Pseudo-Grey Platelet Syndrome: A Rare Artifact

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Abstract Two cases that were identified during routine blood examination, presented an artifact the pseudo-grey platelet syndrome. The platelets from the blood collected with ethylene diamine tetra-acetic acid (EDTA) stained poorly on the blood smear and appeared agranular under the microscope. This artifactual anomaly did not occur when samples were collected in vacutainers containing citrate or collected by finger prick. Reports of pseudo-grey platelet syndrome are few, possibly because of the poorly explained and difficult to diagnose phenomenon.

Keywords Platelet · Ethylene diamine tetra-acetic acid · Artifact · Pseudo-grey platelet syndrome

Introduction

Pseudo-grey platelet syndrome is a rare ethylene diamine tetra-acetic acid (EDTA)-dependent phenomenon that causes platelets to degranulate in vitro, resulting in grey appearance on the peripheral blood (PB) film. On the other hand, the well known Grey platelet syndrome (GPS) is rare bleeding disorder characterised by abnormal platelets with agranular cytoplasm, appearing grey or pale blue on PB film [1, 2]. In pseudo-GPS the platelet count is usually normal, and automated counters may misclassify these abnormal platelets resulting in false thrombocytopenia. To

the best of my knowledge, very few cases of pseudo-GPS have been previously reported [3–6], possibly because of underestimation of this poorly understood artifact. Here, I report two cases of EDTA-induced pseudo-GPS.

Case 1

One year old female child was brought with history of rash all over body predominantly on face and extensor extremities since 1 week. There was also history of puffiness of face and swelling of eyelids. Child's mother also complained of fullness of abdomen. She had been having similar episodes on and off since 3 months of age. Rash appeared on crying and when child was hungry. She was adequately immunised as per schedule. On examination she had a maculopapular rash on face and extremities, periorbital swelling, generalised anasarca, scratch marks were also noted. She was 50th percentile for weight and 75th percentile for height. There was no pallor, icterus or lymphadenopathy. Her initial CBC carried out in EDTA sample revealed Hb 10.8 mg/dl (10.1–12.3), RBC count 3.98 million/mm³ (4.1–5.0), normal RBC indices, WBC count 24,100/mm³ (6,000–18,000), with DLC comprising of N 12%, Eo 40% (AEC—9,640/mm³), L 47%, M 0.1%. She had a platelet count of 2.02 lakhs/mm³ and MPV was 12.1 fl (7–11). The microscopic examination of the PB smear confirmed eosinophilia but also revealed the presence of hardly visible platelets (Fig. 1). Hence, blood was collected in citrate anti-coagulant and a finger prick PB film was also made. These showed normal staining (magenta colour) platelets. Routine stool and routine urine examination were normal. X-ray chest showed bilateral minimal pleural effusion. Her total protein was 6.0 gm/dl, IgE was 15,266 IU/ml. IgG, IgA, IgM were normal. She was diagnosed with infantile eczema and treated with wet

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Fig. 1 Smear from EDTA blood showing *light grey* agranular platelets and a mature eosinophil also seen $\times 100$ oil immersion

dressings and a topical corticosteroid to which she responded.

Case 2

A 45 year-old male was admitted for suspected non-Q-wave myocardial infarction. The past medical history included diabetes mellitus, angina on effort. There was no reported history of bleeding or bruising. The diagnosis was confirmed by appropriate tests (clinical examination, biochemical investigations and electrocardiographic tests). Chest X-ray demonstrated a pulmonary oedema. The initial complete blood count was carried out with an EDTA anti-coagulated specimen, which revealed haemoglobin of 11.1 g/dl, leucocytes $13,300/\text{mm}^3$ (neutrophils 87%, lymphocytes 10%, and monocytes 3%), platelets 1.32 lakh/ mm^3 , MPV 11.6 fl (7–11). The microscopic examination of the PB smear confirmed the leukocyte differential but also highlighted the presence of platelets which were light grey in colour and appeared as agranular ‘ghosts’ and had giant size (Fig. 2). During patient’s follow-up, careful checking of the platelet count was performed. Discrepancies were recorded for platelet count, size, staining property and mean platelet volume (MPV). Usual staining and regular morphology was obtained using citrate anti-coagulated samples.

Discussion

In both cases the patients peripheral blood specimens were collected in vacuum tubes (Vacutainer; BD, San Jose, CA, USA) containing anti-coagulants: K3 EDTA, sodium citrate. The tubes were maintained at room temperature until analysis. Blood cell counts were performed using (LH750, Beckman–Coulter, USA). Platelet counts were checked in

Fig. 2 Smear from EDTA blood showing large *light grey* agranular platelets appearing as ghosts and a neutrophil with vacuoles $\times 100$ oil immersion

Fig. 3 Smear from citrate blood showing large normal magenta staining platelets $\times 100$ oil immersion

improved Neubauer’s hemocytometer. PB films were prepared from both anti-coagulated specimens and finger prick blood, and were stained with Wright’s stain. Electron microscopy was not carried out. Bleeding time was normal in both the patients. Platelet function tests were normal (Case 2). In both cases the platelets did not stain from EDTA blood, but stained in blood collected in citrate and from finger prick PB film (Fig. 3).

Unlike pseudo-GPS, grey platelet syndrome is a very rare congenital disorder characterised by large platelets; mild to moderate thrombocytopenia; mild hemorrhagic tendency; prolonged bleeding time; and abnormal platelet function [7]. Under light microscopy, classic findings include large agranular platelets with a grey to grey-blue colour. On electron microscopy there is severe reduction or complete absence of α -granules.

Platelets in pseudo-GPS do not markedly impair automated platelet count. Though there may be low platelet count and slight increase in MPV [4, 6]. Much more common causes of the low counts of platelets that are often encountered on automated analyzers are due to partial clotting of the specimen, platelet satellitism [8] or anti-coagulant-induced agglutination, mainly EDTA [9]. The platelet agglutination in the presence of EDTA is because of a reaction with circulating EDTA-sensitive antibodies. Platelet clumping is then caused by an immunoglobulin recognising an epitope exposed on platelets in vitro [10]. EDTA-induced degranulation has been very rarely reported [11].

Crossover experiment was carried out wherein normal platelets of healthy control subject were resuspended in patient's plasma (Case 2) and this confirmed that there was a plasma factor responsible for EDTA-induced artifact [5, 6]. The PBF specimens were collected both in EDTA and citrate tubes during the follow-up of the patient (Case 2). All measurements from citrate anti-coagulated samples were normal, histograms were not altered no clumps or other anomalies were flagged. Platelets in EDTA anti-coagulated specimens had a higher MPV when compared with citrate specimens.

A pseudo-GPS was observed because of early platelet degranulation in EDTA-collected blood. Platelet swelled ghosts resulted in moderately decreased platelet counts (Case 2) as estimated by automated analyser and also increased MPV as was seen in both the cases. Platelet skeletons without granules were visible on the stained blood smear (Figs. 1, 2). EDTA-induced degranulation can be confirmed by electronic microscopy that can demonstrate immediate dense bodies release [3, 4, 6]. Moreover, release of CD62P which is a surface platelet glycoprotein P-selectin, a marker of platelet activation, can be observed using a flow cytometric assay which confirms the activated status of the platelets, with α -granule release [6].

In conclusion, EDTA-induced pseudo-GPS seems to be a very rare phenomenon and is poorly known. It should be

considered when platelets appear pale on the peripheral blood smear. Clinical history, bleeding time, platelet function tests, if possible electron microscopy can help to differentiate it from the rare grey platelet syndrome.

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