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Original Article

Study of Ethylenediaminetetraacetic acid (EDTA) - Dependent Pseudothrombocytopenia

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Ethylenediaminetetraacetic acid (EDTA);
CPT (trisodium citrate, pyridoxal 5'-phosphate and Tris);
Pseudothrombocytopenia (PTCP);
Platelet count

Abstract

Objectives: To evaluate and compare the cases of EDTA-PTCP with actual platelet counts at different time intervals with new anticoagulant.

Methods: This cross–sectional study was carried out in rural tertiary centre in central India. Blood samples were collected in K3-EDTA and CPT vials separately and subjected for peripheral smear examination and manual platelet counts. Comparison of platelet counts obtained by automated cell counter at 30 minutes, 3-4 hours and at 24 hours of blood collection from both anticoagulants and with manual counts was done.

Results: The platelet counts in EDTA anticoagulated blood on suspicion of thrombocytopenia, as well as even after collection of fresh blood sample from same patient in EDTA and the counts at different time intervals showed significantly lower counts than that observed in CPT anticoagulated blood at the parallel time intervals and than that in the manual platelet counts.

Conclusions: Unrecognized PTCP may result in unnecessary laboratory testing, bone marrow aspiration and unwarranted transfusions and will prevent needless evaluations of thrombocytopenia and related therapeutic decisions.

1. Introduction

Spurious thrombocytopenia, also called Pseudo-thrombocytopenia (PTCP), results from low platelet counts due to *in vitro* platelet clumping [1-7]. Platelet clumping in PTCP results in inaccurate platelet concentration, which leads to misdiagnosis of thrombocytopenia when analyzed with hematology analyser [7-8].

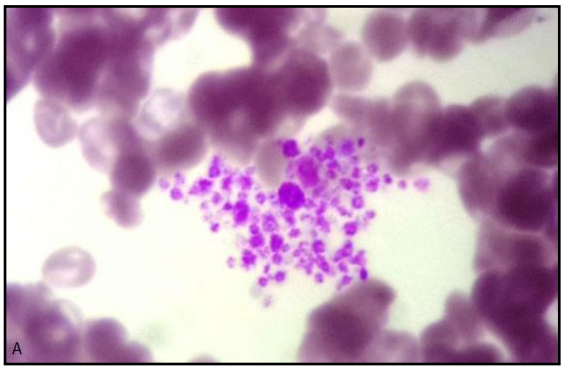
Frequency of 0.3% and 1.2% respectively was reported by Mant *et al*[9] and Manthorpe *et al*[10], which referred however to a small case series. This type of alteration is now familiar to the clinical pathologist; and consequently it has been evaluated more accurately, and at present its frequency is considered to be within range of 0.09 – 0.11% [11]. Although PTCP is an infrequent condition, it accounts for a sizable fraction of all the cases of "thrombocytopenia" that are referred to for further evaluation [12].

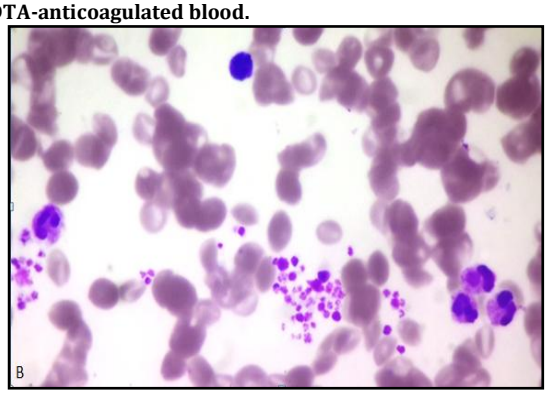
Pseudothrombocytopenia (PTCP) is an immunologically mediated phenomenon caused by the presence of EDTA – dependent cold anti-platelet auto-antibodies in blood that cause *in vitro* platelet clumping as shown in figure 1 – A, B [5,7,13-15].

In automated electronic cell counting of blood samples anticoagulated with edetic acid, PTCP was observed due to *in vitro* platelet clumping induced by edetic acid. It is caused by activation of an abnormal protein identified as an immunoglobulin/ agglutinin [5, 7, and 16]. The mechanism of EDTA induced platelet clumping may be related to the physiological function of the platelet membrane, as EDTA modifies platelet and red blood cell membranes, in presence of reduced calcium concentration [17]. The phenomenon appears to involve a protein fraction with some relation to fibrinogen, which in some cases, is an IgM or IgG antibody against platelet antigens that is maximally reactive at low calcium concentrations [12, 16, 17].

Pseudothrombocytopenia may lead to the erroneous diagnosis of thrombocytopenia, with resultant unnecessary and costly additional laboratory testing, inappropriate treatment with delay of surgery and unwarranted exposure to transfusion-related complications; all being the potential outcomes for an individual with this form of *in vitro* artefact [15,18,19].

Fig.1 (A, B): Platelet clumps in EDTA-anticoagulated blood.





2. Methods

This Cross sectional study was carried out in the Hematology division of the Department of Pathology, over a period of 2 years from May 2011 to May 2013, in a medical institute in central India.

Subjects:

Inclusion criteria:

All the cases in which the automated counter report showed thrombocytopenia with platelet counts less than 130 x 10^9/litre with peripheral blood film examination showing platelets in fair number, either diffusely distributed or in clumps or aggregates and appeared to be within normal limits; were considered as Pseudothrombocytopenia and included in the study.

Exclusion criteria:

1) The platelet counts between 130-150 x 10^9 /litre were excluded.
2) The cases with known cause for thrombocytopenia as obtained from history, clinical examination and medical records, were excluded.

Study methodology:

Blood samples were collected in K3-EDTA and CPT (trisodium citrate, pyridoxal 5'-phosphate and Tris) vials separately and examination of well prepared, air-dried, labeled peripheral smear stained with Leishman was done. Examination was done using light microscope under oil immersion (100x) with (10x objectives) for evaluation of platelet morphology, clumps, and counts. For EDTA-PTCP cases, the manual platelet count is considered 'gold standard' for this comparison as reported by Hyun-Sook Chi[15] and Lippi *et al*[17]. This method was performed using improved Neubauer's chamber. Convenient procedure is to count five groups of 16 small squares in the central area (0.02µl).

$$\text{Platelet count per litre} = \frac{\text{Number of cells counted x Dilution x } 10^6}{\text{Volume counted (µl)}}$$

To ensure a coefficient of variation of 8-10 %, the total number of platelet count should always exceed 200.

Using automated blood analyser, platelet counts were obtained at 30 minutes, 3-4 hours and at 24 hours of blood collection.

Thus, the platelet counts obtained by manual method; by automated counter at 30 minutes, 3-4 hours and at 24 hours of blood collection using two different anticoagulants were compared and these were also compared with the initial platelet counts on which pseudothrombocytopenia was suspected..

The data is recorded and findings were analyzed statistically using z-test and test statistics. The software used in the analysis is SPSS 17.0 version and graph pad prism 5.0. The p-value of less than 0.05 is considered as statistically significant.

3. Results

In the present study, we assessed the cases of suspected EDTA - dependent Pseudothrombocytopenia (showing thrombocytopenia on initial automated platelet counts from EDTA anticoagulated blood with adequate platelet count and presence of platelet clumps in the peripheral blood smear) for accurate platelet count with manual method and also using EDTA and CPT as anticoagulants with automated platelet counts at different time intervals.

Study included 43 males and 60 females with M: F ratio of 1:1.3. The patient's age varied from 3-85 years with the mean age of 36.78 ± 20.33 years. No significant association was found with respect age or distribution of cases. EDTA-PTCP cases were found to be associated both in health and disease state.

The present study compared the mean platelet counts in EDTA anticoagulated blood on initial suspicion of pseudothrombocytopenia and at different time intervals after collection of fresh blood sample from same patients in EDTA anticoagulant with parallel platelet counts in CPT anticoagulated blood and also with manual platelet counts.

The study observed the mean initial platelet counts in EDTA (103.67 ± 25.34 x 109/l) to be much lower than the mean manual platelet count (222.63 ± 85.22 x 10^9/l) and the difference was statistically significant. The mean platelet count in EDTA anticoagulated blood at 0-30 minutes and at 3-4 hours (171.40 ± 78.10 x 10^9/l) and (171.63 ± 81.16), though it was in the normal range, was also significantly lower than mean manual platelet count and the parallel automated platelet count in CPT anticoagulated blood (226.63 ± 93.25 x 10^9/l) and (230.25 ± 97.57) at 0-30 minutes and 3-4 hours respectively (Table 1).

Table 1: Showing comparison of initial platelet count suspicious of pseudothrombocytopenia with manual platelet count and platelet count in EDTA and CPT anticoagulated blood at 0-30 minutes and at 3-4 hours.

Number of cases	Mean initial platelet count (x 10^9/l)	Mean manual platelet count (x 10^9/l)	Mean automated platelet count (x 10^9/l) at 0-30 minutes		Mean automated platelet count (x 10^9/l) at 3-4 hours	
			EDTA	CPT	EDTA	CPT
103	103.67 ± 25.34	222.63 ± 85.22	171.40 ± 78.10	226.63 ± 93.25	171.63 ± 81.16	230.25 ± 97.57
Initial Platelet Count		p-value	0.000, S, p<0.05	0.000, S, p<0.05	0.000, S, p<0.05	0.000, S, p<0.05
Manual Platelet Count		p-value	0.000, S, p<0.05	0.74, NS, p>0.05	0.000, S, p<0.05	0.55 NS, p>0.05
Initial Vs Manual Platelet Count		p-value	0.000,S,p<0.05		0.000,S,p<0.05	
S: Significant; NS: Not significant						

The present study also assessed the changes in the platelet counts in anticoagulated blood samples after preserving the blood samples at 3-4°C in a refrigerator for 24 hours, in total 74 cases. The mean platelet counts in EDTA and CPT anticoagulated blood were found to be 183.70 ± 100.21 x 10^9/l and 266.04 ± 103.51 x 10^9/l respectively. In the same cases, the mean initial platelet count was 100.51 ± 26.57 x 10^9/l and the manual platelet count was 240.68 ± 89.24 x 10^9/l, as shown in table 2.

Table 2: Showing comparison of initial platelet count suspicious of pseudothrombocytopenia with manual platelet count and platelet count in EDTA and CPT anticoagulated blood at 24 hours.

Number of cases	Mean initial platelet count (x 10^9/l)	Mean manual platelet count (x 10^9/l)	Mean automated platelet count (x 10^9/l)	
			EDTA	CPT
74	100.51 ± 26.57	240.68 ± 89.24	183.70 ± 100.21	266.04 ± 103.51
Initial Platelet Count		z-value	6.90	13.32
		p-value	0.000, S, p<0.05	0.000, S, p<0.05
Manual Platelet Count		z-value	3.65	1.59
		p-value	0.000, S, p<0.05	0.11 NS, p>0.05
Initial Vs Manual Platelet Count		z-value	12.95	
		p-value	0.000, S, p<0.05	
S: Significant; NS: Not significant				

By using z-test, statistically significant difference was found between initial platelet count and platelet count in EDTA anticoagulated

blood at 24 hours (z = 6.90; p = 0.000), and between initial platelet count and platelet count in CPT anticoagulated blood at 24 hours (z = 13.32; p=0.000).

Similarly, statistically significant difference was found between manual platelet count and platelet count in EDTA anticoagulated blood at 24 hours (z= 3.65; p = 0.000); but no significant difference was found between manual platelet count and platelet count in CPT anticoagulated blood at 24 hours (z = 1.59; p=0.11) also.

Thus, the platelet counts in EDTA anticoagulated blood on suspicion of thrombocytopenia, as well as even after collection of fresh blood sample from same patient in EDTA and the counts at 0-30 minutes, 3-4 hours and after 24 hours showed significantly lower counts than that observed in CPT anticoagulated blood at the parallel time intervals and than that in the manual platelet counts. Thus, we found the difference of initial mean platelet count in EDTA with manual platelet count of 55% and the difference of mean platelet count in EDTA and CPT anticoagulated blood of about 25% with counts at 0-30 minutes and at 3-4 hours, and of about 31% at 24 hours. Thus, low platelet counts were probably because of the effect of EDTA anticoagulant on platelets.

The mean automated platelet counts in CPT anticoagulated blood at 0-30 minutes and at 3-4 hours as well as at 24 hours after blood collection were found to be comparable with the mean manual platelet counts. Thus, the CPT anticoagulant was found to be a better anticoagulant for getting correct platelet counts in cases of EDTA-dependent pseudothrombocytopenia.

The present study also compared the initial platelet count in EDTA (103.67 ± 25.34 x 10^9/l) with that at 0-30 minutes (171.40 ± 78.10 x 10^9/l) and at 3-4 hours (171.63 ± 81.16 x 10^9/l) in the same anticoagulant. The statistical analysis is shown in table 3:

Table 3: Showing comparison of initial platelet count in EDTA anticoagulated blood with that at 0-30 minutes and 3-4 hours in same anticoagulant.

Descriptive Statistics:

EDTA	No. of cases	Mean platelet count x 10^9/l	Standard Deviation	Standard Error Mean
Initial	103	103.67	25.34	2.49
0-30 min	103	171.40	78.10	7.69
3-4 hrs	103	171.63	81.16	7.99

Wilcoxon Signed Rank Test:

	Paired Differences					z	df	p-value
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
Initial & 0-30 min	67.72	80.45	7.92	52.00	83.45	8.54	102	0.000, S, p<0.05
Initial &3-4 hrs	67.95	82.375	8.11	51.85	84.05	8.37	102	0.000, S, p<0.05
0-30 min & 3-4 hrs	0.22	28.45	2.80	5.33	5.783	0.08	102	0.937, NS, p>0.05

By using Wilcoxan signed rank test, significant difference was found between initial platelet count and that at 0-30 minutes (z = 8.54; p = 0.000); and between initial platelet count and that at 3-4 hours (z = 8.37; p = 0.000); but, no statistical change was found in platelet count from 0-30 minutes to 3-4 hours (z = 0.08; p = 0.937).

Similarly, the initial platelet count in EDTA was compared with the platelet count in CPT anticoagulated blood at 0-30 minutes and at 3-4 hours. The statistical analysis is shown in table 4:

Table 4: Showing comparison of initial platelet count in EDTA with platelet counts in CPT anticoagulated blood at 0-30 minutes and at 3 - 4 hours.

Descriptive Statistics:

CPT	No. of cases	Mean platelet count x 10^9/l	Std. Deviation	Std. Error Mean
Initial	103	103.67	25.34	2.49
0-30 min	103	226.63	93.25	9.18
3-4 hrs	103	230.25	97.57	9.61

Wilcoxon Signed Rank Test:

	Paired Differences					z	df	p-value
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
Initial & 0-30 min	122.95	96.16	9.47	104.15	141.74	12.97	102	0.000, S, p<0.05
Initial & 3-4 hrs	126.57	100.48	9.90	106.93	146.21	12.78	102	0.000, S, p<0.05
0-30 min & 3-4 hrs	3.62	18.82	1.85	0.05	7.299	1.95	102	0.054, N.S, p>0.05

By using Wilcoxan signed rank test, statistically significant difference was found between initial platelet count in EDTA anticoagulated blood with platelet count in CPT anticoagulated blood at 0-30 minutes (z = 12.97; p = 0.000) and that at 3-4 hours (z = 12.78; p = 0.000); but no statistical change was found in platelet count from 0-30 minutes to 3-4 hours (z = 1.95; p = 0.054) in CPT anticoagulated blood.

4. Discussion

The present study entitled, "Ethylenediaminetetraacetic acid (EDTA) - Dependent Pseudothrombocytopenia", was carried out in the Department of Pathology in a medical institution in central India. Total of 103 cases of suspected EDTA-dependent Pseudothrombocytopenia (EDTA-PTCP) were assessed for its correctness using manual platelet count as the gold standard. The platelet counts in two different anticoagulants (EDTA and CPT) were compared at different times from the time of collection of blood sample.

Prevention of platelet aggregation in EDTA-PTCP cases using CPT anticoagulant (Citric acid tri-sodium salt dehydrate, Pyridoxal 5'-phosphate, Tris/ hydroxymethyl/ aminomethane) is opined by Lippi *et al* [17]; Lam *et al* [20]; Paparo *et al* [21] and Lippi and Faschinetti [22]. Most of the studies have tried different other anticoagulants to get correct platelet count in cases of EDTA-PTCP and have found CPT anticoagulant as a better alternative for EDTA in cases of EDTA-PTCP [15,17,20] similar to the present findings. The present study used manual platelet count to be the gold standard for platelet count in EDTA-PTCP cases [15, 17].

The study included 103 cases, of which 9 cases were found to be incorrect. Incidence of thrombocytopenia in 1.5% cases was noted and that of EDTA-dependent Pseudothrombocytopenia in 0.07% cases of total haemograms and 4.9% of total thrombocytopenia was observed. The study was comparable with incidence of 0.03-1.9% as reported by Sakurai *et al*[23].

The study compared the mean platelet count obtained in EDTA and from CPT anticoagulated blood at different time intervals with that of manual platelet count and with initial platelet count on which suspicion of PTCP was based.

The mean initial platelet counts in EDTA (103.67 ± 25.34 x 109/l) were much lower than the mean manual platelet count (222.63 ± 85.22 x 10^9/l) and the difference was statistically significant. The mean platelet count in EDTA anticoagulated blood at 0-30 minutes and at 3-4 hours (171.40 ± 78.10 x 10^9/l) and (171.63 ± 81.16 x 10^9/l) respectively,

though it was in the normal range, was also significantly lower than mean manual platelet count and the parallel automated platelet count in CPT anticoagulated blood (226.63 ± 93.25 x 10^9/l) and (230.25 ± 97.57 x 10^9/l) at 0-30 minutes and at 3-4 hours respectively (Table 1).

In 74 cases, the platelet counts were compared after 24 hours of preservation of blood sample. Even at this time, the mean platelet count in EDTA anticoagulated blood was significantly lower than that in CPT anticoagulated blood (Table 2).The present study observed the difference of mean initial platelet count in EDTA with manual platelet count of 55% and the difference of mean platelet count in EDTA and CPT anticoagulated blood of about 25% at 0-30 minutes and at 3-4 hours, and of 31% at 24 hours.

None of the studies reviewed have shown the comparison of the mean platelet counts in EDTA with manual platelet counts and with that in other anticoagulants, except for the study of Lippi *et al*[17] where they compared the automated platelet counts in EDTA and CPT anticoagulated blood in 4 different make cell counters with the mean manual platelet counts. The mean automated platelet counts in STKS coulter counter in EDTA and CPT anticoagulated blood were found to be 101.75 ± 49.22 x 10^9/l and 256.25 ± 80.08 x 10^9/l respectively, whereas, the mean manual platelet count was 255.31 ± 82.82 x 10^9/l. These findings well correlate with that of the present study.

Different workers explained this phenomenon in different ways. The basic fact is there is *in vitro* binding of antibodies in the blood with some antigenic determinant on the platelet membrane in presence of EDTA which results in formation of platelet aggregates. The size of these platelet aggregates are beyond the upper limit of discrimination of platelet width of the automated cell counters and hence they are counted in the WBC channel and omitted from the platelet channel resulting in low platelet counts shown by automated blood cell counters.

The target antigen on platelet is a cryptic epitope that is normally hidden in platelet membrane glycoprotein; the glycoprotein being GP IIb/IIIa[24]. Although EDTA-PTCP have been reported in variety of diseases (autoimmune, neoplastic, liver, cardiovascular, viral etc.), a documented trigger for the production of antiplatelet antibodies is unknown[25]. Lelie *et al*[26] showed that the binding of antiplatelet antibodies detected in patients with septicemia and normal platelets is completely or partially EDTA-dependent. They suggested, the damaged platelets in patients with septicemia could expose cryptic antigens and induce the synthesis of antiplatelet antibodies.

The antibodies are autoantibodies of all the major classes. But, IgG antibodies are much more frequently involved than IgM antibodies, and IgA antibodies are rarely involved [14, 27]. These autoantibodies are naturally occurring antibodies with antiplatelet activity, devoid of pathologic significance and are capable of recognizing cryptic antigens expressed by aged or damaged platelets to remove these from circulation[25].

Role of EDTA: The chelating effect of EDTA is in some way responsible for agglutination of platelets. The GP IIb/IIIa glycoprotein complex in platelet membrane requires the presence of calcium ions to maintain its heterodimeric structure. EDTA because of its chelating effect can dissociate GP II b/IIIa complex, resulting in exposure of the target epitopes on GP IIb [15, 28].This alteration in confirmation of GP IIb/IIIa is also associated with temperature[28].

However, EDTA is the most commonly used anticoagulant which prevents aggregation of cells and therefore used for blood cell counts. It does not cause platelet clumping in all the cases, but only in cases of EDTA-PTCP. This is probably related to the concentration of EDTA and represents the characteristic inhibitory effect of EDTA on platelet stickiness at higher concentrations of EDTA [1].

The pseudothrombocytopenia can also be because of technique related variables. Platelet clumping may be the result of poor mixing – too little and/or too late mixing, and/or a small, whole blood clot or small fibrin clots in an EDTA anticoagulated specimen. The improper collection of blood sample may cause thrombin release and a falsely low platelet count due to aggregation [29].

In the present study, the mean automated platelet counts in CPT anticoagulated blood at 0-30 minutes and at 3-4 hours as well as at 24 hours after blood collection were found to be comparable with the mean manual platelet counts (Table: 1, 2). Thus, the CPT anticoagulant was found to be a better anticoagulant for getting correct platelet counts in cases of EDTA- dependent pseudothrombocytopenia. These findings are consistent with that reported by Lippi *et al* [17].

Tri-sodium citrate do not alter cell counting and sizing after sampling in CPT mixture. Pyridoxal 5'- phosphate prevents platelet aggregation as it exhibits remarkable anti-aggregant and dis-aggregant effect *in vitro*. The pH is brought to neutrality by adding Tris to the CPT mixture.

Thus, inhibition of both platelet reaction and aggregation is prevented in CPT anticoagulant [17, 20]. Therefore, in routine hematological practice, CPT can be an alternative anticoagulant to K3.EDTA, most suitable for automated complete blood count and useful in avoiding EDTA-induced platelet clumping.

PTCP is time – dependent phenomenon, gradually developing in 0-2 hours of venepuncture [30-31]. Platelet agglutination is detectable within minutes and maximum after 60-90 min. The magnitude of agglutination and the rate at which the clumping proceeded were strongly affected by the platelet concentration in the mixture. In most, the agglutination persisted without disaggregation for more than 24 hours [30]. The size of the aggregates approximates to that of the lymphocytes; often giving rise to suspect flag "platelet clumping" and /or flagging of the platelet parameters [31].

In the present study, comparison of platelet count in EDTA anticoagulated blood at different time intervals showed significant difference between initial platelet count and that at 0-30 minutes and between initial platelet count and that at 3-4 hours. But, no statistical change was found in platelet count from 0-30 minutes to 3-4 hours in EDTA anticoagulated blood (Table 3).

Similarly, the comparison of initial platelet count in EDTA with platelet counts in CPT anticoagulated blood at different time intervals showed significant difference between initial platelet count in EDTA anticoagulated blood with platelet count in CPT anticoagulated blood at 0-30 minutes and that at 3-4 hours. But no statistical change was found in platelet count from 0-30 minutes to 3-4 hours in CPT anticoagulated blood (Table 4).

The lower mean platelet count in initial EDTA blood sample on which EDTA-PTCP was suspected is probably because these samples were different and collected by the clinical residents and at different time. The samples which we personally collected and used for cell counts at 0-30 minutes and 3-4 hours, did not show significant difference of cell counts with lapse of time over upto 24 hours. However, the counts in EDTA anticoagulated blood were still significantly lower than that in manual counts and with CPT anticoagulated blood.

Most of the studies have tried different other anticoagulants to get correct platelet count in cases of EDTA-PTCP and have found CPT anticoagulant as a better alternative for EDTA in cases of EDTA-PTCP [15, 17,20] similar to the present findings.

Pseudothrombocytopenia can complicate an accurate determination of platelet count even with an underlying thrombocytopenic disorder. Therefore, the presence of apparently obvious cause of thrombocytopenia should not be considered to rule out the diagnosis of EDTA-PTCP, which is confirmed by identifying the platelet clumping in EDTA anti-coagulated blood[6]. It is thus important to be able to distinguish between reduced platelet counts due to technique related variables or due to patient's related medical condition [29].

5: Conclusions

Examination of well drawned peripheral blood smear for every case of thrombocytopenia is mandatory to rule out platelet clumping (PTCP). The new CPT mixture is an effective anticoagulant suitable for routine haematology and can be used as better alternative to EDTA in EDTA-PTCP cases. To get correct platelet count in these cases, the manual platelet count is the 'gold standard'. Unrecognized PTCP may result in unnecessary laboratory testing, bone marrow aspirations and unwarranted transfusions and will prevent needless evaluations of thrombocytopenia and related therapeutic decisions.

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References

[1] Shreiner DP, Bell WR. Pseudothrombocytopenia manifestation of a new type of platelet agglutinin. *Blood* 1973; 42: 541-549.

[2] Wilkes NJ, Smith NA, Mallet SV. Anticoagulant- induced pseudothrombocytopenia in a patient presenting for coronary artery bypass grafting. *Br J Anaesth*. 2000 May;84(5):640-2.

[3] Mori M, Kudo H, Yoshitake S, Ito K, Shinguu C, Noguchi T. Transient EDTA-dependent pseudothrombocytopenia in a patient with sepsis. *Intensive Care Med*. 2000 Feb;26(2):218-20.

[4] van der Meer W, Allebes W, Simon A, van Berkel Y, de Keijzer MH. Pseudothrombocytopenia: a report of a new method to count platelets in a patient with EDTA- and temperature-independent antibodies of the IgM type. *Eur J Haematol*. 2002 Oct;69(4):243-7.

[5] Takeshi Yoshikawa KN, Tsutomu Maruta, Daisuke Takenaka, Shozo Hirota, Shinichi Matsumoto, Katsuyasu Saigo, Yoshiharu Ohno, Masahiko Fujji and Kazuro Sugimura. Anticoagulant induced pseudothrombocytopenia occuring after transcatheter arterial embolization for hepatocellular carcinoma. *Jpn J Clin Oncol*. 2006;36(8):527-31.

[6] Marshal A.Lichtman, Ernest Buetlar, Uri Seligsohn, Kenneth Kaushansky, Thomas O. Kipps: Hemostasis and Thrombosis-Thrombocytopenia, Williams Hematology, Seventh Edition, McGraw Hill Publication New York 2007; pp 2.

[7] Wu Wei, GUO Ye, ZHANG Lin, Cui Wei, Li Wei and Zhang Shuo. Clinical utility of automated platelet clump count in the screening for ethylene diamine tetraacetic acid-dependent pseudothrombocytopenia. *Chinese Medical Journal* 2011; 124(20) 3353-3357.

[8] Gerome P, Cardon N, Crevon L. A laboratory artifact to detect: the EDTA-dependent pseudothrombocytopenia. *Ann Biol Clin* 2003; 61:88-93.

[9] Mant MJ, Doery JC, Gauldie J, Sims H. Pseudothrombocytopenia due to platelet aggregation and degranulation in blood collected in EDTA. *Scand J Haematol.* October 1975; 15(3):161-70.

[10] Manthorpe R, Kofod B, Wiik A, Saxtruo O, Svehag SE. Pseudothrombocytopenia. *In vitro* studies on the underlying mechanism. *Scand J Haematol*. May 1981; 26(5):385-92.

[11] Neville Berkman, Yossef Michaeli, Reuven Or, and Amiram Eldor: EDTA-Dependent Pseudothrombocytopenia: A Clinical Study of 18 Patients and a Review of the Literature. *American Journal of Hematology* 1991; 36:195-201.

[12] David C.Sane, Lakshmi V. Damaraju, Eric J. Topol, Catherine F. Cabot, Mary Ann Mascelli, Robert A. Harrington, Maarten L. Simoons, Robert M. Califf.Occurence and clinical significance of pseudothrombocytopenia during abciximab therapy. *Journal of American College of Cardioloy* July 2000; 36(1): 75-83.

[13] JG Pegels, EC Bruynes, CP Engelfriet and AE von dem Borne. Pseudothrombocytopenia: An immunologic study on platelet antibodies dependent on ethylene diamine tetra-acetate. *Blood* January 1982; Vol. 59, No.1:157-161.

[14] Nicola Bizzaro: EDTA Dependent Pseudothrombocytopenia: A Clinical and Epidemiological Study of 112 Cases, With 10-Year Follow up. *Americal Journal of Hematology* 1995; 50:103-109.

[15] Hyun-Sook Chi: EDTA – dependent Pseudothrombocytopenia: Asia Pacific Preanalytical Notes 2010; 13(1):5-6.

[16] Ohnuma O, Shirata Y, Miyazawa K. Use of theophylline in the investigation of pseudothrombocytopenia induced by edetic acid (EDTA-2K). *Journal of clinical pathology*. 1988 Aug;41(8):915-7.

[17] Lippi U, Schinella M, Nicoli M, Modena N, Lippi G: EDTA-induced platelet aggregation can be avoided by a new anticoagulant also suitable for automated complete blood count. *Haematologica* 1990; 75(1):38-41.

[18] Labrini C. Dalamangus and Thomas F. Slaughter: Ethylenediaminetetraacetic Acid-Dependent Pseudothrombocytopenia in a Cardiac Surgical Patient. *Anesthesia and Analgesia* 1998; 86:1210-11.

[19] Asim Momami, Rame Khasawneh, Ruba Abed. Anticoagulant – Induced Pseudo-thrombocytopenia: A case report. *JRMS* September 2012; 19(3):73-75.

[20] Lam SC, Harfenist EJ, Packham MA, Mustard JF. Investigation of possible mechanisms of pyridoxal 5'-phosphate inhibition of platelet reactions. *Thromb Res*. 1980 Dec 1-15;20(5-6):633-45.

[21] Paparo C TE, Meda R, Scott CS, Navaro O. Improved stability of leukocytes in aged samples: Investigation of an alternative anticoagulant strategy in hematology for use with the Abbott CELL-DYN CD 3500 hematology analyzer. *Eur J Lab Med*. 1998;6(16).

[22] G. Lippi, R. Facchinetti. Accurate platelet count avoiding platelet clumping. *Lab Hematol*. 1998;4:225-6.

[23] Sakurai S, Shiojima I, Tanigawa T, Nakahara K. Aminoglycosides prevent and dissociate the aggregation of platelets in patients with EDTA-dependent pseudothrombocytopenia. *Br J Haematol*. 1997 Dec;99(4):817-23.

[24] Chun-Hsien Tu and Stone Yang: Olanzapine-Induced EDTA – Dependent Pseudothrombocytopenia. *Psychosomatics* 2002; 43:421-423.

[25] Mori M, Kudo H, Yoshitake S, Ito K, Shinguu C, Noguchi T. Transient EDTA-dependent pseudothrombocytopenia in a patient with sepsis. *Intensive Care Med.* 2000 Feb;26(2):218-20.

[26] Van Der Lelie J, Lange JM, Vos JJ, Van Dalen CM, Danner SA, Von Dem Borne AE. Autoimmunity against blood cells in human immunodeficiency-virus (HIV) infection. *Br J Haematol*. 1987 Sep;67(1):109-14.

[27] Von Dem Borne AE, Van Der Lelie H, Vos JJ, Van Der Plas-Van Dalen CM, Risseeuw-Bogaert NJ, Ticheler *et al*. Antibodies against cryptantigens of platelets. Characterization and significance for the serologist. *Curr Stud Hematol Blood Transfus*. 1986; (52): 33-46.

[28] Pidard D, Didry D, Kunicki TJ and Nurden AT: Temperature – dependent effects of EDTA on the membrane glycoprotein IIb-IIIa complex and platelet aggregability. *Blood* 1986; 67 (3):604-611.

[29] Narayan Patel. Tech Talk. January 2009; Volume 1, No.1.

[30] O Onder, A Weinstein and LW Hoyer: Pseudothrombocytopenia caused by platelet agglutinins that are reactive in blood anticoagulated with chelating agents. *Blood* 1980; 56: 177-182.

[31] Arnold J.P.F. Lombarts, Jelle J. Zijlstra, Richard H.M.Peters, Cees G. Thomasson and Paul F.H.Franck: Accurate Platelet Counting in an Insidious Case of Pseudothrombocytopenia. *Clin Chem Lab Med* 1999; 37(11/12):1063-1066.