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Platelet aggregability in patients with interstitial pneumonias

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Abstract. *Background:* Recent epidemiological studies have shown that patients with interstitial pneumonia have an increased risk of cardiovascular events. Although the presence of a coagulation/fibrinolysis abnormality in idiopathic pulmonary fibrosis (IPF) has been reported, platelet aggregability has not been evaluated in interstitial pneumonias. This study aimed to investigate platelet aggregability in patients with interstitial pneumonias. *Methods:* This observational cohort study included 59 patients with interstitial pneumonias [19 with IPF, 23 with other idiopathic interstitial pneumonias (IIPs), and 17 with connective tissue disease-associated interstitial pneumonias (CTD-IPs)] and 23 healthy control subjects. ADP- and collagen-induced platelet aggregability was measured together with coagulation/fibrinolysis markers. Whole blood (WB) and platelet rich plasma platelet aggregation were measured using the screen filtration pressure and optical aggregometer techniques, respectively. The platelet aggregation threshold index (PATI) was calculated; a lower PATI indicated enhanced platelet aggregability. *Results:* ADP-induced WB-PATI was significantly decreased in CTD-IPs [log WB-PATI median 0.31 (inter-quartile range, 0.07–0.34) μM, n = 17] compared with that in controls [0.35 (0.32–0.45) μM, n = 23] ($p < 0.05$). However, there was no significant difference in platelet aggregability between the other patient groups and controls. In contrast, d-dimer, thrombin–antithrombin complex, and von Willebrand factor levels were significantly higher in all patient groups compared with those in controls ($p < 0.001$). Platelet aggregability was not associated with either disease severity or survival. *Conclusions:* Serum coagulation and fibrinolysis markers significantly increased in IIPs and CTD-IPs. In contrast, platelet aggregability was only weakly enhanced in CTDs, but not in IIPs. *(Sarcoidosis Vasc Diffuse Lung Dis 2016; 33: 143-150)*

Key words: coagulation factor, connective tissue disease, fibrinolysis, idiopathic pulmonary fibrosis, screen filtration method, thrombosis

Introduction

Idiopathic pulmonary fibrosis (IPF) is defined as a specific form of chronic, progressive fibrosing interstitial pneumonia (1). It is characterized by progressive worsening of dyspnea and lung function, and it is associated with a poor prognosis. Previous

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epidemiological studies showed that patients with IPF have an increased risk of cardiovascular events and deep venous thrombosis (2-4). It has been reported that patients with IPF have abnormalities in the coagulation/fibrinolysis system (5-8), which are associated with a poor prognosis (5). Platelets interact with coagulation factors or leukocytes and also play an important role in thrombus formation (9-11). Activated platelets secrete several cytokines and growth factors [e.g., platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), and basic fibroblast growth factor (bFGF)] that have been suggested to be involved in the pathogenesis of lung fibrosis (9, 12). Furthermore, inherited storage pool defects in platelets cause secondary pulmonary fibrosis (e.g., Hermansky-Pudlak syndrome) (13). Recently, we found that endothelial function was impaired in idiopathic interstitial pneumonias (IIPs) including IPF (14). The impaired endothelial function can also influence the activity of platelets (10, 15). These facts suggest that platelet activation or dysfunction is involved in the pathogenesis of interstitial pneumonias and its associated thrombosis.

In response to vascular injury, altered blood flow, or chemical stimuli, platelets are activated with a functional triad of adhesion, secretion, and aggregation (9). There are several kinds of platelet function tests, among which platelet aggregability tests are, historically, the gold standard (16). Using flow cytometry, the same group recently showed an increased capacity of platelets derived from IPF patients to express P-selectin and to bind to monocytes (17, 18). However, platelet aggregability has not been evaluated in interstitial pneumonias. This study aimed to investigate platelet aggregability in patients with interstitial pneumonias. The levels of coagulation/fibrinolysis markers [D-dimer, thrombin-antithrombin complex (TAT), plasminogen activator inhibitor-1 (PAI-1), and von Willebrand factor (vWF)] were also measured to comprehensively investigate the hemostatic system in interstitial pneumonias.

Methods

Study subjects

This is an observational cohort study that included 59 patients with IIPs and connective tissue disease-associated interstitial pneumonias (CTD-IPs) who visited Kyoto University Hospital. The study population comprised 19 patients with IPF, 23 with other IIPs, 17 with CTD-IPs, and 23 healthy control subjects. IIPs were diagnosed and classified according to the American Thoracic Society (ATS)/European Respiratory Society (ERS)/Japanese Respiratory Society/Latin American Thoracic Association diagnostic criteria for IPF (1) and the ATS/ERS consensus statement on IIPs (19, 20). Patients who had antiplatelet medications and malignancy within the past 5 years and those with liver cirrhosis were excluded. This study was approved by the Kyoto University Graduate School and Faculty of Medicine Ethics Committee (E438), with written informed consent obtained from all patients.

Measurement of Platelet aggregability

Blood samples were collected from each patient in the supine position from an antecubital vein using a 21G needle with a quick tourniquet into a glass tube containing 0.313% sodium citrate. The aggregability of whole blood (WB) and platelet-rich plasma (PRP) was measured by WBA-neo (ISK, Tokyo) using the screen filtration pressure (SFP) method. The platelet aggregation threshold index (WB-PATI or PRP-PATI) was calculated as described previously (21, 22). PATI is a putative agonist for inducing half maximal aggregation, and a lower PATI indicated enhanced platelet aggregability.

Measurement of coagulation/fibrinolysis markers

Blood samples were collected in siliconized tubes that contained 3.8% trisodium citrate. They were centrifuged at 2300*g* for 15 min, and the supernatant was used for measurements. D-dimer, TAT, PAI-1, and vWF were measured by the latex agglutination assay, chemiluminescent enzyme immunoassay (CLEIA), latex photometric immunoassay (LPIA), and fixed platelet aggregation assay, respectively. The normal values were as follows: d-dimer, lower than 1.0 μg/ml; TAT, lower than 4.0 ng/ml; PAI-1, lower than 50 ng/ml; and vWF, 60%-170%.

Physiological assessments

In all the patients, pulmonary function tests were performed using the CHESTAC system (Chest M.I.

Inc., Tokyo, Japan). The diffusing capacity for carbon monoxide (DL_{CO}) was measured using the single-breath technique. Percent-predicted values were used for analyses. The six-minute walk test (6MWT) was performed in a subset of the subjects (IPF, n = 13; other IIPs, n = 16; CTDs, n = 10). It was performed as recommended by the ATS guidelines (23) with oxygen saturations being continuously monitored using a pulse oximeter during the 6MWT (Pulsox-300i, Konica Minolta Inc., Osaka, Japan).

Statistics

All statistical analyses were performed using JMP version 9 software (SAS Institute, Cary, NC, USA). Platelet aggregability was expressed as the logarithm of the PRP-PATI and WB-PATI values because the PATI values were skewed. A χ^2 test or Fisher's exact test was used to compare the results of categorical variables between the groups. Mann–Whitney U test was used to compare continuous variables between patients and controls, or IPF and CTD-IPs. Relationships between pairs of variables were analyzed by Spearman's rank correlation tests. For survival analysis, patients undergoing lung transplantation during follow-up (n = 1) were censored, and then, the duration from blood sampling to death, transplant date, or last visit was recorded. Mortality was first assessed for all risk factors using univariate Cox proportional hazard analysis. Multivariate Cox proportional hazard analyses were then used to examine the prognostic predictive value of platelet aggregability indices and d-dimer while adjusting for disease category. A p value less than 0.05 was considered to indicate statistical significance.

Results

Patient characteristics

The clinical characteristics of all subjects are summarized in Table 1. Among the 19 patients with IPF, three patients underwent surgical lung biopsy (SLB), and others had a typical usual interstitial pneumonia (UIP) pattern on high-resolution computed tomography (HRCT). Other IIPs comprised 23 patients, among which two patients had histologically confirmed nonspecific interstitial pneumonia (NSIP), and 21 patients had unclassifiable IIPs without SLB. On HRCT, two of the unclassifiable cases showed a possible UIP pattern, while the other 19 cases showed an inconsistent UIP pattern (1). CTD-IPs comprised seven patients with polymyositis/dermatomyositis (PM/DM), six patients with rheumatoid arthritis (RA), two patients with primary Sjogren's syndrome, one patient with systemic sclerosis, and one patient with overlap syndrome. Two

Table 1. Characteristics of the study population

	Control	IPF	Other IIPs	CTD-IPs
Number	23	19	23	17
Age	65(59-69)	69 (61-76)	70 (61-77)	63 (53-70)
Male	11 (48)	15 (79)	10 (43)	9 (53)
Current or former smokers	8 (35)	16 (84) †	11 (48)	11 (65)
Immunosuppressive drugs	0 (0)	2 (11)	3 (13)	3 (5)
Hypertension	12 (52)	4 (21)	7 (30)	1 (6) †
Ischemic heart disease	0 (0)	1 (5)	0 (0)	0 (0)
Diabetes mellitus	3 (13)	3 (16)	1 (4)	0 (0)
Hyperlipidemia	9 (39)	4 (21)	6 (26)	6 (35)
Chronic kidney disease	0 (0)	1 (5)	0 (0)	0 (0)
Other lung disease §	0 (0)	3 (16)	4 (17)	4 (24) *
Pulmonary hypertension ||	-	5/12 (42)	3/13 (23)	0/8 (0)
FVC, % predicted	-	81.9 (39.3-115.0)	90.7 (37.9-132.1)	78.5 (48.0-130.3)
DL_{CO}, % predicted ¶	-	46.4 (16.2-73.9)	48.6 (24.3-76.0)	54.9 (31.4-96.9)
Six-minute walk distance, m**	-	397 (180-593)	436.5 (220-565)	482.5 (227-548)
End-exercise oxygen saturation, % **	-	89 (79-95)	92 (77-97)	90 (80-96)
KL-6, U/ml	215 (171-241)	895 (677-1460)‡	1190 (435-1775) ‡	1325 (856-1780) ‡

Data are expressed as a median (interquartile range) or a number (percentage). *p < 0.05, †p < 0.01, ‡p < 0.0001 vs control. § bronchial asthma 6, chronic obstructive pulmonary disease 5. || estimated systolic pulmonary arterial pressure > 40 mmHg. ¶ IPF, n = 15; other IIPs, n = 15; CTDs, n = 13. ** IPF, n = 13; other IIPs, n = 16; CTDs, n = 10

cases with PM/DM underwent SLB, both of which showed NSIP and organizing pneumonia (OP) pattern. On HRCT, all patients had fibrotic interstitial pneumonias. There were no significant differences in age and gender between the patients groups and controls. The frequency of current or former smokers was higher in IPF patients (16/19, 84%) compared with that in controls (8/23, 35%, $p < 0.01$). At the time of blood sampling, 5%-13% of patients were being treated with corticosteroids (prednisolone 5-60 mg/day), and one patient with CTD-IP was treated with prednisolone in combination with tacrolimus 2 mg/day. Comorbid diseases and physiological test results are also shown in Table 1.

Platelet aggregability and coagulation/fibrinolysis markers

ADP-induced WB-PATI was significantly decreased in CTD-IPs {log WB-PATI median 0.31 μM [inter-quartile range (IQR) 0.07-0.34 μM], n = 17} compared with that in the controls [median 0.35 (IQR 0.32-0.45) μM, n = 23) ($p < 0.05$) (Table 2, Figure 1). There were no significant differences in other parameters of platelet aggregability, although patients with CTD-IPs had the lowest mean values in all these indices. In contrast, d-dimer, TAT, and vWF were significantly higher in all the patient groups compared with those in the controls ($p < 0.001$; Table 2, Figures 2 and 3). In CTD-IPs, there was a marginal decrease in collagen-induced PRP-PATI ($p = 0.05$) and a significant decrease in collagen-induced WB-PATI ($p < 0.05$) when compared with IPF (Table 2, Figure 1). There was no significant difference in the PAI-1 levels between the patients and controls.

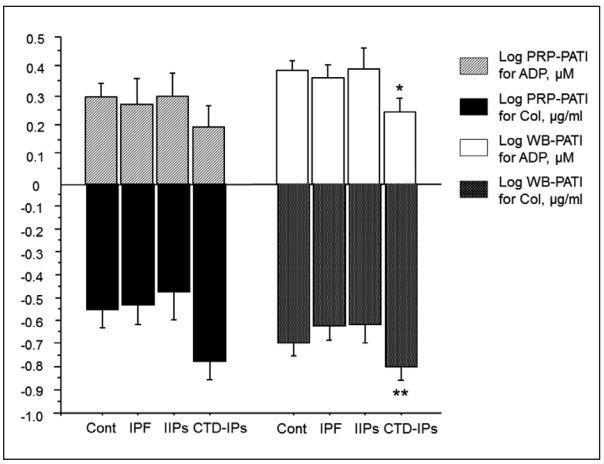

Fig. 1. Comparison of platelet aggregability between patients and controls. ADP-induced whole blood-platelet aggregation threshold index (WB-PATI) was significantly decreased in CTD-IPs compared with controls ($p < 0.05$). Collagen-induced platelet rich plasma-PATI (PRP-PATI) was marginally decreased ($p = 0.05$) and collagen induced WB-PATI was significantly decreased ($p < 0.05$) in CTD-IPs compared with that in IPF. Error bars represent standard errors of the mean. *$p < 0.05$ compared with controls. **$p < 0.05$ compared with IPF. Mann-Whitney U test was used in the statistical analyses. Cont, controls; IPF, idiopathic pulmonary fibrosis; IIPs, other idiopathic interstitial pneumonias; CTD-IPs, connective tissue disease-associated interstitial pneumonias

Correlation between platelet/coagulation markers and disease severities

There was no significant correlation between platelet aggregability parameters and pulmonary function, 6MWD, or KL-6 in each patient group. There was a significant negative correlation between d-dimer and 6MWD in patients with IIPs [*Rs* (correlation coefficient) = –0.527, $p = 0.005$, n = 29) and patients with CTD-IPs (*Rs* = –0.691, $p = 0.038$, n

Table 2

	Control	IPF	Other IIPs	CTD-IPs
Number	23	19	23	17
Log PRP-PATI for collagen, μg/ml	-0.55 (-0.61-0.22)	-0.53 (-0.62- -0.31)	-0.57 (-1.03- -0.01)	-0.59 (-1.02- -0.56)
Log PRP-PATI for ADP, μM	0.35 (0.21-0.41)	0.30 (0.20-0.43)	0.36 (0.19-0.41)	0.28 (-0.03-0.33)
Log WB-PATI for collagen, μg/ml	-0.55 (-1.07- -0.52)	-0.54 (-0.70-0.52)	-0.54 (-1.05-0.51)	-0.73 (-1.06- -0.58) ‡
Log WB-PATI for ADP, μM	0.35 (0.32-0.45)	0.34 (0.26-0.38)	0.39 (0.06-0.54)	0.31 (0.07-0.34) *
D-dimer, μg/ml	0.2 (0.2-0.3)	0.5 (0.3-0.7) †	0.5 (0.3-1.1) †	0.9 (0.4-1.8) †‡
TAT, ng/ml	1.0 (1.0-1.2)	3.3 (2.3-7.3) †	2.5 (1.9-4.8) †	4.7 (2.8-10.4) †
PAI-1, ng/ml	27 (18-39)	28 (20-40)	25 (20-33)	25 (17-32)
vWF, %	103.5 (87-133)	175 (135-279) †	200 (144-245) †	201 (156-293) †

Data are expressed as median (interquartile range). *$p < 0.05$, †$p < 0.001$ vs control. ‡$p < 0.05$ vs IPF. The normal values were as follows: d-dimer, lower than 1.0 μg/ml; TAT, lower than 4.0 ng/ml; PAI-1, lower than 50 ng/ml; vWF, 60%-170%

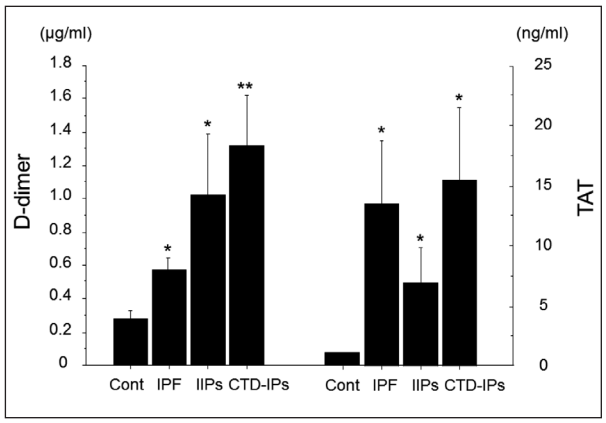

Fig. 2. Comparison of d-dimer and thrombin-antithrombin complex (TAT) between patients and controls. D-dimer and TAT were significantly higher in all patient groups compared with that in controls. Error bars represent standard errors of the mean.
* $p < 0.001$ compared with controls. ** $p < 0.001$ compared with controls, and $p < 0.05$ compared with IPF. Mann-Whitney U test was used in the statistical analyses

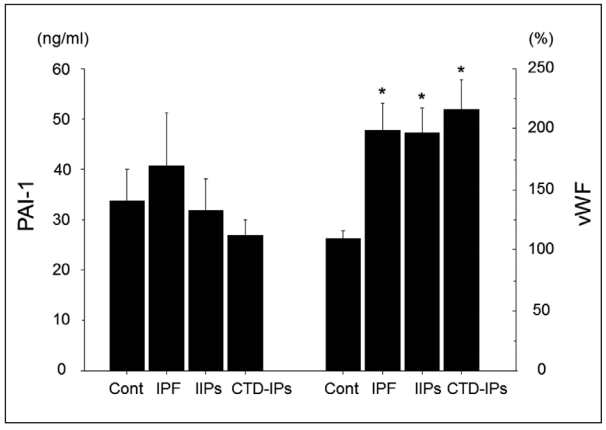

Fig. 3. Comparison of plasminogen activator inhibitor-1 (PAI-1) and von Willebrand factor (vWF) between patients and controls. vWF was significantly higher in all patient groups compared with that in the controls. There was no significant difference in serum plasminogen activator inhibitor-1 (PAI-1) between the patients and controls. Error bars represent standard errors of the mean.
* $p < 0.001$ compared with controls. Mann-Whitney U test was used in the statistical analyses

= 10), although no other significant correlation between TAT, PAI-1, vWF, and disease severity indices was found.

Correlation between platelet/coagulation markers and prognosis

The patients were followed for a median length of 917 days (range 14-2576) from the time of blood sampling. During this period, 22 patients died, and one patient underwent lung transplantation. The result of survival analyses is shown in Table 4. From univariate analysis, male gender [hazard ratio 2.493, 95% CI (1.149-5.417), p = 0.021], IPF diagnosis (hazard ratio 2.899, 95% CI (1.214-6.935), p = 0.017), lower DL_{CO} (hazard ratio 0.963, 95% CI (0.927-0.997), p = 0.031), higher PRP-PATI for collagen (hazard ratio 4.087, 95% CI (1.273-13.410), p = 0.018), higher WB-PATI for collagen (hazard ratio 7.824, 95% CI (1.288-53.115), p = 0.025), and lower DL_{CO} (hazard ratio 0.469, 95% CI (0.155-0.959), p = 0.033) were all significantly associated with shorter survival. The multivariate analysis showed that PRP-PATI for collagen, WB-PATI for collagen, and d-dimer were not associated with survival when adjusted for CTD diagnosis (Table 4).

Discussion

In this study, we showed that platelet aggregability was weakly enhanced in patients with CTD-IPs, but not in IIPs. In contrast, all patient groups showed abnormalities in coagulation/fibrinolysis markers. To the best of our knowledge, this is the first study to investigate platelet aggregability in patients with interstitial pneumonias.

vWF is synthesized by both the vascular endothelium and megakaryocytes (9), with collagen-bound vWF playing a crucial role in the platelet adhesion process to the vessel. Increased vWF in the blood is considered to be a marker of endothelial injury (24). Extravascular coagulation (6) and fibrinolysis (25) are considered to promote lung fibrosis. Increased serum TAT and d-dimer represent the activation of coagulation and fibrinolysis systems, respectively. Previous studies have shown that serum d-dimer is elevated in stable IPF and more significantly in acute exacerbations (7). Kobayashi et al reported that plasma TAT was increased in both IPF and CTD-IPs (8), whereas it has also been reported that vWF is elevated in acute exacerbations of IPF (26). The result of this study further suggested that coagulation/fibrinolysis systems are activated in both IIPs and CTD-IPs. PAI-1 is the primary inhibitor of the fibrinolysis system, and it has a profibrotic role (27). PAI-1 has been reported to be strongly expressed by alveolar epithelial cells in IPF (28), and

Table 3. Cox proportional hazard model results for evaluating the risk of mortality

	Hazard ratio	95% CI	p value
Univariate analysis			
Male, gender	2.493	1.149-5.417	0.021
Age, years	0.999	0.963-1.039	0.943
Smoking history	2.358	0.894-7.456	0.085
IPF diagnosis	2.899	1.214-6.935	0.017
CTD diagnosis	0.204	0.048-0.603	0.003
FVC, % predicted	0.980	0.958-1.002	0.076
DL_{CO}, % predicted	0.963	0.927-0.997	0.031
Serum KL-6, U/ml	1.000	1.000-1.001	0.677
Log PRP-PATI for collagen, μg/ml)	4.087	1.273-13.410	0.018
Log PRP-PATI for ADP, μM	1.215	0.297-5.211	0.790
Log WB-PATI for collagen, μg/ml	7.824	1.288-53.115	0.025
Log WB-PATI for ADP, μM	3.204	0.418-20.239	0.256
D-dimer, μg/ml	0.469	0.155-0.959	0.033
TAT, ng/ml	1.005	0.982-1.022	0.657
PAI-1, ng/ml	1.005	0.989-1.015	0.482
vWF, %	0.999	0.993-1.005	0.801
Multivariate analysis			
Model 1			
CTD diagnosis	0.262	0.060-0.824	0.020
Log PRP PATI for collagen, μg/ml	2.497	0.773-8.435	0.127
Model 2			
CTD diagnosis	0.250	0.057-0.767	0.013
Log WB-PATI for collagen, μg/ml	4.114	0.626-30.345	0.144
Model 3			
CTD diagnosis	0.249	0.055-0.806	0.018
D-dimer, μg/ml	0.727	0.237-1.178	0.302

plasma PAI-1 has been reported to be increased in acute exacerbations of IPF compared with stable IPF (26). Our study showed that serum PAI-1 was not increased in interstitial pneumonias in the stable condition compared with that in the controls. Further investigation is necessary to elucidate the role of PAI-1 in the pathogenesis of interstitial pneumonias.

In this study, we showed that platelet aggregability was weakly enhanced in CTD-IPs, but not in IIPs. Crooks et al and Fahim et al showed an increased capacity of platelets derived from IPF patients to express P-selectin and to bind to monocytes, suggesting that platelet reactivity is increased in IPF (17, 18). The method used in this study examined platelet-to-platelet aggregation, and historically, it has been the gold standard (16). Platelet aggregation has conventionally been examined by the optical aggregometer method, where light transmission of PRP is monitored under agonist stimulation (29). WB aggregation measured by the SFP method reflects a more physiological phenomenon than the aggregability of platelets alone, and we have previously shown that it is useful in monitoring the effects of aspirin (21). Platelets in IIPs may have an increased capacity to express proteins on their surface, although their aggregability may not be enhanced. The reason for this is still unclear.

CTDs are characterized by widespread vascular lesions and a procoagulant state, and increased plasma d-dimer has been reported in different groups of CTDs regardless of comorbid interstitial pneumonias or anti-phospholipid syndrome (30-32). In this study, d-dimer was significantly higher in CTD-IPs compared with that in both controls and IPF (Table 2, Figure 2). In contrast, previous studies showed inconsistent results regarding the platelet aggregability in patients with systemic sclerosis (33-35). Friedhoff reported increased platelet aggregability only in patients with early systemic sclerosis (SSc) (33), while Price et al found no enhancement of platelet aggregability in SSc with disease duration more than 5 years (34). Patients with CTD-IPs in this study had disease duration less than 3 years (median 27, range 27-831 days); this may be one of the reasons for the increased

enhancement of platelet aggregability in this population, although the enhancement was weak.

Further investigation of platelet function is required to assess the effectiveness of antiplatelet drugs for the prevention of vascular events in interstitial pneumonias.

Unexpectedly, the univariate survival analysis showed that a higher WB- or PRP-PATI for collagen, together with decreased d-dimer, was associated with shorter survival. This finding implies that decreased platelet aggregability and d-dimer were associated with a poor prognosis. However, based on the result of the multivariate analysis (Table 4), this could also be attributed to the better prognosis of CTD-IPs compared with IIPs.

There were some limitations in our study. First, the study population was small. The patients with CTD-IPs had the lowest value of all four indices of platelet aggregability evaluated in this study. Further analyses with a larger study population may therefore reveal more significant increases of platelet aggregability in patients with CTD-IPs. In contrast, there was no tendency for increases in platelet aggregability in IIPs. Second, we did not assess inherited or secondary clotting defects by measuring protein S, protein C, lupus anticoagulant, or anticardiolipin antibodies. Third, we did not evaluate the longitudinal change of platelet aggregability. Despite these limitations, this is the first study to shown that platelet aggregability was enhanced in CTD-IPs but not in IIPs.

In conclusion, the result of this study suggested that coagulation and fibrinolysis systems were activated in IIPs and CTD-IPs. In contrast, platelet aggregability was only weakly enhanced in CTD-IPs but not in IPF or other IIPs.

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References

1. Raghu G, Collard HR, Egan JJ, et al. An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. Am J Respir Crit Care Med 2011; 183(6): 788-824.
2. Hubbard RB, Smith C, Le Jeune I, Gribbin J, Fogarty AW. The association between idiopathic pulmonary fibrosis and vascular disease: a population-based study. Am J Respir Crit Care Med 2008; 178(12): 1257-61.
3. Dalleywater W, Powell HA, Hubbard RB, Navaratnam V. Risk factors for cardiovascular disease in people with idiopathic pulmonary fibrosis: a population based study. Chest 2015; 147(1): 150-6.
4. Kim WY, Mok Y, Kim GW, et al. Association between idiopathic pulmonary fibrosis and coronary artery disease: a case-control study and cohort analysis. Sarcoidosis Vasc Diffuse Lung Dis 2015; 31(4): 289-96.
5. Navaratnam V, Fogarty AW, McKeever T, et al. Presence of a prothrombotic state in people with idiopathic pulmonary fibrosis: a population-based case-control study. Thorax 2014; 69(3): 207-15.
6. Ahluwalia N, Shea BS, Tager AM. New therapeutic targets in idiopathic pulmonary fibrosis: aiming to rein in runaway wound healing responses. Am J Respir Crit Care Med 2014; 190(8): 867-78.
7. Bargagli E, Madioni C, Bianchi N, Refini RM, Cappelli R, Rottoli P. Serum analysis of coagulation factors in IPF and NSIP. Inflammation 2014; 37(1): 10-6.
8. Kobayashi H, Gabazza EC, Taguchi O, et al. Protein C anticoagulant system in patients with interstitial lung disease. Am J Respir Crit Care Med 1998; 157(6 Pt 1): 1850-4.
9. Kroll MH, Afshar-Kharghan V. Platelets in pulmonary vascular physiology and pathology. Pulm Circ 2012; 2(3): 291-308.
10. Davì G, Patrono C. Platelet activation and atherothrombosis. N Engl J Med 2007; 357(24): 2482-94.
11. de Witt SM, Verdoold R, Cosemans JM, Heemskerk JW. Insights into platelet-based control of coagulation. Thromb Res 2014; 133 Suppl 2: S139-148.
12. Chaudhary NI, Roth GJ, Hilberg F, et al. Inhibition of PDGF, VEGF and FGF signalling attenuates fibrosis. Eur Respir J 2007; 29(5): 976-85.
13. Pierson DM, Ionescu D, Qing G, et al. Pulmonary fibrosis in hermansky-pudlak syndrome. a case report and review. Respiration 2006; 73(3): 382-95.
14. Aihara K, Handa T, Nagai S, et al. Impaired endothelium-dependent vasodilator response in patients with pulmonary fibrosis. Respir Med 2013; 107(2): 269-75.
15. Ishida K, Taguchi K, Matsumoto T, Kobayashi T. Activated platelets from diabetic rats cause endothelial dysfunction by decreasing Akt/endothelial NO synthase signaling pathway. PLoS One 2014; 9(7): e102310.
16. Michelson AD. Platelet function testing in cardiovascular diseases. Circulation 2004; 110(19): e489-493.
17. Crooks MG, Fahim A, Naseem KM, Morice AH, Hart SP. Increased platelet reactivity in idiopathic pulmonary fibrosis is mediated by a plasma factor. PLoS One 2014; 9(10): e111347.
18. Fahim A, Crooks MG, Morice AH, Hart SP. Increased platelet binding to circulating monocytes in idiopathic pulmonary fibrosis. Lung 2014; 192(2): 277-84.
19. American Thoracic Society/European Respiratory Society International Multidisciplinary Consensus Classification of the Idiopathic Interstitial Pneumonias, This joint statement of the American Thoracic Society (ATS), and the European Respiratory Society (ERS) was adopted by the ATS board of directors, June 2001 and by the ERS Executive Committee, June 2001. Am J Respir Crit Care Med 2002; 165(2): 277-304.
20. Travis WD, Costabel U, Hansell DM, et al. An official American Thoracic Society/European Respiratory Society statement: Update of the international multidisciplinary classification of the idiopathic interstitial pneumonias. Am J Respir Crit Care Med 2013; 188(6): 733-48.
21. Tabuchi A, Taniguchi R, Takahashi K, et al, Action of aspirin on whole blood-aggregation evaluated by the screen filtration pressure method. Circ J 2008; 72(3): 420-6.
22. Oga T, Chin K, Tabuchi A, et al, Effects of obstructive sleep apnea

with intermittent hypoxia on platelet aggregability. J Atheroscler Thromb 2009; 16(6): 862-9.
23. ATS Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories, ATS statement: guidelines for the six-minute walk test. Am J Respir Crit Care Med 2002; 166(1): 111-7.
24. Lavallée PC, Labreuche J, Faille D, et al. Circulating markers of endothelial dysfunction and platelet activation in patients with severe symptomatic cerebral small vessel disease. Cerebrovasc Dis 2013; 36(2): 131-8.
25. Schuliga M, Westall G, Xia Y, Stewart AG. The plasminogen activation system: new targets in lung inflammation and remodeling. Curr Opin Pharmacol 2013; 13(3): 386-93.
26. Collard HR, Calfee CS, Wolters PJ, et al, Plasma biomarker profiles in acute exacerbation of idiopathic pulmonary fibrosis. Am J Physiol Lung Cell Mol Physiol 2010; 299(1): L3-7.
27. Eitzman DT, McCoy RD, Zheng X, et al. Bleomycin-induced pulmonary fibrosis in transgenic mice that either lack or overexpress the murine plasminogen activator inhibitor-1 gene. J Clin Invest 1996; 97(1): 232-7.
28. Kotani I, Sato A, Hayakawa H, Urano T, Takada Y, Takada A. Increased procoagulant and antifibrinolytic activities in the lungs with idiopathic pulmonary fibrosis. Thromb Res 1995; 77(6): 493-504.
29. Born GV, Haslam RJ, Goldman M. Comparative effectiveness of adenosine analogues as inhibitors of blood-platelet aggregation as vasodilators in man. Nature 1965; 205: 678-80.
30. Lippi G, Volpe A, Caramaschi P, Salvagno GL, Montagnana M, Guidi GC. Plasma D-dimer concentration in patients with systemic sclerosis. Thromb J 2006; 4: 2.
31. Mukubo Y, Kawamata M. Higher preoperative D-dimer value remain high postoperatively in patients with rheumatoid arthritis compared with those with osteoarthrosis. J Anesth 2006; 20(1): 51-3.
32. Wu H, Birmingham DJ, Rovin B, et al. D-dimer level and the risk for thrombosis in systemic lupus erythematosus. Clin J Am Soc Nephrol 2008; 3(6): 1628-36.
33. Friedhoff LT, Seibold JR, Kim HC, Simester KS. Simester. Serotonin induced platelet aggregation in systemic sclerosis. Clin Exp Rheumatol 1984; 2(2): 119-23.
34. Price JE, Klimiuk PS, Jayson MI. In vitro platelet aggregability studies: lack of evidence for platelet hyperactivity in systemic sclerosis. Ann Rheum Dis 1991; 50(8): 567-71.
35. Goodfield MJ, Orchard MA, Rowell NR. Increased platelet sensitivity to collagen-induced aggregation in whole blood patients with systemic sclerosis. Clin Exp Rheumatol 1988; 6(3): 285-8.