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CASE REPORT

Multianticoagulant Pseudothrombocytopenia in a Patient with Primary Carcinoma of the Liver with Hypersplenism

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SUMMARY

Background: **Pseudothrombocytopenia (PTCP) is a relatively rare phenomenon *in vitro*, the mechanism is not completely clear, and there is no unified solution for it. How to identify and solve PTCP accurately is a challenge for laboratory personnel.**
Methods: **According to the patient's clinical manifestations, thrombocytopenia caused by hypersplenism was excluded. PTCP was confirmed by platelet volume histograms, scattergrams and platelet clumps on the blood smears. Commonly used alternative anticoagulants such as sodium citrate or heparin were used for platelet counting. The corrective effect of the platelet count was not good, so non-anticoagulant blood was collected and tested immediately, and blood smears were used to count platelets manually.**
Results: **The PTCP of the patient could not be solved using sodium citrate and heparin anticoagulation. By collecting non-anticoagulant blood and testing immediately, the platelet count returned to normal (180 x 10^9/L), which is consistent with the results of manual counting on the patient's blood smears (175 x 10^9/L).**
Conclusions: **When PTCP is confirmed, commonly used alternative anticoagulants can be used. If these do not work, non-anticoagulant blood can be collected and tested immediately, and blood smears can be used to count platelets manually.**
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KEYWORDS

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INTRODUCTION

Gowland [1] first reported pseudothrombocytopenia (PTCP) in 1969, which is a relatively rare phenomenon. The mechanism of PTCP is not completely clear, and there is no unified solution for it. Unlike thrombocytopenia caused by bone marrow suppression, hypersplenism and other reasons, PTCP is an *in vitro* reaction that leads to a pseudodecrease in the platelet (PLT) count due to platelet clumping *in vitro*. Ethylenediaminetetraacetic acid (EDTA) is the preferred anticoagulant for complete blood cell count (CBC), which is recommended by the International Council for Standardization in Hematology (ICSH). However, it is also the most com-

mon factor causing PTCP, which is called EDTA-dependent pseudothrombocytopenia (EDTA-PTCP). In addition, some scholars have reported PTCP caused by other anticoagulants, but the incidence is lower than that of EDTA-PTCP [2]. Although laboratory testing technology has been continuously developed and updated in recent decades, CBC is still the basis of screening, diagnosis, and treatment monitoring in most diseases, so it is crucial to ensure the accuracy of the results of the CBC test. If PTCP is not corrected, it may cause clinicians to misdiagnose patients with thrombocytopenia and then carry out unnecessary interventions, such as bone marrow puncture examination, platelet transfusion treatment, or even splenectomy. In daily laboratory testing, how to quickly and correctly identify PTCP, especially when patients have bone marrow suppression or hypersplenism, how to identify true thrombocytopenia and pseudothrombocytopenia, and how to give an accurate PLT count is a challenge for experimental personnels. In this case report, we focus on how to identify and deal with a multianticoagulant PTCP in a patient with primary carcinoma of the liver with hypersplenism.

CASE PRESENTATION

A 49-year-old female patient was found to have a place-occupying lesion in the liver during CT examination, accompanied by an enlarged spleen and enlarged lymph nodes in the hepatic portal area. Surface antigen of hepatitis B was positive previously, and the patient had a history of allergy to penicillin and oxytetracycline but no history of blood transfusion, trauma or surgery. The preliminary diagnosis was primary carcinoma of the liver, chronic viral hepatitis B, and hypersplenism.

The day after admission, the patient had blood collected for CBC test (hematology analyzer: BC6900, Mindray, ShenZhen, China), and the CBC results showed that the white blood cell (WBC) count was 7.91 x 10^9/L, the red blood cell (RBC) count was 4.96 x 10^{12}/L, hemoglobin (Hb) was 147 g/L, and the PLT count was 25 x 10^9/L, so the PLT count showed an isolated reduction. We communicated with the clinicians and knew that the patient had hypersplenism. Was it thrombocytopenia caused by hypersplenism? However, the patient's skin had no purpura or stasis, and there were no signs of bleeding throughout the body, so it was necessary to further check whether the PLT count was accurate. Combined with the PLT volume histogram and DIFF channel scattergram of the CBC test, we found that the tail of the PLT volume histogram was significantly elevated, with no return to baseline at 20 fL (Figure 1A). The dark blue GHOST region (mainly composed of RBC fragments and PLT with low nucleic acids) of DIFF channel scattergram expanded and contacted the neutrophil region (Figure 1E). Therefore, we considered that the EDTA anticoagulant sample may contain platelet clumps. We immediately made a blood smear and found many platelet clumps (Figure 2A) under the microscope, so EDTA-PTCP was confirmed. According to past experience, most EDTA-PTCP can be solved by replacing anticoagulants, so to obtain accurate results of the PLT count, we replaced sodium citrate and heparin anticoagulation for the CBC test. The PLT count of the sodium citrate anticoagulant group was 21 x 10^9/L and that of the heparin anticoagulant group was 27 x 10^9/L, which were still very low. There were still obvious abnormalities in the PLT volume histograms (Figure 1B, C) and scattergrams (Figure 1F, G). Blood smears were made with sodium citrate and heparin anticoagulation, and many platelet clumps were still found on blood smears under a microscope (Figure 2B, C). Obviously, replacing anticoagulants could not solve this problem. Finally, we decided to collect non-anticoagulant blood and test it immediately. The PLT count was 180 x 10^9/L, and there were no obvious abnormalities in the PLT volume histogram and scattergram (Figure 1D, H) and no platelet clumps on the blood smears (Figure 2D). The modified Fonio method was used to count PLT manually as follows: an area was selected with well-distributed RBC and PLT in the blood smear; at least 1,000 RBC and all PLT encountered were counted. The RBC count given by the hematology analyzer and the ratio of RBC/PLT obtained on the smear were used to count PLT manually. The result of the manual PLT count was 175 x 10^9/L, which is consistent with the result of the PLT count given by the analyzer.

DISCUSSION

At present, most clinical laboratories use electrical impedance technology for the CBC test, and the results can be obtained accurately and quickly most of the time [3]. However, when platelet clumps occur in blood samples, platelet clumps will be misjudged as a single large platelet or a small lymphocyte because platelet clumps are larger than normal platelets, and the accuracy of automatic PLT counting will be compromised, leading to the occurrence of PTCP [4], as described in this case. As the preferred anticoagulant of CBC, EDTA has some advantages, such as its small impact on cell morphology and counting in blood samples. However, when EDTA is used as an anticoagulant, platelet clumps occasionally occur, resulting in a false reduction in the PLT count. The mechanism by which EDTA leads to PTCP is not completely clear. At present, the more unified view is that EDTA can irreversibly chelate Ca_{2+}; when the concentration of Ca_{2+} decreases, the GPIIb/GPIIIa dimer dissociates, revealing the recessive epitope on GPIIb; and some autoantibodies, such as IgM, IgG or IgA, act on the GPIIb epitope, thus causing platelet clumping [5-7]. In this case, in addition to EDTA leading to PTCP, sodium citrate and heparin anticoagulation also led to a similar situation. Because there is less research on PTCP due to sodium citrate or heparin, the mechanism of this phenomenon is not clear. Some scholars have said that conformational changes on the platelet surface

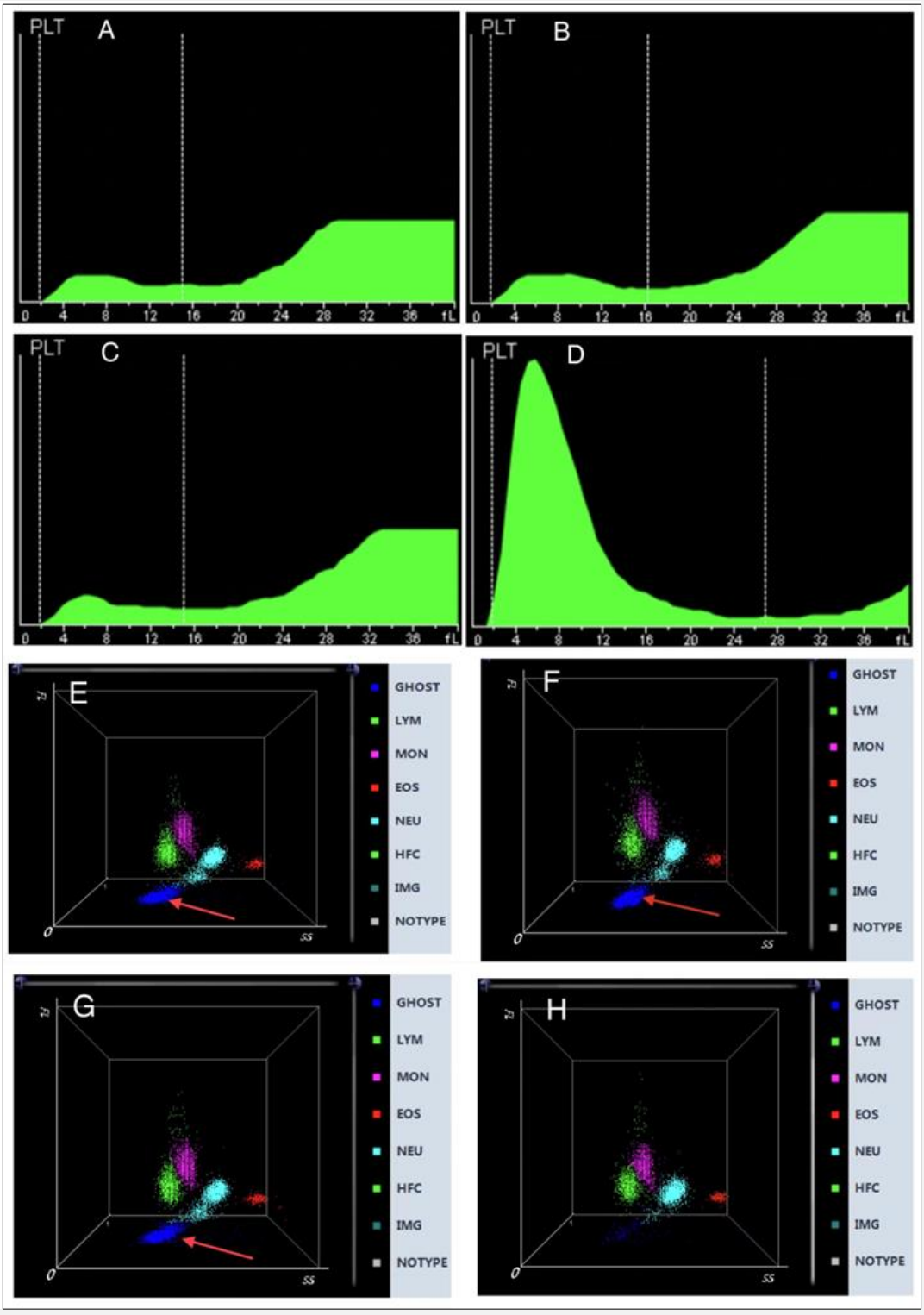

Figure 1. PLT volume histograms, DIFF channel scattergrams.

A, E: EDTA anticoagulation; B, F: sodium citrate anticoagulation; C, G: heparin anticoagulation; D, H: no anticoagulation.
A - C: The tail of the PLT volume histograms is significantly elevated; E - G: The GHOST region of DIFF channel scattergrams are expanded and contact into the neutrophil region (arrow); D, H: PLT volume histograms and DIFF channel scattergrams have no obvious abnormalities.

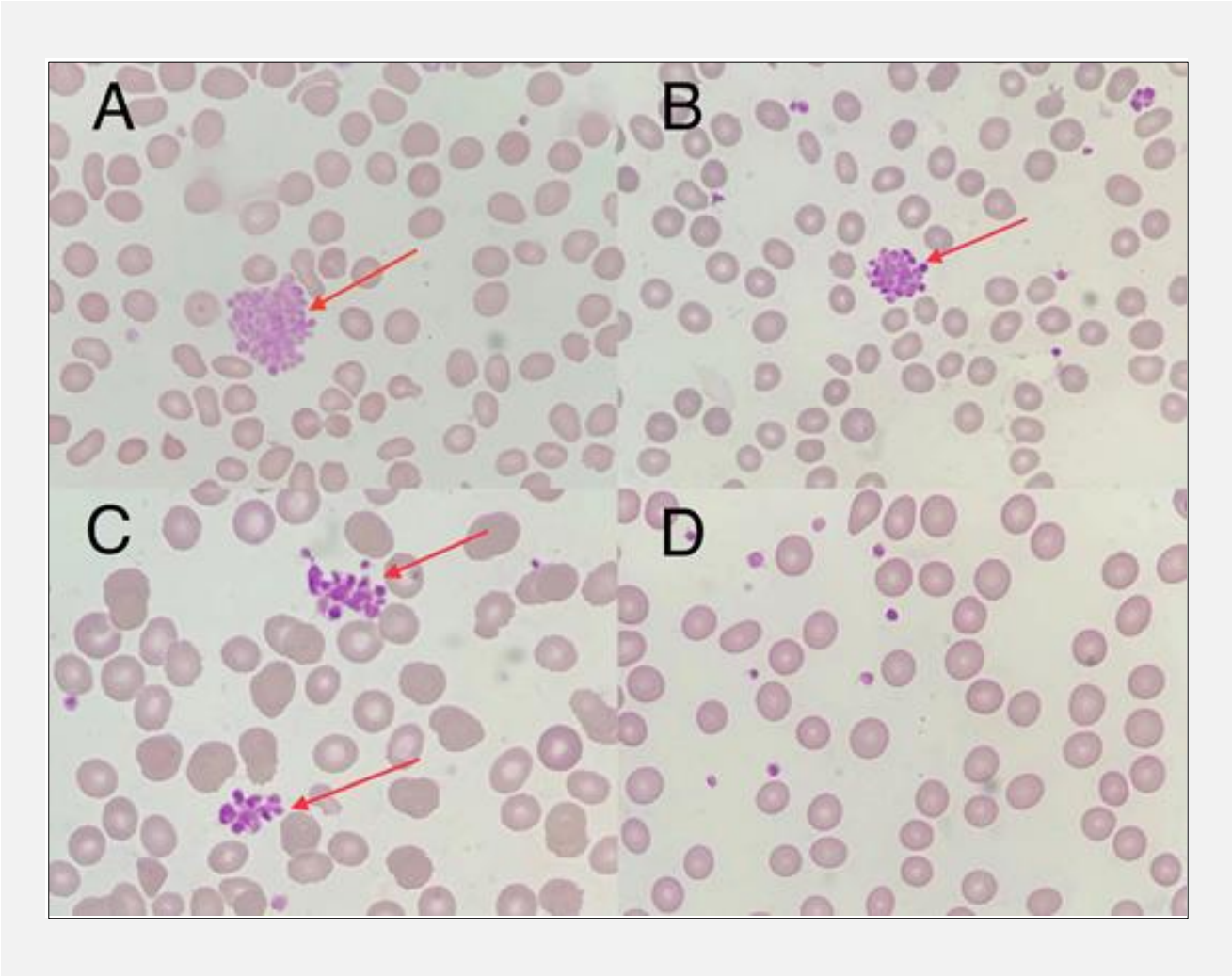

Figure 2. PLT morphology under a microscope (Wright-Giemsa stain, x 1,000).

A: EDTA anticoagulation; B: sodium citrate anticoagulation; C: heparin anticoagulation; D: no anticoagulation.
A - C have platelet clumps (arrow); D has no platelet clumps.

have not been observed in some alternative anticoagulants, such as citrate [8], but a recent report shows that similar conformational changes may occur in citrate and even heparin [7]. In addition, some scholars believe that sodium citrate also achieves anticoagulant effects by chelating Ca_{2+}, so it may also produce conformational changes on the surface of platelets. In the case of heparin, PTCP is caused by platelet endothelial cells and monocyte-activating antibodies that target multimolecular complexes of platelet factor IV and heparin [9].

Although there are many case reports or studies describing different methods for dealing with PTCP, there are few suggestions and no consensus among experts on PTCP detection and management. When the CBC results show that the PLT results are abnormally reduced, we should rule out true thrombocytopenia caused by bone marrow suppression or hypersplenism, pay attention to whether the PLT volume histograms and DIFF channel scattergrams are abnormal, and finally confirm the existence of EDTA-PTCP by blood smears. Most cases of EDTA-PTCP can be corrected by using different anticoagulants. Among them, sodium citrate is the most widely used alternative anticoagulant for EDTA-PTCP because it is the most easily available anticoagulant besides EDTA in the laboratory, and the risk of platelet clumping is low [2]. However, approximately 17% of EDTA-PTCP patients also have PTCP in sodium citrate samples [10]. Heparin is another commonly used alternative anticoagulant that is also easily available in the laboratory, but not all of them are effective in solving PTCP, just as sodium citrate and heparin anticoagulant could not solve PTCP in this report. In addition, sodium fluoride [11], ammonium oxalate [12], magnesium sulfate [13] and other anticoagulants can al-

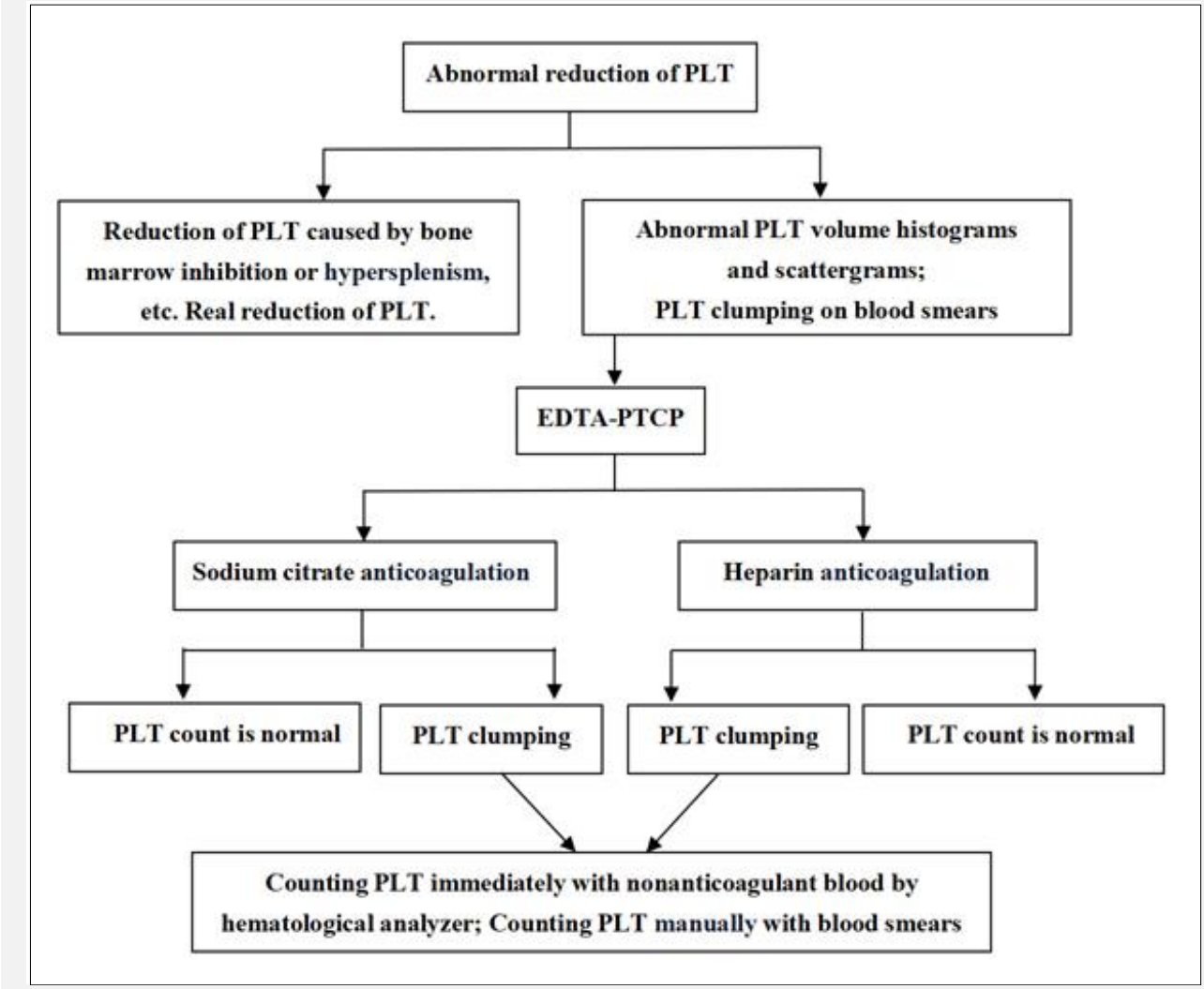

Figure 3. A simple and fast flowchart diagram of solving EDTA-PTCP.

so be used to solve PTCP. However, these anticoagulants are not easy to obtain in some laboratories, which is one reason why these anticoagulants are not used as common alternative anticoagulants in our laboratory; their effects on solving PTCP are uncertain; and they need further study. In addition, supplementing kanamycin or amikacin in EDTA-PTCP samples is considered an alternative method. However, its operation is complicated, and the total cost of preparation and quality management of kanamycin and amikacin must be considered, which is also not applicable to most PTCP [14,15]. When replacing anticoagulants cannot solve PTCP, we can consider collecting non-anticoagulant blood, immediately testing samples by a hematology analyzer, and making blood smears to count PLT manually. This method can solve almost all anticoagulant-dependent PTCP. In summary, a simple and fast processing flow for solving EDTA-PTCP is presented in Figure 3.

CONCLUSION

Although it is generally believed that PTCP itself has no pathological significance at present, PTCT will lead to the wrong diagnosis or treatment by clinicians. Therefore, when the laboratory personnel find that the PLT count are abnormally reduced, after excluding thrombocytopenia caused by bone marrow suppression and hypersplenism, they should combine with the PLT volume histograms, scattergrams and blood smears to confirm

whether PTCP exists. When PTCP is detected, it can be solved with commonly used alternative anticoagulants. If it is ineffective, non-anticoagulant blood should be collected, and PLT should be counted immediately by a hematology analyzer. At the same time, blood smears can be used to count PLT manually for confirmation.

Ethical Approval:
This article does not contain any human participant studies that were performed by any of the authors.

Informed Consent:
Informed consent was obtained from the patient in the study.

Declaration of Interest:
All authors declare that they have no conflicts of interest.

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