Plasma Platelet-derived Microparticles in Patients with Connective Tissue Diseases

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ABSTRACT. Objective. To clarify the role of platelet-derived microparticles (PDMP), which are small vesicles with thrombotic and immunological properties, in systemic lupus erythematosus (SLE), systemic sclerosis (SSc), dermatomyositis/polymyositis (PM/DM), and mixed connective tissue disease (MCTD).

Methods. Plasma levels of PDMP were measured by ELISA, and compared among patients with one of the 4 diseases. Association of PDMP levels with clinical characteristics and medication of the patients was also examined.

Results. PDMP levels were higher in patients with MCTD and SSc than in controls. Multiple linear regression analysis revealed that patients with Raynaud’s phenomenon (RP) showed higher PDMP levels than those without. PDMP levels in individual patients did not fluctuate significantly over several months.

Conclusion. PDMP level is associated with MCTD, SSc, and RP, and could be a novel marker for RP. (First Release Jan 15 2011; J Rheumatol 2011;38:680–4; doi:10.3899/jrheum.100780)

Key Indexing Terms: RAYNAUD’S PHENOMENON MICROPARTICLE PLATELET CONNECTIVE TISSUE DISEASES

Microparticles are small membranes, 0.2–2.0 nm in size, that are released from the plasma membrane following cell activation and apoptosis by various stimuli such as cytokines, thrombin, endotoxins, shear stress, and hypoxia. Microparticles are released by platelets, monocytes, endothelial cells, and erythrocytes, and each microparticle bears antigens from the originating cell. For example, platelet-derived microparticles (PDMP) express platelet-specific antigens, such as CD42b (GPIb) or CD41 (GPIIb/IIIa), while endothelial cell-derived microparticles express CD31 (PECAM-1). These expressions make it possible to identify microparticles originating from various cell types.

PDMP are the most abundant microparticles in plasma and reflect platelet activation. They express an anionic phospholipid, such as phosphatidylserine, on their external surface because asymmetry of the lipid bilayer becomes dysregulated during microparticle formation. Phosphatidylserine on the outer surface facilitates the formation of coagulation complexes and also initiates coagulation by enhancing the capability of tissue factor. PDMP thus play an important role in coagulation and thrombosis. Plasma PDMP levels have been shown to be higher than normal under various clinical conditions, including heparin-induced thrombocytopenia, diabetes, chronic renal failure, acute coronary syndrome, stroke, deep vein thrombosis, sepsis, and atherosclerosis.

Connective tissue diseases are autoimmune inflammatory diseases accompanied by various vascular lesions including vasculitis, thrombosis, atherosclerosis, and Raynaud’s phenomenon (RP). Recent advances have revealed that microparticles have widespread effects on the immune system, including inactivation of pathogens, release of anaphylatoxins, and induction of cytokine production. It has also been demonstrated that microparticles affect immune tolerance and angiogenesis. It has thus been anticipated that microparticles may function as a biomarker of inflammatory and vascular diseases. As for PDMP, an increase in their numbers has been reported in patients with antiphospholipid antibody syndrome (APS), systemic lupus erythe-
matoes (SLE), vasculitides, and rheumatoid arthritis. This suggests that PDMP play a pathological role in vascular lesions as well as immune disorders. Most studies in this area have measured PDMP levels by means of flow cytometry, which is widely used for studying PDMP. However, the flow cytometry method cannot be used in a variety of clinical situations. For this reason, the enzyme-linked immunosorbent assay (ELISA) for PDMP has been developed, and results from the 2 methods have been shown to correlate well (R = 0.722). ELISA is more effective for quantitative measurement of a large number of samples and the samples can be stored until assayed. In an attempt to use ELISA in clinical settings, we reevaluated the role of PDMP in patients with SLE, SSc, mixed connective tissue disease (MCTD), and dermatomyositis/polymyositis (PM/DM) by ELISA; we found that RP is associated with elevated levels of PDMP.

**MATERIALS AND METHODS**

**Patients.** Blood samples were obtained from patients attending the outpatient clinic of Kobe University Hospital; all had provided informed consent. Sample collection was undertaken between February 9 and March 18, 2009. All patients met the criteria for their disease: SLE (n = 88); SSc (n = 33); MCTD (n = 14); and PM/DM (n = 18). Serial samples were collected from 18 randomly selected patients with SLE (n = 9), SSc (n = 3), PM/DM (n = 2), and MCTD (n = 4). RP was positive for 9 patients. The control group consisted of 34 healthy volunteers (age 43.7 ± 9.2 yrs, 25 women, 9 men), who provided informed consent. Clinical manifestations and laboratory data were obtained from the medical charts. Characteristics of patients are summarized in Table 1. RP was considered to be present in 41.6% of the patients, determined on the basis of patients' reports, and defined as positive if it occurred within about a month before the blood draw. Antiplatelet drugs for medication included low-dose aspirin (n = 28), sarpogrelate (n = 26), cilostazol (n = 4), ticlopidine (n = 1), and ethyl icostapentate (n = 1). Vasodilators used included beraprost sodium (n = 18), calcium channel blocker (n = 12), limaprost alfadex (n = 3), and kalligino-genase (kalliginogenase, n = 1).

**Sample preparation.** Two milliliters of blood was drawn with a 21-G needle inserted into an antecubital vein. Samples were mixed with acid-citrate-dextrose ethylenediaminetetraacetic acid in a Neo-Tube (Nipro, Osaka, Japan), and 1.5 ml of each sample was centrifuged at 8000 ¥ 10,000/µl) 21.0 ± 5.6 25.6 ± 6.8 22.5 ± 6.2 24.1 ± 8.3 23.1 ± 6.8

**Anti-platelet drug, +/-**

7/7 4/14 28/60 17/16 56/97

**Vasodilator, +/-**

2/12 4/14 10/78 16/17 32/121

**Prednisolone, +/-**

10/4 14/4 80/8 10/23 114/39

**Prednisolone dose, mg/day**

5.4 ± 5.1 9.8 ± 8.5 7.3 ± 6.4 2.4 ± 5.1 6.3 ± 6.6

MCTD: mixed connective tissue disease; PM/DM: deramatomyositis/polymyositis; SLE: systemic lupus erythematosus; SSc: systemic sclerosis.

**RESULTS**

**PDMP levels in connective tissue diseases.** PDMP levels in patients are shown in Figure 1. The mean (± SD) levels for each disease group and controls were MCTD, 90.6 ± 117.1 U/ml; PM/DM, 27.0 ± 68.91 U/ml; SLE, 23.1 ± 48.3 U/ml; SSc, 52.2 ± 92.8 U/ml; and controls, 7.0 ± 4.6 U/ml. PDMP levels were significantly higher for patients with MCTD and SSc than those for controls. Patients with SLE and PM/DM did not show significantly elevated levels of PDMP, although levels tended to be higher compared to controls.

**Comparison of PDMP level and clinical characteristics.** Clinical characteristics were assessed for associations with PDMP levels in the patient groups (Table 2). Since RP is a common feature of both MCTD and SSc, we focused on RP and use of antiplatelet drugs when evaluating clinical symptoms. Patients with RP showed significantly higher PDMP levels than those without in univariate analysis (t test, p = 0.005). Other factors, such as age, sex, disease, platelet...
count, steroid therapy, fingertip ulcer, and antiplatelet therapy, were not associated with level of PDMP. Multiple linear regression analysis identified the presence of RP as the only significant predictor of PDMP levels (p < 0.01; data not shown). PDMP levels for patients with or without RP and for healthy controls are shown in Figure 2 (58.9 ± 97.5 U/ml, 20.4 ± 43.4 U/ml, and 7.0 ± 4.6 U/ml, respectively). Patients with RP showed significantly higher PDMP levels than those without and controls (p < 0.005). Finally, PDMP levels for patients without RP were not higher than those for controls, again substantiating the association of RP with plasma PDMP levels.

**DISCUSSION**

Previous studies, most using flow cytometry, have reported enhanced plasma PDMP levels in patients with SLE, SSc, PM/DM, and other connective tissue diseases. We used a different assay system but found similar results, thus confirming previous findings. We found the highest PDMP levels in patients with MCTD, and this makes ours the first report of enhancement of PDMP levels in patients with...
Consistent with our results, previous studies reported increased PDMP levels for patients with SSc. Guiducci, et al. reported that PDMP levels are more elevated in patients with limited-type disease than in patients with diffuse disease; and Nomura, et al. reported that PDMP levels are elevated in patients with interstitial pneumonia. These conflicting results indicate that the role of PDMP in SSc remains unclear.

We found that PDMP level is associated with RP, which is a common feature of SSc and MCTD. Factors contributing to RP include vasoconstriction, vasodilation, endothelial cell damage, and procoagulant tendencies, including platelet activation. Since PDMP have been reported to cause vasoconstriction and inhibit nitric oxide-dependent vasodilation in animal models, high PDMP levels may be involved in RP through their effect on vasoconstriction. Although we expected that PDMP might contribute to fingertip ulcers because of their thrombotic capacity, we did not find any such association. Consistent with our results, Guiducci, et al. reported an inverse association of PDMP with skin sclerosis and fingertip ulcer, implying a possibly negative immunoregulatory effect of PDMP. However, the difference between involvement of PDMP in RP and in fingertip ulcers remains to be elucidated.

The causes of high PDMP levels in patients with RP remain unclear. PDMP are released when platelets are activated, and platelet activation in patients with connective tissue diseases has been thoroughly reported. One possible cause is therefore that PDMP are released by increased shear stress or reduced blood flow during activation of RP. However, our results show that PDMP levels are relatively stable over long periods and regardless of climate change, indicating that platelets are continuously activated in these patients. We thus speculate that chronic vascular damage in patients with RP may contribute to the release of PDMP, and that PDMP, in turn, could contribute to the occurrence of this phenomenon.

High levels of PDMP appear to be associated with RP rather than with specific diseases because 2 different diseases, SSc and MCTD, are associated with PDMP. On the other hand, we could not find any association of PDMP levels with RP among patients with SLE. One limitation of our study is that other factors related to PDMP could not be fully evaluated. For example, we could not assess whether there is a link between PDMP levels and atherosclerosis in our patients. Further examination is therefore needed to determine the influence of RP on levels of PDMP.

Both β-thromboglobulin and platelet factor 4 (PF4) are known to reflect platelet activation. An advantage of PDMP as a marker is that, unlike β-thromboglobulin or platelet factor 4, PDMP are not activated by blood collection. The
The effect of antiplatelet therapy, including the use of selective serotonin reuptake inhibitor, on PDMP levels remains an interesting question for future studies. Finally, our findings lead us to conclude that PDMP may be an effective marker for monitoring platelet activation, and that ELISA is a quick and easily administered assay for PDMP.

REFERENCES


Figure 3. Changes in PDMP levels, measured over 6 months for 18 patients. A. 11 patients showed low levels of PDMP (≤ 25 U/ml); B. 7 patients showed high levels of PDMP (> 25 U/ml).