulation of platelet production in the lung is poorly understood.[11] An increase in lung platelets due to megakaryopoiesis activation during decompensated PH may contribute to further lung-capillary thrombosis.

Our study has several limitations. First, we included multiple forms of PH. Including only pulmonary arterial hypertension would have been more compelling, but such patients are rare (12 patients identified over a period of 10 years) and the statistical power would have been too low. Furthermore, our hypothesis relates to a specific subgroup of the most severe patients with decompensated PH requiring catecholamines. It should be interesting to test the hypothesis of the occurrence of lung capillary platelets aggregates in animal models of PH. Second, we acknowledged that despite a careful reading, the quantification of platelet aggregates and megakaryocytes is an approximation, especially in samples taken randomly from different regions of the lungs. However, the counts were performed on similar areas of the tissue section. Third, nearly two-thirds of our patients with PH were receiving parenteral prostacyclin therapy, which may be associated with thrombocytopenia.[2 3] Fourth, the retrospective design precluded an investigation of immunological

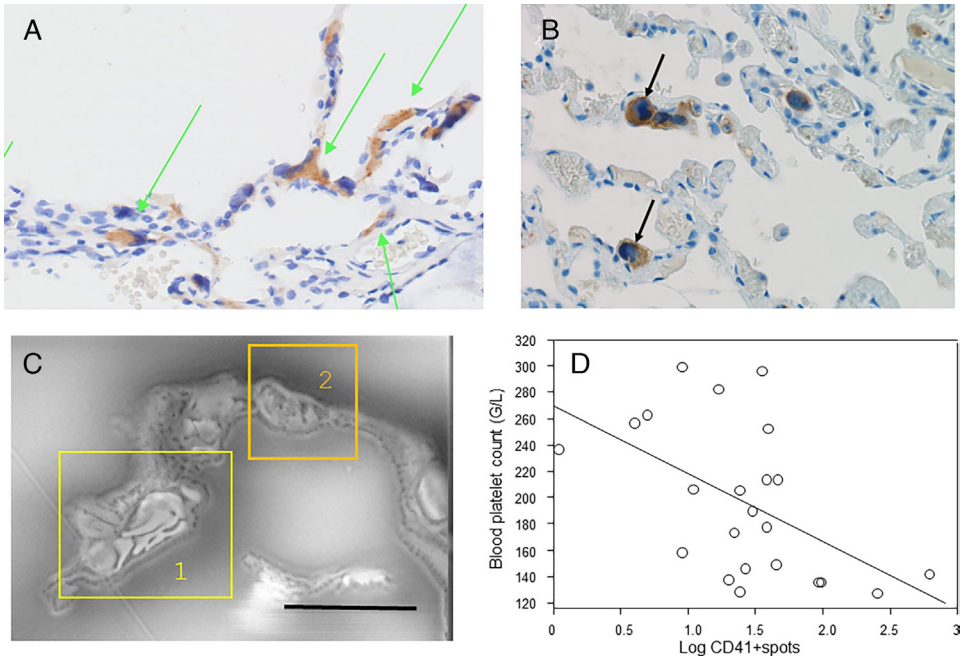

Figure 1 Summary of the main results. (A, B) Immunohistochemistry on lung-tissue sections. For each patient, representative samples of the distal lung parenchyma were subjected to random examination. Four sections were examined, comprising two for anti-CD41 and anti-CD61 immunostaining, along with their respective controls. For each patient, a slide labeled with CD41 and a slide labeled with CD61 were examined. The reading was conducted simultaneously by CM and J-F B. The brown labelling is produced by the final 3,3'-diaminobenzidine staining after incubation with either anti-human CD41 (CD41 clone EP178 diluted at 1:100; Vitro Master Diagnostica) or anti-human CD61 (CD61 clone EP65 diluted at 1:100; Bio S) rabbit monoclonal antibodies. (1): Intracapillary CD41+ platelet aggregates (green arrows). (2): Characteristic intracapillary CD61+ megakaryocytes (black arrows). Controls, especially isotypic, were negative (not shown) (original magnification A X100, B X200). (C) Scanning electron microscopy on paraffin-embedded lung-tissue sections. After deparaffinisation, a second glutaraldehyde fixation was done, and 0.5 µm-thick sections were examined under a Hitachi SU3500 scanning electron microscope (fixation technique developed by Hirsh and Fedorko, J Cell Biol 1968, adapted by Djediat et al., 1990 Haliotis (10): 115–127). Sample from a patient with severe thrombocytopenia; two interalveolar pulmonary capillaries are framed, with frame 1 containing a capillary filled with characteristic red blood cells and frame 2 a capillary filled with material evocative for platelet aggregates [9]. No such features were observed in the sample from the control patient (data not shown) (bar: 15 µm). (D) Scatter diagram illustrating the correlation between (y axis) the blood platelet count (G/L) and (x axis) the CD41+ platelet aggregate count in native lung tissue from the patients in the three groups (R=0.53; P=0.009).

Table 2 Immunohistochemical results

Variables	Group 1(platelet count<150 G/L) (n=8)	Group 2(platelet count≥150 G/L) (n=8)	Controls (n=7)	ANOVA
CD61 and CD41 immunohistochemical results				
Surface area of tissue section (mm^2), mean±SD	475±72	417±75	404±87	0.19
IHC-positive platelet aggregates and megakaryocytes*				
CD61+platelet aggregates, n, median (IQR)	98.2 (14.8–142.6)	32.8 (9.4–51.6)	18.0 (1.8–36.9)	–
CD41+platelet aggregates, n, median (IQR)	147.5 (26.5–203.2)	24.1 (9.3–38.3)	19.4 (3.4–39.3)	–
CD61+megakaryocytes, n, median (IQR)	30.0 (4.2–45.5)	17.4 (0.7–23.2)	3.2 (0.8–5.6)	–
CD41+megakaryocytes, n, median (IQR)	10.5 (2.6–14.7)	4.0 (1.1–5.9)	2.1 (1.2–3.1)	–
CD61+platelet aggregates, log number, mean±SD	1.66±0.59	1.34±0.40	0.91±0.62	0.06
CD41+platelet aggregates, log number, mean±SD	1.86±0.53	1.27±0.37	1.06±0.57	0.013†
CD61+megakaryocytes, log number, mean±SD	1.14±0.61	0.61±0.81	0.32±0.41	0.07
CD41+megakaryocytes, log number, mean±SD	0.79±0.45	0.42±0.43	0.28±0.22	0.04‡
CD 61+platelet aggregates, geometric mean (95% CI)	98.2 (14.8–142.5)	32.8 (9.4–51.6)	18.0 (1.8–36.9)	–
CD 41+platelet aggregates, geometric mean (95% CI)	147.5 (26.5–203.2)	24.1 (9.3–38.3)	19.4 (3.4–39.3)	–
CD 61+megakaryocytes, geometric mean(95% CI)	30.0 (4.2–45.5)	17.4 (0.7–23.2)	3.1 (0.8–5.6)	–
CD 41+megakaryocytes, geometric mean (95% CI)	10.5 (2.6–14.7)	4.0 (1.1–6.0)	2.1 (1.2–3.1)	–

*As shown in figure, the immunohistochemistry results are given as numbers of CD41+or CD61+ platelet aggregates or number of megakaryocytes.
†Group 1 vs. controls, P=0.018; Group 1 vs. group 2, P=0.08.
‡Group 1 vs. controls, P=0.059.
IHC, immunohistochemistry.

mechanisms involving platelet autoantibodies. However, the rapid return to normal of the platelet counts after lung transplantation argues against such a mechanism.

In conclusion, our study suggests that platelets may aggregate within the lungs during acute PH decompensation. If this is indeed the case, platelet transfusion may be hazardous and possibly best avoided, except in patients with active bleeding or at the beginning of the lung transplantation procedure.

Correction notice Since this article first published, the author affiliations for JFB have been updated.

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ORCID iD

Francois Stephan http://orcid.org/0000-0002-6785-5412

REFERENCES

1 Rosenkranz S, Howard LS, Gomberg-Maitland M, *et al*. Systemic Consequences of Pulmonary Hypertension and Right-Sided Heart Failure. *Circulation* 2020;141:678–93.
2 Chin KM, Channick RN, de Lemos JA, *et al*. Hemodynamics and epoprostenol use are associated with thrombocytopenia in pulmonary arterial hypertension. *Chest* 2009;135:130–6.
3 Aytekin M, Aulak KS, Haserodt S, *et al*. Abnormal platelet aggregation in idiopathic pulmonary arterial hypertension: role of nitric oxide. *Am J Physiol Lung Cell Mol Physiol* 2012;302:L512–20.
4 Weyrich AS, Zimmerman GA. Platelets in lung biology. *Annu Rev Physiol* 2013;75:569–91.
5 Happé CM, Szulcek R, Voelkel NF, *et al*. Reconciling paradigms of abnormal pulmonary blood flow and quasi-malignant cellular alterations in pulmonary arterial hypertension. *Vascul Pharmacol* 2016;83:17–25.
6 Savale L, Weatherald J, Jaïs X, *et al*. Acute decompensated pulmonary hypertension. *Eur Respir Rev* 2017;26:170092.
7 Galindo M, Gonzalo E, Martinez-Vidal MP, *et al*. Immunohistochemical detection of intravascular platelet microthrombi in patients with lupus nephritis and anti-phospholipid antibodies. *Rheumatology (Oxford)* 2009;48:1003–7.
8 Bland JM, Altman DG. Statistics notes: Transformations, means, and confidence intervals. *BMJ* 1996;312:1079.
9 Warren BA, de Bono AH. The ultrastructure of initial stages of platelet aggregation and adhesion to damaged vessel walls in vivo. *Br J Exp Pathol* 1970;51:415–22.
10 Zheng Y-G, Yang T, Xiong C-M, *et al*. Platelet distribution width and mean platelet volume in idiopathic pulmonary arterial hypertension. *Heart Lung Circ* 2015;24:566–72.
11 Lefrançais E, Looney MR. Platelet Biogenesis in the Lung Circulation. *Physiology (Bethesda)* 2019;34:392–401.