Research article

Frequency of Causes of Spurious Platelets Count on Routine Complete Blood Count by an Automated Hematology Cell Analyser

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Abstract: The present study was aimed to find out causes of platelet count flagging marked on Complete Blood Count strip. This Cross-sectional study was conducted at Hematology Department, Children Hospital and ICH, Lahore. A total of 350 blood samples were processed for the study and among them a total of 86 samples of blood in EDTA vial were studied with specific tag on platelet count on CBC strip. A specific designed Performa was used for data collection. All the collected data was entered to SPSS 22 (statistical package for social sciences) for analysis. Out of 86 samples 64 samples (74.4%) showed spurious platelet count due to EDTA. Pseudo thrombocytopenia was observed in 9 samples (10.5%). Abnormal CBC 4.7 %, overfilling of sample 4.7%, acute burn anemia with frequency of 3.5%, iron deficiency anemia and clotted samples have 2.3% frequency separately. While 3.5% samples were included in miscellaneous list. We concluded that EDTA dependent pseudo-thrombocytopenia is the most prevalent. Other technical errors (pre-analytical, analytical) can be reduced by proper training and education of laboratory staff. It is very important to rule out the correct cause of spurious platelet count on CBC as otherwise it will lead to unnecessary medication and investigations for patient.

Keywords: Pseudo-thrombocytopenia, EDTA, frequency, spurious platelet count.

Introduction

Automated hematology analyzers are widely used in clinical practice and research settings to characterize blood cells for disease detection and monitoring of blood cell counts. Sophisticated analyzers

can measure cell mor- phology and can also detect small cell populations to diagnose rare blood conditions1. Its widespread use plays a major role in improvement of cellular hematology as it gives quick and accurate results2. When platelet count on the CBC strip goes out of range, the automated analyzer have ability to tag them on the strip. This tagging can be of any type according to the software of automated hematology cell analyzer2,3. Automated cell analyzer may tag them with a flag, a dot or mark it on histogram etc.

All the hematology instruments use software generated flagging to identify abnormal platelet distributions that may indicate presence of platelet specific interference or the presence of platelet clumping. The reporting hematologist must review the blood smear before sending out the complete blood cell count reports when the flag of platelet clumping occurred. Hematologist have to observe the platelet in direct blood smear for approximate quantity and shape or detected by hematology analyzer. In both systems, the collected blood is diluted and counted by passing blood through an electric counter4.

Platelet tagging can be due to some pathological reasons but mostly platelet tags are due to spurious count of platelets which lead to pseudo thrombocytopenia or pseudo thrombocytosis. In these cases, following are the main reasons (5-11)

1. Platelet clumping due to EDTA anticoagulant.
2. Adherence of platelet to mature polymorphonuclear neutrophils
3. Clotting within the sample.
4. Fragmented nucleated cells.
5. Lipids (sample taken after meals, lipid drips)
6. Micro-organisms

Pseudo thrombocytopenia and pseudo thrombocytosis are very common in routine complete blood counts, which can have deleterious effects on patients, resulting in getting even platelet transfusion if it is not clearly checked. To analyze the cause of platelet flagging is very vital in dealing with automated hematology analyzer. It is an important aspect of quality assurance related to complete blood count automated analyzer. The objective of this study was to find out the causes of platelet count flagging marked on Complete Blood Count strip.

Materials and Methods

It is a cross-sectional study conducted in Hematology & Transfusion medicine Department, The chil- dren's hospital & ICH Lahore from July 2018 to Jan 2019. Data was collected with the help of designed proforma. A sample size of 350 blood samples was collected. Sample was collected by aseptic technique. 5 cc of blood was drawn in sterile syringe and promptly poured in EDTA containing anticoagulant vial. This anticoagulated blood was received in hematology

department and processed within 2-3 hours. Those samples giving flag on platelet counts were further investigated by manual checking of samples and peripheral films to find out causes of platelet flagging. Samples were processed first by gently mixing of the vial, and then the blood was brought near the sucking tip of the Hematology analyzer Celltac MEK-6450. The machine processes, counts the cells according to their size and granular morphology. Data was presented in tables, graphs or pie diagrams and analyzed by using Statistical Package for the Social Sciences (SPSS) software computer program version 20.

Results

Out of total 350 samples that were analyzed, 86 samples showed platelet flags. All the 86 samples were considered for analysis making the response rate 100%. Regarding the type of tag on CBC strip and the platelet count the frequency of "*" (Flag) type tag is 82 (95.3%) (Table 1). The most comment platelet count range was 150-400 × 10^9/L (Table 2, Figure 1). The Distribution of causes of Spurious Platelet counts are shown in Figure 2. EDTA was the cause in 74.4% samples. EDTA was the most common and highest causes of platelet count tag on CBC in range of 150-400x10^9. When platelet count is <150x 10^9 then again EDTA the common cause. When platelet count is >400 x 10^9 i.e., the Upper limit PU, the most common cause of spurious count is multiple transfusions (Figure 3). The most common platelet appearance on the slide was platelet clumps (39, 45.3%) followed by Platelet satellitism around WBC (9, 10.5%) (Table 3).

Table 1. Type of tag on complete blood count strip

Tag type	Frequency	Percent
*	82	95.3
PU*	2	2.3
AG*	2	2.3
Total	86	100.0

*Flag; PU=Platelet Upper Limit; AG= Aggregates

Table 2. Platelet count of the samples enrolled in the study

	Frequency	Percent
150-400 × 10^9	66	76.7
<150 × 10^9	8	9.3
>400 × 10^9	12	14.0
Total	86	100.0

Table 3. Platelet appearance on Slide and their frequency and percentages

Platelet Appearance	Frequency	Percent
Platelet Clumps	39	45.3
Platelet satellitism around WBC	9	10.5
Platelet neutrophil clumps	7	8.1
Coagulation within sample	6	7.0
RBC fragments	5	5.8
Platelet Aggregates	4	4.7
Anistocytosis	4	4.7
Normal platelets	4	4.7
Small aggregates	3	3.5
Miscellaneous	3	3.5
Large platelets	2	2.3
Total	86	100

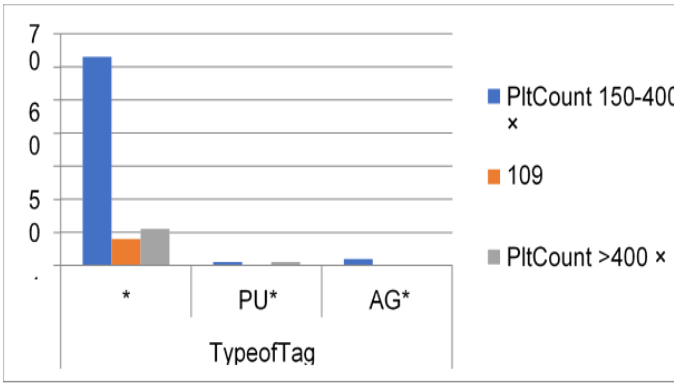

Figure 1. Comparing platelet count and type of tag

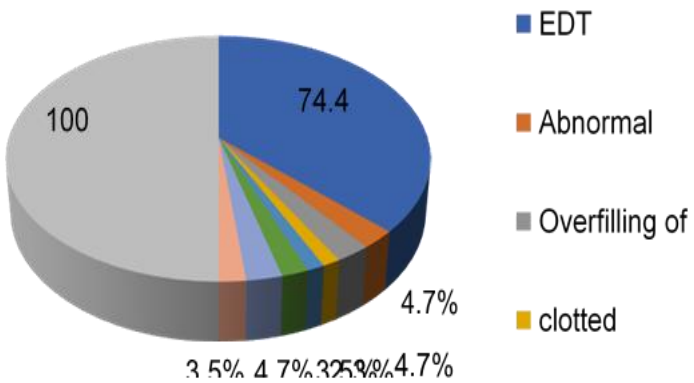

Figure 2. Distribution of causes of spurious platelet

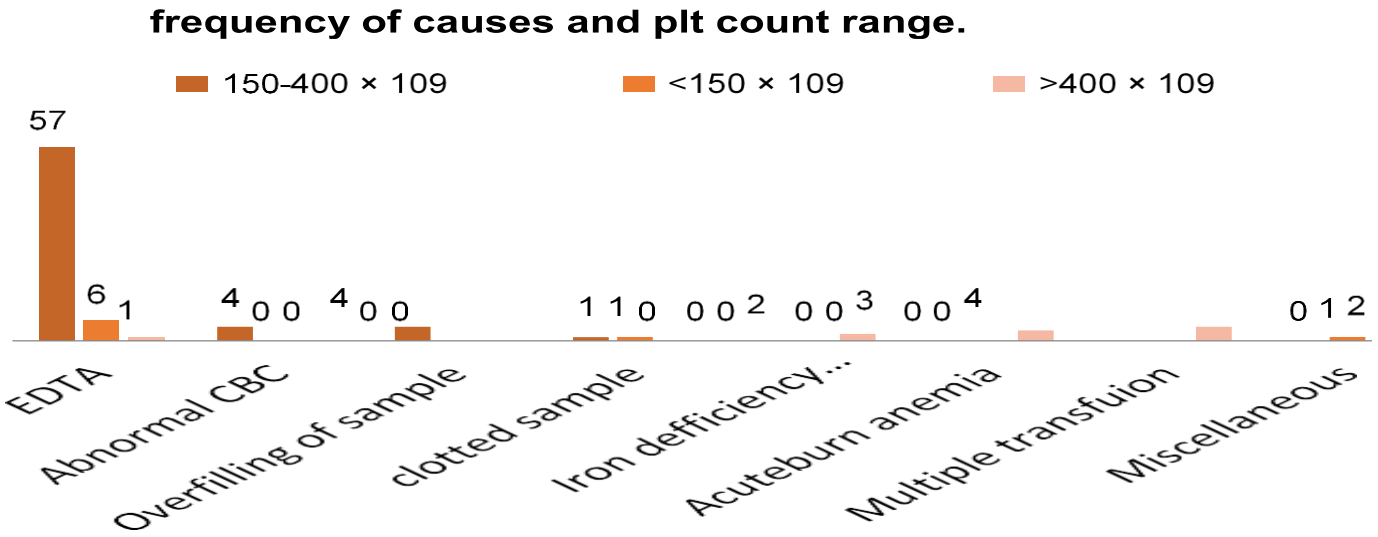

Figure 3. Comparing Platelet Counts and Causes of Platelet Flags

DISCUSSION:

Automated hematology analyzers have great contribution in hematology department as they can cope up with high workload. However Pseudo-thrombocytopenia remains a challenge in hematology laboratory. The pre-analytical problem that platelets tend to easily aggregate in vitro, giving rise to lower platelet counts, has been known since EDTA and automated platelet counting procedures were introduced in the hematology laboratory. The present study was aimed to know the frequency of causes of spurious platelet counts on CBC by automated hematology cell analyzer. Out of 86 samples 82 (96%) were tag by "*" 2 (2%) with "PU*" and the remaining 2 (2%) with "AG*". Samples were divided into three categories on the basis of platelet count range. Firstly 150-400* 109 i.e., normal range, secondly platelet count<150 * 109 (below lower limit) and thirdly platelet count >400 * 109 (above upper limit).

Eighty-six samples were considered in the current study. Different causes of spurious platelet counts and these causes were based upon the platelet appearance on smear slide as mentioned in table 3, the most common cause with highest percentage was EDTA. Out of 86 samples 64 samples (74.4%) were giving spurious platelet count due to EDTA. EDTA is the highest frequency cause of spurious platelet counts on CBC. As regard with the previous studies EDTA dependent pseudo thrombocytopenia is a common laboratory phenomenon with most prevalence rate (25%) according to a study conducted by peter Keller in 2014. In some situations, platelet satellitism around WBC was also the cause of pseudo thrombocytopenia. In the current study this cause was observed in 9 samples (10.5%). This observation was the same as presented by Dr. Nicola bizzaroet al., (1991) which showed platelet satellitisms to polymorphonuclears. Seven samples gave pseudo thrombocytopenia due to platelet neutrophils clumps (8%). Other causes are minor but also of great importance. As causes for spurious counts vary from sample to sample. Abnormal CBC 4.7%, overfilling of sample 4.7%, acute burn anemia with frequency of 3.5%, iron deficiency anemia and clotted samples have 2.3% frequency separately. So, with all this frequency distribution of causes, it is clear that the mostly spurious counts

of platelets is because EDTA anti-coagulant.Spurious platelet counts may be induced in other situations as well and for one mechanism it can be different, ranges between the true and spurious counts, from the patient to patient. It is also pertinent to note that the tags in the results are generated because of other unusual findings in other parameters of the CBC as well.

Peter keller et.al in 2014 presented a research report named as "A case of EDTA-dependent pseudo-thrombocytopenia: simple recognition of an underdiagnosed and misleading phenomenon" in which they stated that EDTA-dependent pseudo-thrombocytopenia (EDTA-PTCP) may be a usual laboratory finding which can range from 0.1-2% in case of hospitalized subjects. This is commonly unnoticed because the blood smear films are not usually assessed neither the histograms in the analyzers are correctly interpreted by the lab staff. Jianhua Lin et.al in 2014 also found that the incidence of EDTA – PTCP was 0.09% in the duration ranges from 02 weeks to 6 months and found that it was time dependent which can be occurred as early as 10 minutes after sample collection. Dr. Nicola Bizzaro et.al in April 1991 conducted a study on "platelet Satellitisms to polymorphonuclears, cytochemical, immunological and ultrastructural characterization of eight cases" and concluded that Satellitisms of platelets to polymorphonuclears was observed in 08 of the study subjects. They reported that this occurred only in the blood anticoagulated at room temperature with EDTA. However function of the platelets and the neutrophils remained normal both in vivo and in vitro. A report was presented in (2015) named as "EDTA dependent pseudo thrombocytopenia'' in this report it is stated that since 1973, EDTA dependent PTCP is approximately 0.1-2%in hospitalized patients. The primary antibodies involved in this mechanism are immunoglobulins IgG, IgM, IgA and combinations of them are also observed. In a study by the van der Meer and colleagues the incidence pseudo thrombocytosis was found in 43 patients out of 169 patients with acute leukemia and among them 07 were categorized as patients with major bleeding risk because of actually low platelet counts. Due to time and resource limitation, the study do not elaborate the procedures to reduce these spurious results. As this study is conducted on limited sample size. It should be conducted on vast level and solutions to minimize these spurious results should also be studied deeply for the betterment and reliability of CBC results by automated hematology analyzer.

Conclusion

The present study concluded that EDTA dependent pseudo thrombocytopenia is the most prevalent. Other technical errors (pre-analytical, analytical) can be reduced by proper training and education of laboratory staff. It is important to rule out the correct cause of spurious platelet count on CBC. Otherwise, it will lead to unnecessary medication and procedures for patient's treatment.

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