Pseudothrombocytopenia induced by incubation at 37 ℃ in ethylenediaminetetraacetic acid tubes

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Abstract: Ethylenediaminetetraacetic acid (EDTA) is the anticoagulant currently used for cell blood counts. Several studies showed that exposure of platelets (PLTs) to EDTA for a short period of time promote tyrosine phosphorylation of certain proteins, events that are related to activation. Spuriously low PLT counts were observed in a patient with cold agglutinins after the blood being warmed at 37 ℃ in EDTA tubes. Some cloudy polymers were found on peripheral blood smear after incubation at 37 ℃ for 15 minutes. These data suggest that PLT were activated after being warmed to 37 ℃ with EDTA. This process initiated coagulation and generated fibrin and PLT polymers from PLT agglutination and release. This case report hence describes a very rare phenomenon, which produces a spuriously low PLT counts caused by warming of EDTA tubes at 37 ℃.

Keywords: Ethylenediaminetetraacetic acid (EDTA); platelet (PLT); pseudothrombocytopenia; incubation; cold agglutinins

Received: 16 November 2016; Accepted: 16 December 2016; Published: 05 January 2017.

doi: 10.21037/jlpm.2016.12.03

View this article at: http://dx.doi.org/10.21037/jlpm.2016.12.03

Introduction

The widespread use of haematology analyzers has led to a major improvement of blood cell analysis. However, spurious results are occasionally observed, so that operators must be able to recognize and circumvent anomalous results (1-3).

Current anticoagulants for cell blood counting mainly include ethylenediaminetetraacetic acid (EDTA) (4). However, several studies point out that it is far from optimal for the preservation of platelet (PLT) ultrastructural and functional capabilities (5-7).

Cold agglutinins are red blood cell (RBC) specific autoantibodies that optimally agglutinate RBC at 4–22 ℃ *in vitro*. It often leads to spurious low RBC. The RBC level often increases after being warmed at 37 ℃ (8). PLT cold agglutinin is a rare phenomenon and it may produce a pseudothrombocytopenia at temperatures below 37 ℃ (9). However, pseudothrombocytopenia after incubation at 37 ℃ has not been previously described to the best of our knowledge. We herein describe the case of a patient with cold agglutinins, in whom we observed PLT activation and decrease of PLT count in an EDTA sample incubated at 37 ℃ for 15 min.

Case presentation

A 56-year-old man presented to our hospital for lymphoproliferative disease, and finally diagnosed with angioimmunoblastic T-cell lymphoma.

The blood cell count was as follows: RBC: 1.6×10^{12}/L, hemoglobin: 114 g/L, PLT: 159×10^{9}/L. The blood EDTA tube was incubated at 37 ℃ for 15 minutes to reduce the effect of cold agglutinins and RBC count was then increased to 3.06×10^{12}/L. Nevertheless, the PLT count decreased to 12×10^{9}/L.

A microscopic review of the warmed blood sample showed the presence of some cloudy polymers (*Figure 1A*). PLT were

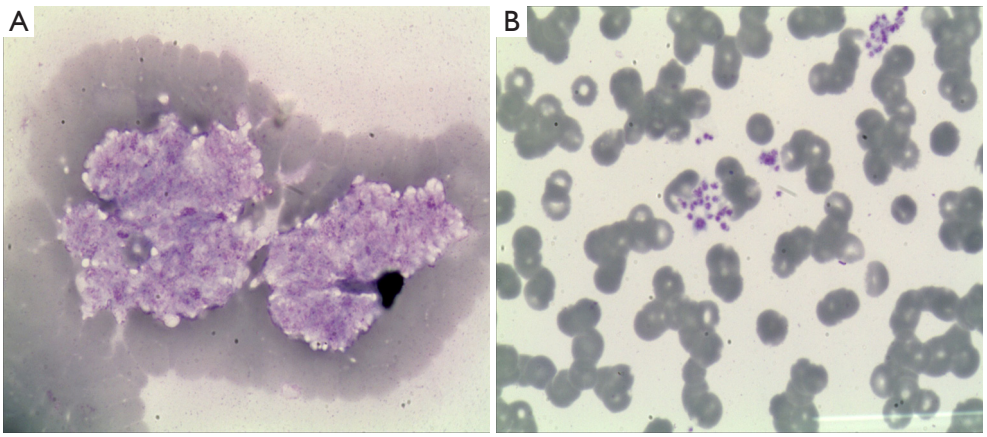

Figure 1 A microscopic review of the blood sample (Wright-Giemsa stain; 1,000×). (A) Cloudy polymers were observed on peripheral blood smear after being warmed at 37 ℃ for 15 minutes with EDTA; (B) PLT clumps on the blood smear without anticoagulant and incubation. EDTA, ethylenediaminetetraacetic acid; PLT, platelet.

activated and triggered the formation of a fibrin clot.

A blood smear was quickly prepared after drawing blood without anticoagulants to assess the PLT count (*Figure 1B*). PLT clumps were observed on the blood smear and normal quantitative PLT were present.

Discussion

Haematology analyzers provide quick and accurate results in most situations. However, spurious counts may be found because of agglutination, cryoglobulins, dyslipidemia, etc. (1-3).

Cold agglutinins usually lead to spuriously low RBC counts and to abnormally high mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). However, hemoglobin value is substantially unaffected. These anomalies generally disappear when the sample is pre-incubated at 37 ℃ (8).

In this case report, the RBC count was 1.6×10^{12}/L and Hb 114 g/L. The RBC count then increased to 3.06×10^{12}/L after warming EDTA blood at 37 ℃ for 15 minutes, thus suggesting that the spuriously RBC counts may have been caused by cold agglutinins.

After additional analyses we found that the serum of this patient contained high values of immunoglobulin (Ig)G: 34.5 g/L (range, 7–16 g/L), IgA: 4.79 g/L (range, 0.7–4 g/L) and IgM: 13.1 g/L (range, 0.4–2.3 g/L), which might be the cause of cold agglutination.

However, PLT count unexpected decreased after incubation. PLT may be activated, thus triggering blood coagulation as confirmed by the microscopic analysis (*Figure 1*).

Contact of PLTs with EDTA for prolonged periods results in extreme distortion of their morphology, including dilation of the open canalicular system, a progressive tendency to shape change, and even formation of PLT agglutinates. It has been demonstrated that addition of inhibitors of protein phosphorylation significantly prevents the degranulation caused by EDTA, especially at 4 ℃ (10).

In this case report, we incubation at 37 ℃ promoted PLT activation in EDTA blood, which further triggered coagulation and formation of fibrin and PLT polymers. Temperature-induced PLT activation with EDTA is a very rare phenomenon, which has not been previously described to the best of our knowledge.

Spuriously low PLT counts usually occur after difficult venipuncture, in the presence of EDTA, or in samples with PLT satellitism and formation of PLT-WBC aggregates.

Conclusions

In this report, we describe a unique example of spuriously low PLT counts caused by being warming EDTA tubes at 37 ℃ in. It is a very rare phenomenon and laboratory professionals must be able to recognize and circumvent these anomalous results.

Acknowledgments

Funding: None.

Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/jlpm.2016.12.03). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This is a case report concerning the laboratory data, with no information related to patients' information. Informed consent would not be required in this case.

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doi: 10.21037/jlpm.2016.12.03
Cite this article as: Zhang L, Wang L, Gao L, Xu J, Pan S. Pseudothrombocytopenia induced by incubation at 37 ℃ in ethylenediaminetetraacetic acid tubes. J Lab Precis Med 2017;2:2.