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CASE REPORT

A Case of Pseudothrombocytopenia due to Pre-Analytical Issues Revealed by the Presence of Fibrin in a Blood Film

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SUMMARY

Background: **Pseudothrombocytopenia (PTCP) can be caused by anticoagulants or pre-analytical issues. The authors present a case of PTCP attributed to pre-analytical issues in a 68-year-old male patient.**
Methods: **The platelet count results were obtained using both the impedance and fluorescence channels of Sysmex XN-10. The blood film was scanned using both Cellavision DM96 and a microscope.**
Results: **The flag for PLT-Clumps and the scattergram from the PLT-F channel indicated the presence of platelet aggregation. Fibrin could be observed at the feathered end of the blood film. A diagnosis of PTCP resulting from pre-analytical issues was made.**
Conclusions: **The presence of fibrin in a blood film is a critical indicator for diagnosing PTCP due to pre-analytical issues.**
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KEYWORDS

pseudothrombocytopenia, pre-analytical issues, fibrin, blood film

INTRODUCTION

Pseudothrombocytopenia (PTCP), a phenomenon first reported in 1969, refers to a situation where the results of platelet counts obtained from a hematology analyzer are significantly lower than the actual values. This discrepancy arises due to platelet clumping *in vitro* [1,2]. PTCP can be caused by anticoagulants, with the most common one being EDTA. Alternatively, it may be attributed to pre-analytical issues. PTCP may mislead clinical conclusions if it is not recognized by the laboratory doctor or technician [3,4]. This phenomenon can be confirmed by examining a blood film under the microscope [5]. As a result of misdiagnosis, excessive medical treatment and unnecessary tests may be performed. Since 1969, numerous cases of PTCP caused by EDTA have been reported [6-9]. In contrast, there have been few reported cases of PTCP caused by pre-analytical issues.

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CASE PRESENTATION

A 68-year-old male patient of Han ethnicity, who underwent rectal cancer surgery three years ago, recently sought radiation therapy and chemotherapy at a general hospital's outpatient department for anastomotic recurrence that occurred two weeks ago. His hematology analysis was conducted on Sysmex XN-10 (Sysmex Corporation, Kobe, Japan) to assess whether radiotherapy and chemotherapy had induced bone marrow suppression. His blood sample was treated with EDTA as an anticoagulant. The platelet count (PC) measured by the impedance channel (PLT-I) was 63 x 10^9/L, while the PC obtained through the fluorescence channel (PLT-F) was 66 x 10^9/L. The flag for PLT-Clumps from the WNR/WDF channel was positive. The scattergram from the PLT-F channel indicated the presence of platelet aggregation (Figure 1). No macroscopic clots were observed in the anticoagulated blood sample after it was examined. In the blood film, platelet aggregation was not observed from the tail to the head area, including both side edges. Cellavision DM96 (CellaVision AB, Lund, Sweden) did not detect any evidence of platelet aggregation on all the screens. However, Fibrin with several platelets wrapped inside could be observed at the feathered end of the blood film (Figure 2).

Another blood sample from the patient was drawn into a EDTA anticoagulant tube after an hour to retest the hematology analysis. The PLT-I and PLT-F results were 151 x 10^9/L and 155 x 10^9/L, respectively, and there were no flags for PLT-clumps. No abnormalities were observed in the PLT-F scattergram and blood film. The bias in the two platelet count measurements was significantly greater than the natural physiological variation. The anticoagulant was not changed, and fibrin was observed. Therefore, a diagnosis of PTCP resulting from pre-analytical issues was made.

DISCUSSION

Radiotherapy and chemotherapy in rectal cancer patients can lead to varying degrees of bone marrow suppression, which is often manifested as thrombocytopenia [10]. Before making a diagnosis, the first step is to determine whether the patient has PTCP [11]. This approach can assist in avoiding misdiagnosis and the unnecessary use of excessive medication.

Modern instruments play a crucial role in the accurate diagnosis of PTCP, although they cannot guarantee 100% sensitivity in the identification of PTCP. Both the WNR/WDF channel and the PLT-F channel of the Sysmex XN series hematology analyzer are capable of flagging samples when platelet aggregation is suspected. Lunde HE et al. study revealed that PLT-F has superior diagnostic accuracy in identifying platelet clumps when compared to the WNR/WDF channel and the PLT-F channel can achieve 100% sensitivity in identifying platelet clumps only when using the strictest PTCP definitions [12]. FSCW represents the duration of each platelet passing through the aperture. When platelet clumps pass through the aperture, FSCW values are higher. Therefore, in one of the scattergrams of PLT-F, where FSC is on the Y-axis and FSCW on the X-axis, high FSCW values can indicate platelet aggregation. The CellaVision DM96 is an automated image-analysis system capable of displaying images of a blood film, providing insights into white blood cells, red blood cells, and platelets through dedicated screens. Gene Gulati reported an 82.8% sensitivity of the Cellavision DM96 in detecting platelet clumps by scanning all screens [13].

According to the guidelines, if clots are not observed in the sample, the investigation for PTCP should involve a microscopic examination of blood films. The presence of platelet clumps, but not fibrin, occurring more than occasionally in a blood film is considered a positive finding [14]. In addition to anticoagulants, pre-analytical issues can also lead to the formation of platelet clumps *in vitro*, such as the blood sample quality. While observing platelet aggregation in a blood film is obvious evidence of PTCP, it may not provide sufficient information to determine the type of platelet aggregation conclusively. When only focusing on platelet clumps while ignoring fibrin, PTCP may be misdiagnosed.

Blood sample quality is crucial because most testing errors, totaling 85%, occur in the phases surrounding the actual analysis. Of these errors, 65% take place in the pre-analytical phase, while 20% happen in the post-analytical phase [15]. Undue clotting is a significant reason for samples being rejected in a clinical hematology laboratory. Recognizing pre-analytical errors, such as sample clotting, is essential for improving the overall quality of laboratory management [16].

In cases of inadequate mixing between the sample and anticoagulant, the activation of the coagulation system occurs. This activation leads to the proteolysis of fibrinogen by thrombin, resulting in soluble fibrin monomers. These monomers then polymerize to form a fibrin clot, which ultimately undergoes covalent crosslinking [17]. In cases of undue clotting, macroscopic clots may not always be easily observable; however, fibrin may be detected in the blood film. During the transformation of fibrinogen into fibrin, the bridging of fibrinogen between αIIbβ3 receptors on adjacent platelets leads to the crosslinking of the platelets, resulting in the formation of a robust platelet aggregate [18,19]. Consequently, in such samples, several platelets or platelet clumps encased in fibrin may also be visible in the blood film.

CONCLUSION

Regardless of whether obvious platelet clumps are visible, the presence of fibrin in a blood film is a critical indicator for diagnosing PTCP due to pre-analytical issues.

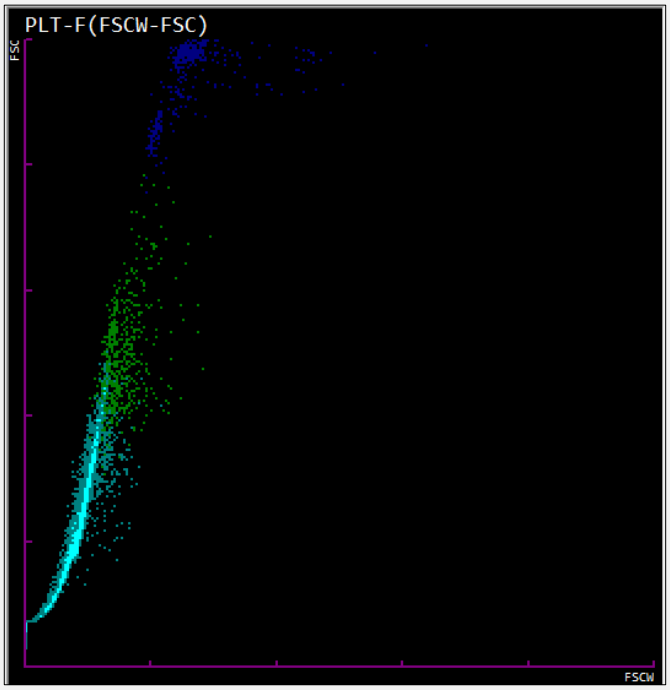

Figure 1. The scattergram of PLT-F (FSC-FSCW).

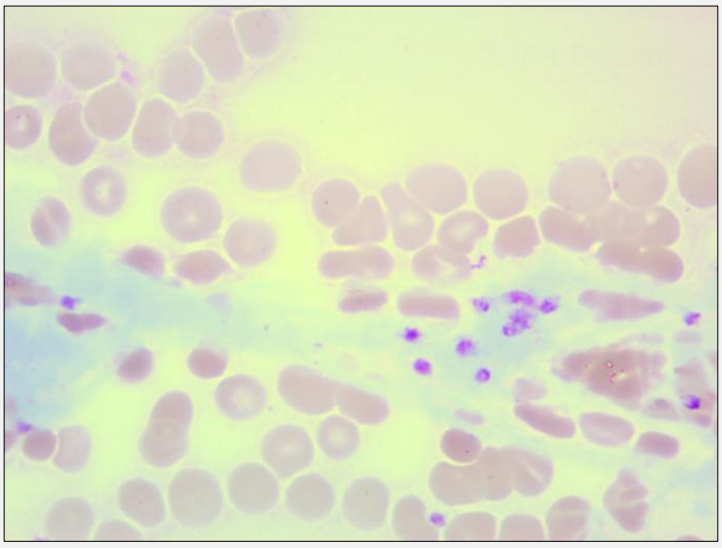

Figure 2. Blood film of the patient (Wright's stain, x 1,000).

Declaration of Interest:

All authors have no competing interests. Written informed consent was obtained from the patient for publication of this case report.

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