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A case of adult-onset Still's disease accompanied by pseudo-gray platelet syndrome

TO THE EDITOR: A 39-year-old man with a 1-week history of fever, polyarthralgia, sore throat, and a salmon pink rash was admitted to our hospital. Laboratory findings based on blood samples, which were collected with ethylenediaminetetraacetic acid (EDTA) and counted 30 min after venipuncture, were as follows: white blood cell (WBC) count, 38,840/μL (neutrophils, 94.0%; eosinophils, 1.0%; monocytes, 1.0%; lymphocytes, 1.0%; basophils, 0.8%; metamyelocytes, 2.2%); red blood cell (RBC) count, 4.19×10^6/μL; hemoglobin level, 12.1 g/dL; platelet count, 138×10^3/μL; immunoglobulin (Ig)G, 1.050 g/dL; IgM, 0.184 g/dL; IgA, 0.264 g/dL; and C-reactive protein, 25.3 mg/dL. The antinuclear antibody titer was 1:40. The ferritin level was 953.5 ng/mL (normal range, 5–157 ng/mL). Finally, the patient was diagnosed with adult-onset Still's disease (AOSD). On day 5 after admission, the patient was treated with prednisolone (PSL, 40 mg/day) and subsequently with PSL in combination with tacrolimus (TAC, 2 mg/day). On the day after admission, the platelet count in EDTA-anticoagulated blood samples examined at 60–120 min after venipuncture remained low ($21–87\times10^3$/μL). On day 11 after admission, the platelet count in EDTA- and heparin-anticoagulated blood samples examined at 120 min after venipuncture was 33×10^3/μL and 320×10^3/μL, respectively. The EDTA blood film showed gray, aggregated agranular platelets, whereas the heparin blood film showed normal platelets. On day 14 after admission, complete blood counts were obtained in blood samples collected with EDTA, sodium citrate, and heparin and examined at 0 min, 30 min, and 120 min after venipuncture. The platelet counts in EDTA-anticoagulated blood examined at 0 min, 30 min, and 120 min after venipuncture were 111, 82, and 26×10^3/μL, respectively (Fig. 1A). These blood films showed agranular platelets (also called gray platelets) partly aggregated and normal granular platelets, middle-sized aggregated agranular platelets, and giant aggregated agranular platelets, respectively (Figs. 2A-C). On the other hand, platelet counts of blood collected with heparin and examined at 0 min, 30 min, and 120 min after venipuncture were 320, 329, and 288×10^3/μL, respectively (Fig. 1A). Similarly, platelet counts of blood collected with sodium citrate and examined at 0 min, 30 min, and 120 min after venipuncture were 333, 221, and 266×10^3/μL, respectively (Fig. 1A). All the heparin and sodium citrate blood films showed normal platelets. Based on these findings, the patient was diagnosed with pseudo-gray platelet syndrome (PGPS). On day 21 after admission, serotonin, a monoamine neurotransmitter stored in δ-granules of platelets and released in large amounts after platelet activation, was measured. The blood samples collected with EDTA and left for 30 min and 90 min after venipuncture were separated into platelet-poor plasma and other components by centrifugation at 3,000 rpm for 30 min at room temperature. The serotonin levels in the plasma of the patient, determined by high-performance liquid chromatography (Hitachi, L-6200, Tokyo, Japan), were found to be 59.0 and 147.4 ng/mL at 30 min and 90 min after venipuncture, respectively (normal value, <262.0 ng/mL) (Fig. 1B). The serotonin levels in blood samples of a normal control subject were also measured following the aforementioned procedure, and the levels were found to be 20.5 and 22.9 ng/mL at 30 min and 90 min after venipuncture, respectively (Fig. 1B). However, on mixing the EDTA-anticoagulated plasma of the patient with the platelets of the normal control subject, neither degranulation nor aggregation was found. On day 28 after admission, the AOSD was almost controlled with PSL (30 mg/day) in combination with TAC (2 mg/day) and the patient was discharged. Approximately 2 months after discharge, the platelet counts in EDTA-coagulated blood examined at 120 min after venipuncture was 208×10^3/μL without degranulation and aggregation on PSL (10 mg/day) in combination with TAC (2 mg/day) (Fig. 2D); in other words, an amelioration of

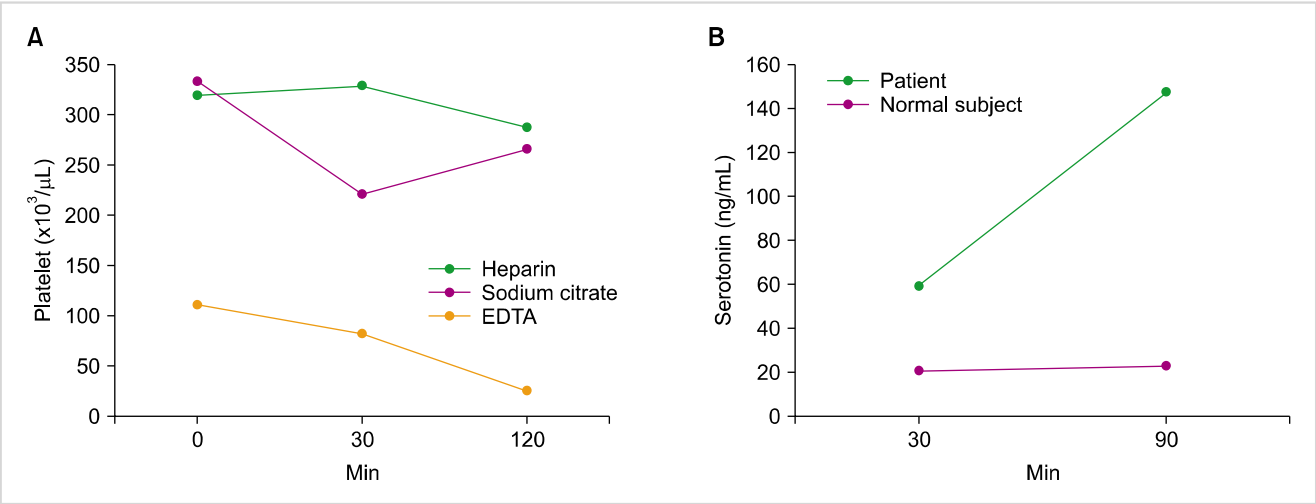

Fig. 1. **(A)** Platelet counts of blood collected with ethylenediaminetetraacetic acid (EDTA), heparin, and sodium citrate and examined at 0 min, 30 min, and 120 min after venipuncture. **(B)** Serotonin levels of the patient and a normal control subject in blood collected with EDTA examined at 30 min and 90 min after venipuncture.

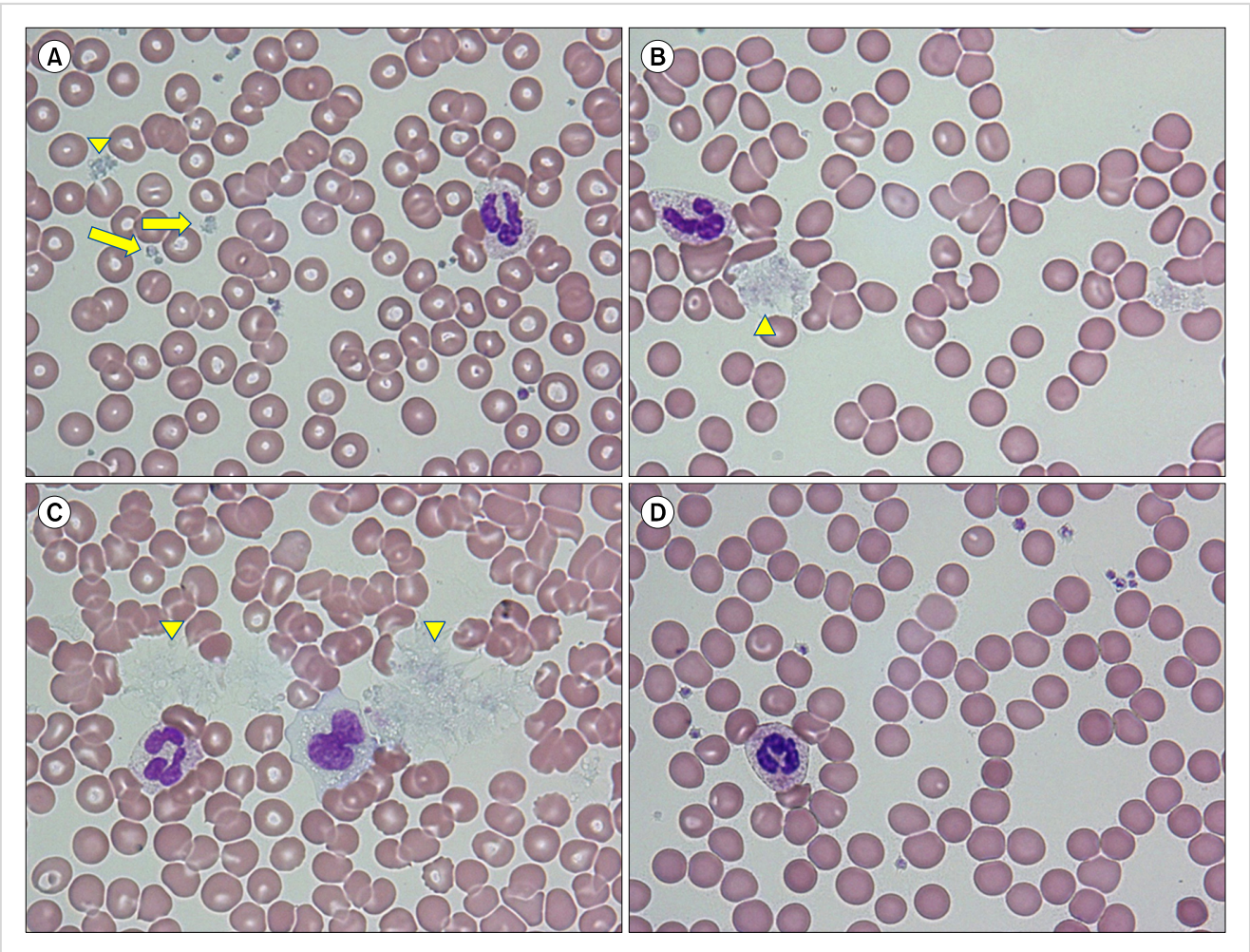

Fig. 2. **(A)** An ethylenediaminetetraacetic acid (EDTA) blood film 0 min after venipuncture shows agranular platelets (also called gray platelets) (arrow) partly aggregated (arrowhead) and normal platelets (×400, Wright-Giemsa stain). **(B)** An EDTA blood film 30 min after venipuncture shows middle-sized, aggregated agranular platelets (arrowhead) (×400, Wright-Giemsa stain). **(C)** An EDTA blood film 120 min after venipuncture shows giant, aggregated agranular platelets (arrowhead) (×400, Wright-Giemsa stain). **(D)** An EDTA blood film 120 min after venipuncture shows normal platelets, approximately 2 months after discharge (×400, Wright-Giemsa stain).

PGPS was observed.

PGPS is a rare EDTA-dependent phenomenon that causes platelets to degranulate in vitro, resulting in a gray appearance on the blood film, similar to that observed in a case of gray pseudo-syndrome (GPS). However, unlike GPS, this phenomenon occurs only when the blood is collected with EDTA as an anticoagulant and not when it is collected with sodium citrate or heparin as the anticoagulant. Typical electron microscopy of PGPS reveals the degranulation of EDTA-exposed platelets. Although we did not perform electron microscopy, we measured serotonin levels and confirmed the degranulation of δ-granules. The pathophysiology of degranulation appears to result from a humoral factor (presumably an antibody) that induces the release of α- and δ-granule contents without platelet aggregation [1]. Crossover studies using the EDTA-anticoagulated plasma of a patient with PGPS mixed with normal patients' platelets demonstrated the degranulation of normal patients' platelets, just as the PGPS platelets degranulate in EDTA. Therefore, it is speculated that some factor in the plasma of the patient with PGPS is responsible for the degranulation. This factor is possibly an antibody against a hidden site in the platelet that is exposed after the reaction with EDTA [2, 3]. The present case is particularly unusual in that platelet aggregation was present in addition to PGPS; these findings have been previously reported [1, 4, 5]. Mant *et al.* [5] reported on cases of EDTA-induced platelet degranulation with aggregation caused by a plasma factor, which was not IgG, IgM, fibrinogen, or albumin; they considered that it was an undefined abnormal plasma component that, on exposure to EDTA, develops antiplatelet activity.

In the present case, although the normal platelets of a healthy control subject were mixed with the patient's plasma, neither degranulation nor aggregation was found. Moreover, PGPS completely ameliorated approximately 2 months after discharge, when AOSD was controlled with a combination of PSL and TAC. Based on these results, we speculate that hyper-inflammation-associated platelet dysfunction and plasma factor may cause degranulation and aggregation. However, the obvious mechanism by which platelet degranulation and aggregation occur remains unclear.

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Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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