THE INCIDENCE OF PSEUDO THROMBOCYTOPENIA IN SAMPLES ANALYSED BY AUTOMATED ANALYSERS AND ROLE OF MICROSCOPIC EXAMINATION OF PERIPHERAL SMEAR.

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Abstract:

Introduction: Complete blood count (CBC) is a blood test which is commonly requested by clinicians in a haematology laboratory. It is used to measure various components of blood, out of which the most frequently asked isolated parameter is Platelet count. The platelet count is evaluated by the automated analysers in the laboratory, which shows histograms and warning flags about the platelet's interpretation. But the validity of the automated analyser results are sometimes not reliable. Pseudo-thrombocytopenia (PTCP) is an in-vitro phenomenon of false low platelet count, given by automated analyser. PTCP if not detected leads to unnecessary diagnostic tests and mismanagement occurs. Hence study is done to stress the role of visual inspection of all the blood smears with thrombocytopenia before alerting the clinician about it.

Objectives: To evaluate the percentage of pseudothrombocytopenia cases in the total thrombocytopenia cases and to evaluate the role of peripheral smear examination as confirmatory test for platelet count in cases of thrombocytopenia.

Methodology: Platelet count of EDTA samples determined by the automated haematology analyser Sysmex XN – 550 showing values less than one lakh were collected. Leishman stained peripheral smear were visually inspected for any presence of platelet clumps, giant platelets and manual count done to identify cases of pseudo-thrombocytopenia and results documented.

Results: The incidence of PTCP in our study was 18.4%. The machine did not flag all cases with platelet clumps and manual examination of smears were very helpful in identifying those cases and avoiding false report of low platelet count.

Key words: PTCP, pseudothrombocytopenia. Low platelets

INTRODUCTION:

The Complete Blood Count (CBC) is a fundamental blood test frequently requested by clinicians for evaluation within haematology laboratories. This test serves to assess various vital aspects of blood's cellular components, including red blood cells, white blood cells, and platelets. While deciphering the implications of these values may necessitate some level of expertise, accurate interpretation plays a pivotal role in enhancing patient management by healthcare professionals[1]. Among the array of parameters, the platelet count stands out as one of the most commonly isolated metrics. Its significance extends to both chronically ill patients and the prognosis assessment of those acutely unwell.

Traditionally, the assessment of platelet count involves automated analyzers in laboratory settings, presenting valuable histograms and warning indicators to aid in interpretation. Paradoxically, this technological advancement has led to a notable reduction in the volume of blood samples forwarded to hematology labs for subsequent peripheral smear examinations[2]. However, this convenience does not come without its drawbacks. The consistency and reliability of automated analyzer results are occasionally questionable, potentially contributing to the misdiagnosis of cases with low platelet counts. Unfortunately, the manual method of platelet count correction, a practice involving blood samples and human intervention, is not universally applied due to logistical constraints in many medical centers.

Pseudo-thrombocytopenia (PTCP) emerges as a noteworthy in-vitro phenomenon wherein automated analyzers generate false low platelet counts. The oversight of PTCP's occurrence may inadvertently lead to unwarranted diagnostic procedures and suboptimal patient management. Instances within the medical literature emphasize how PTCP-induced inaccuracies have misled clinicians[3,4]. This underscores the urgency for not only pathologists but also clinicians to recognize and comprehend this phenomenon. Consequently, this study aims to underscore the indispensable role of visually examining peripheral smears as a confirmatory measure to validate platelet counts reported by automated analyzers.

The primary objective of this study is twofold: firstly, to quantify the prevalence of pseudo-thrombocytopenia cases within the realm of encountered thrombocytopenia instances in our hospital, and secondly, to comprehensively assess the utility of peripheral smear examination as a pivotal confirmatory tool for platelet counts in situations involving thrombocytopenia. This investigation seeks to shed light on the significance of manual verification in an era dominated by automated technology, thereby emphasizing the importance of maintaining a balance between technological advancements and clinical acumen.

METHODOLOGY:

This study follows an observational approach and was conducted at two tertiary referral centers over a span of one year, commencing from January 2022 and concluding in February 2023. Ethical approval from the institutional review board was secured, and it was determined that explicit participant consent was unnecessary due to the study's alignment with routine laboratory protocols governing blood sample assessment. The investigation focused on the platelet count of samples preserved in ethylenediaminetetra-acetic acid (EDTA) and analyzed using the Sysmex XN-550 automated hematology analyzer.

Cases were included in the study if their platelet count was less than 100,000 per microliter, irrespective of whether the automated analyzer signaled any red flags. These selected cases underwent further scrutiny via Leishmann-stained peripheral smear examination, facilitated by a qualified pathologist, to identify the presence of platelet clumps.

In instances where platelet clumps were absent in a well-prepared smear, platelet estimation was executed by quantifying the average number of platelets visible within a 100x oil immersion field in the monolayer. Typically, ten oil immersion fields were assessed,

and the outcomes were averaged. Subsequently, the estimated platelet count per microliter was calculated using the formula: Estimated Platelet Count/µL = Average Count in 10 Fields x 15,000, as referenced by a previous study[5].

Conversely, when platelet clumps were detected, the attending clinician was duly informed, and platelet adequacy was affirmed without specifying the numerical count. Following this step, a capillary blood sample was collected to facilitate manual platelet count determination using an alternative method.

The study's participant pool encompassed both pediatric and adult populations, with blood samples procured from outpatient and inpatient settings within the hospital premises during the designated study period. Specifically, only blood samples preserved in EDTA anticoagulant were eligible for inclusion, with samples collected in alternate anticoagulants like sodium citrate and heparin, as well as cord blood samples, being excluded from the analysis. Additionally, cases exhibiting a corrected manual platelet count below 100,000 per microliter were excluded from the study cohort, as their condition persisted as thrombocytopenic despite the correction of platelet counts, rendering them unsuitable for pseudothrombocytopenia labeling.

RESULTS:

The study revealed a total of 565 instances of thrombocytopenia among the subjects. Among these, 110 patients underwent manual platelet count correction, resulting in the exclusion of 6 cases where the corrected count remained below 100,000 per microliter. Consequently, the calculated prevalence of pseudothrombocytopenia cases within our study stood at 18.4%.

The age distribution of the patients afflicted by thrombocytopenia spanned a wide spectrum, ranging from newborns to individuals as old as 93 years. Similarly, those affected by pseudothrombocytopenia exhibited an age range from newborns to individuals of 84 years. These findings emphasize the occurrence of these conditions across diverse age groups.

Upon closer examination, the data unveiled a distinct age pattern. Specifically, the age group of 21 to 40 years exhibited the highest prevalence of both thrombocytopenia and pseudothrombocytopenia, closely followed by the 41 to 60-year age bracket. This insight underscores a noteworthy trend in the manifestation of these conditions, potentially offering valuable clues for further exploration.

Table 1: Age prevalence of thrombocytopenia and pseudothrombocytopenia

Age in years	Thromocytopenia	Pseudothrombocytopenia	Percentage
0-20	142	17	11.98
21-40	158	35	22.15
41-60	139	28	20.14
>60	126	24	19.04

Among the 104 cases studied, it was observed that 49 instances of pseudo-thrombocytopenia (PTCP) affected male patients, while 55 cases were identified in female patients. Upon conducting a detailed peripheral smear examination of the PTCP cases, distinct patterns emerged.

Out of the PTCP cases, a substantial 80 cases exhibited the presence of platelet clumping within the peripheral smear. Additionally, 15 cases displayed the occurrence of giant platelets, while a further 9 cases exhibited a combination of both clumping and giant platelets in the smear.

These findings underscore the gender distribution of PTCP cases, indicating a comparable occurrence between males and females. Furthermore, the microscopic analysis of peripheral smears revealed a diverse range of anomalies within the platelet morphology, with clumping, giant platelets, and their coexistence representing distinct patterns. Such insights contribute to a deeper comprehension of the underlying mechanisms and characteristics of pseudo-thrombocytopenia, potentially informing future diagnostic and management approaches.

ANALYSIS:
The prevalence of pseudo-thrombocytopenia (PTCP) has been a subject of investigation in numerous studies conducted worldwide, revealing a fluctuating range of incidence rates. Silvestri et al. conducted a study attributing PTCP to ethylenediaminetetra-acetic acid (EDTA) anticoagulant, highlighting a decline in platelet count within EDTA-anticoagulated samples over time. Their research reported a PTCP incidence of approximately 15.3%[6]. Building on this foundation, Cohen et al. observed a higher PTCP prevalence of 17%, ranking it as the second most common cause of thrombocytopenia in their study cohort[7]. Similarly, Froom et al. explored PTCP's landscape and found a prevalence of 12.8% in their investigation[8].

A distinctive perspective on PTCP emerged from the study conducted by Choe et al. Their research exhibited a contrasting incidence pattern, particularly among patients with viral infections. The study disclosed a higher prevalence of PTCP, specifically 13.8%, within patients afflicted by viral infections compared to a lower 6.5% in patients with other non-viral ailments[9]. Remarkably, within the subset of viral-infected patients, those affected by cytomegalovirus (CMV) demonstrated a disproportionately elevated PTCP occurrence, reaching an astounding 72% prevalence within this subgroup.

Aligning with this diverse landscape of PTCP prevalence, the present study contributes to this evolving narrative. Our investigation revealed a comparable incidence of PTCP, totaling 18.4% cases within EDTA-anticoagulated samples. This finding positions our study's observations within the spectrum of PTCP prevalence reported in the existing literature, reaffirming the intricate interplay between EDTA anticoagulant, platelet counts, and the occurrence of pseudo-thrombocytopenia. These varying incidence rates across multiple studies collectively underline the significance of understanding and addressing PTCP, offering valuable insights for clinical practitioners to navigate and interpret platelet-related data accurately.

DISCUSSION:

Thrombocytopenia, characterized by a reduced platelet count, is a frequent concern in the clinical realm, impacting a diverse range of patient populations. Its prevalence is well-documented, with studies indicating approximately 15% prevalence within the general population[10] and a narrower range of 5-12% among pregnant women[11,12,13]. The causes of thrombocytopenia are multifaceted, encompassing conditions that hinder platelet production, such as drug-induced effects, infections, and nutritional deficiencies, as well as scenarios that accelerate platelet destruction, including immune thrombocytopenic purpura, systemic lupus erythematosus, and autoimmune disorders. Furthermore, thrombocytopenia can stem from splenic sequestration and dilution due to massive transfusion[14].

The clinical implications of thrombocytopenia are profound, as evidenced by its association with sepsis and critical care outcomes. In the context of sepsis, thrombocytopenia emerges as an independent predictor of poor prognosis and prolonged stays in intensive care units[15]. Notably, Vanderschueren et al. highlighted that a significant portion (41%) of non-surviving intensive care patients exhibited at least one platelet value below normal limits, correlating thrombocytopenia with increased incidence of multiple organ failure and mortality[16].

However, amidst the imperative to identify genuine cases of thrombocytopenia, the concept of pseudo-thrombocytopenia assumes significance as a potential contributor to misdiagnosis and inappropriate management. Pseudo-thrombocytopenia (PTCP) operates as an in-vitro phenomenon, inducing a false low platelet count when analyzed by automated analyzers. The repercussions of failing to detect PTCP are substantial, encompassing unwarranted diagnostic tests, misguided treatment decisions, possible surgical postponements and unnecessary platelet transfusions[17]. This accentuates the significance of recognizing and addressing PTCP not solely among pathologists but also among clinicians.

The origins of PTCP are diverse, encompassing factors such as improper blood sample collection[18], coagulant-induced effects, EDTA-independent cold agglutinins[19], medication influence[20], platelet satellitism, and the presence of giant platelets that may elude automated counting. While mitigating pre-analytical errors through technician education is a straightforward measure, a structured approach is essential to identify and rectify other contributing factors.

A deeper exploration of the pathophysiology behind coagulant-induced in-vitro platelet aggregation reveals the pivotal role of agglutinins present in patient sera, activated by coagulants. EDTA, a commonly associated anticoagulant, triggers the exposure of antigens within susceptible individuals, potentially harboring antiplatelet antibodies[21]. These antigens, identified as part of the GpIIb-IIIa complex, remain cryptic until interaction with EDTA at lower temperatures, such as 0-4^{o} C, exposes them[22]. Clinical suspicion of EDTA-associated PTCP involves several criteria, including a platelet count below 100×109/l, improved counts upon sample warming, declining counts over time, evidence of platelet clumping under microscopic analysis, and the absence of platelet disorder symptoms[23].

While PTCP may manifest in isolation, certain conditions amplify its occurrence, including viral infections, cardiovascular diseases, liver disorders, gastrectomy, autoimmune

conditions, and neoplastic diseases[24,25,26,27,]. Autoantibody formation in these contexts, cross-reacting with platelet antigens, contributes to the observed PTCP phenomenon. Additionally, platelet satellitism, characterized by platelet aggregation around leukocytes forming rosettes, further adds to the complexity. This phenomenon stems from the presence of an antibody binding both platelet glycoprotein IIb/IIIa complex and neutrophil Fc gamma receptor III[28].

Previously, anticoagulant-induced PTCP was linked to EDTA, spurring recommendations for alternative anticoagulants. However, recent studies have dispelled this notion, highlighting that while at lower rates, aggregation may still occur with alternatives like sodium citrate, sodium oxalate, and heparin[29,30].

Furthermore, the interplay between anticoagulants, processing time, and storage temperature adds another layer of complexity. The time-dependent decline in platelet count, particularly pronounced after 2 hours, raises questions about the potential for aggregation even within the initial two hours after sample withdrawal. Temperature emerges as a crucial factor, with platelet aggregation primarily observed at temperatures below 37°C and reaching its peak at 0-4°C, with IgG-type antibodies playing a prominent role[22].

CONCLUSION:

In conclusion, pseudo-thrombocytopenia (PTCP) represents a prevalent occurrence in EDTA anticoagulated samples, and the present circumstances often necessitate the use of this anticoagulant. The option of obtaining new samples using alternative anticoagulants for these patients presents challenges in terms of complexity and time consumption. While routine hematology analyzers offer a straightforward approach through analyzer flags, solely relying on these flags proves inadequate due to instances of platelet clumping going unnoticed by the machine. As a result, we emphasize that a manual assessment of platelet counts in peripheral smears should be obligatory prior to releasing platelet counts in cases of thrombocytopenia. The incremental time required for this step is modest and holds considerable value for clinicians, offering a crucial opportunity to ensure accurate diagnosis and informed treatment decisions for thrombocytopenic patients. This multifaceted strategy, combining automated analysis with visual inspection, stands as a practical and judicious means to mitigate the potential pitfalls of PTCP, ultimately enhancing the quality of patient care and medical decision-making.

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