HABX SCIENTIFIC NEWSLETTER

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Pre analytical induced Thrombocytopenia

Platelets or thrombocytes are anucleated disc shaped cell fragments (diameter = 2-4 µm) derived from the megakaryocytes of the bone marrow responsible for hemostasis. A normal platelet count ranges from 150,000 to 450,000 platelets per microliter of blood.

Thrombocytopenia: A condition in which platelet count decreases to less than 1,50,000/µL of blood is termed as Thrombocytopenia. Thrombocytopenia occurs generally due to decrease in platelet production or increased destruction of the platelets. Thrombocytopenia is reported in many clinical conditions such as - infections, malignancy, liver disease, autoimmune disorders, disseminated intravascular coagulation, pregnancy, menstruation, medications, and coagulation disorders.

Within the wide normal reference range, there are some ethnic differences such as – in healthy West Indians and Africans platelet counts can be 10–20% lower than those in Europeans living in the same environment.[15]. There may be a sex difference also as in women, the platelet count has been reported to be about 20% higher than in men.[16]

Pseudothrombocytopenia: Pseudo thrombocytopenia can be defined as a situation in which thrombocytocytopenia is reported without any underlying clinical condition but induced mainly due to preanalytical errors.

Pseudothrombocytopenias correspond to 15% to 30% of the cases of isolated thrombocytopenia detected in the routine laboratory (5). Pseudo thrombocytopenia occurs generally due to problems in collection and processing of samples (improper tube shaking, sample dilution, peripheral blood collection difficulties); giant platelet syndrome; induction by anticoagulants, such as ethylenediaminetetraacetic acid (EDTA), citrate, oxalate and heparin; autoimmune diseases; drugs (abciximab, valproic acid, mexiletine and olanzapine); solid tumors, myeloproliferative and lymphoproliferative syndrome(6).

When a patient present with isolated thrombocytopenia, with no family history of thrombocytopenia, hematological diseases or manifestation of bleeding episodes, a case of pseudo thrombocytopenia should be suspected, & it should not be confused with other serious clinical conditions, such as disseminated intravascular coagulation, idiopathic thrombocytopenic purpura or heparin-induced thrombocytopenia(12).

Blood volume and storage conditions: The addition blood volume should be added accurately in tubes as instructed by the manufacturer and then the container with anticoagulant should be repeatedly inverted to ensure the thorough mixing of blood to anticoagulant. The main reason behind this is that improper blood volume or lack of proper mixing causes cells to either swell and disintegrate or result in platelet clumps which in turn results in thrombocytopenia.

The platelet count and red cell indices are usually stable for 8 h after blood collection, When the blood is kept at 4°C. the effects on the blood count are not usually significant for up to 24 h. Thus, for many purposes blood may safely be allowed to stand overnight in the refrigerator if precautions against freezing are taken.[17]

Blood collection: Blood collection method is another possible pre-analytical factor that induce platelet clump formation and ultimately pseudo thrombocytopenia. Capillary collections are more prone to clotting and formation of platelet clumps. [18].

EDTA induced Pseudo thrombocytopenia:

Pseudo thrombocytopenia induced by the anticoagulant ethylenediaminetetraacetic acid (EDTA) is one of the most regular cases in which the presence of anticoagulant in blood samples can lead to platelet aggregation or platelet satellitism.

EDTA is the preferred anticoagulant used to perform blood counts, and its exposure to the blood samples from some patients may induce platelet aggregation or, less frequently, platelet aggregation around neutrophils (platelet satellitism), by an apparently immune-mediated process(7,8). This phenomenon, which is the most frequently associated with cases of pseudo thrombocytopenia, requires that appropriate laboratory procedures be performed, since it represents a major laboratory and clinical problem(6-10).

Confirmatory test: Other procedures such as checking for clotting in the sample, repeating the sample and viewing a peripheral blood smear, requesting a new sample taken with another type of anticoagulant (e.g.- citrate) are imperative for the confirmation of cases of pseudo thrombocytopenia.

Histograms: In such cases, careful observation of data emitted by auto analysers, such as platelet and flag histogram are crucial.

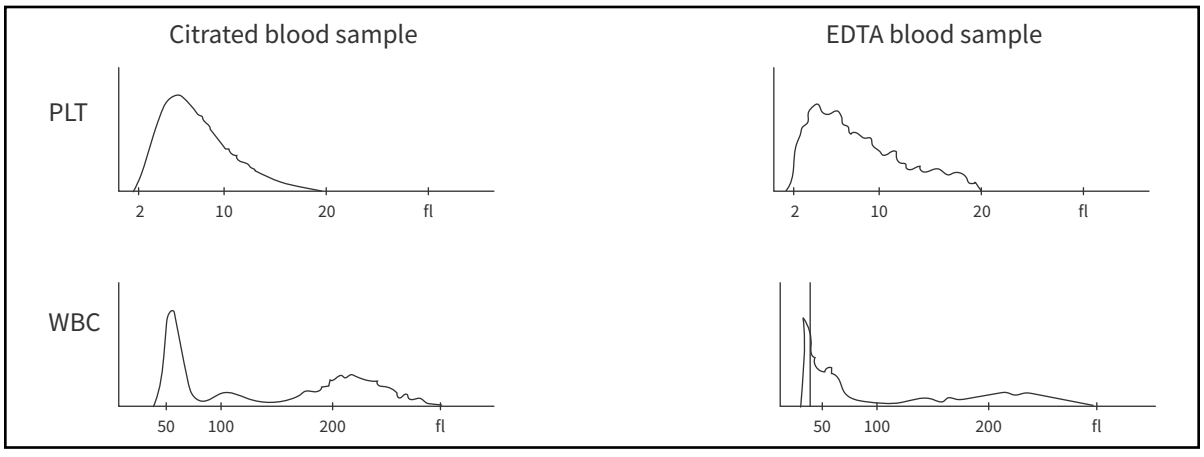

Figure 1 Histogram of PLT & WBC in citrated and EDTA blood sample

Figure 1 shows automated hematology analyzer generated typical histograms of pseudo thrombocytopenia in an EDTA sample (right) in contrast to normal histograms of a citrated (CPT) sample of the same patient (left). This blood cell histograms are generated by plotting the sizes of different blood cells on X-axis and their relative number on Y-axis.

Aggregated platelets are plotted as a serrated (“saw-teeth”) curve in the PLT histogram. In the WBC histogram, the largest aggregates are displayed as a peculiar peak on the left side (arrowhead).

Corrective action: The use of alternative anticoagulants to EDTA, such as sodium citrate, oxalate or heparin, can be used as an alternative method to rule out the phenomenon of EDTA-induced pseudo thrombocytopenia.

To confirm the cases of pseudo thrombocytopenia, the EDTA sample should be incubated for 37°C for about 30 minutes and re-analysis should be performed. The incubation results in dissociation of the platelet aggregates.

Certain aminoglycosides such as kanamycin and amikacin can be added (these do not interfere with cell counts and prevent the formation of platelet aggregates, although their mechanism of action is still not well known)(11) to the sample harvested with EDTA.

Conclusion: Preanalytical errors such as errors during blood collection, storage condition, volume of blood or use of EDTA vial can induce thrombocytopenia even in a normal patient. Hence, the data emitted by auto analyzers (such as platelet histogram, flags suggestive of pseudo thrombocytopenia) should be cautiously observed to verify the presence of a clot in the sample, sample can be re-analyzed, or Peripheral blood smear can be visualized under microscope for further confirmation. The collection of a new citrate sample may be sufficient to confirm the finding of pseudo thrombocytopenia.

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