Ntobongwana M, J Hematol Blood Transfus Disord 2019, 6: 024
DOI: 10.24966/HBTD-2999/100024

HSOA Journal of Hematology, Blood Transfusion and Disorders

Case Report

Staining Artefact Presumed to be Pathology in a Patient Investigated for Megaloblastic Anaemia and Myelodysplastic Syndrome: A Case Study at Groote Schuur Hospital

Monalisa Ntobongwana[1,2*]

[1]*Division of Haematology, Department of Pathology, National Health Laboratory Services, Groote Schuur Hospital, Cape Town, South Africa*

[2]*Faculty of Health Sciences, University of Cape Town, South Africa*

Abstract

Artefacts are structures that are not normally present in well prepared smears. Well stained smears are the cornerstone of diagnostic haematology and this requires properly stained smears achieved by adherence to Standard Operating Procedures (SOPs) to ensure reliability of results. Artefacts on smears may baffle the examiner and may in fact be assessed as real pathology by an in experienced examiner or conceal real pathology. This case report describes a patient who was referred to the haematology department for work-up of a macrocytic anaemia to exclude megaloblastic anaemia and myelodysplastic syndrome. The initial blood smear processed consisted of numerous basophilic stippling-like inclusions which were perplexing and this together with the drying artefact seen on the smear prompted a repeat of the blood smear which showed resolution of the artefacts. Basophillic stippling can be seen in megaloblastic anaemia and myelodysplastic syndrome.

Keywords: Basophilic stippling; Megaloblastic anaemia; Myelodysplastic syndrome; Staining artefact

Abbreviations

BS: Basophilic Stippling
SOPs: Standard Operating Procedures
PAS: Periodic Acid Schiff
RBC: Red Blood Cell

***Corresponding author:** Monalisa Ntobongwana, Division of Haematology, Department of Pathology, National Health Laboratory Services, Groote Schuur Hospital, Cape Town, South Africa, Faculty of Health Sciences, University of Cape Town, South Africa, Tel: +27 0214044244 / +27 0785594277; Email: monalisa.ntobongwana@nhls.ac.za/monalisantobs@yahoo.com

Citation: Ntobongwana M (2019) Staining Artefact Presumed to be Pathology in a Patient Investigated for Megaloblastic Anaemia and Myelodysplastic Syndrome: A Case Study at Groote Schuur Hospital. J Hematol Blood Transfus Disord 6: 024.

Received: October 10, 2019; **Accepted:** October 18, 2019; **Published:** October 25, 2019

Background

Laboratory work up of a case to exclude pathology requires adherence to Standard Operating Procedures (SOPs) in order to produce reliable and accurate results. Non adherence to SOPs can produce erroneous results with can lead to misdiagnosis and improper treatment of patients. Stains are used in haematology pathology to highlight cell morphology. The stain used in our laboratory for staining peripheral blood and bone marrow aspirate smears is the Diff-Quick stain which is a brand of the Romanowsky stain.

The main components of a Romanowsky stain are:

- A cationic or basic dye (methylene blue or its oxidation products such as azure B), which binds to anionic sites on proteins and gives a blue-grey colour to nucleic acids (DNA or RNA), nucleoproteins, granules of basophils and weakly to granules of neutrophils.
- An anionic or acidic dye such as eosin Y or eosin B, which binds to cationic sites on proteins and gives an orange-red colour to haemoglobin and eosinophil granules [1,2].
- The correct procedure for preparation of smears for cell morphology is to prepare smears of adequate length and to allow the smear to air dry properly before staining [1,2]. Figure 1 shows properly dried and improperly dried blood smears

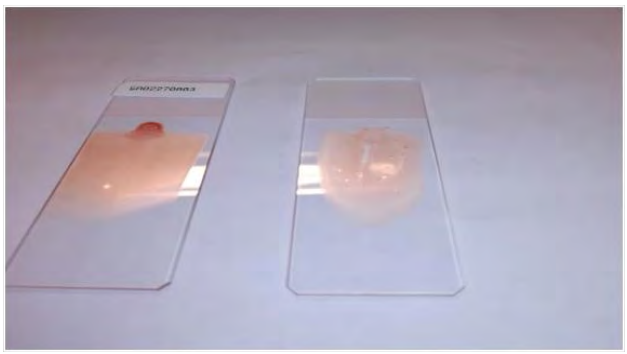

Figure 1: A properly dried blood smear (left) and an improperly dried smear with small clear punched out spaces (right).

- Improper smear processing in this case resulted in an artefact inside red blood cells that was presumed to be Basophilic Stippling (BS). Though very occasional cells with basophilic stippling can be seen in normal people, increased numbers are seen in megaloblastic anaemia, dyserythropoietic states, thalassaemia, lead poisoning, a variety of haemolytic anemias, Pyramidine 5 nucleotidase deficiency etc. [3-6]. Figure 2 shows true basophilic stippling inside red blood cells.

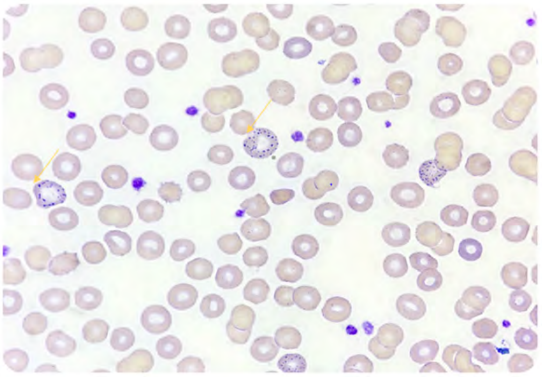

Figure 2: Basophilic stippling inside red blood cells. (Chan NCN, Chan KP).

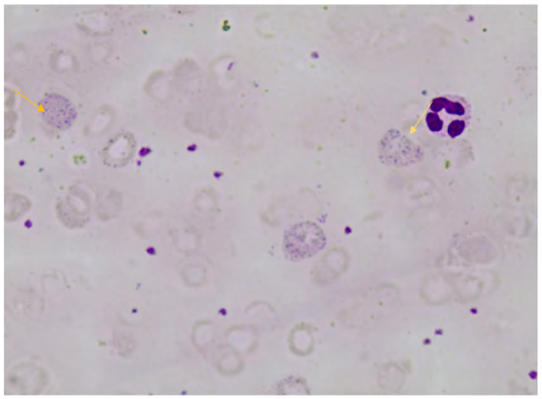

Figure 3: Patient peripheral blood smears with red blood cells containing basophilic stippling -like inclusions. (Magnification x 50).

Case Report

A 49-year-old female patient was referred to haematology for a work-up of a long standing macrocytic anaemia to rule out megaloblastic anaemia or a myelodysplastic syndrome. She had a background history of diabetes, hypertension, and nephrotic syndrome and is partially blind. She was on no drugs that could cause the anaemia and there was no history of haemorrhage.

Laboratory testing

Table 1 shows a full blood count and differential count.

FBC		Differential Count	
WCC	4.25x10^9/L	Neutrophils	62%
Hb	5.2 g/dL	Lymphocytes	29.4%
MCV	108.2fL	Monocytes	4.9%
PLT	189x10^9/L	Eosinophils	1.6%
		Basophils	0%
		Immature granulocytes	2.1%

Table 1: Full blood count and differential count, previous lower hemoglobin levels were in the range of 3-3.4g/dL.

Peripheral blood smear

Of note on the peripheral blood smear were numerous basophilic stippling like inclusions which were identified as basophilic stippling initially in a background of drying artefact/water artefact. The water artefact was not of concern to me as it has no related real pathology and it is seen frequently in the laboratory. My concerns were the numerous red cell inclusions (Figures 3 and 4). At this stage the results for the B12 and folate levels became available and showed no deficiencies in the two vitamins. Other possible causes of BS were entertained at this point and the clinicians were advised on further investigations. A decision was eventually made to repeat the peripheral blood smear.

The peripheral blood smear was repeated and the correct procedure was followed in the preparation and processing of the smear and showed resolution of both the drying artefact and the basophilic stippling artifact (Figure 5).

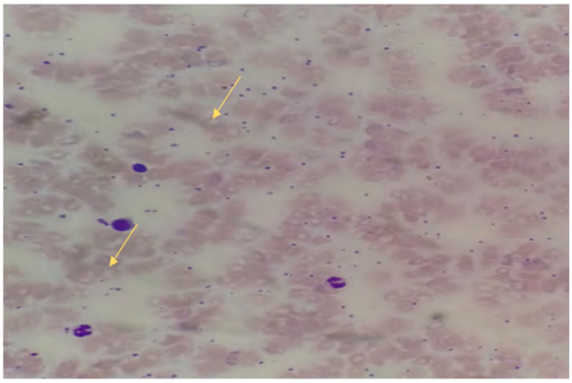

Figure 4: Patient smear with drying artefact which appears as clear punched out lesions. (Magnification x 50).

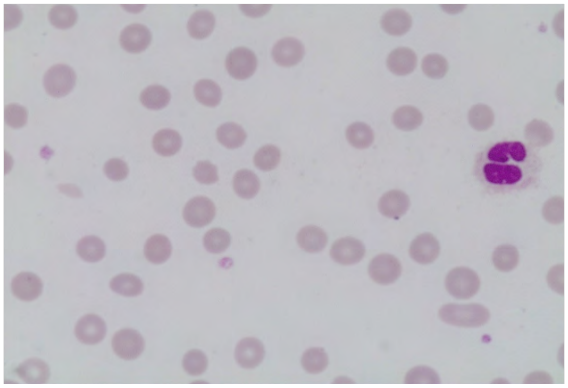

Figure 5: Repeat peripheral blood film properly processed with resolution of drying artefact and basophilic stippling artefact. (Magnification x 50).

Other tests and results relevant to the case were

- Coombs test: negative (repeated twice)
- LDH: 231
- Haptoglobin: 0.15g/L

- Ferritin 1684
- Antibody screen: negative
- Complement screen: negative
- PNH screen: Negative
- TSH: Normal
- Creatinine 231
- Absolute reticulocyte count: Slightly elevated
- Bone marrow biopsy: hyper cellular with erythroid hyperplasia and dysplasia in the erythroid and granulocytic series (Figures 6 and 7).
- Cytogenetics showed a normal female karyotype

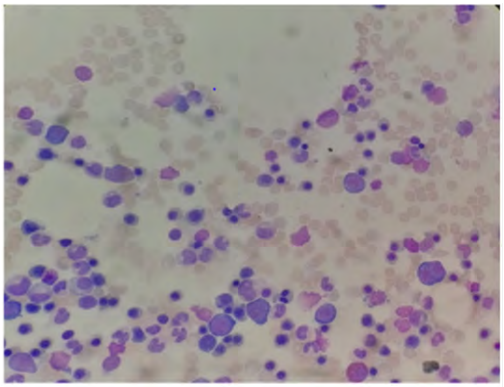

Figure 6: Bone marrow biopsy, Bone marrow aspirate showing erythroid hyperplasia, (Magnification x 500).

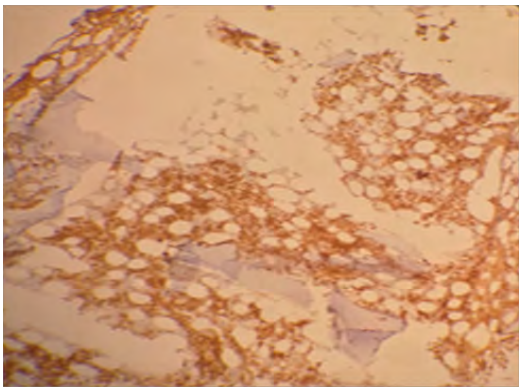

Figure 7: Glycophorin stain (brown staining) highlighting increased erythroid precursors in the marrow. (Magnification x 50).

A PAS (periodic acid Schiff) stain was also performed and showed fine granular staining on erythroid precursors. Normal erythroid cells are PAS negative. Positivity is seen in disease states such erythro leukemias, acute lymphoblastic leukemias, Thalassemia, certain lymphomas etc [2]. A diagnosis of MDS with multilineage dysplasia with possible underlying low grade hemolysis was made. The patient is managed by the clinical hematologists.

Discussion

Blood smear assessment in a patient investigated for a haematological problem forms a fundamental step in overall patient health assessment. Abnormal red cell morphology can suggest a variety of haematological conditions acquired, inherited and clonal.

Artefacts on peripheral blood smears may come from improper processing of smears and can be mistaken for real pathology. Drying artefact results if a smear is not air dried sufficiently before it is stained. The artefact is recognised in red blood cells as round to crescent shaped punched out regions or refractile vacuole like structures. The eosin component of the stain precipitates around red blood cell areas that are inadequately dried creating an artefact that could be mistaken for basophilic stippling, erythroparasites or other cytoplasmic inclusions [7].

Conclusion

Improper processing of smears leads to a waste of time trying to find a cause for an assumed pathology and suggestions of unnecessary further investigations to the clinicians which can be costly with severe negative impact in centres with budgetary constraints. This can lead to improper management of patients with devastating consequences. This case was a learning curve for technologists, technicians and registrars in my laboratory and validated the fact that Standard Operating Procedures (SOPSs) should always be adhered to by the laboratory staff to yield accurate and reliable results.

Declarations

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

Availability of data and materials: National Health Laboratory Services, Laboratory Information System (LIS).

Patient consent to a written case report has been obtained.

Conflict of Interest

None

References

1. Bain J B, Bates I, Laffan MA (2017) Dacie and Lewis Practical Haemalology, (12th edn). Elsevier, Amsterdam, Netherlands.
2. Bone Marrow SOP Number HAE1301: Cytochemistry. National Health Laboratory Services.
3. Knollmann-Ritschel BEC, Markowitz M (2017) Lead Poisoning. Acad Pathol 4: 2374289517700160.
4. Chan NCN, Chan KP (2017) Coarse basophilic stippling in lead poisoning. Blood 129: 3270.
5. Vander Meeren S, Van Damme A, Jochmans K (2015) Prominent basophilic stippling and hemochromatosis in glucose-6-phosphate dehydrogenase deficiency. Int J Hematol 101: 112-113.
6. Bain BJ (2006) Blood Cells: A Practical Guide, Fourth Edition . Wiley-Blackwell, New Jersey, United States.
7. Walker D (2016) Veterian Key: Fastest Veterinary Medicine Insight Engine. Chapter 26. Peripheral Blood Smears.

Journal of Anesthesia & Clinical Care

Journal of Addiction & Addictive Disorders

Advances in Microbiology Research

Advances in Industrial Biotechnology

Journal of Agronomy & Agricultural Science

Journal of AIDS Clinical Research & STDs

Journal of Alcoholism, Drug Abuse & Substance Dependence

Journal of Allergy Disorders & Therapy

Journal of Alternative, Complementary & Integrative Medicine

Journal of Alzheimer's & Neurodegenerative Diseases

Journal of Angiology & Vascular Surgery

Journal of Animal Research & Veterinary Science

Archives of Zoological Studies

Archives of Urology

Journal of Atmospheric & Earth-Sciences

Journal of Aquaculture & Fisheries

Journal of Biotech Research & Biochemistry

Journal of Brain & Neuroscience Research

Journal of Cancer Biology & Treatment

Journal of Cardiology: Study & Research

Journal of Cell Biology & Cell Metabolism

Journal of Clinical Dermatology & Therapy

Journal of Clinical Immunology & Immunotherapy

Journal of Clinical Studies & Medical Case Reports

Journal of Community Medicine & Public Health Care

Current Trends: Medical & Biological Engineering

Journal of Cytology & Tissue Biology

Journal of Dentistry: Oral Health & Cosmesis

Journal of Diabetes & Metabolic Disorders

Journal of Dairy Research & Technology

Journal of Emergency Medicine Trauma & Surgical Care

Journal of Environmental Science: Current Research

Journal of Food Science & Nutrition

Journal of Forensic, Legal & Investigative Sciences

Journal of Gastroenterology & Hepatology Research

Journal of Gerontology & Geriatric Medicine

Journal of Genetics & Genomic Sciences

Journal of Hematology, Blood Transfusion & Disorders

Journal of Human Endocrinology

Journal of Hospice & Palliative Medical Care

Journal of Internal Medicine & Primary Healthcare

Journal of Infectious & Non Infectious Diseases

Journal of Light & Laser: Current Trends

Journal of Modern Chemical Sciences

Journal of Medicine: Study & Research

Journal of Nanotechnology: Nanomedicine & Nanobiotechnology

Journal of Neonatology & Clinical Pediatrics

Journal of Nephrology & Renal Therapy

Journal of Non Invasive Vascular Investigation

Journal of Nuclear Medicine, Radiology & Radiation Therapy

Journal of Obesity & Weight Loss

Journal of Orthopedic Research & Physiotherapy

Journal of Otolaryngology, Head & Neck Surgery

Journal of Protein Research & Bioinformatics

Journal of Pathology Clinical & Medical Research

Journal of Pharmacology, Pharmaceutics & Pharmacovigilance

Journal of Physical Medicine, Rehabilitation & Disabilities

Journal of Plant Science: Current Research

Journal of Psychiatry, Depression & Anxiety

Journal of Pulmonary Medicine & Respiratory Research

Journal of Practical & Professional Nursing

Journal of Reproductive Medicine, Gynaecology & Obstetrics

Journal of Stem Cells Research, Development & Therapy

Journal of Surgery: Current Trends & Innovations

Journal of Toxicology: Current Research

Journal of Translational Science and Research

Trends in Anatomy & Physiology

Journal of Vaccines Research & Vaccination

Journal of Virology & Antivirals

Archives of Surgery and Surgical Education

Sports Medicine and Injury Care Journal

International Journal of Case Reports and Therapeutic Studies

Journal of Ecology Research and Conservation Biology

Submit Your Manuscript: http://www.heraldopenaccess.us/Online-Submission.php

Herald Scholarly Open Access, 2561 Cornelia Rd, #205, Herndon, VA 20171, USA.
Tel: +1 202-499-9679; E-mail: info@heraldsopenaccess.us
http://www.heraldopenaccess.us/