

To Test the Accuracy of Platelet Count, as Corrected by Citrate Anticoagulant in EDTA Induced Pseudo-Thrombocytopenia

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Abstract: *Background:* EDTA- induced pseudothrombocytopenia (EDTA-PTCP) is a common laboratory phenomenon with a prevalence ranging from 0.1-2% in hospitalized patients to 15-17% in outpatients evaluated for isolated thrombocytopenia. (EDTA-PTCP) is falsely low platelet count due to antibodies directed against glycoprotein (gp) IIB-IIIa complex leading to agglutination. Misinterpretation of artificial low platelet count lead to unnecessary transfusion of platelets increasing the risk of associated morbidity. *Methodology & Results-* A Total of 100 Cases of EDTA induced Pseudothrombocytopenia as corrected by Citrate anticoagulant is studied. The PLT Count by Abotts Cell DYN Ruby using EDTA as anticoagulant is (55 ± 25.9) & citrate anticoagulant is $(217.8 \pm 61.16) \times 10^3/\text{mm}$. Manual platelet count = $222 \pm 58.7 \times 10^3/\text{mm}$. P value < 0.05 which is statistically significant. This results clearly shows manual platelet count very well correlated with citrated blood platelet. *Conclusion:* EDTA induced PTCP can be corrected by immediate blood analysis in hematology analyzers with Citrate as anticoagulant. So it can be confidently concluded that EDTA induced pseudothrombocytopenia can be effectively corrected by citrate anticoagulated blood.

Keywords: platelet aggregation, pseudothrombocytopenia, anticoagulation, EDTA, citrate, agglutination.

1. Introduction

EDTA-dependent pseudo-thrombocytopenia (EDTA-PTCP) is a common laboratory phenomenon. Its prevalence is reported to vary between 0.1-2% among hospitalized patients and 15-17% in outpatients evaluated for isolated thrombocytopenia. ¹(EDTA-PTCP) is the result of spuriously low platelet count induced by agglutinating antibodies that recognize cytoadhesiverceptor on platelet glycoprotein IIb-IIIa which causes platelet clumping. ²

Platelet clumping also termed as pseudo-thrombocytopenia is very commonly encountered in clinical practice especially in Obstetrics & Gynaecology section. The pregnancy related thrombocytopenia should be distinguished from pseudo-thrombocytopenia. A very easy safe & reliable method is to use Citrated blood for platelet count. This study was undertaken to assess the correlation of corrected citrate utilized platelet count & manual platelet count which will have a great diagnostic implication.

2. Methodology & Results

This study includes a total of 100 cases of EDTA-PTCP.

The criteria for case selection:

- Clinically suspected Pseudo-thrombocytopenia
- when flagged as suspicious for platelet aggregates by Autoanalyzers

For such cases slides were stained with Giemsa stain & reviewed for platelet aggregates or clumping. The platelet clumps are studied for their number and size in detail. The blood is collected in Citrate-Anticoagulated tube and immediately run in Abotts Cell Dyn Ruby. Precaution should be taken that there should be no time lapse between blood collection & blood testing in Ruby. The sensitivity & p value is calculated to test the correlation of corrected platelet count in Citrated plasma and by manual platelet count.

The manual platelet count is done by using 1% ammonium oxalate as diluent (for lysing RBC) with 1:100 Dilution. 20 microlitre of blood is taken in a pipette & 1.98 ml of diluent is added. Platelets are counted in Neubauer Hemacytometer. This manual platelet count is cross checked with the platelet count obtained by Giemsa stained smears.

Formula for Corrected PLT Count Obtained By Citrated Blood

$$\text{In Cell Dyn Ruby} = (N \times 1.1) \times 10^3/\text{mm}$$

N = Citrated blood platelet count

3. Results

A Total of 100 CASES of EDTA induced Pseudothrombocytopenia as corrected by Citrate anticoagulant is studied

Table: Comparison of platelet count in EDTA & Citrate as Anticoagulant

ANTICOAGULANT	EDTA – PTCP (PLT Count by Abotts Cell DYN Ruby)	CITRATE (PLT Count by Abotts Cell DYN Ruby)	MANUAL PLT COUNT	P
PLATELET COUNT = (mean ± standard deviation)	$(55 \pm 25.9) \times 10^3/\text{mm}$	$(198 \pm 55.6) \times 10^3/\text{mm}$	$(222 \pm 58.7) \times 10^3/\text{mm}$	< 0.05
PLT COUNT RANGE	$(29.1 – 80.9) \times 10^3/\text{mm}$	$(142.4 – 253.6) \times 10^3/\text{mm}$	$(163.3 – 280.7) \times 10^3/\text{mm}$	

Corrected PLT Count Obtained By Citrated Blood
 IN CELL DYN RUBY = (198 X 1.1) = Average PLT
 Count = 217.8x10³/mm

Corrected Standard Deviation (SD) = 55.6 x 1.1 = 61.16x10³/mm
Range of PLT Count = (156.64 – 278.96) x10³/mm
Manual platelet count = 222± 58.7 x10³/mm and the range = (163.3 – 280.7) x10³/mm
P value < 0.05

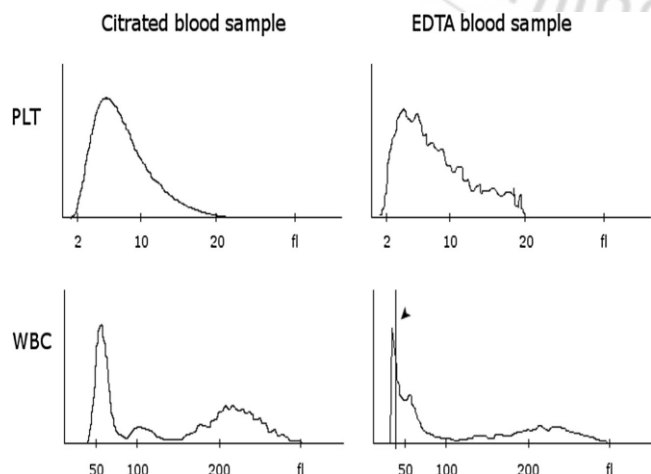
4. Discussion

EDTA-dependent pseudothrombocytopenia (EDTA-PTCP) is a common laboratory phenomenon. Its prevalence is reported to vary between 0.1-2% among hospitalized patients and 15-17% in outpatients evaluated for isolated thrombocytopenia. ¹(EDTA-PTCP) is the result of spuriously low platelet count induced by agglutinating antibodies that recognize cytoadhesive receptors on platelet gpIIb-IIIa which causes platelet clumping. ²

International Council for Standardization in Haematology (1993) ³ & the NCCLS has recommended EDTA (salts) as the anticoagulant of choice for CBC, principally for its cell preservation properties. EDTA chelates divalent cations including calcium, thus avoiding coagulation and stabilizing the sample for subsequent haematological analysis (Banfi et al, 2007). ⁴

Visual evaluation of blood smears is regarded as gold standard for detection of EDTA-PTCP, but only a limited amount of smears will be performed in routine laboratories.

A simpler approach for detection of EDTA-PTCP is to inspect the histograms and flags of hematology analyzers & run the blood sample in hematology analyzers with Citrate as anticoagulant. ⁵ PTCP is a time- and temperature-dependent phenomenon. Therefore with citrate direct sampling and immediate analysis results in higher platelet counts as compared to those obtained after delay. ⁵



Aggregated platelets are plotted as a serrated (“saw-teeth”) curve in the PLT histogram. ^{6,7}

In this study a total of 100 Cases of EDTA induced Pseudothrombocytopenia corrected by Citrate anticoagulant were studied

The PLT Count by Abotts Cell DYN Ruby using EDTA as anticoagulant is (55 ± 25.9) & citrate anticoagulant is (198 ± 55.6).

Corrected PLT Count Obtained by Citrated Blood
 IN CELL DYN RUBY = (198 X 1.1) = Average PLT
 Count = 217.8x10³/mm
 Corrected Standard Deviation (SD) = 55.6 x 1.1 = 61.16
 x10³/mm

Corrected platelet count = mean ± SD = (217.8 ± 61.16) x10³/mm
Range of PLT Count = (156.64 – 278.96) x10³/mm
Manual platelet count = 222± 58.7 x10³/mm and the range = (163.3 – 280.7) x10³/mm
P value < 0.05 .it is statistically significant

This results clearly shows manual platelet count very well correlated with citrated blood corrected platelet. It can be confidently concluded that EDTA induced pseudothrombocytopenia can be effectively corrected by citrate anticoagulated blood by multiplying with diluent factor 1.1

Platelet aggregates were present in EDTA samples and their number and size were inversely related to the fall in platelet count. The most pronounced decrease in platelet count was associated with the presence of few and large aggregates composed of 10 to 20 platelets. These facts very well correlated with other studies. ^{8,9}

Possible Causes of Platelet Clumping

- 1) The most common cause of platelet clumping in an EDTA anticoagulated specimen is improper mixing of the tube.
- 2) Improper collection of the blood sample may cause thrombin release and a falsely low platelet count due to platelet aggregation. This may be due to an excessively traumatic venipuncture or inadequate anticoagulation.
- 3) Blood collection tubes should be filled to their stated draw volume. Overfilling an EDTA tube can result in an improper blood to additive ratio. Insufficient EDTA in the sample may contribute to platelet clumping and/or clotting of the blood.
- 4) Another cause of platelet clumping is EDTA-induced pseudothrombocytopenia. This phenomenon is believed to be caused by EDTA-dependant platelet agglutinins or antibodies present in the plasma. The clumping occurs due to an alteration of the platelet surface glycoproteins when they are incubated with a calcium chelator such as EDTA. These modified platelet antigens then react to anti-platelet autoantibodies (immunoglobulins of both IgG and IgM types to form the large agglutinates.
- 5) EDTA-induced pseudothrombocytopenia can be exhibited in normal, healthy individuals and those with diseases such as human immunodeficiency virus (HIV), rubella, cytomegalovirus (CMV), autoimmune disorders, thrombotic disorders and infectious mononucleosis. In infectious mononucleosis, the patient may have increased

levels of cold agglutinins, which may contribute to platelet agglutination^{10, 11,12}

Managing Platelet Clumping in the Lab

When a platelet, white cell and/or red cell count is flagged on an electronic counter, there are several steps that can be taken in order to get an accurate platelet count.

It is recommended that the EDTA tube be inverted eight to 10 times immediately after the specimen is collected. Tube with EDTA should be inverted 10 times after collection, and mixed 10 times prior to testing.

Examine a stained blood smear microscopically to verify the platelet clumping.

Re-collect the patient's blood into a sodium citrate tube. Platelets will generally not exhibit clumping in sodium citrate. The sample in the citrate tube should be run immediately on the instrument, & then multiply the platelet count by 1.1 to get the correct value.^{11,12}

If the patient has infectious mononucleosis, or a cold agglutinin is suspected as the reason for platelet clumping, collect the blood into an EDTA tube that has been pre-warmed to 37°C in a water bath.¹³

The sample can be vortexed, which will cause the platelet clumps to break apart and they can then be counted more accurately. However, be aware that too vigorous mixing can, by itself, cause platelet activation.

Aminoglycosides (e.g. kanamycin) have been reported to be effective in dissociating platelet clumps in cases of EDTA-induced pseudothrombocytopenia.¹⁴

Once laboratory personnel understand the reasons and possible causes for platelet clumping in an EDTA-anticoagulated blood specimen, it can be much easier to deal with clumped platelets when they occur, and in many circumstances, prevent them from occurring in the first place.^{11,12,15,16}

5. Conclusion

A simpler approach for detection of EDTA-PTCP is to inspect the histograms and flags of hematology analyzers & run the blood sample in hematology analyzers with Citrate as anticoagulant. PTCP is a time- and temperature-dependent phenomenon. Therefore with citrate direct sampling and immediate analysis, results in higher platelet counts as compared to those obtained after delay. The results of this study clearly shows manual platelet count very well correlated with citrated blood corrected platelet

It can be confidently concluded that EDTA induced pseudothrombocytopenia can be effectively corrected by citrate anticoagulated blood

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