Co-existent Platelet Phagocytosis, Satellitism and Clumping Causing Spurious Thrombocytopenia

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Received: 24 January 2012 / Accepted: 23 April 2012 / Published online: 26 May 2012
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Introduction

Manual platelet counts have a high coefficient of variability. Increasing use of automated hematology analyzers for blood cell counts has increased the reproducibility and accuracy of platelet counts. In general, thrombocytopenia is more alarming in clinical practice than thrombocytosis. However, at times, artefactual thrombocytopenia may mislead the clinician into unnecessary investigations or management. We present a patient with three platelet-related artefacts leading to spurious thrombocytopenia.

Case Summary

A 35-year-old gentleman presented to the ENT outpatient service with complaints of pain in the left side of tongue since 5 months. Examination showed an irregular 2×1.5 cm ulcer with raised and indurated borders over the left lateral margin of the tongue. The patient had undergone renal transplantation for end stage renal disease 7 years ago and was on Azathioprine, Cyclosporine and Prednisolone. A diagnosis of malignancy of the tongue was considered and excision biopsy was planned.

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The complete blood count requested as part of the pre-operative work up showed hemoglobin of 10.8 gm/dl, total leukocyte count of 5,300/cu mm and platelet count of 59,000/cu mm. The red cell indices showed mean corpuscular volume (MCV) of 106.1 fl. The peripheral blood smear showed macrocytic red cells and normal white cell count. Many neutrophils showed platelet satellitism (Fig. 1), while nearly a third of the neutrophils showed platelet phagocytosis, with 1–4 platelets within the cytoplasm (Fig. 2). Small platelet clumps were also seen in the background (Fig. 1). Although the automated platelet count was low, the platelet count was adequate on peripheral smear examination. Ethylenediamine tetraacetic acid (EDTA)-induced pseudothrombocytopenia causing platelet phagocytosis, satellitism and platelet clumping was considered.

Repeat blood samples were taken using heparin and sodium citrate as anticoagulants. The automated platelet count in the repeat samples still showed thrombocytopenia although the platelet counts were marginally higher than that seen in the EDTA sample (Table 1). Smears made from blood collected in these anticoagulants showed complete abolition of platelet satellitism and phagocytosis. However the platelet clumps still persisted (Fig. 3) and the platelets were adequate on the blood smear. A review of previous blood counts showed normal platelet counts documented on many occasions, with mention of platelet clumps on peripheral smear examination.

Thus platelet satellitism and platelet phagocytosis were considered EDTA-dependant, while the platelet clumping was considered to be an EDTA-independent immune phenomenon. The presence of this phenomenon was intimated to the surgeons and the patient underwent partial glossectomy successfully in view of adequate platelet counts on the peripheral blood smear. Histopathology

Fig. 1 Peripheral blood smear from the EDTA-anticoagulated blood sample showing platelet satellitism. Leishman $\times 1000$

Fig. 2 Peripheral blood smear from the EDTA-anticoagulated blood sample showing platelet phagocytosis. Leishman $\times 1000$

Table 1 Comparison of platelet counts in EDTA with heparin and sodium citrate

	Anticoagulant used		
	EDTA	Heparin	Sodium citrate
Automated platelet count (/cu mm)	59,000	62,000	66,000
Platelet satellitism	Present	Absent	Absent
Platelet phagocytosis	Present	Absent	Absent
Platelet clumps	Present	Present	Present

EDTA ethylenediamine tetraacetic acid

confirmed the clinical suspicion of squamous cell carcinoma. On follow up, the platelet phagocytosis and platelet satellitism disappeared. However, platelet clumps continued to persist. The manual platelet count was adequate (140,000/cu mm) at 7 months follow up.

Fig. 3 Peripheral blood smears from citrated (a) and heparinised (b) blood samples showing abolition of platelet phagocytosis and satellitism with persistence of platelet clumps. Leishman $\times 1000$

Discussion

Pseudothrombocytopenia, a relatively uncommon phenomenon, refers to a spuriously low automated platelet count. Pseudothrombocytopenia is commonly seen when EDTA is used as an anticoagulant, or with platelet cold agglutinins [1]. The most common cause of pseudothrombocytopenia with use of EDTA is platelet clumping or platelet satellitism. EDTA-induced spurious thrombocytopenia occurs in about one in 1,000 persons, irrespective of the presence or absence of any disease [2]. As compared to EDTA-induced platelet clumping and pseudothrombocytopenia due to platelet satellitism, platelet phagocytosis is an extremely rare phenomenon [3].

Platelet phagocytosis by neutrophils is an uncommon occurrence which is usually dependent on the presence of EDTA and patient autoantibody. Criswell et al. [4] reported a rare case of EDTA-dependant platelet phagocytosis of nearly all the circulating platelets by leukocytes. Marked phagocytosis of platelets can be an extension of the more classic platelet satellitism phenomenon, along with presence of vacuolization, apparent degeneration, and digestion of platelets by the polymorphs [5]. The interaction between platelets and neutrophils has been demonstrated on electron microscopy [6]. The platelet count in patients with phagocytosis or satellitism may be spuriously low. Accurate platelet count in these subjects can be achieved by adding aminoglycosides to the EDTA-anticoagulated blood which causes disaggregation [7].

Platelet-leukocyte satellitism refers to adherence of platelets to neutrophils or less commonly, monocytes and is an EDTA-dependant in vitro phenomenon [1]. The platelets typically form a rosette around the periphery of the leukocyte [8]. The pathogenesis of platelet satellitism is explained by crypt-antigens or hidden antigens that are

present on the platelet glycoprotein (GP) IIb–IIIa complex which react simultaneously with the leukocyte Fc γ receptor III (CD16) resulting in platelet satellitism to leukocytes [9]. These hidden antigens are exposed only in the presence of calcium in EDTA resulting in a conformational change in the platelet membrane. Exposure of cryptic antigens on EDTA-treated platelets and leukocytes may trigger this phenomenon.

Although platelet-leukocyte satellitism and platelet phagocytosis have been considered to be a transient in vitro phenomenon to EDTA, it may also be an expression of a person's thrombotic tendencies in vivo [10]. These phenomena have been reported in association with underlying systemic diseases like malignant lymphoma [11] or autoimmune disease [10].

Clumping or agglutination of platelets occur due to their activation during a difficult venepuncture or mediated by either an EDTA-dependent IgG or IgM antibody, or an EDTA-independent antibody. Platelet clumping in blood samples collected in EDTA occurs due to the chelating effect of EDTA, which alters the GPIIb–IIIa molecule on the platelet surface, thereby leading to binding of a naturally occurring IgG antibody to GPIIb [12]. Other anticoagulants, such as sodium citrate or heparin, do not affect GPIIb–IIIa, and therefore do not lead to platelet agglutination in these cases [13]. Enhanced platelet activation and subsequent aggregation has been reported in post renal transplant patients especially those on Cyclosporine and/or Azathioprine therapy [14]. Our patient was also on Cyclosporine and Azathioprine and the persistent platelet clumps even when repeat samples were drawn in heparin and sodium citrate can be possibly attributed to the effect of these drugs.

Conclusion

Spurious thrombocytopenia must be considered in EDTA-anticoagulated blood samples when unexpectedly low automated platelet counts are obtained. Peripheral blood film examination is warranted in these cases to exclude platelet satellitism, phagocytosis and clumping.

Conflict of interest None.

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