LEADING ARTICLE

VALIDATING THE PLATELET COUNT

S L Tien

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Introduction

Platelet count is an integral component of the full blood count. Unexpectedly abnormal platelet counts are sometimes obtained in clinical practice. For example, a falsely low platelet count could be due to platelet aggregation which can be easily picked up on a blood film. Failure to recognise this phenomenon has sometimes led to the misdiagnosis of a patient as suffering from idiopathic thrombocytopenic purpura and has led to corticosteroid therapy and even splenectomy in one case[1]. Before any clinical decision on diagnosis or management of the patient is made, the abnormal platelet count has to be validated. In interpreting the platelet count result it is important to understand the physiological and various technical factors that could affect the platelet count such as the method of collecting blood specimen, preparation of the platelet sample, the type of instrument and the calibration method used.

Normal Platelet Count

The normal range of platelet count is 150,000 to 450,000 / mm^3 and in health these levels are usually constant. Physiological variables affecting the platelet count have to be considered when interpreting the result. There are no obvious age differences; at birth and in the first few weeks of infancy, however, the platelet count tends to be at the lower level of the adult normal range, rising to adult values at about 6 months[2]. A recent study found children to have higher platelet count than adults[3]. The platelet count has been reported to be about 20% higher in women than in men[4]. A fall in platelet count may occur in normal women at about the time of menstruation and there is some evidence of a cycle with a 21-35 day rhythm[5]. The platelet count has been observed to fall during pregnancy[6] but if subjects with pregnancy-related hypertension are excluded no fall is observed[7].

Venous Versus Capillary Platelet Count

The method of collecting blood influences the platelet count. Capillary blood obtained by finger-prick generally has lower platelet count than blood obtained by venepuncture. This is also shown in the paper by Tai et al in this issue[8]. The lower platelet count in capillary blood is probably due to the adhesion of some platelets at the site of the wound and the diluting effect of the tissue fluid[9] occurring while squeezing the finger. The small amount of blood collected in capillary blood does not allow for a recheck on the same sample if the initial result is doubtful. In practice a venous blood is preferable and more reliable. Capillary blood may be used when there is poor venous access or if the patient requires daily blood count monitoring as in patients with haematological malignancies who are undergoing chemotherapy.

Department of Haematology
Singapore General Hospital
Outram Road
Singapore 0316.

S L Tien, M Med (Int Med), FAMS, FRCPA
Consultant

Effect of Anticoagulant in Blood Collection

Although ethylene-diamine-tetra-acetic acid (EDTA) is a very convenient anticoagulant to use for routine platelet counts, occasionally the presence of EDTA causes the platelets to clump and the count to be falsely low. The platelet agglutinins responsible for this may be IgG or IgM antibodies active in the presence of EDTA. Such antibodies may be optimally active at lower temperature (0-4°C)[1] and the aggregation may be time-dependent. Platelet satellitism may be formed with adhesion of platelets around the neutrophil.

In the presence of EDTA-dependent agglutinins a repeat platelet count using citrate or heparin as an anticoagulant is necessary for an accurate result. Platelet aggregation may also be due to an antibody acting independently of EDTA. Some of these agglutinins are also cold agglutinins and an accurate result may be produced by warming a fresh sample.

Platelet Preparation for Automated Counting

Platelets may be prepared in 3 ways for automated counting depending on the type of counter used. It can be counted in platelet-rich plasma, in whole blood in the presence of intact red cells or in whole blood following lysis of red cells. When platelets are counted in platelet-rich plasma, falsely low platelet count may be due to loss of platelets during preparation. Inaccuracy may also be consequent to failure to correct for the platelet-free plasma which is trapped in the red cell column. When platelets are counted in whole blood in the presence of red cells, falsely high platelet count may be due to red cell fragments or microcytic red cells counted as platelets. When platelet count is taken in whole blood following lysis of red cells, falsely high platelet count may be due to the presence of malarial parasites, Howell-Jolly bodies, Heinz bodies, Pappenheimer bodies, erythrocyte debris formed when red cells are aggregated by antibodies or agglutinating paraproteins[10].

Platelet Size Calibration

Most instruments measure cells falling between 2 and 20 femtolitres as platelets. This raw data is plotted on the platelet histogram. While the bulk of the platelet population falls within a fixed threshold, others exist outside these boundaries, microthrombocytes on the low end and macrothrombocytes on the high end. Cytoplasmic fragments, microcytic red cells and debris can masquerade as platelets. To overcome this problem, mathematical curve fitting is applied to the raw data histogram to deliver a more reliable platelet count, eliminating non-platelet particles. The raw data (2-20 fl) is tested against a set of criteria and fitted to a log normal curve from 0 to 70 fl. The curve must be positive, the mode must fall between 3 and 15 fl and the coefficient of variation of platelet size or

platelet distribution width (PDW) is less than 20%. If these criteria are met, the computer uses the fitted curve to determine the platelet count (equal to area under the curve from 0 to 70 fl). If one of the above criteria is not met, an "R" flag appears next to platelet count and no log normal curve will be fitted. The platelet count is then derived using the area under the curve of raw data from 2 to 20 fl, which may be less than accurate. In the presence of an instrument flag the platelet count has to be verified by a peripheral blood film. The setting of the upper threshold of platelet count is less critical with instruments counting on platelet-rich plasma as contaminating red cells are not usually present.

In instrument such as Coulter, the application of sweep flow in the counting chamber improves the accuracy of the platelet count. It was found that red cells produced characteristic swirling patterns as they passed through the aperture. They may re-enter the sensing zone and produce small pulses that could be counted as platelet. To prevent this, sweep flow lines carrying diluent are attached to the bottom of the counting chamber. Vacuum pulls the diluent from these lines past the aperture outlets, preventing re-entry of the cells into the sensing zone.

In Technicon instrument the platelets and red blood cells are analysed by a single optical cytometer. A Mie Scatter computer programme determines the platelet and red blood cell counts. Counts are corrected for coincidence. Platelets will have a greater high:low angle ratio while red blood cells have a greater low:high angle light scatter.

Falsely Low Platelet Count

Falsely low platelet counts are more common than falsely high counts. The error is most often due to partial clotting of the specimen or platelet aggregation. The platelets may surround the neutrophil uniformly to form platelet satellitism. This effect is sometimes seen in EDTA-anticoagulated blood. Proper collection technique and prompt counting will reduce blood clotting and platelet aggregation. Presence of giant platelets may cause falsely low platelet counts as these large platelets will not be counted as they fall outside the upper threshold of platelet calibration.

Falsely High Platelet Count

Contamination of capillary samples with skin debris, food remnants with fat globules or subcutaneous fat droplets may lead to falsely high platelet count[11]. Thorough cleaning of the site of capillary puncture is essential.

Microcytic or red cell fragments less than 20 fl will be counted as platelets and platelet count will be spuriously elevated. The red cell volume and the platelet histogram, together with instrument flag, provide clues to this artefact. Blood film examination is necessary and manual count is indicated. Blast fragments or cytoplasmic fragments of white cells can simulate platelets as well.

Presence of cryoglobulin[12] result in pseudothrombocytosis. Correct counts can often be obtained by warming the sample to 37°C. Bacteria[13] in blood in patients with septicaemia may be counted as platelets leading to spuriously high count. In-vitro haemolysis can result in red-cell ghosts counted as platelets.

Manual Platelet Count

Although electronic counting systems have greatly improved counting precision, visual methods are still indicated in situations where falsely abnormal platelet count is suspected. The standard method of doing a manual platelet count is the use of hemocytometer counting chamber, with Neubauer or improved Neubauer ruling. Accuracy in visual counting can be achieved only by paying attention to detail with regard to the cleanliness of the preparation and by experience. There is a possibility that red cell debris may be mistaken for platelets but with some experience this should not cause any difficulties. The use of phase-contrast microscopy helps considerably in recognising and counting platelets.

Peripheral Blood Film

Examination of the peripheral blood film is an important step in validating an abnormal platelet count. If a platelet count is very inaccurate, the inspection of the blood film will be sufficient to reveal the inaccuracy. If the degree of error is less, then it may be useful to estimate the platelet count from the blood film. This can be done by either counting the platelets in relation to red cells and calculating the platelet count from this ratio and the red blood cell or counting the number of platelets per high power field. In one study[14] it was found that subjects with a normal platelet count had 7-21 platelets per oil immersion field. In another study[15] it was found that the sum of platelets in ten immersion fields multiplied by two approximated to the platelet count. These relationships will differ somewhat between different microscopes.

Besides looking for artefacts causing a low platelet count, a full examination of the blood film may reveal the underlying cause of the thrombocytopenia, for example the presence of blast cells in leukaemia or the presence of microangiopathic haemolytic anaemia in disseminated intravascular coagulation.

Conclusion

A platelet count can only be considered validated when all unexpectedly abnormal counts and all instrument flagged counts have been verified on a blood film. The blood sample should be checked for small clots and fibrin strands. A repeat sample and a manual platelet count may be necessary.

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