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【 CASE REPORT 】

Acquired Gray Platelet Syndrome Associated with Primary Myelofibrosis

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Abstract:
A 53-year-old man presented with uncontrolled bleeding caused by acquired platelet dysfunction accompanied by calreticulin-mutated primary myelofibrosis. Based on the detection of abnormal platelets, including large gray platelets, under light microscopy and the loss of the second wave of aggregation observed by light transmission aggregometry, the patient was diagnosed with platelet dysfunction accompanied by myeloproliferative neoplasms (MPNs). In addition, the absence of platelet α-granules was confirmed by electron microscopy. Therefore, this condition may be termed "acquired gray platelet syndrome." Acquired platelet dysfunction must be ruled out when abnormal platelets are observed in patients with MPNs.

Key words: acquired platelet dysfunction, primary myelofibrosis (PMF), myeloproliferative neoplasms (MPNs)

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Introduction

Philadelphia chromosome-negative myeloproliferative neoplasms (Ph-negative MPNs), including polycythemia vera, essential thrombocythemia (ET), and primary myelofibrosis (PMF), are clonal hematopoietic cell disorders characterized by the proliferation of cells of one or more myeloid lineages (1, 2). Patients with Ph-negative MPNs frequently experience thrombotic complications and unexpected bleeding (3, 4) and exhibit the following quantitative and qualitative platelet disorders: acquired von Willebrand syndrome; the administration of aspirin; acquired coagulopathies including liver dysfunction and acquired hemophilia; vascular alterations; platelet dysfunctions due to clonal hematopoiesis; and thrombocytopenia caused by progressive disease, splenomegaly, cytoreductive therapies, and the administration of ruxolitinib (5-7). A platelet function analysis, flow cytometry, and thromboelastography might be useful for diagnosing these disorders (5). However, no tests that effectively screen for the risk of bleeding among Ph-negative MPNs and the treatment for reducing the risk of bleeding have yet been established.

We herein report a case of acquired platelet dysfunction associated with calreticulin (CALR)-mutated PMF that was detected using light microscopy and light transmission aggregometry (LTA).

Case Report

A 53-year-old man had been diagnosed with CALR-mutated PMF 2 years before admission and had been monitored carefully without any treatment. However, the patient's PMF progressed to the intermediate-2 risk category on the basis of the refined dynamic international prognostic scoring system (DIPSS plus) and the accelerated phase according to his level of circulating blastoid cells (Table 1); he also exhibited palpable splenomegaly (2, 8, 9). Therefore, allogeneic hematopoietic cell transplantation (allo-HCT) was indicated. In order to control disease progression, ruxolitinib and hydroxycarbamide administration had been initiated three months before admission. Despite these treatments, he

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Table 1. Laboratory Findings upon Admission.

Peripheral blood	Reference range	Values upon admission
White-cell count (/μL)	4,300-8,000	16,500
Neutrophils (%)		39.0
Immature granulocytes (%)		3.0
Eosinophils (%)		1.0
Basophils (%)		12.0
Lymphocytes (%)		31.0
Monocytes (%)		1.0
Blastoid cells (%)		13.0
Red-cell count (/μL)	4,500,000-5,100,000	3,090,000
Reticulocytes (‰)	5-20	22.0
Hemoglobin (g/dL)	12.4-17.2	8.0
Hematocrit (%)	38.0-54.0	25.9
Erythroblasts (/100 cell count)		18
Platelet count (/μL)	180,000-340,000	151,000
Prothrombin time (s)	11.5-14.5	13.8
PT-INR	0.9-1.1	1.18
Activated partial-thromboplastin time (s)	25.0-40.0	37.6
Fibrinogen (mg/dL)	200-400	409
FDP (μg/mL)	0-10.0	8.2
WT1 mRNA (copies/μg RNA)		1100
JAK2 V617F mutation		(-)
CALR exon9 mutation		(+), type1 (del52)
MPL W515L/K mutation		(-)
G-banding, peripheral blood		46, XY, add(12)(q11), del(13)(q?) [3] 46, XY [17]

FDP: fibrin and fibrinogen degradation products, PT-INR: prothrombin time international normalized ratio

required admission to our hospital due to the onset of massive ascites. At the time of admission, 25 mg ruxolitinib was administered twice a day and 1,500 mg hydroxycarbamide once a day; however, no platelet-interfering drugs such as aspirin or clopidogrel had been administered.

Using invasive procedures, we estimated the risk of bleeding to be low at that time because general laboratory examinations did not suggest hemostatic disorders, although the platelet count was slightly below the lower limit of normal (Table 1). The peritoneum was punctured to obtain a peritoneal effusion specimen and reduce the volume of the abdomen both on the day of admission and again five days after admission. At seven days after admission, the patient developed severe anemia (hemoglobin: 5.4 g/dL). A dynamic contrast-enhanced computed tomography scan revealed uncontrolled bleeding of the abdominal wall, which was stopped by embolizing the responsible artery. Furthermore, at 12 days after admission, a central venous catheter was inserted through the right internal jugular vein. However, it became necessary to remove the catheter and suture the hole immediately because of uncontrollable bleeding at the insertion site around the catheter.

Due to these unexpected bleeding episodes, a hemostatic or coagulation disorder was suspected; therefore, various laboratory tests were performed. Detailed laboratory findings are shown in Table 2. The bleeding time was normal, but von Willebrand disease or acquired von Willebrand syndrome (vWD/AvWS) type 2A, 2B, or 2M could not be ruled out because of the low level of von Willebrand factor (vWF):ristocetin cofactor (RCo) and the low vWF:RCo/vWF:antigen ratio (10). In contrast, large gray platelets were observed using May-Giemsa staining under light microscopy (Fig. 1A, B). In LTA (Born's method), a lag phase of normal duration and a normal level of aggregation (%) were observed with 2.0 μM collagen, and a normal level of aggregation (%) was observed with 1.5 mg/mL ristocetin. However, the loss of the second wave of aggregation and a tendency toward deaggregation was observed with 2.0 μM adenosine diphosphate (ADP) (Fig. 2). Ristocetin-induced platelet aggregation (RIPA) with two fold-diluted ristocetin did not indicate platelet hyperreactivity; therefore, vWD/AvWS type 2B or platelet-type was ruled out (Fig. 3) (10, 11). However, we could not determine the type of vWD/AvWS, such as the type 2A and 2M, because no further vWF multimer analysis was performed, and there was no decrease in RIPA with use of the standard concentration of ristocetin; this decrease is typically seen in type 2 A or 2M vWD/AvWS (Fig. 2) (12). Electron microscopy demonstrated that platelets lacked α-granules and contained abundant channels of the open canalicular system (Fig. 1C, D). Therefore, the patient was diagnosed with acquired platelet dysfunction accompanied by PMF. After the

Table 2. Coagulation Test Results.

Variable	Reference range	After bleeding episodes
Bleeding time, Duke method (min)	1.00-5.00	3.00
Prothrombin time (s)	11.5-14.5	13.0
PT-INR	0.9-1.1	1.11
Activated partial-thromboplastin time (s)	25.0-40.0	38.1
Fibrinogen (mg/dL)	200-400	468
FDP (μg/mL)	0-10.0	20.7
TAT (ng/mL)	<3.0	1.8
PIC (μg/mL)	<0.8	1.1
Antithrombin (%)	70-120	115
Protein C activity (%)	64-146	78
Protein S activity (%)	67-164	59
Factor II activity (%)	74-146	98
Factor V activity (%)	70-152	54
Factor VII activity (%)	63-143	107
Factor VIII activity (%)	80-140	95
Factor IX activity (%)	80-120	70
Factor X activity (%)	71-128	111
Factor XIII activity (%)	70-140	95
vWF:antigen (%)	50-155	74
vWF:RCo (%)	60-170	29

FDP: fibrin and fibrinogen degradation products, PIC: plasmin-α_2 plasmin inhibitor complex, PT-INR: prothrombin time international normalized ratio, RCo: ristocetin cofactor, TAT: thrombin-antithrombin complex

diagnosis of platelet dysfunction was made, prophylactic platelet transfusion was administered to the patient, and invasive procedures could be performed safely. Additionally, a transjugular liver biopsy revealed that portal hypertension caused by extramedullary hematopoiesis in the liver had led to the onset of massive ascites.

At one month after admission, the patient underwent allo-HCT with the intention to suppress extramedullary hematopoiesis and obtain normal trilineage hematopoiesis. Neutrophil engraftment was achieved at 46 days after allo-HCT, whereas platelet engraftment was not achieved before he expired, namely at 112 days after allo-HCT due to idiopathic pneumonia syndrome. Therefore, platelet transfusion could not be stopped by performing allo-HCT in the present case.

Discussion

We herein describe a case of acquired platelet dysfunction that mimicked gray platelet syndrome (GPS) accompanied by CALR-mutated PMF. The detection of abnormal platelets, including large gray platelets, under light microscopy and the loss of the second wave of aggregation observed by LTA was an important clue for the diagnosis of platelet dysfunction, which was further confirmed by electron microscopy. In addition, prophylactic platelet transfusion could reduce the risk of bleeding in patients suffering from platelet dysfunction.

Morphologically unusual platelets with an abnormal function may be observed in CALR-mutated MPNs because thrombopoietin-independent megakaryopoiesis through the thrombopoietin receptor has been reported to be activated by mutant CALR (13). Another study reported that CALR-mutated platelets were less activated following ADP stimulation (14). They speculated that the result could explain the lower risk of thrombosis in CALR-mutated ET patients compared with JAK2-mutated ET patients. This result might partially account for the bleeding that occurred in the present case. In the present PMF case, there existed the possibility that platelet dysfunction due to a mutation in CALR and the development of an increased bleeding risk, which was compensated by his relatively high platelet count (31.0×10^4/μL) before cytoreductive therapy was started, might have occurred according to the decreasing platelet count. Our case suggests that we should examine the platelet function in MPN patients in order to assess the risk of bleeding if invasive procedures or cytoreductive therapies are planned.

In LTA, ADP-induced aggregation is mediated by G protein-coupled receptors named $P2Y_1$ and $P2Y_{12}$. The activation of G_q protein via $P2Y_1$ leads to a change in the platelet shape and the first wave of aggregation, and the activation of G_i protein via $P2Y_{12}$ leads to a second wave of aggregation through granule release (15). In the case of congenital pathogenesis, the presence of the first wave and the loss of the second wave suggests the existence of platelet secretion disorders including abnormalities of receptors for soluble agonists, abnormalities of platelet granules, impaired liberation of arachidonic acid, cyclo-oxygenase deficiency, and defective thromboxane synthetase (11, 16). However, the loss of the second wave of aggregation by ADP in adults can be caused by a wide variety of acquired platelet dys-

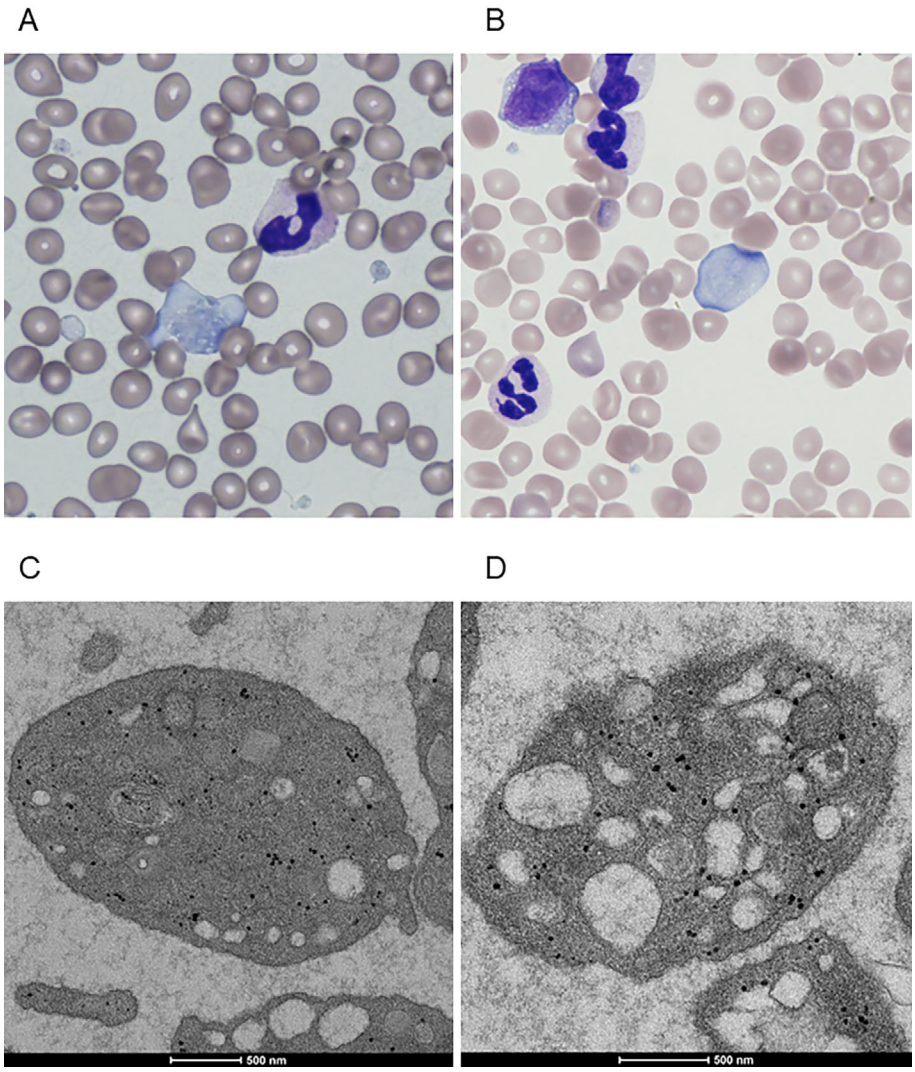

Figure 1. **Large gray platelets were observed with May-Giemsa staining under light microscopy (A and B). Electron microscopy demonstrated that platelets lacked α-granules (C) and contained abundant channels of the open canalicular system (D).**

functions, among them platelet-interfering drugs, such as aspirin and thienopyridines, clonal hematopoiesis of MPNs, monoclonal protein, liver disease, uremia, cardiopulmonary bypass, and antiplatelet antibodies (17). In this case, the loss of the second wave of aggregation was attributed to the clonal hematopoiesis of MPNs that mimicked the congenital pathogenesis of platelet secretion disorders because the patient did not have liver disease except for extramedullary hematopoiesis and uremia without platelet-interfering drugs, monoclonal protein, cardiopulmonary bypass, and antiplatelet antibodies. Moreover, typical platelet secretion disorders showed a decreased aggregation response to collagen, as well as a loss of the second wave by ADP, but the hemostatic patterns of clonal hematopoiesis of MPNs were reported to be heterogeneous (7, 18).

GPS is an inherited platelet dysfunction disorder characterized by levels of platelet α-granules which are less than 15% of normal (19-21). Mutations in the genes encoding several proteins, such as *NBEAL2*, *GATA1*, and *VPS33B/VIPS39,* in arthrogryposis, renal dysfunction, and cholestasis syndrome, respectively, and *GFI1B* were reported to be responsible for GPS, although the mutation sites are heterogeneous (22, 23). The LTA pattern of GPS is reported to be heterogenous, although a decreased aggregation response to collagen and the loss of the second wave by ADP are typical (18, 19, 24, 25). Therefore, the diagnosis of GPS was further confirmed morphologically by electron microscopy (26). Additionally, several reports have shown that congenital GPS can cause secondary myelofibrosis (25, 27). However, this PMF patient's hemostatic disorder was not considered to be congenital because he had no family history of hemostatic disorders and had experienced no bleeding events before developing PMF. Based on these considerations, we thought that he had likely developed "acquired GPS" associated with PMF.

In this case, "acquired GPS" seemed to be the main cause of uncontrolled bleeding because prophylactic platelet transfusion prevented uncontrolled bleeding during invasive procedures; however, the possibility of concomitant AvWS was not completely ruled out owing to the decreased in vWF:

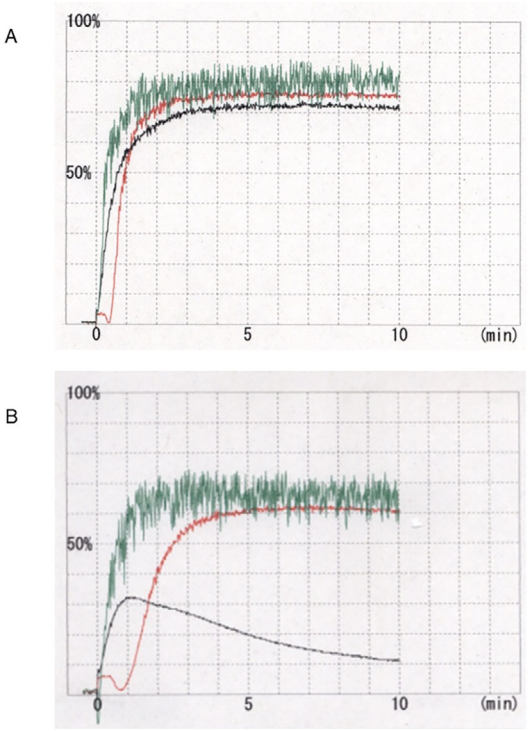

Figure 2. **Light transmission aggregometry (LTA; Born's method) by regular concentrations of agonist: normal control (A); this case (B). In this case, a lag phase of normal duration and a normal level of aggregation (%) were observed with 2.0 μM collagen (red line), and a normal level of aggregation (%) was observed with 1.5 mg/mL ristocetin (green line). However, the loss of the second wave of aggregation and a tendency toward deaggregation was observed with 2.0 μM adenosine diphosphate (ADP) (black line).**

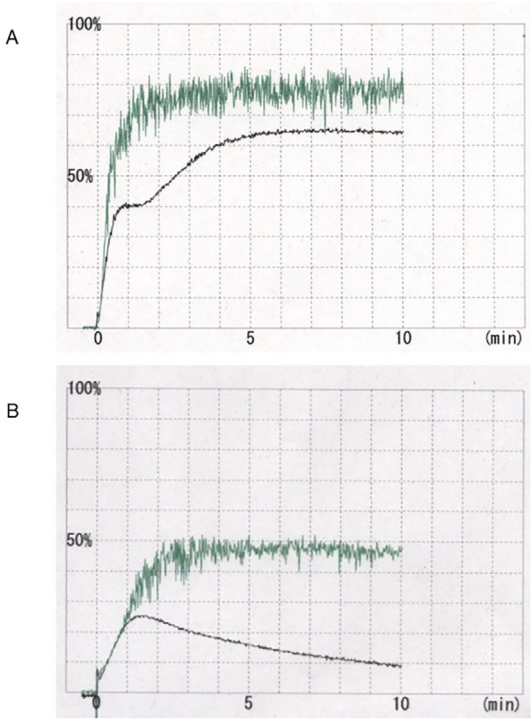

Figure 3. **Light transmission aggregometry (LTA; Born's method) by two-fold-diluted agonists: normal control (A); this case (B). In this case, a relatively low level of aggregation (%) was observed with ristocetin compared with the normal control (green line), and the aggregation pattern with adenosine diphosphate (ADP) was similar to that observed for the standard concentration of ADP (black line).**

RCo, and AvWS has been reported in patients with MPN even without extremely high platelet counts (10, 28). AvWS should also be considered as one of the causes of uncontrolled bleeding in patients with MPN.

Prophylactic platelet transfusion could reduce the risk of bleeding in patients suffering from platelet dysfunction. Platelet transfusion is reported to improve aspirin-induced platelet dysfunction (29, 30). In fact, prophylactic platelet transfusion enabled the patient to undergo an invasive procedure. Furthermore, it was previously reported that allo-HCT in a patient with acquired platelet dysfunction owing to PMF resulted in normal trilineage hematopoiesis, although, like the present case, that patient also died of transplant-related complications (31).

This report is associated with several limitations. First, we did not examine the genetic mutations associated with congenital GPS. Second, we could not sufficiently rule out the possibility of "pseudo-GPS" with platelet dysfunction by chance. Pseudo-GPS is known as an *in vitro* phenomenon in which degranulated platelets are observed through ethylenediaminetetraacetic acid (EDTA) sampling, and it does not develop into a bleeding risk by itself (32, 33). Although we identified platelet dysfunction in our patient based on an abnormal wave pattern of aggregation by LTA in samples collected with sodium citrate rather than EDTA, we confirmed the presence of large gray platelets by light microscopy and the absence of platelet α-granules by electron microscopy only in his EDTA sample. In order to make a more accurate diagnosis of "acquired GPS," we require additional examinations, including genetic analyses and morphological findings on blood samples collected by using sodium citrate or heparin.

In conclusion, the detection of large gray platelets under light microscopy and abnormal wave patterns of aggregation by LTA may therefore be an important clue for the diagnosis of hemostatic disorders accompanied by MPNs. In addition, prophylactic platelet transfusion could reduce the risk of bleeding in patients suffering from platelet dysfunction. When morphologically unusual platelets are observed in MPN patients, platelet dysfunction disorders, including acquired GPS, should therefore be ruled out.

The authors state that they have no Conflict of Interest (COI).

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