NORMAL PLATELET COUNT IN THE HIV POSITIVE PREGNANT PATIENT

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Submission in fulfilment of the requirement in respect of the Master's Degree MMed in the Department of Obstetrics and Gynaecology in the Faculty of Health Science at the University of the Free State.

SUBMISSION:

24 April 2019

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I, Bianca van Wyngaard, declare that the coursework Master's Degree mini-dissertation that I herewith submit in a publishable article format for the Master's Degree qualification MMed at the University of the Free State is my independent work, and that I have not previously submitted it for a qualification at another institution of higher education.

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ABSTRACT

Background. Thrombocytopenia complicates 6.6% – 11.6% of all pregnancies. Gestational Thrombocytopenia accounts for the majority of cases, it is usually mild with spontaneous resolution postpartum. It is estimated that more than 25% of pregnant South African patients are HIV positive.

Objectives. The objective of the study was to determine the platelet count for HIV positive pregnant patients. This will lead to minimizing unnecessary invasive testing and workup for other pathologies.

Method. This was a prospective descriptive study in which low and mild risk pregnant patients with WHO stage 1 HIV disease were recruited for participation. In patients that needed routine blood tests drawn, a platelet count was added. Patients were either first visit patients or following up for routine antenatal care.

Results. For all participants (n = 120), the mean platelet count was 270.9 x 10^9/L (range 91 – 488). For first trimester participants (n = 37), the mean platelet count was 282.3 x 10^9/L (range 103 – 441). For the second trimester participants (n = 28), the mean platelet count was 263.7 x 10^9/L (range 91 - 470). For the third trimester participants (n = 34), the mean platelet count was 260.2 x 10^9/L (range 99 - 488). For the participants where no trimester was indicated (n = 21), the mean platelet count was 278.1 x 10^9/L (range 181 - 426).

Discussion and Conclusion. Stage 1 HIV does not have a clinical significant impact on the platelet count in pregnant patients.

KEYWORDS

Thrombocytopenia, Pregnancy, HIV, Platelet

LIST OF ABBREVIATIONS

ADAMTS 13	A disintegrin and metalloproteinase with thrombospondin type 1 motif, member 13
AFLP	Acute Fatty Liver of Pregnancy
AIDS	Acquired Immunodeficiency Syndrome
AKI	Acute Kidney injury
APLS	Antiphospholipid Syndrome
aPTT	activated Partial Thromboplastin Time
AST	Aspartate Transaminase
DNA	Deoxyrubunucleac Acid
DIC	Disseminated Intravascular Coagulopathy
FFP	Fresh Frozen Plasma
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HELLP	Haemolysis, Elevated Liver Enzymes, Low Platelets
HIV	Human Immunodeficiency Virus
HUS	Haemolytic Uraemic Syndrome
ICU	Intensive Care Unit
IgG	Immunoglobulin G
ITP	Immune Thrombocytopenic Purpura
IVIG	Intravenous Immunoglobulin
LDH	Lactate Dehydrogenase
LMWH	Low Molecular Weight Heparin
PHAT	Primary HIV – associated Thrombocytopenia
Plt	Platelet(s)
PRBC	Packed Red Blood Cells
PT	Prothrombin Time
PTT	Partial Thromboplastin Time
SLE	Systemic Lupus Erythematosus
TGF beta	Transforming Growth Factor Beta
TTP	Thrombotic Thrombocytopenic Purpura
VWF	Von Willebrand Factor

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CHAPTER ONE

LITERATURE REVIEW

INTRODUCTION

Thrombocytopenia is defined as a platelet count of less than 150 x 10^9/L, and it is the second most common hematological abnormality encountered during pregnancy. It is estimated that 6.6 – 11.6% of all pregnancies are affected.[2] Thrombocytopenia in pregnancy is part of the physiological phenomenon of the dilutional effect due to the increase in plasma volume and consumption by aggregation of platelets in peripheral tissues.[1]

Thrombocytopenia of <100 x 10^9/L is encountered in only 1% of pregnancies. It remains the task of the Obstetrician to determine the pathophysiological nature of the thrombocytopenia in order to determine the risks posed to the mother and the fetus. Treatment goals change as the pregnancy progresses and particularly before and during delivery.[1,2,3]

PLATELET PHYSIOLOGY

Megakaryocytes are precursor cells derived from stem cells in the bone marrow. Platelets are formed from the fragmentation of megakaryocytes into smaller cell fragments (figure 1). They form an integral part in the process of coagulation after blood vessel injury.

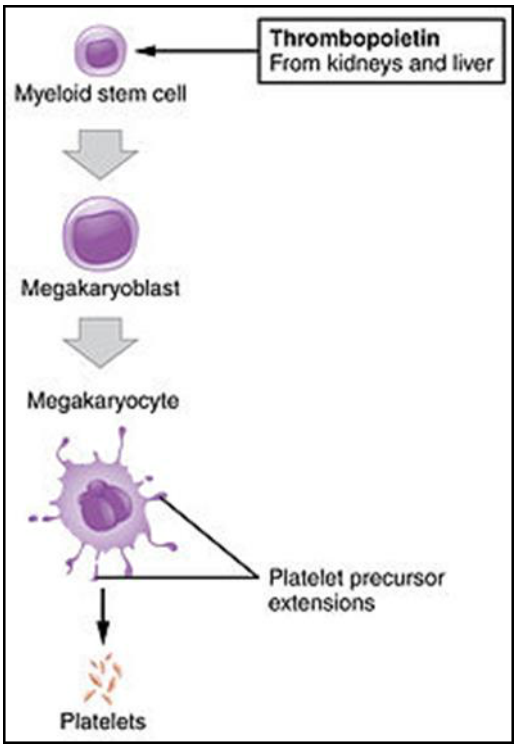

Figure 1. Illustration of normal platelet development (illustration available from URL: https://za.pinterest.com/pin/553450241685541930/?autologin=true).

PLATELET STRUCTURE AND DISTRIBUTION

Thrombocytes are irregular in shape, are without nuclei and measure 2 - 3 µm in diameter (Figure 2). They are classified as cell fragments due to the absence of a nucleus, but they do have mitochondria and mitochondrial DNA as well as endoplasmic reticulum and granules from the initial megakaryocyte.[12]

Adhesive proteins within the platelet give them the ability to adhere to fibrin mesh, vascular endothelium, microtubule and microfilament skeleton that extends into filament during activation secondary to vessel injury. Platelets account for <1% of whole blood.

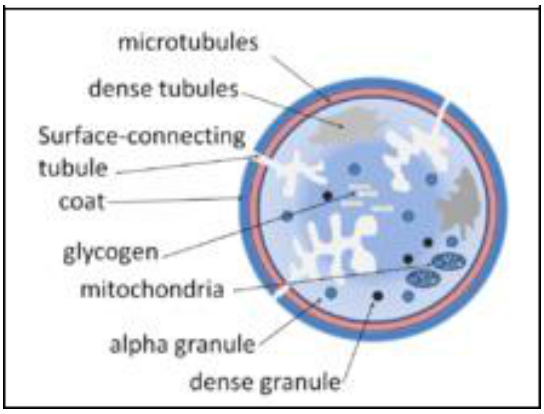

Figure 2. Illustration of normal platelet structure (illustration available from URL: https://en.wikipedia.org/wiki/Platelet).

PLATELET FUNCTION

Platelets circulate in the plasma where their primary function is coagulation with secondary wound healing properties. Vessel injury leads to adherence of platelets to the fibrin mesh that forms a platelet plug and results in clotting of the blood. Once a blood clot has formed, wound healing can start.

Substances involved in coagulation and wound healing are released from the activated platelets. For coagulation these substances include the following:

Thromboxane A: Increased local aggregation on platelets.

Serotonin: Mediates the inflammatory response.

Thromboplastin: Increases thrombin and fibrin levels that promote coagulation.

For wound healing the following growth factors are released:

Platelet-derived growth factor: Directs cell movement.

TGF beta: Deposition of extracellular matrix into the affected area.

Vascular endothelial growth factor: Regeneration of new blood vessels.

Having mentioned the above, it is clear that thrombocytopenia has a significant effect on both coagulation and wound healing.

PLATELET FORMATION

Platelets are produced continuously from megakaryocytes. Protoplatelets are formed within the cytoplasm of the megakaryocytes which then fragment into small irregular cells called platelets. In response to thrombocytopenia, the liver and kidneys release the hormone thrombopoietin, which stimulates precursor cells within the megakaryocyte to release platelets. Platelet levels regulate the production of thrombopoietin by a process of negative feedback.

Between 5000 and 10 000 platelets are produced from each megakaryocyte before its cellular components are depleted and then consumed by macrophages. In normal physiological circumstances each adult produces around 10^{11} platelets daily with a lifespan of between 5 – 10 days. Old platelets are destroyed in the liver and the spleen. The spleen is the reservoir for up to 40% of platelets. The release of platelets is mediated by splenic muscle contraction in response to severe vessel injury.[12]

For the purpose of this article we will focus on disease processes, particularly in pregnancy, that result in thrombocytopenia.

ETIOLOGY OF THROMBOCYTOPENIA

Table 1 contains the specific etiological factors involved in thrombocytopenia

Table 1: Etiological factors involved in thrombocytopenia

Pregnancy Specific	**Non – Pregnancy Specific**
Isolated: Gestational Thrombocytopenia	**Isolated:** Primary ITP Secondary ITP Drug induced Thrombocytopenia Type IIB Von Willebrand's Disease Congenital
Systemic Disorders: Preeclampsia HELLP Syndrome Acute Fatty liver of Pregnancy	**Systemic Disorders:** TTP/HUS SLE APLS Viral infections – HIV Bone Marrow Disorders Nutritional Deficiency DIC

GESTATIONAL THROMBOCYTOPENIA[2, 3]

Gestational thrombocytopenia is a physiological phenomenon which occurs in the second and third trimester of pregnancy. It accounts for about 70 – 80% of cases of isolated thrombocytopenia in pregnancy.[1] The platelet count is usually between 130 – 150 x 10^9/L. In less than 10% of cases the platelet count drops below 100 x 10^9/L. The patient will either have no history of previous thrombocytopenia or she will report thrombocytopenia during her previous pregnancy. Gestational thrombocytopenia usually resolves spontaneously within eight weeks postpartum with no fetal or neonatal involvement. The optimal mode of delivery remains a normal vaginal delivery unless an obstetric indication for a caesarean section arises. Gestational thrombocytopenia is a diagnosis of exclusion.

IMMUNE THROMBOCYTOPENIC PURPURA[2,3]

Immune Thrombocytopenic Purpura (ITP) accounts for 3% of cases of isolated thrombocytopenia in pregnancy. It is an autoimmune disorder whereby antiplatelet antibodies stimulate platelet destruction in the spleen.[1]

Diagnosis of ITP is made on a clinical basis:

- Moderate thrombocytopenia, usually <100 x 10^9/L.
- Symptoms in direct relation to the platelet level.
- Asymptomatic or ecchymosis, petechia, purpura or gum bleeding.
- Prior history of thrombocytopenia or other immune-mediated diseases.
- Does not resolve spontaneously postpartum.
- Therapeutic response to steroids/ intravenous immunoglobulin.

As a rule of thumb, ITP usually presents with platelets less than 100 x 10^9/L during early pregnancy and declines as the pregnancy progresses. Table 2 illustrates the differences between Gestational Thrombocytopenia and ITP.

Pregnancy does not affect the course of ITP, but the thrombocytopenia may cause considerable anxiety, especially around the time of delivery with regards to the possibility of severe

haemorrhage. The antiplatelet IgG crosses the placenta and causes fetal thrombocytopenia. The risk of the fetal platelet count being below 50 x 10^9/L is 5 - 10%, and may be higher if the patient had ITP prior to pregnancy or if she was symptomatic during the index pregnancy. There is a 0 - 1.5% risk of antenatal or neonatal intracranial haemorrhage with delivery. The best predictor of severe neonatal thrombocytopenia is a previously affected child.[1,4,5]

Management

MATERNAL CONSIDERATIONS[3,4]

It is important to first exclude other associated autoimmune conditions like SLE or APLS as part of the diagnostic process. If ITP is confirmed, patients require monthly platelet counts and then more frequently, as delivery approaches, to decide when to initiate treatment. Spontaneous maternal haemorrhaging remains a risk. However, prior to the third trimester, therapy is only indicated in the following cases:

- Bleeding.
- Platelet count below 20 x 10^9/L.
- Prior to any invasive procedures.

Prior to 36 weeks' gestation, steroids or intravenous immunoglobulin can be administered as treatment. The goal for delivery is a platelet level of at least 50 x 10^9/L, which is considered safe for both vaginal and caesarean delivery.[1] For the safe administration of regional anaesthesia, a platelet level above 75 x 10^9/L is generally required.[1] There is no contraindication to vaginal delivery, with caesarean section only recommended per obstetric indication.

Corticosteroids

Prednisone may be administered at a dose of 1 mg/kg in daily doses. It generally takes between 2–14 days to get a response and is thus not sufficient for the acute setting.

Intravenous Immunoglobulin

Intravenous immunoglobulin is indicated in resistant cases, in the acute setting, where prolonged therapy is required or when high maintenance doses of Prednisone is needed. At a

dose of 1 g/kg the response is rapid; usually within 1 – 3 days. It delays the clearance of IgG-coated platelets from the circulation, thus lasting for 2 – 3 weeks.

Anti-D immunoglobulin

Anti-D immunoglobulin is indicated in Rh+ women who have not had a splenectomy. It competitively inhibits the destruction of antibody–coated platelets. It is given at a dose of 50 – 70 mcg/kg and is safe and effective in the second half of pregnancy. Effects in the neonate include neonatal jaundice, anaemia, and a positive direct antiglobulin test.

Splenectomy:

Splenectomy in pregnancy is only indicated in severe cases and can be performed with safety during the second trimester. Minimally invasive techniques are still acceptable during the second trimester.

Previous Splenectomy:

In patients who have had a previous splenectomy, penicillin prophylaxis needs to be continued throughout pregnancy.

Platelet transfusion:

Transfusion of platelets would be the last line of treatment and is indicated in cases of active bleeding or before any invasive procedure or surgery with platelet counts of less than 50 x 10^9/L. In any other situation, it should be avoided as the transfusion of platelets increases the antibody titres in patients with ITP.

Analgesia:

ITP has an increased risk of postpartum haemorrhage in the 48 hours following birth. Drugs that inhibit platelet function, such as Ibuprofen, should best be avoided during this time.[2]

FETAL CONSIDERATIONS[4]

Towards the end of the third trimester the placental transfer of IgG to the fetus increases. During the antenatal period there is no increased risk for fetal haemorrhage and no attempt should be made to determine fetal platelet levels.[2,4] At the time of delivery, cord blood needs

to be taken for baseline platelet counts. The neonatal platelet count may only reach a nadir 2 – 5 days post-delivery so continuous monitoring is indicated as the risk for neonatal haemorrhaging persists.[2,4] Intravenous Immunoglobulin is indicated in the event of bleeding, severe thrombocytopenia or prophylactically if the cord platelet count is less than 20 x 10^9/L.

Table 2: Differential diagnosis of Gestational thrombocytopenia versus ITP[3]

Characteristic	Gestational Thrombocytopenia	ITP
Onset during Pregnancy	Mid to late trimester 2 and trimester 3 Frequency increased closer to term	Anytime
Evidence for alternate etiologies	No	No
Platelet count	> 50 x 10^9/L Progressively decreases closer to term	Any count < 100 x 10^9/L
Thrombocytopenia outside Pregnancy	No	Possible
Neonatal Thrombocytopenia	No	Possible (10% platelets < 50 x 10^9/L)
Postpartum resolution	Yes	Possible

THROMBOTIC MICROANGIOPATHIES OF PREGNANCIES

Thrombotic microangiopathies of pregnancy results from thrombotic occlusion of the microvasculature leading to red cell fragmentation, profound thrombocytopenia and macroangiopathic haemolytic anaemia with elevated LDH levels and negative Direct Coombs tests.

Pre eclampsia, HELLP Syndrome and Acute Fatty Liver of pregnancy, have overlapping clinical and laboratory features with thrombotic microangiopathies not specific to pregnancy.[3]

PREECLAMPSIA[3]

The diagnosis of preeclampsia is made with the new onset of hypertension after 20 weeks' gestation. It is usually accompanied by proteinuria of more than 300 mg/day, but this is not

mandatory for the diagnosis. 15 – 25% of patients with gestational hypertension develop preeclampsia. It may also develop during the postpartum period, usually within 4–6 weeks.

Preeclampsia is a multisystem disease specific to pregnancy. It causes widespread circulatory disturbances involving among others the coagulation system. It is the second most common cause of thrombocytopenia in the late second and third trimester of pregnancy. And it is estimated to be responsible for 21% of thrombocytopenia cases at delivery. Thrombocytopenia may be the initial manifestation. Less than 5% of patients with preeclampsia will have a platelet count lower than 50 x 10^9/L. Coagulation abnormalities are unlikely if the platelet count is more than 100 x 10^9/L.

The pathophysiological process could either be that of a genetic predisposition, as the risk for development of preeclampsia is increased three fold in women with a first degree relative with a history of preeclampsia, or, secondly, due to abnormal placentation resulting in placental ischaemia leading to the clinical syndrome of preeclampsia.

The diagnosis of preeclampsia is made with the recognition of signs and symptoms, there is no specific diagnostic test. Table 3 illustrates the clinical features used in the diagnosis of preeclampsia.

Table 3: Clinical features of preeclampsia. Reproduced from Nelson-Piercy (2015)

SYMPTOMS	Headache/Flashing light Epigastric/right upper quadrant pain Nausea/vomiting Rapidly increasing/severe swelling of face, fingers or legs
SIGNS	Pregnancy-induced hypertension New onset proteinuria Rapidly progressive oedema Epigastric/right upper quadrant tenderness Convulsions, mental disorientation Fetal growth restriction/ Intrauterine death

	• Placental abruptio
INVESTIGATIONS	□ 24-hour urinary protein excretion >0.3g □ Protein creatinine ration (PCR) >30mg/mmol □ Thrombocytopenia □ Prolonged clotting times (if concomitant DIC in HELLP syndrome) □ Raised serum creatinine □ Increased haematocrit and haemoglobin levels □ Anaemia if haemolysis, associated with raised LDH and bilirubin □ Abnormal liver function tests, particularly raised transaminases □ Reduced foetal growth, oligohydramnios □ Abnormal uterine artery Doppler (bilateral notches and increased resistance/pulsatility index at 24 weeks predict preeclampsia) □ Abnormal umbilical artery Doppler (reduced, absent or reversed end diastolic flow indicating foetal compromise) □ Low placental growth factor (PIGF) (reduced in preeclampsia and predictive of delivery for preeclampsia within 2 weeks)

Antenatal care is directed at detecting women that may be asymptomatic initially by measuring blood pressure and testing urine for the development of proteinuria.

Management of preeclampsia comprises of the following:

- Monitoring of preeclampsia.
- Treatment of hypertension.
- Fetal surveillance.

- Decision regarding delivery.

HELLP SYNDROME[2, 3, 4]

HELLP Syndrome is an acronym for **H**aemolysis, **E**levated **L**iver Enzymes and **L**ow **P**latelets. 10 – 20% of patients presenting with preeclampsia go on to develop HELLP Syndrome. In 15 – 20% of patients with HELLP Syndrome there was no antecedent hypertension or proteinuria.

Clinical features of HELLP syndrome include:

- Epigastric of right upper quadrant pain
- Nausea and vomiting
- Tenderness in the right upper quadrant
- Hypertension – proteinuria not necessarily present
- Other features of preeclampsia
- Acute renal injury
- Abruptio placenta
- Metabolic acidosis

Martin et al. (2006) proposes the following criteria for the diagnosis of HELLP Syndrome:[3]

- LDH > 600 U/L
- AST > 70 U/L
- Plt < 100 x 10^9/L

The severity of HELLP syndrome is classified by the level of thrombocytopenia, according to the Mississippi-Triple Class System, as it is the first coagulation abnormality to present.[2] In severe cases the platelet count falls below 30 x 10^9/L and 20% of women may develop DIC.[4] Profound thrombocytopenia of < 10 x 10^9/L, is unusual in preeclampsia and HELLP syndrome. 70% of patients develop this syndrome antenatally, and 30% only develop these signs during the postpartum period.

The haemolysis developing in HELLP syndrome has features of macroangiopathic haemolytic anaemia, including:

- Red Cell fragmentation (schistocytes)
- Elevated serum bilirubin (> 1.2 mg/dl)
- Low serum haptoglobin levels (≤ 25 mg/dL)
- Elevated LDH (≥ 600 IU/L).

It is important to differentiate HELLP Syndrome from TTP/HUS, since the first line of management of HELLP syndrome will be prompt delivery or termination of the pregnancy rather than plasmapheresis. According to Nelson-Piercy (2015) TTP and HUS are both rare compared to HELLP syndrome. Abnormal liver function and coagulopathy suggest HELLP rather than TTP, even in the presence of frank heamolysis.[4]

Co-existence of AKI is well recognized in HELLP syndrome and does not necessarily imply a diagnosis of HUS. As the conditions are closely related, HUS may evolve from HELLP syndrome. Patients usually recover rapidly from HELLP Syndrome with no hepatic impairment. The thrombocytopenia takes longer to normalize than the liver enzymes.[4]

ACUTE FATTY LIVER OF PREGNANCY[3, 4, 5]

Acute Fatty Liver of Pregnancy is a life-threatening condition that typically develops in the third trimester of pregnancy. It affects 1 in 7000 to 1 in 20 000 pregnancies.[4] There is an association with male fetuses and multiple pregnancies in the development of AFLP. 50% of patients presenting with AFLP meet the criteria of preeclampsia and it overlaps with HELLP syndrome. [2] The pathophysiology of AFLP is a variant of preeclampsia.

Clinically the patients present with severe vomiting and abdominal pain. It should result in a high index of suspicion by the medical team. Jaundice and pruritis are typical symptoms, but they generally appear two weeks after the onset of symptoms. In severe cases, patients develop hepatic encephalopathy. Due to hepatic failure patients are at high risk of severe hypoglycaemia, which affects about 70% of patients. DIC affects about 90% of patient with AFLP, which might be the presenting feature postpartum.

LABORATORY INVESTIGATIONS:[2]

The following laboratory features are indicative of AFLP:

- Normochromic, normocytic anaemia
- No/mild evidence of macroangiopathic haemolysis
- Elevated WCC
- Thrombocytopenia
- Elevated serum transaminase (constant feature)
- Conjugated Bilirubin levels > 5 mg/dL
- Other common features include:
    - Metabolic acidosis
    - Raised creatinine
    - Hypoglycaemia
    - High ammonia
    - Concomitant pancreatitis with elevated amylase and lipase.

MANAGEMENT:[4]

The management relies on prompt delivery of the fetus with subsequent improvement in maternal and fetal prognosis. The involvement of a multidisciplinary team approach is mandatory to ensure a good maternal outcome. Critically ill patients necessitate admission to an intensive care facility, around 65% of cases may require ICU admission and up to 7% may require ventilatory support. Early liaison with a gastroenterology team is advised in patients with hepatic failure and encephalopathy. The associated coagulopathy has to be treated prior to delivery of the foetus by the administration of Fresh Frozen Plasma and vitamin K 10 mg IVI. Hypoglycaemia should be corrected prior to delivery. Often large volumes of 10% or 50% dextrose is required.

The best markers for the severity of AFLP include:

- Increased PT
- Hypoglycaemia
- Acidosis and raised lactate

- Encephalopathy
    - ask the patient to draw a clock face or a 5-point star
    - asterixis

There should be a low threshold for the administration of antibiotics as ASLP carries a significant sepsis risk. In the event of Diabetes Insipidus with polyuria, Desmopressin intranasally or subcutaneously should be administered. Repeat doses are indicated once urine production exceeds 400 ml/h, serum Na > 140 mmol/L of plasma osmolality > 290 mOsmol/L.

AFLP usually resolves within 2 – 3 day postpartum, but patients may deteriorate up to one week post-delivery. It is expected that the platelet count should increase by day four postpartum and reach levels of 100 by day six post-delivery. The mainstay of treatment for all thrombotic microangiopathies of pregnancy is delivery of the foetus.

THROMBOTIC THROBOCYTOPENIC PURPURA/HAEMOLYTIC URAEMIC SYNDROME[1,4,11]

TTP/HUS is a continuum with both manifesting due to microvascular platelet aggregation. The clinical features are thrombocytopenia and microangiopathic haemolytic anaemia. However, both are rare occurrences in pregnancy and the puerperium.

HUS[4,11]

HUS is defined by haemolytic anaemia, thrombocytopenia and acute renal failure.[11] The clinical features overlap with that of pre-eclampsia and the clinical difference is based on the acute renal failure associated with HUS. Hypertension is not common in the presentation of patients with HUS. Haemolytic anaemia is identified by low haptoglobin levels, high LDH levels and schistocytes.[11] The incidence is only around 1 in 25 000 pregnancies with variable timing of onset. Only a minority of cases are seen in the first trimester.

Early recognition and diagnosis is essential as in most fatal cases, death occurs within 24 hours from presentation. Thrombocytopenia and macroangiopathic haemolytic anaemia in the absence of an obvious precipitating condition should be classified as TTP/HUS.

LABORATORY FINDINGS:[3]

The laboratory features of HUS include:

- Macroangiopathic haemolytic anemia
- Negative direct antiglobulin test
- Normal coagulation screen

There are two forms of HUS described, typical and atypical.

Typical:

Infection with Shiga-toxin producing E.Coli. It is the most common occurring form of HUS in children and accounts for approximately 90% of cases. There is typically a history of bloody diarrhoea.

Atypical:

Associated with congenital defects in the complement pathway. ADAMTS 13 deficiency and intracellular defect of vitamin B12 metabolism have been attributed to the development of atypical HUS.[11] The atypical form of HUS is usually associated with pregnancy and occurs mostly postpartum.

MANAGEMENT:

The outcome of the disease is not affected by delivery of the fetus. The first line of treatment is the administration of Fresh Frozen Plasma and plasmapheresis. Plasmapheresis is continued until platelets and LDH levels have normalized.[1] Dialysis may be required for the acute kidney injury.

THROMBOTIC THROMBOCYTOPENIC PURPURA[1, 2, 4]

Two types of TTP have been identified, namely familial and non-familial.

Familial Type:

The familial type is caused by a hereditary deficiency of the von Willebrand cleaving protease ADAMTS13.

Non-familial Type:

Non-familial TTP is caused by the formation of anti–ADAMTS13 antibodies. ADAMTS 13 cleaves the von Willebrand factor and prevents thrombosis by impaired platelet aggregation.[1]

There is a deficiency for ADAMTS13 in DIC, HUS, preeclampsia and HELLP syndrome. It is less than 40%, but more than 10%. Severe deficiency with <5% of activity in normal plasma, is specific for TTP. Baseline estimations of ADAMTS13 activity needs to be done prior to initiation of treatment.[1]

TTP is characterized by a Pentad:[4]

- Microangiopathic haemolytic anemia
- Marked thrombocytopenia
- Neurological deficit
- Fever
- Renal Dysfunction

MANAGEMENT:

Delivery is only indicated if TTP is associated with preeclampsia. Plasma exchange benefit exceeds the risk if the diagnosis is missed. Important is a virology screen including HIV, HBV and HCV.

Plasma exchange is done daily until platelet count exceeds 150 x 10^9 /L for three days and LDH is within normal limits. British guidelines advocate aspirin 75 mg daily and LMWH at prophylactic doses once platelets are above 50 x 10^9/L.[2]

If TTP presents in trimester one, regular plasma exchanges are necessary to ensure delivery of a live infant. The frequency of exchange transfusions will be quided by blood counts and LDH

levels. Women who are resistant to plasma exchange qualify for delivery. A plasma exchange prior to delivery will ensure adequate levels of ADAMTS13.[2]

Subsequent Pregnancies:[3]

In the case of inherited TTP the risk of relapse is 100% in the absence of prophylactic exchange transfusion. The risk of relapse with acquired TTP with severe ADAMTS13 deficiency is 20%. There are currently no reports of transmission of TTP to infants of affected mothers.

DISSEMINATED INTRAVASCULAR COAGULATION[1,4,13]

Aetiology for Disseminated Intravascular Coagulopathy (DIC) in pregnancy include:[4]

- Haemorrhage
- Preeclampsia/HELLP Syndrome
- Amniotic fluid embolism
- Sepsis, especially chorioamnionitis
- Intrauterine foetal death

Depending on the degree of the DIC, patients may either be asymptomatic or present with massive haemorrhaging.

PATHOGENESIS

Upon vessel injury, procoagulant substances are released from the injured endothelium. These substances stimulate the coagulation with increased production and destruction of coagulation factors. Subsequent consumption of coagulation factors, including platelets, leads to bleeding.

DIC has the following laboratory features:[1,4]

- Increased PT and aPTT
- Thrombocytopenia
- Decreased Fibrinogen (< 2 g/L)

- Increased fibrin degradation products
- Presence of D-dimers

MANAGEMENT

Management is directed at treatment of the underlying cause. It usually necessitates delivery of the fetus and evacuation of the uterus.

The coagulopathy is treated with the following blood products:[4]

- Fresh Frozen Plasma
- Packed Red Cells
- Platelets
- Cryoprecipitate

Rapid response with active management of precipitating event and correction of coagulation improves morbidity and decreased mortality. Obstetric Massive Transfusion Protocol is given in table 3 below.[13]

Table 3: Obstetric Massive Transfusion Protocol. Reproduced from Pacheco LD et al(2013)

	PRBC	FFP	Platelets	Cryoprecipitate
Round 1	6U	6U	6U	10U
Round 2	6U	6U		20U
Round 3	Recombinant activated factor VII 40mcg/kg			
Round 4	6U	6U	6U	10U
Round 5	6U	6U		10U
Round 6	Recombinant activated factor VII 40mcg/kg			

HIV AND THROMBOCYTOPENIA[5, 7, 8]

The prevalence of HIV infection in the South African pregnant population, is between 28% and 30%.[10] HIV is a retrovirus that invades and destroys the CD4 lymphocytes. HIV contributes to a defective immune system with an increased risk of infection and malignancy.[10] In asymptomatic HIV infection, thrombocytopenia is present in between 5 – 9% of patients but increases to up to 40% in patients with AIDS. There have been reports of a direct correlation between thrombocytopenia and the CD4 counts and an accelerated progression to AIDS with a poorer prognosis in those patients with thrombocytopenia.

PATHOPHYSIOLOGY

There are two mechanisms involved in the development of thrombocytopenia in HIV positive individuals.

1) Primary HIV - associated thrombocytopenia (PHAT) occurs during all stages of the disease, most commonly early in the process. Immune-mediated destruction of platelets accounts for PHAT. Anti-HIV antibodies cross react with the platelet membrane glycoproteins.[8] During the later stages of the disease, defective platelet production contributes to thrombocytopenia.

2) HIV infection has a direct effect on the megakaryocytes in the bone marrow, where it causes apoptosis and destruction of megakaryocytes.

ALGORITHM BASED ON BLOOD FILM[3]

To assist in the differential diagnosis of the cause of thrombocytopenia based on the blood film, an algorithm was developed by Gernsheimer T, James AH, Stasi R(figure 4).

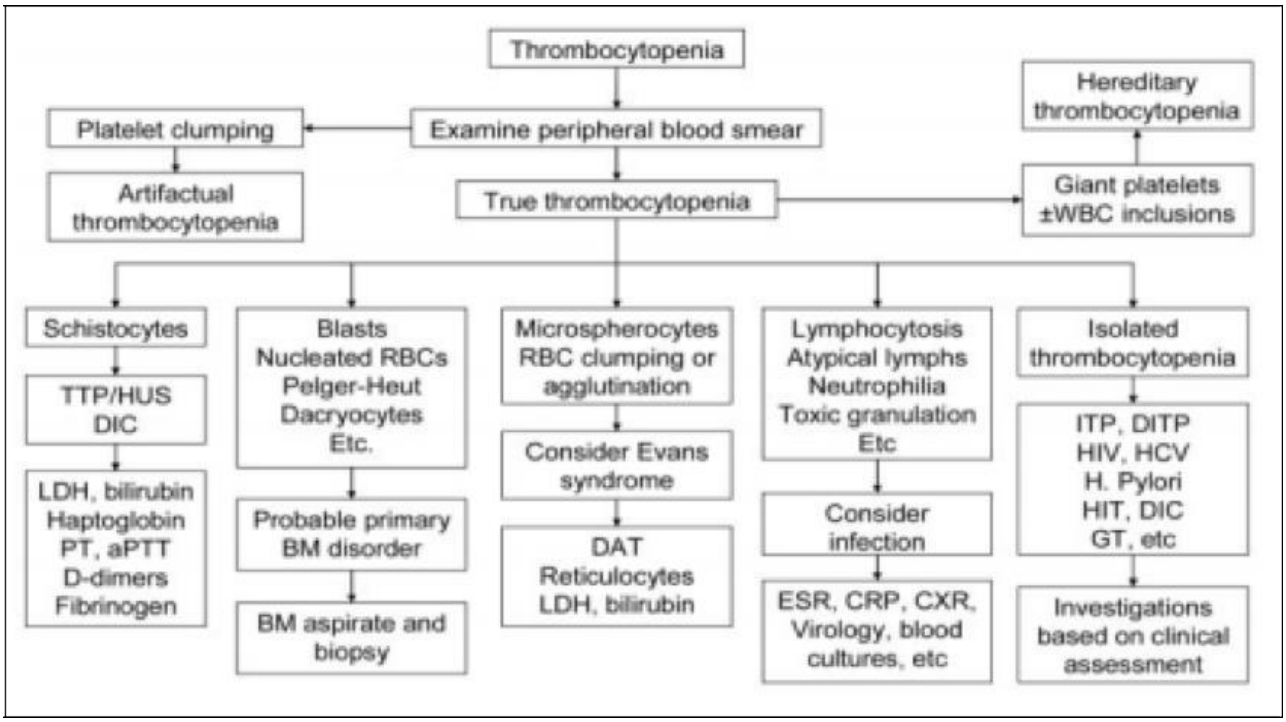

Figure 4. Algorithm based on blood film. Reproduced from Gernsheimer T, James AH, Stasi R (2013).

CONCLUSION

Thrombocytopenia is a relatively common occurrence during pregnancy and diagnostic workup is essential for appropriate treatment and minimizing the risk of bleeding for both the mother and the fetus.

Diagnosis depends on the timing of onset, the severity of the thrombocytopenia and the association with other abnormalities. The diagnosis and management require optimal interdisciplinary collaboration.

But what is the normal platelet count for a HIV positive pregnant patient? Currently there is limited data available for normal platelet count in HIV positive pregnant patients. Knowing the normal platelet count will guide investigation and thus limit unnecessary resource use.

Therefore, the primary objective of this study is to determine the normal platelet count for pregnant patients who are HIV positive. The secondary objectives are to determine the difference in the average platelet count in the different trimesters and possible motivation for routine platelet count screening in HIV positive pregnant individuals.

REFERENCES:

1. Ciobanu AM, Colibaba S, Cimpoca B, Peltecu G, Panaitescu AM. Thrombocytopenia in Pregnancy. MAEDICA – a Journal of Clinical Medicine 2016; 11(5): 55 - 60
2. Bergman F, Rath W. The Differential Diagnosis of Thrombocytopenia in Pregnancy - an interdisciplinary challenge. Dtsch Arztebl Int 2015; 112: 795 - 802
3. Gernsheimer T, James AH, Stasi R. How I treat thrombocytopenia in pregnancy. Blood 2013; 121(1): 38 - 47
4. Nelson-Piercy, C. Handbook of Obstetrics Medicine. Haematological Problems. 5th ed. CRC press; 2015: 269 - 272
5. Sebitloane HM, Thrombocytopenia during pregnancy in women with HIV infection receiving no treatment. South African Medical Journal 2016; 106(2): 210-213.
6. Vaughan J, Wiggil T, Munster M, Immature platelet fraction levels in a variety of bone marrow pathologies in South African HIV-positive patients with thrombocytopenia. Hematology 2014 Vol. 19 No. 7
7. O'Bryan TA, Okulics JF, Bradley WP, Ganesan A, Wang X and Agan BK, Impact of the highly active antiretroviral therapy era on the epidemiology of primary HIV thrombocytopenia. O'Bryan et al. BMC Res Notes (2015) 8: 595
8. Wondimeneh Y, Muluye D, Ferede G, Prevalence and associated factors of thrombocytopenia among HAART naïve HIV positive patients at Gondor university hospital, northwest Ethiopia. BMC Research Notes 2014 7:5
9. Franzetti M, Adorni F, Oreni L, Van Den Bogaart L, Resnatti C, Milazzo L, Antinori S, Galli M and Ridolfo A, Changes in the Incidence of Severe Thrombocytopenia and its predisposing Conditions in HIV-infected Patients Since the Introduction of Highly Active Antiretroviral Therapy. J Acquired immune Defic Syndr 15 December 1014, Vol. 67 No. 5
10. Cluver CA, Theron GB. Human Immunodeficiency virus infection in pregnancy. In: Cronje HS, Cilliers JBF, Du Toit M editors. Clinical Obstetrics a South African Perspective. Fourth edition. Van Schaik Publishers 2016: 476 - 484.

11. Boyer O, Niaudet P. Hemolytic Uremic Syndrome: New Developments in pathogenesis and treatment. International journal of Nephrology volume 2011 (2011), article id 908407, 10 pages. Available from: URL: http://dx.doi.org/10.4061/2011/908407
12. Lumen. Platelets. Available from: https://courses.lumenlearning.com/boundless-ap/chapter/platelets/. Accessed on 25 February 2019
13. Pacheco LD et al. The Role of Massive Transfusion Protocols in Obstetrics. Am J Preinatol. 2013; Jan; 30(1): 1-4

CHAPTER TWO

NORMAL PLATELET COUNT IN THE HIV POSITIVE PREGNANT PATIENT

B van Wyngaard: MB ChB

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ABSTRACT

Background. Thrombocytopenia complicates 6.6% – 11.6% of all pregnancies. Gestational Thrombocytopenia accounts for the majority of cases, it is usually mild with spontaneous resolution postpartum. It is estimated that more than 25% of pregnant South African patients are HIV positive.

Objectives. The objective of the study was to determine the platelet count for HIV positive pregnant patients. This will lead to minimizing unnecessary invasive testing and workup for other pathologies.

Method. This was a prospective descriptive study in which low and mild risk pregnant patients with WHO stage 1 HIV disease were recruited for participation. In patients that needed routine blood tests drawn, a platelet count was added. Patients were either first visit patients or following up for routine antenatal care.

Results. For all participants (n = 120), the mean platelet count was 270.9 x 10^9/L (range 91 – 488). For first trimester participants (n = 37), the mean platelet count was 282.3 x 10^9/L (range 103 – 441). For the second trimester participants (n = 28), the mean platelet count was 263.7 x 10^9/L (range 91 - 470). For the third trimester participants (n = 34), the mean platelet count was 260.2 x 10^9/L (range 99 - 488). For the participants where no trimester was indicated (n = 21), the mean platelet count was 278.1 x 10^9/L (range 181 - 426).

Discussion and Conclusion. Stage 1 HIV does not have a clinical significant impact on the platelet count in pregnant patients.

INTRODUCTION

Thrombocytopenia in pregnancy is defined as a platelet count of less than 150 x 10^9/L. It can be classified as pregnancy specific (e.g. Gestational thrombocytopenia), pregnancy related but not specific, or, independent of pregnancy (e.g. HIV).[1,2,3,4] Thrombocytopenia is the second most common haematological abnormality encountered during pregnancy with a prevalence of 6.6% – 11.2% during the third trimester.[2,3] The physiological mechanisms include pregnancy induced hemodilution with an increased mean platelet volume and increased consumption of platelets in the peripheral tissue and increased aggregation due to higher levels of thromboxane A2 during pregnancy.[1,2,4] Physiological thrombocytopenia in pregnancy is mild and has no effect for the mother or the fetus.[1] Gestational thrombocytopenia is a diagnosis of exclusion, meaning pathological causes of low platelet count must be investigated and excluded. Keeping this in mind, 5% – 9% of patients who are HIV positive have thrombocytopenia albeit asymptomatic. The prevalence of thrombocytopenia increases to 21% – 40% in patients with AIDS.[4] The prevalence of HIV in South Africa in women during their reproductive years has remained stable at 17% since 2008. However, the prevalence of HIV in pregnant women is between 28% and 30%.[9] Primary HIV associated thrombocytopenia may occur at any stage during the disease and has a multifactorial pathology. It correlates with a poorer prognosis and a more rapid progression from HIV to AIDS.[4] As the CD4 count decreases the more likely patients are to have thrombocytopenia, possibly because platelets have immunological functions and therefore participate in the interaction between pathogens and host defences.[8] Typically, during the early stages of the disease thrombocytopenia mimics Immune Thrombocytopenic Purpura (ITP) with accelerated platelet antibodies.[4,5,6,7] As the disease progresses, thrombocytopenia may develop by other virus-related mechanisms including suppression of platelet replication, shortening of platelet life-span and direct destruction of megakaryocytes by apoptosis and dysmegakaryopoiesis.[4,5,6,7] The diagnosis of the cause of thrombocytopenia is highly important as the risk for maternal and fetal morbidity and even mortality differ from one underlying factor to another as does the treatment.[2] In our setting where patients often only present when the pregnancy is already advanced with no or minimal previous medical history available, it becomes important to know the correlation between HIV, pregnancy and thrombocytopenia. It is important to distinguish between pathological and physiological thrombocytopenia as to not perform unnecessary, painful, costly and risky procedures or for instance, to terminate a pregnancy without reason. But we should also not

just ascribe thrombocytopenia in pregnancy to HIV and fail to investigate and diagnose life threatening pathologies.

METHODS

This was a descriptive study to determine the average platelet count in HIV positive pregnant patients. The cohort compromised low and medium risk HIV positive pregnant patients attending antenatal clinics. Patients were either first visit patients or follow up patients for routine antenatal care. Patients with WHO stage 1 disease were eligible for recruitment into the study. Patients were between 20 and 41 years of age.

No randomisation regarding the study population was done and all patients who were found to be eligible were recruited for voluntary participation. Patients signed a written consent form and received an information document with contact details should they have any further enquiry. No study participants were lost or withdrew from the study.

Inclusion Criteria:

All patients that were pregnant and HIV positive and WHO stage 1 disease.

Exclusion Criteria:

Patients known or previously diagnosed with:

- Preeclampsia
- Pregancy Induced Hypertension
- Systemic Lupus Erythromatosus
- Idiopathic Thrombotic Purpura
- TTP
- Hepatitis B Infection
- Splenomegaly
- Sepsis
- DIC
- AIDS
- HIV stage 2&3
- Malignancies
- Drugs: Heparin

Ethical approval for the study was obtained from the Health Sciences Research Ethic Committee: Faculty of Health Sciences of the University of the Free State (Ref:HSD2017/1431).

For statistical analysis we used MedCalc (version 18.11.6) statistical analysis software.

RESULTS

Results were available for a total of 120 patients. There were 37 participants in trimester 1, 28 in trimester 2 and 34 in trimester 3. In 21 cases the trimester, during which the platelet count was obtained, was not recorded (Figure 1).

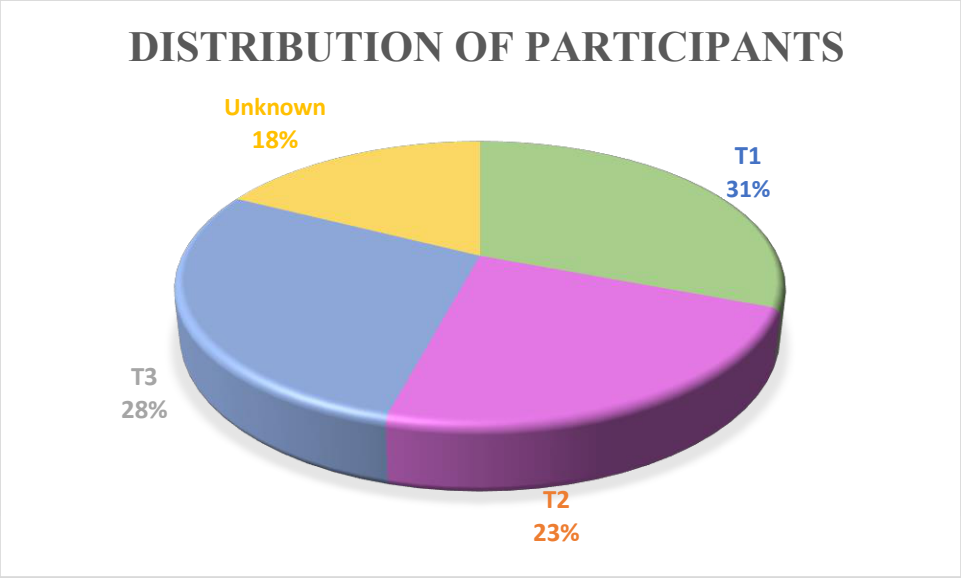

Figure 1: Distribution of participants based on trimester.

The reference intervals for each group are summarized in table 1.

For all participants (n = 120), the mean platelet count was 270.9 x 10^9/L (range 91 – 488). Using the Robust method (CLSI C28-A3), the lower limit 90% confidence interval (CI) is 83.2 – 125.6. The upper limit 90% confidence interval (CI) is 404.8 – 450.3. The incidence of thrombocytopenia in the cohort was 7/120 (5.8%) and the incidence of thrombocytosis was 9/120 (7.5%).

For first trimester participants (n = 37), the mean platelet count was 282.3 x 10^9/L (range 103 – 441). Using the normal distribution, the lower limit 90% confidence interval (CI) is 78.3 – 157.6. The upper limit 90% confidence interval (CI) is 407.1 – 486.4. The prevalence of thrombocytopenia in this group was 2/37 (5.4%) and the incidence of thrombocytosis was 3/37 (8.1%). The T-test was 0,74 and the p-value was 0.46.

For the second trimester participants (n = 28), the mean platelet count was 263.7 x 10^9/L (range 91 - 470). Using the normal distribution, the lower limit 90% confidence interval (CI) is 80.6 – 160.3. The upper limit 90% confidence interval (CI) is 367.1 - 446.7. The incidence of thrombocytopenia in this group was 2/28 (7.2%) and the prevalence of thrombocytosis was 1/28 (3.6%). The T-test was -0.44 and the P-value 0.66.

For the third trimester participants (n = 34), the mean platelet count was 260.2 x 10^9/L (range 99 - 488). Using the normal distribution, the lower limit 90% confidence interval (CI) is 32.8 – 124.3. The upper limit 90% confidence interval (CI) is 396.1 – 487.6. The prevalence of thrombocytopenia in this group was 3/34 (8.8%) and the incidence of thrombocytosis was 4/34 (11.8%). The T-test was -0.66 and the P-value 0.5

For the participants where no trimester was indicated (n = 21), the mean platelet count was 278.1 x 10^9/L (range 181 - 426). Using the normal distribution, the lower limit 90% confidence interval (CI) is 99.6 – 186.6. The upper limit 90% confidence interval (CI) is 369.6 – 456.7. The prevalence of thrombocytopenia in this group was 0/21 (0%) and the incidence of thrombocytosis was 1/21 (4.8%). The T-test was 0.38 and the P-value 0.7.

Of the 7 patients with thrombocytopenia, one was identified to have had 3 previous miscarriages that could indicate underlying pathology, eg APLS.

Table 1: Reference intervals for the total population, and the three trimesters, and unknown trimester, respectively.

	TOTAL	T1	T2	T3	UNKNOWN
	n = 120	n = 37	n = 28	n = 34	n = 21
MEAN	270.9 x 10^9/L	282.3 x 10^9/L	263.7 x 10^9/L	260.2 x 10^9/L	278.1 x 10^9/L
RANGE	91-488 x 10^9/L	102-441 x 10^9/L	91-470 x 10^9/L	99-488 x 10^9/L	181-426 x 10^9/L
THROMBOCYTOPENIA	7 (5.8%)	2 (5.4%)	2 (7.2%)	3 (8.8%)	1 (4.8%)

DISCUSSION

Thrombocytopenia is defined as a platelet count of less than 150 x 10^9/L, with normal ranges from 150 – 400 x 10^9/L.

Of all pregnant patients, 6.6% – 11.6 % are affected by thrombocytopenia. Gestational thrombocytopenia is due to a physiological phenomenon occurring secondary to the increase in plasma volume with subsequent dilution of the platelets, increased consumption in the peripheral tissues and increased aggregation due to elevated levels of thromboxane A2 during pregnancy. Gestational thrombocytopenia is a diagnosis of exclusion, when all other pathological causes has been investigated first. There are no maternal or fetal risks involved in patients with gestational thrombocytopenia.

In stage 1 HIV disease, 5% – 9% of patients will present with thrombocytopenia[4]. The pathophysiology includes immune mediated destruction of the platelets and a direct effect on the megakaryocytes in the bone marrow, where it causes apoptosis and destruction of the megakaryocytes.

In this study, the mean platelet count recorded in pregnant patients with HIV stage 1 disease was 270 x 10^9/L. This falls into the normal reference ranges for platelet counts. All of the participants were on ARV's at the time the platelet count was drawn.

In the current study 5.8% of the total number of participants had thrombocytopenia, correlating with current literature.

There was no statistical difference in the mean platelet count between the different trimesters, including the unknown group, based on the p-values for each group, suggesting that pregnancy doesn't alter the platelet count with progression.

Although it was not confirmed, it could be argued that for 6 of the 7 patients with thrombocytopenia, is was most likely gestational thrombocytopenia based on the prevalence rates when compare to previous literature. In all of the cases it was a mild thrombocytopenia, which is in keeping with current literature on gestational thrombocytopenia.
Limitations of the study were the absence of a cohort of patients that are HIV negative.

CONCLUSION

The results of this study confirmed that stage 1 HIV disease does not have a clinically significant influence on the platelet count of pregnant patients. With this information, it would be suggested that a platelet count would not be mandatory prior to emergency surgical interventions in an unbooked patient with HIV stage 1 disease, presenting to the labour and delivery room with no other known comorbidities. Furthermore, it confirms that in the event that a thrombocytopenia is discovered on investigations further investigation as to the underlying causes is warranted, and not only to assumed that it is secondary to the HIV infection.
A further or repeat study that includes a cohort of HIV negative participants should be conducted.

REFERENCES

1. Ciobanu AM, Colibaba S, Cimpoca B, Peltecu G, Panaitescu AM. Thrombocytopenia in Pregnancy. MAEDICA – a Journal of Clinical Medicine 2016; 11(5): 55-60
2. Bergman F, Rath W. The Differential Diagnosis of Thrombocytopenia in Pregnancy - an interdisciplinary challenge. Dtsch Arztebl Int 2015; 112: 795 - 802
3. Gernsheimer T, James AH, Stasi R. How I treat thrombocytopenia in pregnancy. Blood 2013; 121(1): 38 - 47
4. Sebitloane HM, Thrombocytopenia during pregnancy in women with HIV infection receiving no treatment. South African Medical Journal 2016; 106(2): 210-213
5. Vaughan J, Wiggil T, Munster M, Immature platelet fraction levels in a variety of bone marrow pathologies in South African HIV-positive patients with thrombocytopenia. Hematology 2014 Vol. 19 No. 7
6. O'Bryan TA, Okulics JF, Bradley WP, Ganesan A, Wang X and Agan BK, Impact of the highly active antiretroviral therapy era on the epidemiology of primary HIV thrombocytopenia. O'Bryan et al. BMC Res Notes (2015) 8: 595

7. Wondimeneh Y, Muluye D, Ferede G, Prevalence and associated factors of thrombocytopenia among HAART naïve HIV positive patients at Gondor University Hospital, Northwest Ethiopia.
8. Franzetti M, Adorni F, Oreni L, Van Den Bogaart L, Resnatti C, Milazzo L, Antinori S, Galli M and Ridolfo A, Changes in the Incidence of Severe Thrombocytopenia and its predisposing Conditions in HIV-infected Patients Since the Introduction of Highly Active Antiretroviral Therapy. J Acquired immune Defic Syndr 15 December 1014, Vol. 67 No. 5
9. Cluver CA, Theron GB. Human Immunodeficiency virus infection in pregnancy. In: Cronje HS, Cilliers JBF, Du Toit M editors. Clinical Obstetrics a South African Perspective. Fourth edition. Van Schaik Publishers 2016: 476 - 484

APPENDICES

APPENDIX A

UNIVERSITY OF THE FREE STATE
UNIVERSITEIT VAN DIE VRYSTAAT
YUNIVESITHI YA FREISTATA

UFS·UV
HEALTH SCIENCES
GESONDHEIDSWETENSKAPPE

Health Sciences Research Ethics Committee

22-Jun-2018

Dear **Dr Bianca Van Wyngaard**

Ethics Clearance: **WHAT IS THE NORMAL PLATELET COUNT IN A HIV POSITIVE PREGNANT PASIENT?**

Principal Investigator: **Dr Bianca Van Wyngaard**

Department: **Obstetrics and Gynaecology (Bloemfontein Campus)**

APPLICATION APPROVED

Please ensure that you read the whole document

With reference to your application for ethical clearance with the Faculty of Health Sciences, I am pleased to inform you on behalf of the Health Sciences Research Ethics Committee that you have been granted ethical clearance for your project.

Your ethical clearance number, to be used in all correspondence is:**UFS-HSD2017/1431**

The ethical clearance number is valid for research conducted for one year from issuance. Should you require more time to complete this research, please apply for an extension.

We request that any changes that may take place during the course of your research project be submitted to the HSREC for approval to ensure we are kept up to date with your progress and any ethical implications that may arise. This includes any serious adverse events and/or termination of the study.

A progress report should be submitted within one year of approval, and annually for long term studies. A final report should be submitted at the completion of the study.

The HSREC functions in compliance with, but not limited to, the following documents and guidelines: The SA National Health Act. No. 61 of 2003; Ethics in Health Research: Principles, Structures and Processes (2015); SA GCP(2006); Declaration of Helsinki; The Belmont Report; The US Office of Human Research Protections 45 CFR 461 (for non-exempt research with human participants conducted or supported by the US Department of Health and Human Services-(HHS), 21 CFR 50, 21 CFR 56; CIOMS; ICH-GCP-E6 Sections 1-4; The International Conference on Harmonization and Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH Tripartite), Guidelines of the SA Medicines Control Council as well as Laws and Regulations with regard to the Control of Medicines, Constitution of the HSREC of the Faculty of Health Sciences.

For any questions or concerns, please feel free to contact HSREC Administration: 051-4017794/5 or email EthicsFHS@ufs.ac.za.

Thank you for submitting this proposal for ethical clearance and we wish you every success with your research.

Yours Sincerely

Dr. SM Le Grange
Chair : Health Sciences Research Ethics Committee

Health Sciences Research Ethics Committee
Office of the Dean: Health Sciences
T: +27 (0)51 401 7795/7794 | E: ethicsfhs@ufs.ac.za
IRB 00006240; REC 230408-011; IORG0005187; FWA00012784
Block D, Dean's Division, Room D104 | P.O. Box/Posbus 339 (Internal Post Box G40) | Bloemfontein 9300 | South Africa
www.ufs.ac.za

APPENDIX B

Informed Consent:

Dear Participant

I am conducting a study to establish the average platelet count in HIV positive pregnant patients.

Platelets are cells in our bloodstream that assist in the clotting of blood during injury and in pregnancy particularly during the delivery of the baby to ensure the patients do not bleed ~~excessively~~.too much. Both pregnancy in itself and HIV can cause these platelets to have lower levels in the blood. The aim of this study is to determine what is the average platelet count for HIV pregnant patients in order to prevent unnecessary further testing but also to identify patients at high risk of bleeding and other complications during pregnancy and delivery.

You will be interviewed and examined, as is routine on the antenatal visit, and your stage of disease will be determined based on the examination and history. This will happen in a closed examination room to ensure utmost confidentiality.

Every HIV positive pregnant patient has their blood drawn at the Antenatal clinic for certain routine tests. These include the CD4 count, Viral Load to determine the stage and progression of the disease, the Creatinine to evaluate Kidney function, the Hemoglobin to evaluate for anemia, RPR to determine a current syphilis infection. On the same blood that goes to the lab we will add the platelet count.

No additional blood will be drawn.

A data sheet will be completed with the information obtained during the interview and examination. No names will be used on the form to ensure confidentiality. A barcode will be placed on the form to trace the results from the blood. The same barcode is placed in your antenatal book so the results will be available to you on the next antenatal visit. In the event of significant abnormalities the sister at the clinic will be able to refer you appropriately.

If during the collection of results, I detect a severe abnormality I will trace the results back to you to ensure you are referred appropriately and receive the management required.

Once enough samples have been evaluated we will be able to analyze the results and to determine the average platelet count for HIV positive pregnant patients.

Participation in this study is voluntary and you may withdraw at any point during the study. There is no benefit or risks involved in participating in this study nor is there any remuneration for participation.

Your contribution towards this study is greatly appreciated. There is unfortunately no reimbursement for participation in this study.

You are welcome to contact me through the Department of Obstetrics and Gynaecology at any time during the study if you have additional questions.

Kind Regards

Dr Bianca van Wyngaard

O&G Registrar

University of the Free State

Tel : 051 405 3272

Health Science Research Ethics Committee

Tel: 051 401 7794

CONSENT TO PARTICIPATE IN RESEARCH

You have been asked to participate in a research study.

You have been informed about the study by Dr Bianca van Wyngaard.

You have been informed about any available compensation or medical treatment if injury occurs as a result of study-related procedures.

You may contact **Dr van Wyngaard at 051 405 3272** any time if you have questions about the research or if you are injured as a result of the research.

You may contact the Secretariat of the **Health Sciences Research Ethics Committee**, UFS at t**elephone number (051) 4017794/5** if you have questions about your rights as a research subject.

Your participation in this research is voluntary, and you will not be penalized or lose benefits if you refuse to participate or decide to terminate participation.

If you agree to participate, you will be given a signed copy of this document as well as the participant information sheet, which is a written summary of the research.

The research study, including the above information has been verbally described to me. I understand what my involvement in the study means and I voluntarily agree to participate.

Signature of Participant	Date
Signature of Witness	Date
Signature of Translator	Date

APPENDIX C

health
Department of Health
FREE STATE PROVINCE

25 May 2018

Dr B Van Wyngaard
Dept. of Obstetrics and Gynaecology
UFS

Dear Dr B Van Wyngaard

Subject: WHAT IS THE NORMAL PLATELET COUNT IN A HIV POSITIVE PREGNANT PASIENT?

- Permission is hereby granted for the above – mentioned research on the following conditions:
- Participation in the study must be voluntary.
- A written consent by each participants must be obtained.
- Serious adverse events to be reported and/or termination of the study.
- Ascertain that your data collection exercise neither interferes with the day to day running of the selected facilities nor the performance of duties by the respondents or health care workers.
- Confidentiality of information will be ensured and no names will be used.
- Research results and a complete report should be made available to the Free State Department of Health on completion of the study (a hard copy plus a soft copy).
- Progress report must be presented not later than one year after approval of the project to the Ethics Committee of the University of Free State and to Free State Department of Health.
- Any amendments, extension or other modifications to the protocol or investigators must be submitted to the Ethics Committee of the University of Free State and to Free State Department of Health.
- **Conditions stated in your Ethical Approval letter should be adhered to and a final copy of the Ethics Clearance Certificate should be submitted to** lithekom@fshealth.gov.za or sebeelats@fshealth.gov.za **before you commence with the study**
- No Financial liability will be placed on the Free State Department of Health
- Please discuss your study with the institution managers/CEOs on commencement for logistical arrangements
- Department of Health to be fully indemnified from any harm that participants and staff experiences in the study
- Researchers will be required to enter in to a formal agreement with the Free State department of health regulating and formalizing the research relationship (document will follow)
- You are encouraged to present your study findings/results at the Free State Provincial health research day

Trust you find the above in order.

Kind Regards

Dr D Motau
HEAD: HEALTH
Date: 6/6/18

Head: Health
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APPENDIX D

UNIVERSITY OF THE FREE STATE
UNIVERSITEIT VAN DIE VRYSTAAT
YUNIVESITHI YA FREISTATA

UFS·UV
HEALTH SCIENCES
GESONDHEIDSWETENSKAPPE

23 October 2017

The Chairman
Ethics Committee
Faculty of Health Sciences
University of the Free State
Bloemfontein

Dear dr Le Grange

RESEARCH PROJECT : DR B VAN WYNGAARD
WHAT IS THE NORMAL PLATELET ACCOUNT IN HIV POSITIVE PREGNANT PATIENTS?

Permission is granted to Dr Bianca van Wyngaard to perform a prospective clinical research project: "WHAT IS THE NORMAL PLATELET ACCOUNT IN HIV POSITIVE PREGNANT PATIENTS" in the Department of Obstetrics and Gynaecology.

Yours sincerely

SM BALOYI
HEAD & CHAIR OF CLINICAL DEPARTMENT
OBSTETRICS & GYNAECOLOGY

Departementshoof / Head of Department: **Dr SM Baloyi**
Konsultante / Consultants: Prof BF Cooreman, drr EW Henn, BW Richter, T Nondabula, I Zondagh, T Leroko, NN Uzoho

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Koördinate/Coordinates: S 29° 06 53.7" E 26° 11'02.6"

APPENDIX E

WHAT IS THE NORMAL PLATELET COUNT IN A HIV POSITIVE PREGNANT PATIENT?

RESEARCH TEAM

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1. INTRODUCTION

Thrombocytopenia in pregnancy is defined as a platelet count of less than 150 x 10^9/L. It can be classified as pregnancy specific (eg. Gestational thrombocytopenia), pregnancy related but not specific, or, independent of pregnancy (eg. HIV). [1,2,3,4]

Thrombocytopenia is the second most common haematological abnormality encountered during pregnancy with a prevalence of 6.6 – 11.2 % during the third trimester. [2,3] The physiological mechanisms include pregnancy induced hemodilution with an increased mean platelet volume and increased consumption of platelets in the peripheral tissue and increased aggregation due to higher levels of thromboxane A2 during pregnancy. [1,2,4] Physiological thrombocytopenia in pregnancy is mild and has no effect for the mother or the fetus.[1]

Gestational thrombocytopenia is a diagnosis of exclusion, meaning pathological causes of low platelet count must be investigated and excluded. Keeping this in mind, 5 – 9% of patients who are HIV positive have thrombocytopenia albeit asymptomatic. The prevalence of thrombocytopenia increases to 21 – 40% in patients with AIDS. [4]

The prevalence of HIV in South Africa in women during their reproductive years has remained stable at 17% since 2008. However, the prevalence of HIV in pregnant women in between 28 and 30%. [10]

Primary HIV associated thrombocytopenia may occur at any stage during the disease and has a multifactorial pathology. It correlates with a poorer prognosis and a more rapid progression from HIV to AIDS. [4] As the CD4 count decreases the more likely patients are to have thrombocytopenia, possibly because platelets have immunological functions and therefore participate in the interaction between pathogens and host defences. [8] Typically, during the early stages of the disease thrombocytopenia mimics Immune Thrombocytopenic Purpura (ITP) with accelerated platelet antibodies.[4,5,6,7] As the disease progresses the thrombocytopenia may develop by other virus-related mechanisms including suppression of platelet replication, shortening of platelet life-

span and direct destruction of megakaryocytes by apoptosis and dysmegakaryopoiesis.[4,5,6,7]

The diagnosis of the cause of thrombocytopenia in highly important as the risk for maternal and fetal morbidity and even mortality differ from one underlying factor to another as does the treatment.[2] In our setting where patients often only present when the pregnancy is already advanced with no or minimal previous medical history available, it becomes important to know the correlation between HIV, pregnancy and thrombocytopenia.

It is important to distinguish between pathological and physiological thrombocytopenia as to not perform unnecessary, painful, costly and risky procedures or for instance, to terminate a pregnancy without reason. But we should also not just ascribe thrombocytopenia in pregnancy or HIV and fail to investigate and diagnose life threatening pathologies.

But what is a normal platelet count for a pregnant HIV positive patient? Currently there is limited clinical data available for normal platelet count in HIV positive pregnant patients. Knowing the normal

platelet count in HIV positive pregnant patients will guide investigations and thus limit unnecessary resource use.

2. STUDY OBJECTIVE

2.1. The primary objective is to determine the normal platelet count for pregnant patients who are HIV positive.

2.2. The secondary objectives are to determine the difference in the average platelet count in the different trimesters and possible motivation for routine platelet count screening in HIV positive pregnant individuals.

3. METHODOLOGY

3.1.STUDY DESIGN

A prospective descriptive study to determine the average platelet count in HIV positive pregnant patients.

3.2.STUDY POPULATION

We will identify patients in low and medium risk antenatal clinics who are HIV positive and are either first visit patients or following up for routine antenatal care.

3.3.SAMPLE COLLECTION

The patients will be seen in a private room to ensure confidentiality. The aim will be to obtain specimens from a100 patients form each of the three trimesters. (Total 300 patients)

3.4.INCLUSION CRITERIA

All patients who are pregnant and HIV positive and WHO stage 1 will be considered for the study.

3.5.EXCLUSION CRITERIA

Patients known or previously diagnosed with:

- Preeclampsia
- Pregancy Induced Hypertension
- Systemic Lupus Erythromatosus
- Idiopathic Thrombotic Purpura
- TTP
- Hepatitis B Infection
- Splenomegaly
- Sepsis
- DIC
- AIDS
- HIV stage 2&3
- Malignancies
- Drugs: Heparin

4. MEASUREMENT

Interviews and examinations will be conducted in a private room to ensure confidentiality. A data sheet will be completed.

The consent forms will be in English, Afrikaans and Sesotho. In order to secure accurate translation, the consent form was translated back to English by an independent Sesotho doctor. This assures accuracy and validity in the consent form for Sesotho speaking patients. A Sesotho speaking colleague will assist in doing the interview when necessary.

After consent is gained, a venous blood sample will be drawn from the patient. It is routine to test Hemoglobin levels as well as CD4 counts, Viral load and Creatinine levels and will therefore not add and additional procedure. Hemoglobin testing is an automated test and platelet counts are automatically done by the machine. In case of an abnormality which need to be verified the cost related to a manual platelet count would be R20.24.

The patient will then be examined to:

1) Determine gestational age in order to group the patient to a specific trimester. (Trimester 1: From conception to 12 weeks 6 days, Trimester 2: 13 weeks 0 days to 26 weeks 6 days and Trimester 3: 27 weeks 0 days until delivery)

2) Stage of HIV according to the WHO classification

3) Exclude signs and symptoms of any underlying disease which may cause thrombocytopenia

We will collect the following information from each patient:

- Age
- Gravidity/Parity
- HIV WHO clinical staging
- HIV treatment regime

- HIV treatment duration
- CD4 Count
- Viral Load
- Recent Infections
- TB Exposure/ Co Infection
- Gestation at which patient booked het first antenatal visit.
- Blood Pressure and Urine dipstick to rule out proteinuric hypertension (pre-eclampsia)

After data is collected platelet counts will be grouped according to trimesters. Data will then be sent for statistical analysis.

5. STATISTICAL ANALYSIS

With the assistance of the department of biostatistics we will determine the mean and range of normal platelet counts for each trimester.

We expect approximately 100 patients per trimester to be included in our study (300 total) depending on the numbers required.

6. OUTCOME OF THE STUDY

We currently regard thrombocytopenia in pregnancy as a platelet count lower than 150 x 10^9/L. We know that both HIV and pregnancy can cause lower platelet counts. With our study, we aim to establish the normal platelet count values for HIV positive pregnant patients.

7. DURATION OF THE STUDY

March 2018: Submission to ethics Committee
April 2018: Permission from Department of Health
May 2018: Pilot Study
June 2018 – November 2018: Data Collection
December 2018: Data Analysis
January 2019: Report Writing

8. BUDGET

Submission to ethics Committee

Permission from Department of Health

Pilot Study Data Collection Data Analysis Report writing

Since all patients who are pregnant require a Hemoglobin test (which is automated and automatically includes a platelet count) no extra cost would be incurred. Should platelet counts be done and quoted separately at R20.24 per specimen, 300 specimens would amount to R6072.00. In the event of extra costs, it would initially be incurred by the researcher and then application for postgraduate funding for reimbursement at the department of Obstetrics and Gynecology would be made.

We aim to print 300 data collection and consent forms at R1 per sheet. The cost of printing will be incurred by the researcher.

	Unit Price	Units	Total cost
Platelet count	R20,24	300	R6072
Consent forms	R1	600	R600
Total			R6672

9. ETHICAL CONSIDERATIONS

Ethical approval will be sought from the ethics committee.

Application for permission from the Department of Health will be made after clearance by the ethics committee.

Patients will be seen in a private consultation room. If the patient is found to be HIV positive she will be given the opportunity to participate in the study. The study aim and method will be presented to the participants with no coercion or undue influence. This will assist that the participants autonomy and freedom of choice is maintained.

Consent and information will be collected during the consultation period. In this way no other patient or staff member will be aware of her

HIV status or even that she was approached to participate in a research study. Data will be collected anonymously. Only tracking numbers (laboratory stickers) will be used to correlate the results and patient names will be withheld from the data forms.

The participants will be informed that they may withdraw from the study at any point, there are no benefits or risk involved in participating in the research study.

10. LITERATURE REVIEW

1. Ciobanu AM, Colibaba S, Cimpoca B, Peltecu G, Panaitescu AM. Thrombocytopenia in Pregnancy. MAEDICA – a Journal of Clinical Medicine 2016; 11(5): 55-60
2. Bergman F, Rath W. The Differential Diagnosis of Thrombocytopenia in Pregnancy - an interdisciplinary challenge. Dtsch Arztebl Int 2015; 112: 795 - 802.
3. Gernsheimer T, James AH, Stasi R. How I treat thrombocytopenia in pregnancy. Blood 2013; 121(1): 38-47
4. Sebitloane HM, Thrombocytopenia during pregnancy in women with HIV infection receiving no treatment. S Afr Med J 2016;106(2):210-213.
5. Vaughan J, Wiggil T, Munster M, Immature platelet fraction levels in a variety of bone marrow pathologies in South African HIV-positive patients with thrombocytopenia. Hematology 2014 Vol. 19 No. 7
6. O'Bryan TA, Okulics JF, Bradley WP, Ganesan A, Wang X and Agan BK, Impact of the highly active antiretroviral therapy era on the epidemiology of primary HIV thrombocytopenia. O'Bryan et al. BMC Res Notes (2015) 8:595
7. 7.Wondimeneh Y, Muluye D, Ferede G, Prevalence and associated factors of thrombocytopenia among HAART naive¨ HIV positive patients at Gondor university hospital, northwest Ethiopia.
8. 8. Franzetti M, Adorni F, Oreni L, Van Den Bogaart L, Resnatti C, Milazzo L, Antinori S, Galli M and Ridolfo A, Changes in the Incidence of Severe Thrombocytopenia and its predisposing Conditions in HIV-infected Patients Since the Introduction of

Highly Active Antiretroviral Therapy. J Acquired immune Defic Syndr 15 December 1014: Vol. 67 No. 5

9. 9.Cluver CA, Theron GB. Human Immunodeficiency virus infection in pregnancy. In: Cronje HS, Cilliers JBF, Du Toit M editors. Clinical Obstetrics a South African Perspective. Fourth edition. Van Schaik Publishers 2016: 476 - 484

APPENDIX F

DATA COLLECTION SHEET

Clinic: _____

Age: ___

Gravidity: ____ Parity: _____ Miscarriages: _____

T1: ___ T2: ___ T3: ___

BP: _____ Urine Dipstix: _____

WHO staging: 1: ___ 2: ___ 3:___ 4: ___

CD4 count: _____

Viral Load: _____

FDC: ___ Alternative: ___ Duration: ___

TB exposure: ___ TB co- infection: ____ TB Prophylaxis: _____ Recent infection: _____

HB: _____

Platelets: _____

RPR: _____

Hep B: _____

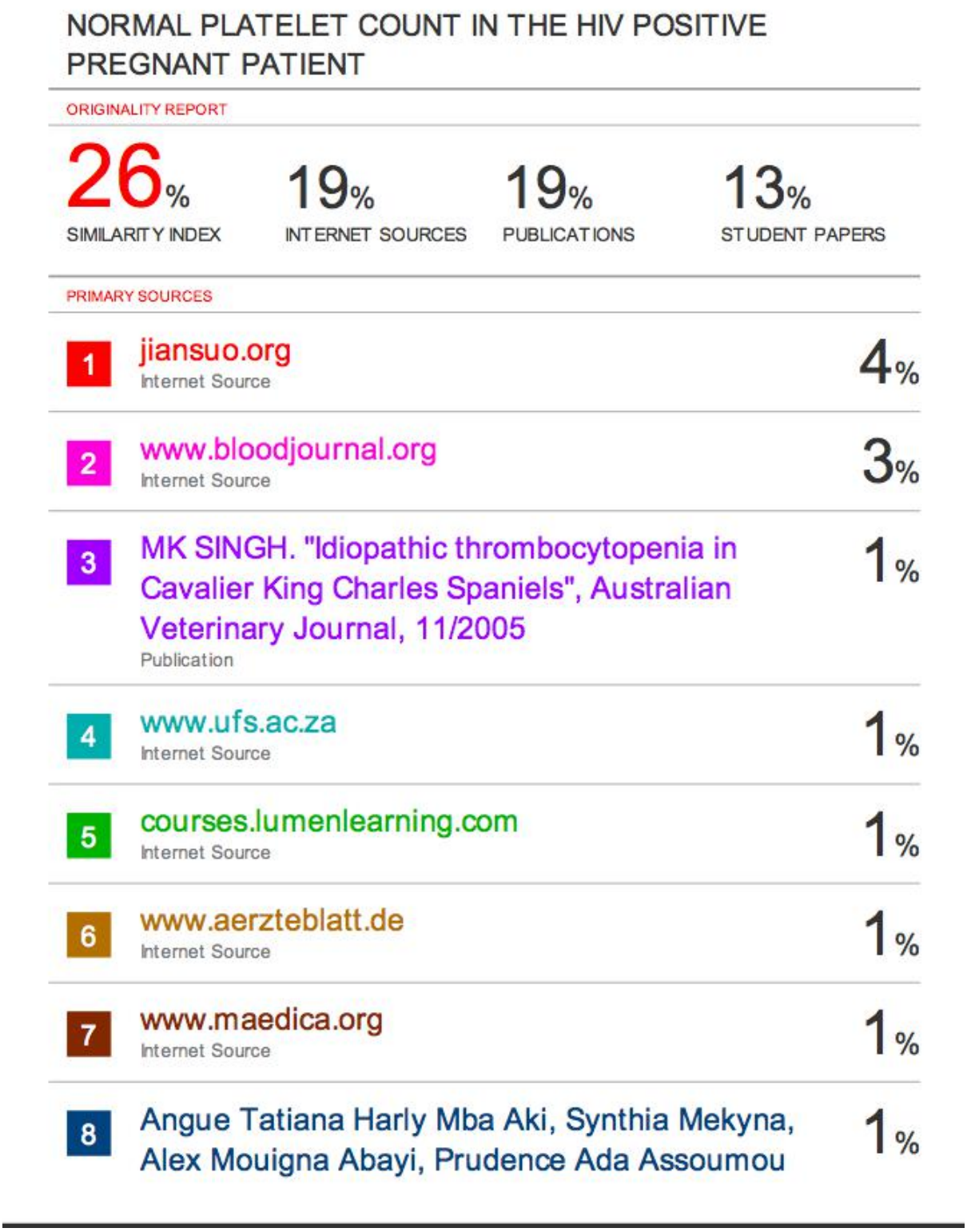
Creatinine: _____

WHO STAGING:

Stage 1	Stage 2	Stage 3	Stage 4
Clinically well Lymphadenopathy	Mild weight loss Repeated URTI Angular cheilitis Recurrent oral Ulceration Papular pruritic eruptions Fungal nail infections of fingers Herpes Zoster(shingles)	Moderate unexplained weight loss Unexplained chronic diarrhea >1 month Unexplained persistent fever >1 month Oral candidiasis Oral hairy leukoplakia PTB in last 2 years Severe Bacterial infections	Severe weight loss HIV associated opportunistic infections: • Oesophageal Candidiasis • Extrapulmonary TB • Cryptococcal Meningitis • Toxoplasmosis Encephalitis • Malignancies, eg Karposi

Barcode: _____

APPENDIX G



NORMAL PLATELET COUNT IN THE HIV POSITIVE PREGNANT PATIENT

ORIGINALITY REPORT

26%	19%	19%	13%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	jiansuo.org Internet Source	4%
2	www.bloodjournal.org Internet Source	3%
3	MK SINGH. "Idiopathic thrombocytopenia in Cavalier King Charles Spaniels", Australian Veterinary Journal, 11/2005 Publication	1%
4	www.ufs.ac.za Internet Source	1%
5	courses.lumenlearning.com Internet Source	1%
6	www.aerzteblatt.de Internet Source	1%
7	www.maedica.org Internet Source	1%
8	Angue Tatiana Harly Mba Aki, Synthia Mekyna, Alex Mouigna Abayi, Prudence Ada Assoumou	1%

	et al. "Bilateral Macular Hemorrhage Revealing Severe Thrombocytopenia in an AIDS Context about a Case Report", Open Journal of Ophthalmology, 2019 Publication	
9	www.sareh-abdollahi.ir Internet Source	1%
10	Submitted to Sheffield Hallam University Student Paper	1%
11	Submitted to University of the Free State Student Paper	1%
12	scholar.ufs.ac.za:8080 Internet Source	1%
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14	issuu.com Internet Source	<1%
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17	Submitted to Institute of Biomedical Science Student Paper	<1%

18	bloodjournal.hematologylibrary.org Internet Source	<1%
19	Submitted to National postgraduate Medical College of Nigeria Student Paper	<1%
20	T. Gernsheimer, A. H. James, R. Stasi. "How I treat thrombocytopenia in pregnancy", Blood, 2012 Publication	<1%
21	Submitted to University of Wales Institute, Cardiff Student Paper	<1%
22	innspub.net Internet Source	<1%
23	intl-pedsinreview.aappublications.org Internet Source	<1%
24	Submitted to University of KwaZulu-Natal Student Paper	<1%
25	www.ukessays.com Internet Source	<1%
26	Frauke Bergmann, Werner Rath. "The Differential Diagnosis of Thrombocytopenia in Pregnancy", Deutsches Aerzteblatt Online, 2015 Publication	<1%

27	files.fwgbd.gethifi.com Internet Source	<1%
28	Submitted to Mansoura University Student Paper	<1%
29	Karamjit K Gill, John G Kelton. "Management of idiopathic thrombocytopenic purpura in pregnancy", Seminars in Hematology, 2000 Publication	<1%
30	Submitted to University of Witwatersrand Student Paper	<1%
31	journals.sagepub.com Internet Source	<1%
32	haem2.net Internet Source	<1%
33	Daniel, Koshy, and Robert Dunn. "Comparison of platelet count in tuberculosis spine to other spine pathology", European Spine Journal, 2013. Publication	<1%
34	Nelson-Piercy, Catherine. "Haematological problems", Handbook of Obstetric Medicine Fifth Edition, 2015. Publication	<1%
35	Franzetti, Marco, Fulvio Adorni, Letizia Oreni, Lorena Van Den Bogaart, Chiara Resnati,	<1%

	Laura Milazzo, Spinello Antinori, Massimo Galli, and Anna L. Ridolfo. "Changes in the Incidence of Severe Thrombocytopenia and Its Predisposing Conditions in HIV-Infected Patients Since the Introduction of Highly Active Antiretroviral Therapy :", JAIDS Journal of Acquired Immune Deficiency Syndromes, 2014. Publication	
36	Submitted to St George's Hospital Medical School Student Paper	<1%
37	www.healthplexus.net Internet Source	<1%
38	Submitted to University of Sydney Student Paper	<1%
39	www.sajaa.co.za Internet Source	<1%
40	Butwick, Alexander J., and Lawrence T. Goodnough. "Transfusion and coagulation management in major obstetric hemorrhage :", Current Opinion in Anaesthesiology, 2015. Publication	<1%
41	Nelson-Piercy, Catherine. "Hypertension and pre-eclampsia", Handbook of Obstetric Medicine Fifth Edition, 2015. Publication	<1%

42	Submitted to Western Governors University Student Paper	<1%
43	aoczone.net Internet Source	<1%
44	Submitted to University of Liverpool Student Paper	<1%
45	Neslihan Karakurt, İlker Uslu, Canan Albayrak, Leman Tomak, Elif Ozyazici, Davut Albayrak, Canan Aygun. "Neonates born to mothers with immune thrombocytopenia", Blood Coagulation & Fibrinolysis, 2018 Publication	<1%
46	www.ontca.ca Internet Source	<1%
47	www.patientfromhell.org Internet Source	<1%
48	Submitted to University of Southern Queensland Student Paper	<1%
49	O'Bryan, Thomas A., Jason F. Okulicz, William P. Bradley, Anuradha Ganesan, Xun Wang, and Brian K. Agan. "Impact of the highly active antiretroviral therapy era on the epidemiology of primary HIV-associated thrombocytopenia", BMC Research Notes, 2015. Publication	<1%

50	preview-bmcresnotes.biomedcentral.com Internet Source	<1%
51	gidiv.ucsf.edu Internet Source	<1%
52	Submitted to Cardiff and Vale College Student Paper	<1%
53	Yazan Daaboul, Serge Korjian, Lamis Khalil, Rita Nemr. "Pheochromocytoma Presenting as Partial HELLP Syndrome", Case Reports in Obstetrics and Gynecology, 2015 Publication	<1%
54	Nkambule, Bongani B., Glenda Davison, and Hayley Ipp. "The value of flow cytometry in the measurement of platelet activation and aggregation in human immunodeficiency virus infection", Platelets, 2015. Publication	<1%
55	"Nonmalignant Hematology", Springer Nature, 2016 Publication	<1%
56	Submitted to De Montfort University Student Paper	<1%
57	flore.unifi.it Internet Source	<1%
	Segal, A.I.. "Physician attitudes toward human	

58	immunodeficiency virus testing in pregnancy", American Journal of Obstetrics and Gynecology, 199606 Publication	<1%
59	Submitted to University of Greenwich Student Paper	<1%
60	Submitted to King's College Student Paper	<1%
61	"Practice Bulletin No. 166", Obstetrics & Gynecology, 2016 Publication	<1%
62	Hema Priya L, Ambarish Bhandiwad. "IDIOPATHIC THROMBOCYTOPENIC PURPURA IN PREGNANCY & NEONATAL EFFECTS – A CASE REPORT", Journal of Evolution of Medical and Dental sciences, 2013 Publication	<1%
63	Submitted to The University of Manchester Student Paper	<1%
64	archive.org Internet Source	<1%
65	K KROK. "Liver Disease in Women", Principles of Gender-Specific Medicine, 2010 Publication	<1%
66	Vesna Elveđi-Gašparović, Petrana Beljan, Snježana Gverić-Ahmetašević, Snježana	<1%

Schuster, Snježana Škrablin. "Fetal-maternal complications and their association with gestational thrombocytopenia", Ginekologia Polska, 2016
Publication

67	Olivia Boyer. "Hemolytic Uremic Syndrome: New Developments in Pathogenesis and Treatment", International Journal of Nephrology, 2011 Publication	<1%
68	Grzegorz Jakiel, Michał Ciebiera, Aneta Słabuszewska-Jóźwiak, Bartosz Horosz et al. "Successful obstetric and hematologic outcome of aplastic anemia in a pregnant Jehovah's Witness", International Journal of Immunopathology and Pharmacology, 2016 Publication	<1%
69	Nelson-Piercy, Catherine. "Liver disease", Handbook of Obstetric Medicine Fifth Edition, 2015. Publication	<1%
70	"Obstetric Anesthesia for Co-morbid Conditions", Springer Nature America, Inc, 2018 Publication	<1%

APPENDIX H

SAMJ Article Guidelines

Accepted manuscripts that are not in the correct format specified in these guidelines will be returned to the author(s) for correction, which will delay publication.

General:

- Manuscripts must be written in UK English.
- The manuscript must be in Microsoft Word format. Text must be single-spaced, in 12-point Times New Roman font, and contain no unnecessary formatting (such as text in boxes).
- Please make your article concise, even if it is below the word limit.
- Qualifications, ***full*** affiliation (department, school/faculty, institution, city, country) and contact details of ALL authors must be provided in the manuscript and in the online submission process.
- Abbreviations should be spelt out when first used and thereafter used consistently, e.g. 'intravenous (IV)' or 'Department of Health (DoH)'.
- Include sections on Acknowledgements, Conflict of Interest, Author Contributions and Funding sources. If none is applicable, please state 'none'.
- Scientific measurements must be expressed in SI units except: blood pressure (mmHg) and haemoglobin (g/dL).
- Litres is denoted with an uppercase L e.g. 'mL' for millilitres).
- Units should be preceded by a space (except for % and ºC), e.g. '40 kg' and '20 cm' but '50%' and '19ºC'.
- Please be sure to insert proper symbols e.g. µ not u for micro, a not a for alpha, b not B for beta, etc.
- Numbers should be written as grouped per thousand-units, i.e. 4 000, 22 160.
- Quotes should be placed in single quotation marks: i.e. The respondent stated: '...'
- Round brackets (parentheses) should be used, as opposed to square brackets, which are reserved for denoting concentrations or insertions in direct quotes.
- If you wish material to be in a box, simply indicate this in the text. You may use the table format –this is the *only* exception. Please DO NOT use fill, format lines and so on.

SAMJ is a generalist medical journal, therefore for articles covering genetics, it is the responsibility of authors to apply the following:

- Please ensure that all genes are in italics, and proteins/enzymes/hormones are not.

- Ensure that all genes are presented in the correct case e.g. TP53 not Tp53.

**NB: Copyeditors cannot be expected to pick up and correct errors wrt the above, although they will raise queries where concerned.

- Define all genes, proteins and related shorthand terms at first mention, e.g. '188del11' can be glossed as 'an 11 bp deletion at nucleotide 188.'

- Use the latest approved gene or protein symbol as appropriate:

- Human Gene Mapping Workshop (HGMW): genetic notations and symbols
- HUGO Gene Nomenclature Committee: approved gene symbols and nomenclature
- OMIM: Online Mendelian Inheritance in Man (MIM) nomenclature and instructions
- Bennet et al. Standardized human pedigree nomenclature: Update and assessment of the recommendations of the National Society of Genetic Counselors. J Genet Counsel 2008;17:424-433: standard human pedigree nomenclature.

Preparation notes by article type

Research

Guideline word limit: 4 000 words

Research articles describe the background, methods, results and conclusions of an original research study. The article should contain the following sections: introduction, methods, results, discussion and conclusion, and should include a structured abstract (see below). The introduction should be concise – no more than three paragraphs – on the background to the research question, and must include references to other relevant published studies that clearly lay out the rationale for conducting the study. Some common reasons for conducting a study are: to fill a gap in the literature, a logical extension of previous work, or to answer an important clinical question. If other papers related to the same study have been published previously, please make sure to refer to them specifically. Describe the study methods in as much detail as possible so that others would be able to replicate the study should they need to. Results should describe the study sample as well as the findings from the study itself, but all interpretation of findings must be kept in the discussion section, which should consider primary outcomes first before any secondary or tertiary findings or post-hoc analyses. The conclusion should briefly summarise the main message of the paper and provide recommendations for further study.

Select figures and tables for your paper carefully and sparingly. Use only those figures that provided added value to the paper, over and above what is written in the text.

Do not replicate data in tables and in text .

Structured abstract

- This should be 250-400 words, with the following recommended headings:
  - **Background:** why the study is being done and how it relates to other published work.
  - **Objectives:** what the study intends to find out
  - **Methods:** must include study design, number of participants, description of the intervention, primary and secondary outcomes, any specific analyses that were done on the data.
  - **Results:** first sentence must be brief population and sample description; outline the results according to the methods described. Primary outcomes must be described first, even if they are not the most significant findings of the study.
  - **Conclusion:** must be supported by the data, include recommendations for further study/actions.
- Please ensure that the structured abstract is complete, accurate and clear and has been approved by all authors.
- Do not include any references in the abstracts.

Here is an example of a good abstract.

Main article

All articles are to include the following main sections: Introduction/Background, Methods, Results, Discussion, Conclusions.

The following are additional heading or section options that may appear within these:

- Objectives (within Introduction/Background): a clear statement of the main aim of the study and the major hypothesis tested or research question posed
- Design (within Methods): including factors such as prospective, randomisation, blinding, placebo control, case control, crossover, criterion standards for diagnostic tests, etc.
- Setting (within Methods): level of care, e.g. primary, secondary, number of participating centres.
- Participants (instead of patients or subjects; within Methods): numbers entering and completing the study, sex, age and any other biological, behavioural, social or cultural factors (e.g. smoking status, socioeconomic group, educational attainment, co-existing disease indicators, etc)that may have an impact on the study results. Clearly define how participants were enrolled, and describe selection and exclusion criteria.
- Interventions (within Methods): what, how, when and for how long. Typically for randomised controlled trials, crossover trials, and before and after studies.
- Main outcome measures (within Methods): those as planned in the protocol, and those ultimately measured. Explain differences, if any.

Results

- Start with description of the population and sample. Include key characteristics of comparison groups.
- Main results with (for quantitative studies) 95% confidence intervals and, where appropriate, the exact level of statistical significance and the number need to treat/harm. Whenever possible, state absolute rather than relative risks.
- Do not replicate data in tables and in text.
- If presenting mean and standard deviations, specify this clearly. Our house style is to present this as follows:
- E.g.: The mean (SD) birth weight was 2 500 (1 210) g. Do not use the ± symbol for mean (SD).
- Leave interpretation to the Discussion section. The Results section should just report the findings as per the Methods section.

Discussion

Please ensure that the discussion is concise and follows this overall structure – sub-headings are not needed:

- Statement of principal findings
- Strengths and weaknesses of the study
- Contribution to the body of knowledge
- Strengths and weaknesses in relation to other studies
- The meaning of the study – e.g. what this study means to clinicians and policymakers
- Unanswered questions and recommendations for future research

Conclusions

This may be the only section readers look at, therefore write it carefully. Include primary conclusions and their implications, suggesting areas for further research if appropriate. Do not go beyond the data in the article.

Editorials

Guideline word limit: 1 000 words

These opinion or comment articles are usually commissioned but we are happy to consider and peer review unsolicited editorials. Editorials should be accessible and interesting to readers without specialist knowledge of the subject under discussion and should have an element of topicality (why is a comment on this issue relevant now?) There should be a clear message to the piece, supported by evidence.

Please make clear the type of evidence that supports each key statement, e.g.:

- expert opinion
- personal clinical experience
- observational studies
- trials
- systematic reviews.

CME (by invite only)

CME is intended to provide readers with practical, up-to-date information on medical and related matters. It is aimed at those who are not specialists in the field.

From January 2016, all CME articles will be printed in full in the *SAMJ*. Please try to adhere strictly to the guidelines on word count as we have a page limit for the print issue of the *SAMJ*. We reserve the right to place some tables and reference lists online if this is necessary for space.

In practice, this means that each CME topic usually covers two issues of the print issue of the *SAMJ*.

The guest editor, in consultation with the editor, is responsible for convening a team of authors, deciding on the subjects to be covered and for reviewing the manuscripts submitted. The suggestion is for 4 - 5 articles, although there is some room for flexibility contingent on discussions with the editor.

For queries about these guidelines please feel free to contact the CME editor, Dr Bridget Farham, by email (ugqirha@iafrica.com) or telephone (+27 (0)21 789 2331).

Review process

The guest editor reviews the articles and returns them to the CME editor for review and final approval.

Guest editorials

Guideline word limit: 1 000 words

- Include the guest editor's personal details (qualifications, positions, affiliation, e-mail address, and a short personal profile (50words)).
- If possible, include a photograph of the author(s) at high enough resolution for print. It is preferable to provide two guest editorials, one for each issue, so that the content of the articles in each issue is covered.

Articles

Guideline word limit: 2 000 - 3 000 words

- Each article requires an abstract of ±200 words.
- The editor reserves the right to shorten articles but will send a substantially shortened article back for author approval.

Personal details

Please supply: Your qualifications, position and affiliations and MP number (used for CPD points); Address, telephone number and fax number, and your e-mail address; and a short personal profile (50words)and a few words about your current fields of interest.

In Practice

Guideline word limit: 2 000 - 3 000words

This section includes articles that would previously have been accepted into the Forum section, and case reports.

In practice articles are those that draw attention to specific issues of clinical, economic or political interest regarding medicine and healthcare in southern Africa. They are assigned to a topic:

Case report
Clinical practice
Clinical alert
Issues in medicine
Issues in public health
Healthcare delivery
Consensus/Position statement
Medicine and the environment
Medicine and the law

Cochrane corner

An In Practice article should follow the following format – sub-headings are not necessary, but may be used for clarity:

- Author affiliations and qualifications: to be the same as for Research. Provide all authors' names and initials, qualifications and full affiliations, and corresponding author.
- Short abstract: does not need to be structured, but should capture the essential features of the article
- Introduction: the reason for the article and the issue being addressed
- Recent research, discussion, local policy around the issue – include your own research where appropriate
- All statements should be referenced and, if opinion only, this should be stated
- Discussion: how this article adds to the discussion around a particular topic
- If a clinical practice or policy point is at issue, this needs to be emphasised, using a box with highlights if appropriate.

Essentially In practice is an opportunity for a more discursive approach to topics of clinical, economic or political importance in southern African health systems. It is not an opportunity to put forward unsubstantiated opinions!

Case reports

The *SAMJ* has recently started to accept case reports. The cases must come from Africa, preferably southern Africa unless the condition is common to all African countries, and must be either a completely new description of a clinical condition or result (use Google!) or a case that highlights important practice or management issues.

Please use the following format for case reports:

- Title of case: do not include the words 'a case report' in the title
- Summary/abstract: up to 150 words summarising the case presentation and outcome
- Background: why is this case important and why did you write it up?
- Case presentation: presenting features, medical, social, family history as appropriate
- Case management: should be according to best practice, and if not, please explain why
- Investigations, if relevant: save space by simply saying 'normal' if, for example, renal function was completely normal, rather than listing normal results, highlight the abnormal – or indeed the normal if this is clinically significant
- Differential diagnosis, if relevant
- Treatment, if relevant
- Outcome and follow-up
- Discussion – a VERY BRIEF review of similar published cases
- Teaching points: 3 - 5 bullet points
- References: as per the *SAMJ* house style
- Tables and figures: keep to a minimum. Use clinical images where relevant – we need hi-res versions for print, and identifiable persons must have a consent form
- Patient consent: please include a statement about patient consent to a written case report. This should be uploaded as a supplementary file.