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Case Report Open Access

Iron Stain on Bone Marrow Aspirate Smears Interfering with Assessment of Iron Stores and Sideroblasts

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Abstract

Bone marrow examination for assessment of iron status is an important part of the evaluation of patients with both haemopoietic and non haemopoetic disorders. An iron stain is the gold standard for the assessment of the body's iron status. It also allows the detection not only of an increased or decreased proportion of sideroblasts (iron containing nucleated red blood cells) but also of abnormal sideroblasts hence is valuable in the diagnosis of various hematological diseases. The quality of the stain is hence pivotal in hematology.

In my laboratory, I have noticed for a prolonged period of time an artefact on our iron stain which made it difficult to assess iron stores and impossible to assess the percentage of sideroblasts. I undertook this troubleshooting investigation to determine the possible cause and solution to these artefacts. In literature there is paucity of information about the causes of Perl's Prussian blue stain artefacts in hematology specimens most suggestions attributing the artefact to the age of the stain and suggesting making up fresh stain to filtering the stain reagent Saffranin-O to remove any impurities from the stain as this reagent tends to form precipitates. Such measures were attempted but were unhelpful in resolving the artefact. The results of this troubleshooting showed that storing Saffranin-O in a plastic contained after reconstituting the powder to a solution was the cause of the artefact and the practice has since been changed to storing the Saffranin-O stock solution in a glass bottle.

Keywords: Iron stain artefacts; Perl's Prussian blue stain; Saffranin-O

Introduction

The iron stain used in our laboratory is called the Perl's stain or Perl's Prussian blue stain. Examination of the Perl's stain of a bone marrow is the classic method for demonstrating iron in tissues; it allows adequate assessment of erythroblasts iron (sideroblasts), free lying iron and grading of iron stores which is iron contained in bone marrow macrophages. This method stains mostly iron in the ferric state which includes ferritin and haemosiderin. Iron in haemosiderin is chemically reactive and turns blue-black when exposed to potassium ferrocyanide, which forms the basis for the Prussian blue stain [1,2].

A proportion of normal erythroblasts have a few iron containing granules [3-5] distributed randomly in the cytoplasm. In haematologically normal subjects with adequate iron stores, 20%-50% of bone marrow erythroblasts are sideroblasts [1]. The sideroblasts ratio can change in various pathological states (decreased in anaemia of inflammation, thermal injury, hyposiderosis), (moderately increased in thalassaemia, pyridoxine deficiency, drugs used in TB therapy such as INH, lead poisoning, haemolytic anaemias, megaloblastic anaemias, Haemochromatosis), markedly increased in refractory sideroblastic anaemia [2]. The distribution and size of the siderotic granules is also important and can have diagnostic implications [2].

Artefacts may interfere with an accurate diagnosis or may render the specimen useless for diagnostic purposes [6], as it was the case with the particular artefacts experienced in our laboratory.

In practice, staining to demonstrate iron stores in marrow fragments and siderotic granules in erythroblasts is a simple and valuable diagnostic procedure and in our laboratory we apply it to bone marrows films as a routine. In addition to siderotic granules within erythroblasts, haemosiderin can normally be seen in marrow films as accumulation of small granules, lying free or in macrophages in marrow fragments. The amount of haemosiderin will be markedly increased in patients with increased iron stores, whereas haemosiderin is absent in iron deficiency anaemia. In sideroblastic anaemia and myelodysplastic syndromes siderotic granules have a particular ring like pattern around at least 1/3 of the nucleus, these can also be seen in non-clonal causes.

Case Study

Several bone marrows aspirate smears stained for assessment of iron as part of the work up of cases referred for bone marrow biopsy were reviewed at the NHLS laboratory in Groote Schuur Hospital during a month period August 2018 [6]. The slides were stained as per method described below with reagents stored at room temperature.

Iron stain methodology GSH NHLS as per standard operating procedure (SOP)

Unstained bone marrow aspirate smears are fixed in methanol for 10 minutes then air dried. Smears are then dipped in a solution of

Potassium Ferrocyanide for 10 minutes then rinsed with running tap water for 15 minutes, then counterstained with Saffranin-O for 15 seconds then rinsed with distilled water and air dried and ready for microscopy (ferric iron will appear blue and the nuclei red) (Figures 1-4). The artefact that appeared as red crystal was noted after counterstaining with Saffranin-O (Figure 5). Stock solutions of the reagents used in the staining procedure were stored in plastic bottles and are stable for several weeks [7]. The issue with our stained slides were sharp needle-like (Figure 5) which made them useless for assessing the iron stain.

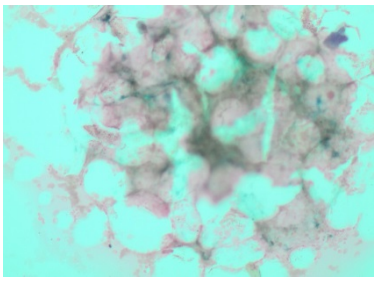

Figure 1: Bone marrow particle highlighting iron stores-bluish black iron in macrophages.

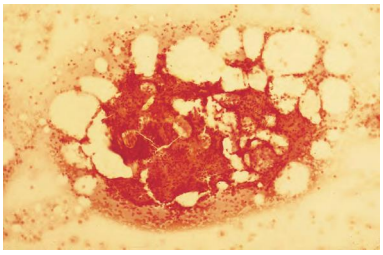

Figure 2: Perl's iron stain with no stainable iron (iron deficiency).

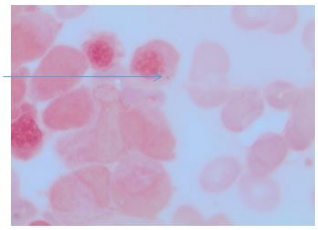

Figure 3: Normal sideroblasts with an iron granule in the cytoplasm (arrow).

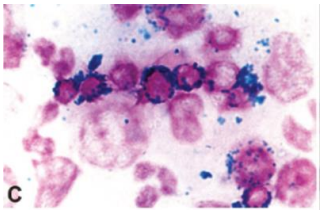

Figure 4: Ring sideroblasts [5].

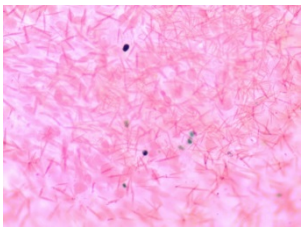

Figure 5: Sharp needle-like artefacts on bone marrow aspirate smear after counterstaining with Saffranin-O.

Over the 1-month period several slides were viewed under the microscope after each stage of the staining process to detect at which stage these artefacts appeared and eventually one of the reagents the Saffranin-O which was stored in a plastic bottle as a stock solution after reconstitution of the powder form. The other variable that was investigated was age of specimen where fresh stain was prepared (Table 1).

Air drying of slides	Heat block drying of slides	Saffranin-O stored in plastic container	Saffranin-stored in a glass bottle	Filtering of the Saffranin-O through a micron filter
X	X	X	resolution	X
X=not effective in resolving the artefacts.				

Table 1: A summary of some of the variables that were looked at during the iron staining procedure of bone marrow aspirate slide.

Discussion

The study showed that Saffranin-O stock solution stored in a plastic container caused the artefacts [8]. The other variables (age of the reagents, air drying versus heat block drying of slides, filtering of Saffranin-O solution had no effect in resolving the artifacts. The current practice is now to store the Saffranin in a glass bottle (Figure 6).

Figure 6: The glass bottle currently used for keeping Saffranin-O stock solution.

Conclusion

Artefacts on smears prepared for haematological assessment are a problem for the pathologists as they may make the assessment impossible. This leads to numerous re-processing of smears to try to resolve the problem. In this study it was shown that the artefacts that were seen on our Perl's iron stain were due to storage of Saffranin-O reagent in a plastic bottle and the practise has since been changed to

storage in a glass bottle. Saffranin-O is a biological stain used in histology and cytology; it is used as a counterstain in haematology.

Plastic containers have a basic chemical compound of polyethylene and it is possible that the reaction between chemicals in the plastic and the Saffranin reagent may have caused the artefacts.

Information in literature about artefacts and their causes in haematology smears are very scanty to non-existent and in literature I have not come across such artefacts.

Declarations

Ethics approval and consent to participate: Granted by the University of Cape Town Medical Research Council

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