Ethylene Diaminete Traacetic Acid - Dependent Pseudothrombocytopenia (EDTA-PTCP)

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Abstract
Background
Ethylene diaminete traacetic Acid-dependent pseudothrombocytopenia (EDTA-PTCP) is an in vitro phenomenon of platelet clumping in blood, anticoagulated with EDTA, that leads to spuriously low platelet counts by automatic hematology analyzers. We describe a case of EDTA-PTCP in a 77-year-old woman, who was admitted for total hip replacement surgery. Preoperatively, low level and unknown changes of platelet counts cause confusion and postpone of surgery. A correlative experiment was designed and the results confirmed EDTA-PTCP in the patient. The physician accordingly diagnosed as pseudothrombocytopenia and subsequently followed by surgery.

Methods
We searched "UpToDate" Evidence-Based Medicine database for definition and diagnostic approach of pseudothrombocytopenia, and consequently designed an experiment in this case. We collected 5 blood specimens by venepuncture in different tubes each containing EDTA(tube1), sodium citrate(tube2), heparin(tube3) anticoagulants, and freshly drawn samples without any anticoagulant(tube 4), plus one more EDTA tube(tube 5) with 20 minutes, 37°C incubation prior to platelet measurement. All tubes were measured with a CELL-DYN 3700 hematology analyzer; tube1, 2, 3 were measured at times 0, 5, 10, 20, 30 minutes from venepuncture. Immediately after analysis at these respective times, slides of tube1 sample were also made and inspected. Tube 4 was measured directly as fresh whole blood put in, and Tube 5 were measured immediately after 37°C, 20 minutes incubation.

Results
Three agents(tube1-3) were observed to induce a decreasing platelet count. Platelet counts in the sodium citrate sample also fell, yet this appeared to be considerably less than the other two specimens. Increased clumping could be observed on the PB smears through microscopic examination. The 77-year-old woman in this case was confirmed EDTA-PTCP. The operation was subsequently performed.

Conclusions
Platelet clumping could be avoided in such cases by the use of anticoagulants other than EDTA, but we also found even citrate, and heparin have been implicated as anticoagulants that may cause platelet clumping. The best analytic course appears to be immediately running the freshly drawn samples [11] without the use of anticoagulants at all. To avoid incorrect diagnoses and inappropriate treatment, EDTA-PTCP should always be considered as a possible cause of low platelet count, especially in cases of inconspicuous clinical findings. Appropriate laboratory analysis should be applied.

Keywords: EDTA-PTCP, pseudothrombocytopenia, platelet clumping
Introduction

EDTA-pseudothrombocytopenia (EDTA-PTCP) is a well-known phenomenon within the determination of blood cell counts by automated analysers[1] and results in spurious low platelet counts approximately 0.1 percent[2] in the general population have EDTA-dependent agglutinins, which can lead to platelet clumping and spurious thrombocytopenia and leukocytosis. This phenomenon of EDTA-induced pseudothrombocytopenia has been reported rarely in both normal individuals and in association with a variety of diseases, such as autoimmune diseases, neoplastic diseases, liver diseases, cardiovascular diseases and viral infections[1,3]. The exact mechanism of ETDA-PTCP is not clearly defined. This is thought to result from a "naturally occurring" platelet autoantibody directed against a normally concealed epitope on the platelet membrane glycoprotein (GP) IIb/IIIa, which becomes exposed by EDTA-induced dissociation of GP IIb/IIIa [4-9]. Pseudothrombocytopenia then occurs because EDTA is an anticoagulant employed in the tubes used for routine complete blood counts. In addition, in vitro aggregation of platelets due to IgM and IgG types of cold agglutinins may also lead to PTCP[1, 8,15,16]. These cold agglutinins are no activity at 37℃ but react apparently at room temperature. Platelet clumping in EDTA-PTCP can result in inaccurate platelet concentrations, which easily leads to misdiagnose as true thrombocytopenia if analyzed with hematology analyzer only[10]. Lack of recognition of EDTA-PTCP may lead to additional unnecessary testing, delays in diagnostic or therapeutic procedures, and inappropriate treatments including platelet transfusion, steroid therapy, and Splenectomy. This article reports a case of experimental results confirmed EDTA-PTCP in a 77-year-old woman preoperatively so that the physician was able to start the operation.

Case Report

A 77-year-old woman has history of diabetes mellitus type 2, old pulmonary tuberculosis s/p therapy 6 years ago, valve heart disease and sleepless. She suffered from left hip pain due to left femoral basal neck fracture by vehicle accident. She received open reduction and interal fixation in the next day. Because the pain of left hip always presented and was more severe, she was admitted in April for total hip replacement surgery. Preoperatively, there were great changes in rerunning platelet counts in one day on Apr. 18, 2012; 14.6K/ul at 5:46; 305K/ul, 69.1K/ul at 09:35 and 13:00 respectively. We ruled out the cause of inadequate anticoagulation of the blood sample and hematology tests including Bleeding time, Clotting time, prothrombin time (PT) and activated partial thromboplastin time (APTT) were all normal.

Thereupon we ran the freshly drawn whole blood without any anticoagulants at all immediately after withdrawal, 538 K/ul platelet count result was obtained at 13:50. We confirmed the presence of platelet clumping by examining the PB smear and appropriately noted platelet clumping on the peripheral blood smear, the falsely low platelet count still be recorded and cause confusion and postpone of surgery.

Materials and methods

We searched 「UpToDate」Evidence-Based Medicine Prefiltered (secondary) database for definition and diagnostic approach of pseudothrombocytopenia, results were as below:

Pseudothrombocytopenia was defined as the presence of one or more of the following [11]:
1. Platelet clumping on a peripheral smear made from blood in which the offending anticoagulant was used.
2. A difference between the platelet count in blood samples obtained using different anticoagulants (eg, EDTA, heparin, citrate), with one having a count at least 20 percent lower than that of the comparison anticoagulant. The offending anticoagulant was EDTA in most of the 117 cases, although 14 were documented to occur in the presence of citrate as an anticoagulant.
3. A normal platelet count on a non-anticoagulated blood sample (eg, finger stick) taken directly into platelet count diluent fluid.

We designed an experiment and performed at 8:00 am, Apr. 19, 2012, three different anticoagulants included as above to substantiate the diagnosis in our patient.
We collected 5 blood specimens by venipuncture in different tubes each containing EDTA (tube 1), sodium citrate (tube 2), heparin (tube 3) anticoagulants, and freshly drawn samples without any anticoagulant (tube 4), plus one more EDTA tube (tube 5) with 20 minutes, 37°C incubation prior to platelet measurement. All tubes were measured with a CELL-DYN 3700 hematology analyzer; tube 1, 2, 3 were measured at times 0, 5, 10, 20, 30 minutes from venipuncture. Immediately after analysis at these respective times, slides of tube 1 sample were also made and inspected. Tube 4 was measured directly as fresh whole blood put in, and Tube 5 were measured immediately after 37°C, 20 minutes incubation.

Results

Platelet count (and percentage) of EDTA-anticoagulated tube 1 at different times were as following: 0' 511.0K/ul (100%); 5' 458.0K/ul (90%); 10' 250.0K/ul (49%); 20' 54.0K/ul (11%); 30' 46.0K/ul (9%). The microscopic images of 0 minute, 5 minutes, 10 minutes, 20 minutes PB smears (Figure 1) illustrated a false platelet count with increasing clumping as time increased. Three agents (tube 1-3) were observed to induce a decreasing platelet count (Table 1), especially in the EDTA sample, dropped fast all the way during the interval between 5 and 20 minutes post-venipuncture; platelet count in the heparin sample during the same interval decreased a little, lined a horizontal slope shown in Figure 1, but dropped fast from 0 to 5 minutes and from 20 to 30 minutes post-venipuncture. Platelet counts in the sodium citrate sample also fell, yet this appeared to be considerably less than the other two specimens. Platelet count in EDTA sample at times 10, 20, 30 minutes was more than 20% (49%, 84%, 62% respectively) lower than in sodium citrate sample, platelet count of non-anticoagulant sample was 520K/ul, platelet count of 37°C, 20 minutes incubated. EDTA sample was 482K/ul.

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In this case, examination of the peripheral blood smear provides evidence of EDTA-PTCP in the form of platelet clumping. Furthermore, comparison of platelet count in EDTA to sodium citrate anticoagulant samples appears to enable the diagnosis of EDTA-PTCP in the 77-year-old woman, the operation was subsequently performed. Postoperative course was almost uneventful and the patient was discharged on 10th day after surgery.
Discussion

Given these findings, vitro platelet clumping obviously occur with EDTA anticoagulant, similar agglutination appears to occur with other anticoagulants as citrate and heparin. In such a suspected pseudothrombocytopenia patient, adding a mixture containing 9mmol/L CaCl₂ and 0.1unit/L sodium heparin to collected samples which was designed to dissociate platelet clumps was a novel measure[12], or adding aminoglycosides[13]. In addition to these approaches above, the best analytic course appears to be immediately running the freshly drawn samples[14] without the use of anticoagulants at all. Henceforward we add「EDTA-dependent Pseudothrombocytopenia patient, freshly drawn blood only」on the blood withdrawal sheets of such EDTA-PTCP patients as a notification.

In cases of newly developed thrombocytopenias, EDTA-PTCP should always be considered as a possible cause of low platelet count, in particular case of unapparent clinical findings. In the first step, an appropriate laboratory analysis to evaluate the patient, their medical and family history and the examination of PB smear should be applied[3], particularly, because it is the "gold standard" for evaluation of thrombocytopenia of any cause and for distinction between pseudo- and true thrombocytopenia. Besides, expensive and sophisticated laboratory procedures are not available in all clinics.

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References


個案報告

EDTA-依賴型假性血小板減少

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摘要

背景

EDTA-依賴型假性血小板減少（EDTA-PTCP）是一種在體外以EDTA為抗凝劑時血小板凝集的現象，而造成在血液儀器測量下血小板呈假性低值。我們描述一個EDTA-依賴型假性血小板低下的77歲女性的個案報告，預定做全人工髖關節置換手術而住院。手術前，血小板低值與其數值不明原因的變化造成困惑與手術的延遲，一個相關的實驗被設計，結果確認病患有EDTA-依賴型假性血小板低下。因此醫生診斷為假性血小板減少並在隨後為病患進行手術。

方法

我們在「UpToDate」實證醫學資料庫搜尋假性血小板減少的定義與診斷方法，並隨後設計了一個實驗，由靜脈抽血採檢後分裝五個不同的採血管，五管分別含有EDTA（第1管）、檸檬酸鈉（第2管）、肝素（第3管）等抗凝劑，及採新鮮全血不加任何抗凝劑（第4管），外加一EDTA管（第5管），在測量血小板數值前先孵育於37℃下20分鐘。所有檢體皆由血液自動分析儀CELL-DYN 3700測量。第1、2、3管分別於採血後0分、5分、10分、20分、30分、等時間點即時上機，同時將第1管製做抹片並觀察。

結果

含抗凝劑的1-3管上機測量後可以觀察到血小板減少的狀況，檸檬酸鈉抗凝劑檢體的血小板數亦會減少，但減少的幅度明顯比另外兩管小多了。周邊血液抹片可以觀察到漸漸增大的血小板凝集。在此案例77歲婦人確認為EDTA-依賴型假性血小板低下，手術隨後展開。

結論

在此類案例使用EDTA以外的抗凝劑可避免血小板凝集，但我們也發現即使是檸檬酸鈉與肝素抗凝劑也可能血小板凝集的形成有關。似乎最好的分析方式是抽血後即刻上機而不添加任何抗凝劑。為了避免不正確的診斷與不適當的處置，EDTA-依賴型假性血小板低下應經常被視為血小板低值的可能原因之一，特別是當臨床上有不顯著的發現時，應運用適當的實驗室分析。

關鍵詞：EDTA-依賴型假性血小板減少、假性血小板減少、血小板凝集