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Peprah, M A (Koranteng)

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Platelet count trends in pregnant women who have pre-eclampsia with thrombocytopenia

Dr MA (Koranteng) Peprah

Supervisor: Professor EJ Buchmann



A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, in fulfilment of the requirements for the degree of Master of Medicine in Obstetrics and Gynaecology

MMed (O&G)

Johannesburg, May 2016

DECLARATION

I, Mama-Asu Peprah, declare that this research report is my own work.

It is being submitted for the degree of Master of Medicine in Obstetrics and Gynaecology at the University of the Witwatersrand, Johannesburg.

It has not been submitted before for any degree or examination at this or any other university.

.....

.....day of 2016

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ABSTRACT

Background

Pre-eclampsia can result in abnormal platelet function and count. Due to lack of resources and the subsequent over burden of specimens sent to our National Health Laboratory Services (NHLS), these results may not be obtained on time. Obstetricians and anaesthetists therefore make assumptions about platelet count in pre-eclamptic women – a common assumption is that a low platelet count will drop further as long as the woman remains pregnant. This may lead to unnecessary clinical and anaesthetist decisions in patient management.

Objective

This dissertation questions the predictability, at least while pregnancy continues, of platelet count trends in women who have pre-eclampsia with thrombocytopaenia, and also aims to identify factors associated with trends in platelet count in these women.

Methods

This study had a cohort design with data collected prospectively. Women who had thrombocytopenia as a complication of pre-eclampsia, with follow-up results while still pregnant, were included. Women were recruited at all three academic hospitals attached to the University of the Witwatersrand . No interventions were done, and all platelet count results used were from blood tests done as part of the management of each patient. Follow-up platelet counts were compared with initial platelet counts and observed for changes, using statistical tests for paired data, guided by a significance level of $p<0.05$.

Results

Thirty two women were entered into the study. For all the women, the median first platelet count on admission into the study was $112x10^9$/L and the median follow-up platelet count was $99x10^9$L, with no significant difference (Wilcoxon signed rank test for paired data, p=0.78). However, in the 12 women with raised aspartate aminotransferase (AST) levels on admission (AST≥40 U/L), the platelet count decreased by a mean of 19.7 $x10^9$/L (Student's t-test for paired data; p<0.01). No other measured risk factors were associated with decreases in platelet count.

Conclusion

The platelet count in women with pre-eclampsia who have thrombocytopenia can be predicted only in the groups with raised AST or HELLP syndrome ($p < 0.01$).

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ABBREVIATIONS

ACOG	American College of Obstetricians and Gynecologists
AST	Aspartate aminotransferase
CD63	Glycoprotein 53
CD62P	Platelet surface p selectin
CHBH	Chris Hani Baragwanath Academic Hospital
DIC	Disseminated intravascular coagulopathy
HELLP	Haemolysis, elevated liver enzymes, low platelets
INR	International normalized ratio
ITP	Immune thrombocytopenic purpura
ISSHP	International Society for the Study of Hypertension in Pregnancy
NHLS	National Health Laboratory Services
NO	Nitric oxide
PTT	Partial thromboplastin time
RCOG	Royal College of Obstetricians and Gynaecologists
RMMCH	Rahima Moosa Mother and Child Hospital
SOMANZ	Society of Obstetrics Medicine of Australia and New Zealand
TTP	Thrombotic thrombocytopenic purpura
WITS	University of the Witwatersrand

INTRODUCTION

Pre-eclampsia, commonly defined as new-onset pregnancy induced hypertension with proteinuria that develops after 20 weeks gestation, can result in abnormal platelet function and count.[1] Expectantly managed pre-eclampsia generally deteriorates and platelet counts may fall with time. However, frequent serial testing of pre-eclamptic women may not be feasible due to lack of resources and the subsequent burden and backlog of specimens sent to our National Health Laboratory Services (NHLS). We have observed that obstetricians and anaesthetists therefore make assumptions about thrombocytopenia and platelet count decline in pre-eclamptic women. A common assumption is that a low platelet count will drop further as long as the pre-eclamptic woman remains pregnant. This leads, for example, to the use of pre-operative prophylactic platelet transfusions for pre-eclamptic women who do not have severe thrombocytopaenia. This dissertation questions the predictability of platelet count trends in women who have pre-eclampsia with thrombocytopaenia.

1. LITERATURE REVIEW

1.1 Epidemiology

In 2010 the worldwide incidence of pre-eclampsia in pregnancy was between 2-8%, occurring more commonly in low income areas.[2] The incidence appears to be on the increase in developed countries too.[2,3] Figures differ according to geographic location as pre-eclampsia incidence may be influenced by environmental, socio- economic and genetic factors.[2,3]

In the 2011-2013 "Saving Mothers Report for South Africa", death from hypertension and its associated complications were recorded as the third highest cause of maternal mortality (14.8%) after non pregnancy related infections, and haemorrhage.[4] Mortality from hypertensive disorders in pregnancy occurs most commonly due to neurological complications of eclampsia and cerebral haemorrhage.[4] What was concerning in the report was that majority of the deaths were avoidable and were as a result of failed recognition of diagnosis and severity, lack of resources and delayed intervention.[4] Reduction in maternal death is one of the key goals in the Millennium Development Goals, and can therefore be attained with improved training of health professionals and provision of resources to health care facilities and to the community at large.[5]

Severe thrombocytopenia and HELLP syndrome are well known complications associated with pre-eclampsia, complicating 10% of affected pregnancies.[3,6] Severe complications

occur more frequently in early-onset pre-eclampsia (onset before 34 weeks) and in patients with previous underlying cardiovascular and autoimmune diseases.[6]

Patients at risk for pre-eclampsia

Risk factors include age over 40 years, African descent, low socio-economic status, nulliparity, previous pre-eclampsia, an interval of more than 10 years between antecedent and current pregnancy, obesity, underlying medical conditions such as diabetes, kidney disease and chronic hypertension, and the presence of antiphospholipid antibodies.[7]

1.2 Pre-eclampsia

Definitions and classification

The definitions and classifications of pre-eclampsia and its severity differ between professional groups such as the Society of Obstetrics Medicine of Australia and New Zealand (SOMANZ), the Royal College of Obstetrics and Gynaecology (RCOG), the American College of Obstetricians and Gynecologists (ACOG), the Society of Obstetricians and Gynaecologists of Canada (SOGC) and the International Society for the Study of Hypertension in Pregnancy (ISSHP).[8,9,10,11,12]

The SOMANZ classification uses the definition of pre-eclampsia as the onset of hypertension typically after 20 weeks, but there are cases that may develop before 20 weeks. Hypertension must be associated with features of end organ damage in relation to the renal, haematological, liver, neurological, respiratory, cardiovascular and uteroplacental systems.[8] The

classification does not include proteinuria as mandatory and uses a limit of systolic blood pressure ≥170 mmHg.[8]

The RCOG unlike the other institutions does not include uteroplacental insufficiency in their definition.[9] The ACOG necessitates the definition of pre-eclampsia to include proteinuria and use a lower cut off systolic blood pressure (≥160 mmHg) compared to the SOMANZ classification.[11] The former makes a clear distinction between mild and severe pre-eclampsia.[10] The SOGC published an article in 2014 regarding newest evidence surrounding pre-eclampsia and have recommended complete removal of definitions of mild and moderate pre-eclampsia.[11] The ISSHP aimed to clarify the uncertainties involving the definition and classification of pre-eclampsia by the various societies.[12] One of the questions raised was whether the presence of proteinuria was mandatory for the diagnosis, due to the high false positive readings associated with urine dipsticks, as well as the expense and time taken to collect a 24 hour urine sample.[12] The ISSHP agreed that in clinical practice it is not mandatory to include proteinuria, but in the academic setting where research will occur, inclusion of proteinuria in the diagnosis of pre-eclampsia would make selection more uniform.[12]

The Department of Obstetrics and Gynaecology affiliated to the University of the Witwatersrand (Wits) generally favours the SOMANZ classification of pre-eclampsia, with aspects similar to those of the ACOG and ISSHPP definitions.[13] The Wits definition, which is used in this dissertation includes the 160 mmHg systolic blood pressure cut off for severe hypertension and the inclusion of proteinuria in the definition, as well as the distinction of mild and moderate pre-eclampsia.[13]

1.3 Pre-eclampsia pathogenesis

Pre-eclampsia is described as the "disease of theories" because although research has been conducted for many years it still remains poorly understood and unpredictable, thus management and classification of the condition is still under debate.[14] The disease process, initiated within the placenta and the vascular endothelium, starts early in pregnancy, many weeks before manifestation of clinical disease.[14,15] The common theory that pre-eclampsia is primarily a placental function disorder is further reinforced by cases with molar and anembryonic pregnancies which develop pre-eclampsia that resolves only after the pregnancy has been terminated.[15]

1.3.1 *Early and late onset pre-eclampsia*

The pathophysiologies of early and late onset pre-eclampsia are believed to differ.[15] Early onset disease, which is clinical onset at less than 34 weeks gestation, is believed to occur as a result of abnormal adaptation and functioning of the placenta, immunological factors and abnormal endometrial preparation.[15] This results in impaired fetal growth. Haemodynamically there is typically a low cardiac output, increased peripheral resistance and small left ventricular size.[17] Von Dadelszen et al. refer to it as "placental pre-eclampsia."[11,15] Late onset disease, the clinical onset of pre-eclampsia after 34 weeks, appears to be more influenced by the underlying maternal condition of chronic hypertension or obesity as well as endothelial dysfunction.[11,16] This causes changes in vessels of the decidua, resulting in poor adaption of the spiral arterioles to the pregnancy between 10-15 weeks as well as abnormal cardiovascular response to normal pregnancy.[15,16] Haemodynamic studies reveal reduced peripheral resistance, increased cardiac output and left ventricular enlargement.[17] Von

Dadelszen et al. refer to this as “maternal pre-eclampsia”.[15] In some patients the early and late onset disease processes may be combined.[12,17]

1.3.2 *Abnormal placentation*

The placental insult begins as early as day 11 after fertilization with abnormal formation of the placenta.[18] In normal placental development the first phase of invasion occurs by week 12 where the cytotrophoblast invades the spiral arterioles within the decidua. By week 16 the second invasion occurs where the trophoblast invades even deeper into the myometrium and radial arteries.[18]

Abnormal placental development is believed to be influenced by multiple predisposing factors which include genetics, abnormally functioning natural killer cells, human leukocyte antigen (HLA) types C, D, E (which are the regulators controlling the remodelling of vessels to adapt to pregnancy), reduced levels of CD4+ T regulatory cells and increased levels of T helper 17 (found to be increased in some autoimmune conditions).[17] Decreased release of Notch ligand JAG1, has also been found in placental tissue (notch signals regulate the formation of blood vessels).[17] Abnormal placental formation results in narrow and thick walled spiral arterioles, instead of the large-calibre, virtually amuscular vessels seen in normal pregnancy.[19] The placenta then becomes hypoperfused, with the hypoxic stress adding to local damage.

1.3.3 *Endothelial dysfunction*

Fragments of placental debris may then be released into the maternal blood stream. Examples of the fragments include activated neutrophils, activated monocytes, lipid peroxidase and oxidised syncytiotrophoblast.[19]

The fragments cause an immune response and result in an exaggerated systemic inflammatory response.[17] The maternal vascular endothelium is chiefly affected, with damage especially to vessels in the kidneys, brain and liver, and an abnormal increase in endothelial activation.[17] In pre-eclampsia the balance of maintaining vascular tone is skewed, favouring the release of vasoconstrictive substances and increased vascular tone.[17] Factors that signify damage to the endothelium can be found in the blood stream several weeks before the clinical manifestation of pre-eclampsia. These include Fms-like tyrosine kinase 1 and soluble endoglin.[17] Fms-like tyrosine kinase 1 decreases Vascular endothelial growth factor and Placental growth factor levels (which are factors that maintain endothelial cells) and soluble endoglin inhibits binding of transforming growth factor 1 to the endothelial , impairing the action of vasodilator and antiplatelet aggregator nitic oxide (NO).[17]

1.3.4 *Eicosanoid imbalance*

Maternal vascular tone is maintained by endothelial production of vasoconstrictors and vasodilators. Walsh states that studies showing decreased prostaglandin E and prostacyclin combined with increased prostaglandin F2 alpha and thromboxane production have shown that eicosanoids play a role in pre-eclampsia.[20] This imbalance is triggered by oxidised fragments released by the placenta that activate cyclooxygenase enzymes to produce

thromboxane and inhibit the actions of prostacyclin synthase thus decreasing prostacyclin synthesis.[20] Walsh further suggests that decreased prostacyclin correlates with mild pre-eclampsia whereas increased thromboxane is associated with severe pre-eclampsia.[20] The prostaglandin imbalance contributes to platelet aggregation, a critical component of the pathophysiology of pre-eclampsia.

1.4 Platelet physiology

1.4.1 *Structure*

Platelets are miniscule, anucleated blood cells that consist of cytoplasmic organelles, a cytoskeleton and granules.[21] They generally have a flat surface and are irregular in shape, which allows for haemostasis. The shape of platelets is dynamic and changes depending on the level of activation, where the activated platelet has an irregular branch-like shape and the resting platelet has a smoother surface.[22] The size also differs depending on the maturity of the cell, where the more immature the platelet, the larger the size.[22,23]

Over 200 billion platelets are produced within the cytoplasm of a mature megakaryocyte every day.[23] They are the second most common blood constituent after red blood cells.[23] Their life span ranges from 7 to 10 days and the normal platelet count is between 150 and $400x10^9$/L.[23]

1.4.2 *Platelet production and life cycle*

Megakaryocytes are large cells found within the bone marrow, and a small percentage are also found peripherally in the spleen, blood and respiratory system.[23] Platelets originate from a single pluripotent haematopoietic stem cell which produces an uncommitted common myeloid progenitor cell.[24] This common myeloid progenitor cell has the potential to become any component in the haematological system, such as red and white blood cells as well as platelets.[23,24] Therefore, under the influence of thrombopoeitin, the common myeloid progenitor cell produces megakaryocyte progenitor cells, which can then form immature megakaryocytes, and finally after various structural and biochemical changes, the mature megakaryocyte.[23,24]

These various structural and biochemical changes are divided into two phases that result in platelet formation, in which thrombopoietin is the main initiating and controlling factor.[23] Phase 1 occurs under the influence of growth factors that allow the nucleus and the cytoplasm of the megakaryocyte to enlarge, and at the same time cytoskeletal proteins, alpha (CD62P) and dense (CD63) platelet-specific granules and membrane accumulate within the platelet and aid in development.[22] Phase 2 is where the cytoplasm matures to form proplatelets, which branch outwards to become preplatelets and then finally mature.[23] The products that are released from the tips of these branches are platelets, and the remainder of the cell degenerates.[23]

1.4.3 *Platelet function*

The main role of platelets is protective, as the first line in maintaining primary and secondary haemostasis.[22] In response to endothelial damage, irrespective of the mechanism of injury, platelets are programmed to aggregate rapidly at the site of injury and start formation of the haemostatic fibrin/ platelet plug.[22] The process is initiated when the damaged endothelium releases Von Willebrand Factor (VWF), fibronectin and laminin, causing the platelets to bind either directly to the subendothelial matrix proteins or indirectly by binding to VWF.[22] The latter mechanism slows down the movement of the platelet causing it to "roll" along the vessel wall towards the site of injury.[22,24] Binding is further reinforced when the collagen binds to the platelet surface receptor, resulting in platelet activation.[22] In the inactive form, platelets have glycoproteins on the membrane surface, e.g. fibrinogen receptor and CD62P and CD63.[24] When activated, the platelet increases the expression of membrane glycoproteins and increases the release of granules. There is also an associated change in shape, causing the platelet to develop branch-like outgrowths called filopodia and lamelipodia.[24] The process is further accompanied by increased surface expression of phosphatidylserine and platelet degranulation, resulting in the activated platelet.[24] Activation causes release of thromboxane, ADP, serotonin and VWF with further platelet recruitment, activation, aggregation, spreading, release reactions, and induction of pro-coagulant activity.[23,24] This exposes phospholipid binding sites, eventually resulting in the release of thrombin and formation of the haemostatic plug.[23,24] The physiological role of platelets is not limited to haemostasis. Other functions include roles in inflammation, vasoconstriction, and wound healing.[22]

1.5 Measurement of the platelet count

Platelet count, in the context of pre-eclampsia and its complications, is used to determine the severity of disease, and helps the clinician decide when to expedite delivery. It is also used to determine the mode of anaesthesia as well as whether a platelet transfusion is required.

Measuring the platelet count using full blood count technology is only one example of how platelet function is assessed, acting as a screening tool and raising red flags if an abnormal count is reported.[22] However, the count itself cannot establish the cause of thrombocytopaenia, and further functional tests need to be conducted.[26]

Blood for full blood count is taken in an ethylene diamine tetra-acetic acid (EDTA) tube that contains an anticoagulant (usually buffered sodium citrate).[27] Correct technique is required to ensure that a good sample is taken and accurately measured. This includes use of a tourniquet applying light circumferential pressure to the arm, a 21 gauge needle or larger, and easy aspiration of blood to a specimen volume of at least 2 mL for accurate analysis.[27] A little-known point is that the initial 2 mL aspirated should be discarded. This diminishes the chances of artefacts and platelet clumping as a result of the blood clotting, which would give a falsely low value of platelets.[27]

Where a full blood count shows a very low platelet count or abnormal platelet morphology, manually evaluating and counting of platelets under the microscope can still be used. Both of these factors can be missed or falsely identified with the automated machine.[27]

1.6 Thrombocytopenia in pregnancy

Thrombocytopenia, defined as a platelet count <150 x10^9 /L, affects 7-10% of pregnancies.[28]

1.6.1 Causes are as follows:[21,29]

1. Decreased production in the bone marrow

- Haematological malignancies (e.g. leukaemia)
- Aplastic anaemia
- Myelodysplasia
- Bone marrow infiltration from metastasis
- HIV infection
- Drugs
- Radiation
- Vitamin D deficiencies
- Hereditary thrombocytopenia

2. Increased destruction.

Immune-related peripheral destruction

- ITP (immune thrombocytopenic purpura)
- HIT (heparin induced thrombocytopenia)
- Drug induced
- HIV infection
- Post-transfusion purpura
- Connective tissue diseases (systemic lupus erythematosus,

antiphospholipid syndrome, vasculitis)

- Pre-eclampsia and HELLP syndrome
- Acute fatty liver of pregnancy

Non-immune related peripheral destruction.

- Disseminated intravascular coagulopathy (DIC)
- Sepsis
- Cardiac valves
- Thrombotic thrombocytopenia purpura-haemolytic uraemic syndrome
- Kasabach Merrit syndrome
- Haemodialysis
- Endocarditis

Splenic Sequestration

- Hypersplenism

All of the above causes, other than pre-eclampsia, HELLP syndrome and acute fatty liver, are not specific to pregnancy. The causes in pregnancy are predominantly secondary to increased destruction. The focus of the rest of this section will be on the differential diagnosis of thrombocytopenia in pregnancy.[29]

1.6.2 *Gestational thrombocytopenia*

Gestational thrombocytopenia is the most frequent cause of low platelets that occur after 20 weeks gestation.[28] It typically presents in the third trimester, resulting in a mild form of thrombocytopenia (no clear numerical value) and requires no treatment.[28] The platelet count returns to a normal value within 12 weeks after delivery and has no adverse effect on mother or baby.[28] Gestational thrombocytopaenia is a diagnosis of exclusion that can mimic early features of HELLP syndrome, especially when the blood pressure is not yet elevated. Functionally, it has no clinical significance.[21]

1.6.3 *Thrombotic thrombocytopenic purpura*

Thrombotic thrombocytopenic purpura (TTP) is described as micrangiopathic haemolytic anaemia together with fever, thrombocytopenia and neurological symptoms (headache, seizures, altered levels of consciousness).[21,30] It should always be in the differential diagnosis of thrombocytopenia in pregnancy because of its rapid progress and high mortality rate.[30] Causes can be hereditary as a result of an inherited deficiency in ADAMTS13 (an enzyme needed for cleavage of VWF); or acquired as an autoimmune response against ADAMTS13.[30] The deficiency results in activation of VWF and attracts and activates platelets, causing obstruction of small calibre vessels and ischaemia, resulting in end organ damage.[30] Some patients with the inherited condition can present for the first time in pregnancy as pregnancy is one of the known triggers, causing confusion with features of pre-eclampsia or HELLP syndrome.[21,30] Treatment is by plasma exchange and attention to the precipitating cause.[22,38]

1.6.4 *Haemolytic uraemic syndrome*

The clinical picture of haemolytic uraemic syndrome is similar to TTP except for the presence of significant renal dysfunction.[21] Features of bloody loose stool may also be present if Shigella is the causative organism.[21]

1.6.5 *Immune thrombocytopenic purpura*

Immune thrombocytopenic purpura (ITP) should be the last in the list of differential diagnoses in a patient with thrombocytopaenia in pregnancy.[21] ITP is autoimmune mediated, possibly triggered by drugs, connective tissue diseases or infections.[21] Treatment is achieved by treating the underlying cause as well as platelet transfusion, immunoglobulins, and immunosuppressive agents. Splenectomy is indicated in patients with a poor response to medication.[21]

1.6.6 *Pre-eclampsia*

Thrombocytopaenia is one of many complications associated with pre-eclampsia and occurs typically in 10% of cases.[31] However, a cohort study by Burrows et al. found an incidence approximately five times higher, reporting a 50% occurrence rate in their pre-eclamptic population – this 50% occurrence rate is also supported by Leduc et al.[32,33] The authors of the two studies suggested that the abnormally high incidence was because the study population only included those with pre-eclampsia severe enough to require intensive care admission. The thrombocytopaenia can be isolated (apart from clinical evidence of pre-eclampsia) or can occur as part of the HELLP syndrome.[34] The platelet count improves within a few days after delivery.

1.6.7 *Haemolysis, elevated liver enzymes, low platelets syndrome*

Haemolysis, elevated liver enzymes, low platelets (HELLP) syndrome is a severe form of pre-eclampsia in which the pathophysiology and risk factors are mostly similar.[21] The difference is in the microangiopathy that occurs in HELLP syndrome.[21] In addition to thrombocytopaenia, haemolysis is evident on peripheral smear, showing schistocytes and spherocytes, with elevated serum lactate dehydrogenase. Elevated transaminase levels are observed and hyperbilirubinaemia may occur.[21] Hypertension is not present in up to 20 % of cases.[21] Endothelial dysfunction is believed to cause the reduction in the platelet count by causing platelet aggregation and destruction.[21] Similarly to pre-eclampsia, the platelet count will also improve after delivery.

1.6.8 *Acute fatty liver in pregnancy*

Acute fatty liver, which is also a microangiopathic haemolytic condition, is also in the list of differentials for HELLP syndrome as it presents with right upper quadrant pain, moderate thrombocytopenia, elevated liver enzymes and renal failure, elevated conjugated bilirubin (frequently > 5mg/dL), and coagulopathy.[35]

1.6.9 *Disseminated intravascular coagulopathy*

Disseminated intravascular coagulopathy (DIC) is a consumptive coagulopathy, triggered by an assortment of pathologies. In the obstetric setting the causes include sepsis, haemorrhage, intrauterine fetal death, amniotic fluid embolism, abruptio placentae and eclampsia.[36] These triggers lead to activation of the coagulation system. Haematological parameters in DIC include prolonged International normalised ratio (INR) and partial thromboplastin time

(PTT), thrombocytopenia, low fibrinogen levels and elevated D-dimers.[37] If the triggers of DIC are not treated, pathology may progress and cause consumption of factors V and VIII in the clotting cascade as well as platelets, all resulting in increased fibrin deposition, thromboembolism and tissue ischaemia.[37] Fibrinolysis follows with formation of fibrin degradation products and haemorrhage is consequent on clotting factor and platelet consumption.[37] HELLP syndrome and pre-eclampsia are often included in the differential diagnosis of DIC.[37] In mild cases, the diagnosis may be difficult in pregnancy as some of the clotting parameters change as part of a normal physiological response to pregnancy, for example D-dimers being elevated.[37]

1.7 **Why platelet counts drop in pre-eclampsia**

Investigations concerning the mechanism and pattern of thrombocytopenia in pre-eclampsia were popular, mainly in the 1980s and 1990s, predominantly among American authors. There are few articles published after 1995, and these articles typically quote the older ones. Studies had differing conclusions and about three decades later few newer findings have arisen. Theories for the pathogenesis of thrombocytopaenia include increased platelet activation, increased destruction likened to DIC, an immune component, dysfunctional platelets as one of the causes of pre-eclampsia, reduced platelet life span through microangiopathic haemolytic processes, and increased turnover.[38]

1.7.1 *Abnormal increase in platelet activation*

The most common theory for thrombocytopaenia in pre-eclampsia is that of increased platelet activation.[39] The actual trigger remains unclear, but is thought to be abnormal eicasanoid

production related to placental dysfunction.[39,40] Studies have aimed to prove this theory by demonstrating an increase in markers that rise during platelet activation. Some of the markers include raised levels of the compound urinary 11-dehydrothromboxane b2/mg creatinine, B-thromboglobulin, platelet factor 4, CD63, CD62P, fibrinogen antigens and increased platelet volume.[40,41,42]

Hayashi et al. in 2002 conducted a study on 99 women, investigating five markers that would accurately represent the link between the endothelium, coagulation and pre-eclampsia.[41] With regard to platelet count trends in pregnant women who have pre-eclampsia with thrombocytopenia, Hayashi et al. used urinary 11- dehydrothromboxane b2/mg creatinine, B-thromboglobulin and platelet factor 4 as markers of platelet activation. Thromboxane B2 is a metabolite of thromboxane A2 which is an activator of platelets. Unlike Thromboxane B2 in blood, Urinary 11-dehydrothromboxane B2 is thought to be more stable and a more accurate representation of thromboxane levels as it is not affected by blood aspiration. Creatinine ratio is included in the measurement because urine output can affect the thromboxane levels.[41] The authors found that the median platelet count decreased as it progressed from the non-pregnant controls, to pregnant patients with normal blood pressure, to those with mild and severe pre- eclampsia. These findings where statistically significant ($p<0.05$).[41] Interestingly, the progressive decrease of platelet count coincided with an increase in urinary 11-dehydrothromboxane b2/mg creatinine, B-thromboglobulin and platelet factor 4, suggesting that the decrease in platelet count was related to platelet activation.[41] The increase in platelet factor 4 was only statistically significant in the severe pre-eclamptic group, and the increase in B-thromboglobulin and urinary 11 dehydrothromboxane b2/ mg creatinine was only statistically significant compared to the non-pregnant control ($p<0.05$).[41] Hayashi et al. used strict criteria to decrease the chances of measurement bias by excluding those with

comorbid disease that would impact on hypertension, and ensured a detailed and standardised method for urine and blood collection.[41] On the other hand, Major et al. (2015) found no features suggestive of platelet activation in pre-eclampsia.[42] Major et al. conducted a small study that found an increase in platelet factor 4 levels in both pre-eclamptic and normotensive pregnant patients, suggesting that it was pregnancy rather than pre-eclampsia alone that resulted in an increased level.[42]

Janes et al. in 1994 aimed to clarify the differences in platelet behaviour expressed in various studies in pre-eclampsia by adding whole blood flow cytometry methods to measure platelets, in addition to blood aggregometry and measurement of B-thromboglobulin levels.[40] Blood aggregometry and measurement of B-thromboglobulin levels are the usual methods used by researchers to assess platelet behaviour. The two methods have been criticised because platelet results are affected by the manner in which the blood sample is collected and measured.[40] Flow cytometry is considered superior.[40] Janes et al. analysed the blood of 55 women categorising them into four groups: non pregnant, pregnant normotensive, hypertensive and pre-eclamptic, and measured the platelet count, B-thromboglobulin, as well as CD63 antigen and fibrinogen antigens (the last two are antigens that become exposed on the surface of the platelet membrane after structural changes associated with platelet activation). In agreement with Hayashi et al., Janes et al. found elevated B-thromboglobulin levels in the hypertensive (75 μg/l) and pre-eclamptic groups (81 μg/l) , compared to the non- pregnant group (42 μg/l) ($p < 0.01$). They also found higher B-thromboglobulin levels expressed per platelet in the hypertensive and pre-eclamptic groups (0.39 $\mu g/10^9$ platelets and 0.46 $\mu g/10^9$ platelets) compared to the non-pregnant controls (0.26 $\mu g/10^9$ platelets) ($p-< 0.01$).[40] The criticism of Janes et al. is that their definition of pre-eclampsia was inconsistent with the definitions used by the recognized societies mentioned previously.

It should be noted, however, that this work was published two decades prior to the ISHHP document.

Increased expression of cell adhesion molecules such as CD62P and CD63, as well as increased binding of activated platelets to neutrophils and monocytes, and increased platelet-derived microparticles have also been used as markers of increased platelet activation. Macey et al. in 2010 found higher amounts of CD62P in pre-eclamptic women (0.8%) compared to normotensive pregnant women(0.3%), and higher platelet monocyte aggregate formation of 2.8% in the normotensive compared to 4.8% in the pre-eclamptic group ($p<0.05$).[43] Results from a London-based case control study also supported findings of Macey et al.[40] Evidence of increase in platelet-derived microparticles was not supported by Van Wijk in 2002 nor by Lok et al. in 2008.[45,46] In contrast, they found decreased levels of microparticles.[45,46] Lok et al. suggested that the lower levels of microparticles could have been related to the overall low platelet count in the pre-eclamptic women; both studies used only small samples of women.[45,46]

Ceyhan et al. in 2006 investigated 100 patients comparing full blood count and mean platelet volume in mild and severe pre-eclamptic and normotensive pregnant patients.[47] The increased platelet volume is believed to represent increased production of immature platelets, this increased production in response to increased demand and platelet activation. Ceyhan et al. found no statistically significant difference in the platelet count nor mean platelet volume, in the normotensive or pre-eclamptic groups.[47]

Overall, the results of increased platelet activation as a cause for reduced platelets in pre-eclampsia seem convincing. However, the numerous potential markers make it difficult for researchers to decide which one is most accurate. Research comparing the efficacy of each test may be helpful in the future.

1.7.2 *Initiating factor of pre-eclampsia*

A review by Redman et al. in 1990 raises the question as to whether the reason for a decrease in platelet count in pre-eclampsia is because platelets actually play a role in the aetiology of pre-eclampsia.[48] Redman et al. refer to a study that was conducted by Zemel et al. in 1990 to find out whether increased release of free calcium stored in the cytoplasm of platelets precedes the clinical manifestations of pre-eclampsia.[49] Calcium is a secondary messenger involved in the activation of platelets. Increased release of free calcium within platelets would indirectly imply increased activation of platelets.[48] Redman et al. conducted a follow-up study on 48 women measuring their platelet intracellular ionised calcium levels at the end of the first trimester, at 25 weeks, and at 34-36 weeks, and found that of the 14 women who later developed pre-eclampsia, 11 showed increased intracellular free calcium within platelets after the addition of arginine vasopressin ($p<0.01$), well before the onset of clinical preeclampsia.[48] Konijnenberg et al. in 1997 used a different method to test whether platelets were involved in pre-eclampsia aetiology.[50] They measured P-selectin, platelet endothelial cell adhesion molecule and the cell adhesion molecule CD63, aiming to show that these markers of platelet activation would be increased early in those destined to complicate with pre-eclampsia.[50] Konijnenberg et al. conducted their study on 244 pregnant women from 13 weeks gestation and found that of the 17 who developed pre-eclampsia, eight had increased first trimester CD63 expression, implying that increased CD63 levels could be used to predict

pre-eclampsia. They used a percentage of activated platelets above 2% as reference of a positive test of risk association, and the 2% cut off was considered to yield the highest sensitivity and specificity.[50] However, the sensitivity and specificity were low (47% and 76% respectively) with a poor likelihood ratio of 1.94 for predicting pre-eclampsia.[50] P-selectin and platelet endothelial cell adhesion molecule were not found to be raised early on in the pre-eclamptic group.

It has proved difficult to implicate platelet activation early in the pregnancies, before clinical onset of pre-eclampsia as a cause of pre-eclampsia rather than a complication. If platelets are involved early in the pathogenesis of pre-eclampsia, one would expect an increase in most or all markers of platelet activation in advance of clinical pre-eclampsia disease. The laboratory markers investigated so far do not appear to be useful for screening or early detection of pre-eclampsia.

1.7.3 *Immune component*

The presence of increased platelet associated IgG in Burrows et al.'s prospective study from 1987 may suggest an immune component to platelet destruction.[32] They monitored 61 pregnant patients with pre-eclampsia and 25 normotensive patients, and found that about one-fifth of pre-eclamptic patients had elevated levels of platelet associated IgG, and in this group 42.5% had thrombocytopenia.[32] None of the normotensive patients showed elevated levels of IgG.[32] However, the findings by Burrows et al. failed to support this theory entirely because 25% of patients with pre-eclampsia without thrombocytopenia also demonstrated elevated IgG.[32] Whether this may be an early sign of future thrombocytopenia is not clear. There is

insufficient evidence to suggest an immune component as there appears to be no further research published regarding this matter.

1.7.4 *Subclinical activation of the coagulation system and disseminated intravascular coagulopathy*

A review by Letsky el al. stated that the finding of a low platelet count in the absence of antiplatelet antibodies in pre-eclampsia could suggest the presence of a mild consumptive coagulopathy.[36] This coagulopathy has been likened to DIC in a study conducted by Heilman et al. in 2007 where they measured factors that affect coagulation, including platelet count and D-dimers from the blood of 111 women with early and late onset pre-eclampsia, and compared these to 33 healthy pregnant women.[51] They found that in the group with early onset pre-eclampsia, a low platelet count $<50x10^{9/}L$ corresponded with an increased D-dimer ($r = -0.578$, $p < 0.05$).[51] Heilman et al. failed to demonstrate this correlation in the group with late onset pre-eclampsia and the study did not measure the INR or PTT, which would have provided the other necessary parameters to diagnose a DIC.[51] In their study, Leduc et al. in 1992 also aimed to determine if there were other markers in the coagulation cascade that would be abnormal if the platelet count was low in women with pre-eclampsia.[33] The findings of Leduc et al. disproved DIC as a cause of thrombocytopenia in pre-eclampsia by demonstrating that out of the 50 patients who had platelet counts $<150\ x10^{9}$ /L, 17 had low fibrinogen levels (300mg/dL) and increased INR and increased fibrin degradation products only occurred in 2 of these cases; both of which had platelets below $50\ x10^{9/L}$.[33] Janes et al. also supported the findings of a negative association of DIC and thrombocytopenia by Leduc et al. by demonstrating higher fibrinogen levels in the pre-eclamptic compared to the normotensive and non-pregnant group, (4.8g/dL and 3.8g/dL) ($p< 0.01$). They also found no

other abnormalities in INR or PTT.[40] DIC as the cause of thrombocytopenia in pre-eclampsia appears to be unlikely, but a mild form may contribute in the pathogenesis of pre-eclampsia in its entirety.

1.7.5 *Damage by activated endothelium*

There are suggestions that abnormal endothelial structure and function associated with pre-eclampsia is a cause of a low platelet count[52] In their prospective study analysing the relationship between reduced prostacyclin biosynthesis and pregnancy-induced hypertension, Fitzgerald et al. in 1990 found that the endothelium produced less 2, 3-dinor-6-keto-PGF (a prostacyclin metabolite) and more thromboxane in women with pre-eclampsia compared to their normotensive counter parts.[53] Thromboxane is primarily produced by the endothelium and its main effects include vasoconstriction and platelet activation and aggregation , with increased levels associated with pre-eclampsia all these effects are amplified.[53] Abnormal endothelium may result in platelet adherence to the endothelium destroying the platelets by shear forces, and this is believed to occur as a microangiopathic haemolysis in HELLP syndrome.[53] Another possibility is that the platelet count drops as platelets aggregate in areas of endothelial damage to aid with healing and thrombus formation with increased deposition of thrombin.[52] Another theory is that the life span of the platelet is reduced by 3-5 days because of changes in the structure of the platelet, and that this abnormal morphology results in the premature destruction of platelets.[52,53] All these findings suggests a combination of increased platelet activation, aggregation and destruction as a cause of low platelet count in pre-eclampsia.

1.8 **Platelet count trends in pre-eclampsia**

There is little literature regarding platelet trends in pre-eclampsia. Leduc et al. in 1992 conducted a retrospective study where they reviewed the files of 100 women diagnosed with pre-eclampsia or chronic hypertension with superimposed pre-eclampsia.[33] Platelet counts were measured within 2 hours of admission then 6 hourly thereafter.[33] They found that half of these patients had thrombocytopenia as a complication.[33] They then used a regression equation to analyse the platelet count on admission and see whether it would determine the lowest platelet count drop later on.[33] They found that if the platelet count on admission was $<200x10^9$/L, there was 50% confidence that the lowest platelet count would be $<150x10^9$/L. They also found that if the platelet count was$<100x10^9$/L on admission, the subsequent platelet count had a 75% likelihood of dropping below $100x10^9$/L, even as low as $50x10^9$/L ($p<0.01$).[33] The findings suggested that platelet counts dropped in pre-eclampsia, and that the lower the platelet count, the greater the likelihood of further decreases.

A study conducted by Rinehart et al. in 2001 aimed to determine whether the initial platelet count and LDH level could identify patients in whom disease progression would occur as well as the rate at which it would occur.[55] The study recruited patients with HELLP syndrome and with severe pre-eclampsia.[55] The authors further categorised patients with HELLP syndrome into three groups depending on lowest platelet counts recorded at the time of delivery: class 1 had counts $<50x10^9$/L, class 2 with $50\text{-}100x10^9$/L and class 3 from $100\text{-}150x10^9$/L.[55] They recorded the platelet count on admission, two hours prior to delivery, during labour and in the post-partum period.[55] Firstly, regarding the platelets, they found that only 10% of the 639 patients reached the lowest platelet count prior to delivery, the majority reaching the platelet nadir in the post-partum period (61%).[55] The second finding was that

patients whose platelet count reached <50 x10^9/L prior to delivery had the fastest rate of decline over the 2 days prior to this measurement (a rate of 50x$0^{9/}$L/day).[55] The rate of decline was significantly less if the platelet count was >50x10^9/L. This accords with the findings of Le Duc et al.[33] The study failed to demonstrate what the initial platelet count was. What is therefore missing is prospective work to predict platelet count declines in women with pre-eclampsia and thrombocytopaenia.

1.9 **Antenatal corticosteroid medication**

Glucocorticoid drugs are anti-inflammatory and immune-suppressive in nature, and it is therefore reasonable for researchers to believe that these drugs can counteract the pathophysiological process of pre-eclampsia and its complications.[56] With HELLP syndrome, these steroids are believed to inhibit platelet adhesion and decrease splenic sequestration of damaged platelets.[56] Martin well known for his research in corticosteroids and pre-eclampsia conducted a study in 2003 regarding the benefits of steroids in HELLP syndrome and found that intravenous administration of high dose (10mg) dexamethasone 12 hours apart resulted in fewer maternal complications, including a higher mean platelet count of 65x10^9/L compared to the 55x10^9/L in the group who had received a lower dose of steroids ($p<0.01$).[57] A Cochrane Review did not find such benefit and showed that there were limitations to this study as well as other studies that show benefit of steroids.[58] The review noted that the trials did not compare steroids to placebo, that they gave the patients either medication or none at all, some had small sample sizes, different types and doses of steroids were used, and the definitions of HELLP syndrome differed between the studies.[58] There is however still uncertainty about the therapeutic role of steroids in HELLP syndrome. Improvement of the platelet count due to steroids alone seems unlikely.

1.10 **Platelet count cut-offs for regional anaesthesia**

The platelet cut off for safe administration of regional anaesthesia is controversial. A review by Loo et al. stated that the greatest concern in performing regional anaesthesia on a patient with coagulation abnormalities is the development of haematoma and associated neurological sequela, should the venous plexuses be punctured.[59] What makes the topic controversial is that the incidence of neurological complications appears to be low having been recorded in a few case series and retrospective file reviews with an incidence of 0.2-3.7/100 000 in epidural blocks and 5 per million in a general population undergoing spinal anaesthesia in the United Kingdom.[59] But anaesthetic researchers are understandably reluctant to conduct interventional research to confirm the lowest platelet count cut off, and would rather perform general anaesthesia in these patients. The known cut off of 75-80x10^9/L, is believed to have originated from Orlikowski et al. study from 1996, where they measured the platelet count, bleeding time and thromboelastography(used to monitor the characteristics of a clot) in 49 pre-eclamptic and eclamptic women.[60] Orlikowski et al. found that a platelet count <54x10^9/L suggested a reduced ability for clot formation to be preserved (95% confidence interval of 40-75x10^9/L).[60] Rucklidge and Paech (2001) reviewed the files of 9735 women who had received regional anaesthesia, of whom 47 had platelet counts from 75-100x10^9/L and 13 had platelet counts<75 x10^9/L.[61] The retrospective review by Rucklidge and Paech found that none of these patients had neuraxial haematoma as a complication.[61] Pre-eclampsia was the second most common cause of the thrombocytopenia (26%) in the study population.[61] Beilin suggests a lower platelet count of 50-100x10^9/L as safe for regional anaesthesia, and states that the findings are also recommended by the ACOG.[62]

The majority of anaesthetists agree that regional anaesthesia can be administered with a platelet count below $100x10^9$/L but the lowest value has yet to be confirmed.

1.11 **Platelet count cut-offs for prophylactic platelet transfusion at delivery**

Prophylactic administration of platelets prior to surgery to prevent blood loss in patients with low platelets is also controversial. Platelet transfusions like all blood products has associated risks, is expensive, and supply is limited.[63] The cut off level of $<50x10^9$/L for patients undergoing invasive procedures such as surgery is rather arbitrary and is based on level 4 evidence from the opinion of experts and a small number of case series.[64] Original research concerning prophylactic platelet transfusion, irrespective of subsequent surgery, was conducted by Gaydos et al. in the 1960s where they reviewed the files of 92 patients with leukaemia aiming to see at what level the platelet count would correlate with spontaneous haemorrhage.[65] They found that haemorrhage was least likely to occur if the platelet count was $>20x10^9$/L.[65] Gaydos is credited with the original data that has led clinicians to use a platelet count of $>20x10^9$/L as an indication for prophylactic platelet transfusions to prevent spontaneous bleeding.[65] The suggestion of transfusion with platelet counts $<50\ x10^9$/L for those undergoing surgical intervention remains an arbitrary one based on expert opinion guidelines and a limited amount of research.[66] The most data collected regarding major surgery on patients with thrombocytopenia was in 1987 by Bishop et al.[67] Bishop and colleagues reviewed the files of 95 patients with leukaemia who had a surgical procedure (majority were laparotomies and craniotomies) and found that in the group where platelets were $>50x10^9$/L, significant blood loss of more than 500 mL and need for significant blood transfusion of four units occurred in only 7% of cases.[67] Limitations of Bishop et al. work was its retrospective nature and lack of a comparative arm. In 1990 McVay retrospectively

analysed the files of patients who had liver biopsies and recorded an incidence of significant haemorrhage in 3.4% of the patients with platelets of 50-99x10^9/ L; this figure was similar to those patients who had liver biopsy with normal blood results.[68] These findings, although of poor quality, support a platelet count of >50 x10^9/L as safe to proceed with some surgical procedures, including caesarean section, without needing to resort to prophylactic platelet transfusion.

2. **PROBLEM STATEMENT AND MOTIVATION FOR RESEARCH**

A low platelet count is a marker of disease severity and is an indicator of poor prognosis for both mother and fetus in women with pre-eclampsia and related syndromes. The platelet count is therefore used in clinical management decisions such as admission to high care area and early delivery of the fetus. Once a patient with a low platelet count has been identified, there is understandably concern that the platelet count will fall further, to the point of causing bleeding complications, either during spinal or epidural analgesia, or at caesarean section. In the absence of frequent serial testing of platelet counts in such patients, clinicians may make assumptions about the platelet count trend. Such assumptions can affect clinical management of patients in the following ways:

1. General anaesthesia preferred over regional analgesia in patients with platelet counts >75 $x10^9$/L, based on the concern that such platelet count will have fallen to below this level by the time anaesthesia is given. General anaesthesia itself is not without hazard in these patients, and complications include aspiration of stomach contents as well as acute severe hypertension.[69]

2. Prophylactic platelet transfusions given to patients with platelet counts >$50x10^9$/L before caesarean section. The belief, again, is that the platelet count would have fallen to <$50x10^9$/L by the time caesarean section starts. Besides the cost of transfusion, there is always a small risk of transfusion-related complications.

3. Longitudinal skin incisions made in pre-eclamptic women with a low platelet count to decrease blood loss associated with injury of abdominal wall vessels with a transverse incision. Besides cosmetic objections, higher rate of post-operative pain and incisional hernia have been shown for patients undergoing vertical skin and sheath incisions.[70]

4. The fetus delivered prematurely if the platelet count is $>100x10^9$/L, in anticipation of a drop in platelet count to $<100x10^9$/L. This is done rather than repeating the blood test and waiting for a result. Premature delivery increases the infant's length of admission, likelihood of admission to an intensive care unit and increased risk of preterm birth complications.[71]

2.1 **Purpose of the study**

Only two studies could be found that considered platelet count trends in pregnant women who have pre-eclampsia with thrombocytopaenia. This study was done to prospectively identify patients diagnosed with pre-eclampsia and who have thrombocytopaenia, and follow their platelet counts while still pregnant to identify a predictable trend. Based on the results of this study, more educated predictions or resisting the temptation to predict the platelet count may decrease the need for unnecessary expense involved, such as mentioned in the four situations discussed above.

2.2 **Objectives of the study**

1. To describe platelet counts on admission to hospital in women with pre-eclampsia who have thrombocytopaenia and who have follow-up platelet counts performed while still pregnant.

2. To describe demographic and clinical factors in women admitted with pre-eclampsia who have thrombocytopaenia and who have admission and follow-up platelet counts performed while still pregnant.

3. To describe follow-up platelet counts in these women while still pregnant and observe any trend in the platelet count.

4. To identify factors associated with trends in platelet count in these women.

3. **METHODOLOGY**

3.1 **Definitions of key terms (adapted from the ACOG guidelines)**

Pre-eclampsia: elevated blood pressure of ≥140/90 mmHg on ≥2 occasions (readings should be taken at least 4 hours apart), and significant proteinuria ≥300 mg collected in a 24 hour specimen or ≥1+ proteinuria on test strips, had no history of chronic hypertension and who were normotensive at antenatal booking and non-proteinuric. The definition for this study allows inclusion of women who presented for antenatal care after 20 weeks of gestation and were normotensive at the time.

Severe pre-eclampsia:blood pressure ≥160/110 mmHg on two or more occasions at least 4 hours apart or a once-off diastolic blood pressure ≥120 mmHg, or proteinuria ≥5g over a 24 hour period or persistently ≥3+ proteinuria on test strips.

Or

New-onset elevated blood pressure after 20 weeks of pregnancy with at least one of the following features:

- Features of imminent eclampsia
- Neurological or visual disturbances such as severe headache, hyper reflexia and seizures
- Epigastric or right upper quadrant pain
- Pulmonary oedema or central cyanosis
- Impaired liver function AST ≥40U/L

- Thrombocytopenia <150x10^9/l or evidence of haemolysis (Mississippi classification used).[69]
- Features of placental insufficiency and intra uterine growth restriction
- Renal dysfunction with creatinine >100μmol/L, urine output <500 mL in a 24 hour period or urea >8 mmol/L

3.2 **Research design and ethics**

The study was a cohort design, with data collected prospectively. Patients were recruited at all three academic hospitals attached to the University of the Witwatersrand. No interventions were done, and all platelet count results used were from blood tests done as part of the management of each patient. Permission to undertake the study was obtained from the Human Research Ethics Committee of the University of the Witwatersrand (certificate number M130737, attached as Appendix D), and from the Chief Executive Officers of the three academic hospitals.

3.3 **Study population**

The study population was women with pre-eclampsia, including HELLP syndrome, found to have, on admission to hospital, a platelet count <150x10^9/L (thrombocytopaenia), or who developed thrombocytopaenia while admitted to hospital. Women had to have two or more platelet counts estimated while pregnant, from entry into the study up to delivery. Because of the local high proportion of women who book late (after 20 weeks) for antenatal care, the definition of pre-eclampsia was extended to include women who booked after 20 weeks of

gestation, -who had no history of chronic hypertension and who were normotensive and non-proteinuric at booking.

Exclusion criteria:

- Gestational age <20 weeks
- Intrauterine fetal death
- Abruptio placentae
- Eclampsia
- Connective tissue or autoimmune disorders
- Advanced HIV disease (World Health Organization stage 4)
- Acute infections
- Platelet transfusions received before the second platelet count
- Anticoagulant medication
- Haematologic disorders resulting in low platelet counts, e.g. VWF disease
- Microangiopathies other than HELLP syndrome, e.g. haemolytic uraemic syndrome, thrombotic thrombocytopaenic purpura

3.4 **Sampling**

The researcher, a registrar in Obstetrics and Gynaecology, had time on certain days of her rotation at the various hospitals to collect data for this study. On such days, she identified all women potentially eligible for the study admitted during the previous 24 hours (pre-eclamptic with thrombocytopaenia), and obtained informed consent from them to be included in the study. The women were followed up for later platelet count results and clinical course. The sample was therefore a period sample restricted to days that the researcher was available to collect data. The initial plan was to recruit about 100 participant women for the study.

Gestational age was calculated using the best estimate. Early ultrasound scan (before 24 weeks) was the preferred method for calculation. In the absence of early scanning, the last menstrual period was used next, failing which late ultrasound or clinical assessment was used.

Steroids administered in anticipation of preterm birth were betamethasone 12mg intramuscular injections two doses 12 hours apart. The administration of steroids was recorded in relation to the time that the second platelet count was obtained. Steroids are not used at Wits teaching hospitals to treat HELLP syndrome.

3.5 **Data collection techniques**

The researcher went to the obstetric high care area and identified patients diagnosed with pre-eclampsia who had thrombocytopaenia, and excluded those found under the exclusion criteria list. The included patients were then followed up in the antenatal and post-natal wards.

The researcher explained the research, using an interpreter if needed, and obtained consent to look through patients' hospital files while they were in the ward to make sure that they met the criteria of pre-eclampsia and thrombocytopaenia and recorded this information in the data sheet (Appendix A, B, and C). The researcher then recorded their hospital numbers and checked on the NHLS computer for platelet count results. She continued to check any further results of platelet counts until they were discharged. The patients were chosen from all three Wits academic hospitals, depending on the researcher's work rotation. The time interval taken between subsequent platelet counts was not set, as this was determined by clinical decisions of the staff on duty. The time interval between platelet count tests was determined by using the time that the blood sample specimen was entered on the computer in the NHLS laboratory. The researcher recorded all information on a data collection sheet and transferred it to Microsoft Excel.

The following data were collected:

- Demographics: age, gravidity and parity, gestational age at time of first antenatal booking, blood pressures and urine test strips on booking.
- HIV status, CD4 counts HIV WHO classification, antiretrovirals used.

- Presence of medical disorders such as diabetes mellitus.
- The use of aspirin, nifedipine, methyldopa and/or magnesium sulphate.
- Highest blood pressure and proteinuria during admission, and clinical or biochemical complications associated with pre-eclampsia.
- Features of placental insufficiency.
- Severity of pre-eclampsia.
- Administration of steroids for anticipated preterm birth.
- The platelet count reading on admission and the subsequent platelet count.
- Date and time, mode and outcome of delivery.

3.6 **Data analysis**

Data from Microsoft Excel were imported to Stata 11 statistical software (Statacorp, College Station, Texas, USA). Categorical variables were reported as proportions with percentages, and continuous variables were reported as means with standard deviations or medians with ranges. Differences in platelet counts were treated as continuous variables. Comparisons of first and subsequent platelet counts were made using Student's t-test for unpaired normally distributed data or Wilcoxon's signed rank test for paired skewed data. A p value <0.05 was accepted as indicating statistical significance.

4. **RESULTS**

4.1 **Demographic information**

Thirty two women were included in the study. The mean age of the women was 27.1 years with a range of 18-36 years. Sixteen (50%) were from Rahima Moosa Mother and Child, ten (31%) from Chris Hani Baragwanath Academic and six (19%) from Charlotte Maxeke Johannesburg Academic. Fourteen (44%) were nulliparous. Using the best gestational estimate, 14 women (44%) had booked for antenatal care before 20 weeks gestation. None were hypertensive at booking. All women received anti-hypertensive drugs in the antenatal period, either nifedipine or methyldopa or both. Sixteen of the women (50%) were between 28 to 33 weeks pregnant at the time of admission. Thirteen women had symptoms and signs of imminent eclampsia (Table 1). A diagnosis of HELLP syndrome was made by the attending clinicians in 14 of the women. However, only four women of the 32 were tested for evidence of haemolysis (either lactate dehydrogenase level, haptoglobin level or peripheral smear). Of the four women tested, only one had HELLP syndrome confirmed (on smear and with haptoglobin level).

Table 1. Demographic and obstetric data for pre-eclamptic women with low platelet counts who had repeat platelet counts done before delivery (number (%) or mean±standard deviation) (n=32).

Hospital:	
Rahima Moosa Mother and Child	16 (50.0%)
Chris Hani Baragwanath Academic	10 (31.0%)
Charlotte Maxeke Johannesburg Academic	6 (19.0%)
Age (years)	27.1±5.3
Parity:	
0	14 (44.%)
1	12 (37.5%)
≥2	6 (18.5%)
Women who booked for antenatal care at <20 weeks	14 (44.0%)
HIV infected	4 (12.5%)
Diabetes mellitus	0 (0.0%)
Twin pregnancy	1 (3.0%)
Gestational age on admission:	
<28 weeks	2
28-33 weeks	16
≥34 weeks	14
Women who received antenatal antihypertensive medication	32 (100.0%)
Women who received antenatal corticosteroids (betamethasone):	
No doses	14
Incomplete dosage (12 mg)	6
Full dosage (24 mg)	12
Highest pregnancy systolic blood pressure (mmHg)	175±20
Highest pregnancy diastolic blood pressure (mmHg)	106±13
Proteinuria on urine reagent strips:	
1+	4
2+	7
3+	21
Antenatal pulmonary oedema	0
Antenatal imminent eclampsia symptoms or signs	13

4.2 **Delivery outcomes**

Thirty-one (96.9%) of the women delivered by caesarean section. The median duration between the last platelet count taken and delivery was 3.8 hours. Twenty-five of the babies weighed <2500 g at birth. There were no maternal deaths or admissions to the intensive care unit (Table 2)

Table 2. Delivery data for pre-eclamptic women with low platelet counts who had repeat platelet counts done before delivery (number (%) or median (interquartile range); n=32 women and 33 babies).

Mode of delivery: Caesarean section Vaginal birth	 31 (96.9%) 1
Time interval between last platelet count and delivery (hours)	3.8 (2.1-8.9)
Birth weight categories: <1000 g 1000-2499 g ≥2500 g	 4 21 8
Babies small for gestational age	7
Birth outcome: Live birth Stillbirth Early neonatal death	 31 0 2
Maternal death or admission to adult intensive care unit	0

4.3 **Platelet count results**

The median first platelet count on admission into the study was $112x10^9$/L and the next platelet count was $99x10^9$/L) (p=0.78). The median time interval between first and second platelet counts, and haemoglobin levels, was 19.3 hours (interquartile range 8.8-23.9). Ten percent of time intervals exceeded 48 hours. There was no significant difference for difference between second and third platelet counts (n=14, p=0.49). There was no significant difference in first and second haemoglobin levels (n=27; p=0.62). AST levels were estimated in 24 women, and 12 had AST levels ≥40 U/L (Table 3).

Table 3. Platelet count ($x10^9$/L) at first, second and third testing, haemoglobin levels (g/dL) at first and second testing, and aspartate transaminase levels (IU/L) and creatinine levels (µmol/L during admission to hospital (median and interquartile ranges), with P value given for differences where applicable.

	Median (interquartile range	P value*
First platelet count (n=32) Second platelet count (n=32)	112 (79-137) 99 (70-130)	0.78
Third platelet count (n=14)	100 (76-128)	NA
Fourth platelet count (n=6)	142 (65-163)	NA
First haemoglobin level (n=30) Second haemoglobin level (n=27)	12.7 (11.3-13.5) 12.4 (11.3-13.6)	0.62
Aspartate aminotransferase level (n=24)	45 (27-139)	NA
Creatinine level (n=26)	59 (51-72)	NA

*Wilcoxon's signed rank test for paired data – comparisons between first and second tests, NA=comparison not applicable

4.4 **Change in platelet count according to risk factors**

The mean change in platelet count did not differ by gestational age, level of first platelet count, or use of antenatal corticosteroids. Women who were diagnosed by attending clinicians with HELLP syndrome had a mean change in platelet count of $18x10^9$/L, a significant difference ($p<0.01$) when compared with women who did not have HELLP syndrome, where the platelet count rose on average by $14.5x10^9$/L. When the clinical diagnosis of HELLP syndrome was ignored and only the AST level was considered, an AST level ≥40 U/L was accompanied by a mean decrease of $19.7x10^9$/L, significantly different ($p<0.01$) from women in whom the AST level was normal, where the platelet count increased on average by $17.8x10^9$/L (Table 4). Changes in haemoglobin level were not statistically significantly different in women with and without high AST levels (data not shown).

Table 4. Change in platelet counts from first to second testing by the presence of absence of possible risk factors. Negative signs indicate a mean reduction in platelet count.

Risk factor present:	Mean difference in platelet count		P value*
	Yes	No	
Pregnant <34 weeks	2.1±35.5 (n=18)	-2.0±38.0 (n=14)	0.76
First platelet count $<100x10^9$/L	6.2±15.4 (n=11)	-2,8±43.3 (n=21)	0.51
Clinical diagnosis of HELLP syndrome	-18.0±27.7 (n=14)	14.5±36.0 (n=18)	<0.01
Aspartate transaminase level ≥40 IU/L	-19.7±29.5 (n=12)	17.8±28.8 (n=12)	<0.01
Second platelet count ≥24 hours after first	13.8±48.4 (n=8)	-4.2±30.9 (n=24)	0.23
Any betamethasone received	3.6±36.0 (n=18)	-4.0±37.1 (n=14)	0.56

*Student's t-test

5. **DISCUSSION**

5.1 **Main findings**

The three academic hospitals in this study are managed by the South African Government and serve low income populations in Johannesburg and surrounding areas. This prospective study provides potentially useful findings regarding women with pre-eclampsia who have thrombocytopenia, and may be especially relevant for clinicians who are in the habit of, or are forced into, predicting platelet counts in resource limited settings where rapid platelet count estimation may not be available. The high caesarean section rate in this study (97%) confirms the clinical need to be aware of platelet count levels in pre-eclamptic women. Considerations related to surgical care, such as choice of anaesthesia, choice of abdominal incision, and the possible need for platelet transfusion, apply strongly to the care of such patients.

The main finding of the study is that prediction of platelet count declines may be dependent on the AST level. A normal level suggests that platelet counts will not decline significantly while managing these women in anticipation of delivery. Conversely, a high AST level (or a diagnosis of HELLP syndrome) confers a risk of significant platelet count decline. To the researcher's knowledge, these findings have not been found in previous studies. Interpretation of this finding should perhaps be confined to a raised AST level rather than HELLP syndrome, because the diagnosis of HELLP syndrome was only confirmed in one of the women with raised AST levels. There is, however, a tendency in the Wits teaching hospitals to speak loosely of HELLP syndrome in any pre-eclamptic a woman who has low platelets and a raised AST level.

There was no evidence that the initial platelet count on its own, as suggested by some studies,[33,55] could be used to predict the subsequent count. The study also detected no significant effect with administration of steroids, in keeping with the findings for HELLP syndrome from the Cochrane systematic review.[58] Only the first and second platelet counts could be meaningfully analysed, because too few women had a third platelet count performed. Therefore, a trend over a longer period with three or more counts could not be discerned.

5.2 **Strengths and limitations**

A first strength of this study was its strict inclusion and exclusion criteria, ensuring that even with the constraint of late-booking antenatal care, truly pre-eclamptic women were chosen. A second strength was the prospective recruitment of participants, with all selection and data collection under the control of the researcher, who is a specialist registrar. Selection bias due to misclassification, and information bias due to poor data quality, were therefore minimised. It is however possible that some unbooked women presenting with early-onset pre-eclampsia were missed. They had to be excluded because they had no record of a normal antenatal blood pressure.

There were several important limitations. The small sample size, due in part to the stringent inclusion criteria, did not allow for much confidence in the sample estimates, nor provide much room for subgroup comparisons, nor give information on rate of platelet count reductions per unit time. Nevertheless, the sample size was large enough to detect a predictive effect of AST for the platelet count drop. Another very real reason for the small

sample size is that the researcher, as a full-time registrar, was not always able to find the necessary time to recruit patients for the study. While no priority sample size was specified, owing to lack of data on platelet count drops, it was hoped that at least 50 patients could be recruited. Related to the small sample size was the inclusion of a large number of women with platelet counts $\geq 100 \times 10^9$/L. These women only have mild thrombocytopaenia and are probably of less clinical interest than those with lower platelet counts, where anaesthetic and surgical considerations start to apply.

The finding of the relationship between AST and platelet count drop brought into question the incomplete nature of diagnoses of HELLP syndrome in these hospitals. The study was limited by the available data in the files, with most women who had raised AST levels not further investigated. All blood tests used for analysis in this study were done by the on-duty clinicians, and the researcher had to be content with the results of whatever tests had been requested.

5.3 Implications for practice and research

The study, while limited, achieved its objectives in tracking platelet counts in pre-eclamptic with thrombocytopaenia patients while still pregnant. There is however, no fine detail in the results. Practising clinicians at the three hospitals would be advised to seek urgent platelet counts before surgery in pre-eclamptic women who have recent (8-48 hours prior) thrombocytopaenia $< 100 \times 10^9$/L and a raised AST level. Clinicians can afford to be less concerned in patients who have thrombocytopaenia $> 100 \times 10^9$/L, or have normal AST results.

Specific cut off levels cannot be recommended, and clinical acumen with individual patients is still required.

Further useful research may focus on women with HELLP syndrome and thrombocytopaenia $<100x10^9$/L. A study is needed with adequate power gained from a large enough sample size and clear case definition for HELLP syndrome. Prospective testing at defined intervals would be especially useful. It may then become possible to predict platelet count declines, and therefore provide more precise clinical guidelines for urgent repeat platelet count testing or, in resource-poor settings, using prediction curves to decide on surgical and anaesthetic precautions related to severe thrombocytopaenia.

REFERENCES

1.ACOG committee. Diagnosis and management of pre-eclampsia and eclampsia. ACOG practice bulletin no. 33. Am J Obstet Gynecol 2002; 32:159–-67.

2.Steegers EA, von Dadelszen P, Duvekot JJ, Pijnenborg R. Pre-eclampsia. Lancet 2010; 376:631-44.

3.Duley L. The Global Impact of Pre-eclampsia and Eclampsia. Semin Perinatol 2009; 33:130-137.

4.Department of Health. Saving Mothers 2011-2013: Sixth report on confidential enquiries into maternal deaths in South Africa: Fact sheet. Saving Mothers,; 2015. http://www.kznhealth.gov.za/mcwh/Maternal/Saving-Mothers-2011-2013-short-report.pdf. (Accessed 16/09/15)

5.Ghulmiyyah AL, Sibai B. Maternal mortality from preeclampsia/eclampsia. Semin Perinatol 2012;36:56-9.

6.Haram K, Svendsen E, Abildgaard U. The HELLP syndrome: Clinical issues and management. A Review. BMC Pregnancy Childbirth 2009; 9:8.

7.Roberts M, Cooper DW. Pathogenesis and genetics of pre-eclampsia.

Lancet 2001; 357:53-6.

8.Lowe S, Bowyer L, Lust K, McMahon L, Morton M, North R, et al. The SOMANZ Guidelines for the Management of Hypertensive Disorders of Pregnancy 2014. Aust N Z J Obstet Gynaecol 2015; 55:e1-e29.

9.Royal College of Obstetricians and Gynaecologists. Hypertension in pregnancy: diagnosis and management. Clinical guideline, 2010. http://www.nice.org.uk/guidance/cg107 (accessed 12 September 2015).

10.ACOG Committee on Obstetric Practice. ACOG practice bulletin. Diagnosis and management of preeclampsia and eclampsia. Int J Gynaecol Obstet 2002;77:67-75.

11.Von Dadelszen P, Magee L. Pre-eclampsia:An Update. Curr Hypertens Rep 2014; 16:8.

12.Tranquilli AL, Dekker G, Magee L, Roberts J, Sibai BM, Steyn W, et al. The classification, diagnosis and management of the hypertensive disorders of pregnancy: A revised statement from the ISSHP. Pregnancy Hypertens 2014; 4:97-104.

13.Buchmann E (editor). Wits Obstetrics 2013. Johannesburg: Department of Obstetrics and Gynaecology, University of the Witwatersrand, 2013.

14.World Health Organization. WHO recommendations for prevention and treatment of pre-eclampsia and eclampsia. http://whqlibdoc.who.int/publications/2011/9789241548335_eng.pdf. (accessed 16/10/15)

15.Steegers EA, von Dadelszen P, Duvekot JJ, Pijnenborg R. Pre-eclampsia. Lancet 2010; 376:631-44.

16.Sircar M, Thadhani R, Karumanchi SA. Pathogenesis of preeclampsia. Curr Opin Nephrol Hypertens 2015; 24:131-8.

17.Foo L, Tay J, Lees C,et al. Hypertension in pregnancy: natural history and treatment options. Hypertens Rep 2015; 17:36.

18.TW Sandler. Langman's Essential medical embryology. 8th ed. Philadelphia: Lippincott Williams & Wilkins, 2005.

19.Roberts JM, Cooper DW. Pathogenesis and genetics of pre-eclampsia. Lancet 2001; 357:53-6.

20.Walsh SW. Eicosanoids in preeclampsia:Prostaglandins. Leukot Essent Fatty Acids. 2004;70:223-32.

21.Kappler S, Ronan-Bentle S, Graham A. Thrombotic microangiopathies (TTP, HUS, HELLP). Emerg Med Clin North Am 2014;32:649-71.

22.Cantor AB. Thrombocytopoiesis. In: Hoffman R, Edward J, Leslie E, et al. Hematology: Basic Principles and Practice. 6th ed. Canada: Churchill Livingstone, 1991: 292-306.

23.Italiano JE, Hartwig JH. Megakaryocyte and Platelet structure. In: Hoffman R, Edward J, Leslie E, et al. Hematology: Basic Principles and Practice. 6th ed. Canada: Churchill Livingstone, 1991: 1797-1808.

24.Cardigan R, Turner C, Harrison P. Current methods of assessing platelet function: relevance to transfusion medicine. Vox Sang 2005;88:153–163.

25.Holthe MR, Staff AC, Berge LN, Lyberg T. Different levels of platelet activation in preeclamptic, normotensive pregnant, and nonpregnant women. Am J Obstet Gynecol 2004 ;190:1128-34.

26.Bath PM, Butterworth RJ. Platelet size: measurement, physiology and vascular disease. Blood Coagul Fibrinolysis 1996;7:157-61.

27.Jurk K, Kehrel BE. Platelets: physiology and biochemistry. Semin Thromb Hemost 2005;31:381-392.

28.Adams TM, Allaf MB, Vintzileos AM. Maternal thrombocytopenia in pregnancy: diagnosis and management. Clin Lab Med 2013;33:327-41.

29.Sekhon SS, Roy V. Thrombocytopenia in adults: A practical approach to evaluation and management. South Med J 2006 ;99:491-8; quiz 499-500, 533.

30.George JN, Nester CM. Syndromes of thrombotic microangiopathy. N Engl J Med 2014; 371:654-66.

31.Bockenstedt PL. Thrombocytopenia in pregnancy. Hematol Oncol Clin North Am 2011;25:293-310.

32.Burrows RF, Hunter DJ, Andrew M. A prospective study investigating the mechanism of thrombocytopenia in preeclampsia. JG.Obstet Gynecol 1987;70:334-8.

33.Leduc L, Wheeler JM, Kirshon B, Mitchell P, Cotton DB. Coagulation profile in severe preeclampsia. Obstet Gynecol. 1992;79:14-8.

34.Parnas M. Moderate to severe thrombocytopenia during pregnancy. Eur J Obstet Gynecol Reprod Biol 2006;125:59-65.

35.Pourrat O, Coudroy R, Pierre F. Differentiation between severe HELLP syndrome and thrombotic microangiopathy, thrombotic thrombocytopenic purpura and other imitators. Eur J Obstet Gynecol Reprod Biol 2015;189:68-72.

36.Letsky EA. Disseminated intravascular coagulation. Best Pract Res Clin Obstet Gynaecol 2001; 15:623-44.

37.Thachil J, Toh CH. Disseminated intravascular coagulation in obstetric disorders and its acute haematological management. Blood Rev 2009; 23:167-76.

38.Kenny LC, Mccrae KR, Cunningham FG. Platelets, Coagulation, and the Liver. In: Taylor RN, Roberts JM, Cunningham FG, et al. Chesley's Hypertensive Disorders in Pregnancy. Oxford:Appleton & Lange A Pearson Education Company, 1978: 379-396.

39.Kam PC, Thompson S A, Liew AC. Thrombocytopenia in the parturient. Anaesthesia 2004;59:255-64.

40.Janes SL, Goodall AH. Flow cytometric detection of circulating activated platelets and platelet hyper-responsiveness in pre-eclampsia and pregnancy. Clin Sci 1994; 86:731-9.

41.Hayashi M, Inoue T, Hoshimoto K, Negishi H, Ohkura T, Inaba N. Characterization of five marker levels of the hemostatic system and endothelial status in normotensive pregnancy and pre-eclampsia. Eur J Haematol 2002; 69:297-302.

42.Major H, Campbell R, Weyrich A. Platelet activation in preeclampsia: myth or fact? Am J Obstet Gynecol 2015; 212: 318-318.

43.Harlow F, Brown M, Brighton T, Smith SL, Trickett AE, Kwan YL, et al. Platelet activation in the hypertensive disorders of pregnancy. Am J Obstet Gynecol 2002;187:687-695.

44.Macey MG, Bevan S, Alam S, Verghese L, Agrawal S, Beski S, et al. Platelet activation and endogenous thrombin potential in pre-eclampsia. Thromb Res 2010;125:e76-81.

45.VanWijk MJ, Nieuwland R, Boer K, van der Post JA, Van Bavel E, Sturk A. Microparticle subpopulations are increased in preeclampsia: possible involvement in vascular dysfunction? Am J Obstet Gynecol 2002;187:450-6.

46.Lok CA, Van Der Post JA, Sargent IL, Hau CM, Sturk A, Boer K, et al. Changes in microparticle numbers and cellular origin during pregnancy and preeclampsia. Hypertens Pregnancy 2008;27:344-60.

47.Ceyhan T, Beyan C, Başer I, Kaptan K, Güngör S, Ifran A. The effect of pre-eclampsia on complete blood count, platelet count and mean platelet volume. Ann Hematol 2006;85: 320-2.

48.Redman CW. Platelets and the beginnings of preeclampsia. N Engl J Med. 1990 16; 323: 478-80.

49.Zemel MB, Zemel PC, Berry S, Norman G, Kowalczyk C, Sokol RJ, et al. Altered platelet calcium metabolism as an early predictor of increased peripheral vascular resistance and preeclampsia in urban black women. N Engl J Med 1990;323:434-8.

50.Konijnenberg A, van der Post JA, Mol BW, Schaap MC, Lazarov R, Bleker OP, et al. Can flow cytometric detection of platelet activation early in pregnancy predict the occurrence of preeclampsia? A prospective study. Am J Obstet Gynecol 1997;177:434-42.

51.Heilmann L, Rath W, Pollow K. Hemostatic abnormalities in patients with severe preeclampsia. Clin Appl Thromb Hemost 2007;13:285-91.

52.Magann EF, Martin JN Jr. The laboratory evaluation of hypertensive gravidas. Obstet Gynecol Surv 1995;50:138-45.

53.Fitzgerald DJ, Rocki W, Murray R, Mayo G, Mayo, FitzGerald GA. Thromboxane A2 synthesis in pregnancy-induced hypertension. Lancet.1990;335:751-4.

54.Levi M. Disseminated intravascular coagulation. Crit Care Med 2007; 35:2191-5.

55.Rinehart BK, Terrone DA, May WL, Magann EF, Isler CM, Martin JN Jr. Change in platelet count predicts eventual maternal outcome with syndrome of hemolysis, elevated liver enzymes and low platelet count. J Matern Fetal Med 2001;10:28-34.

56.Katz L, Amorim M, Souza JP, Haddad SM, Cecatti JG. COHELLP Study Group. COHELLP: collaborative randomized controlled trial on corticosteroids in HELLP syndrome. COHELLP Study Group. Reprod Health 2013;10:28.

57. Martin JN Jr, Thigpen BD, Rose CH, Cushman J, Moore A, May WL. Maternal benefit of high-dose intravenous corticosteroid therapy for HELLP syndrome. Am J Obstet Gynecol 2003;189:830-4.

58. Woudstra DM, Chandra S, Hofmeyr GJ, Dowswell T. Corticosteroids for HELLP (hemolysis, elevated liver enzymes, low platelets) syndrome in pregnancy. Cochrane Database Syst Rev 2010;9:CD008148.

59.Loo CC, Dahlgren G, Irestedt L. Neurological complications in obstetric regional anaesthesia. Int J Obstet Anesth 2000;9:99-124.

60.Orlikowski CE, Rocke DA, Murray WB, Gouws E, Moodley J, Kenoyer DG, et al. Thrombelastography changes in pre-eclampsia and eclampsia. Br J Anaesth 1996;77:157-61.

61.Rucklidge M, Paech M. Regional anaesthesia in parturients with low platelet counts

Int J Obstet Anesth 2002; 11:5-5.

62.Beilin Y, Zahn J, Comerford M. Safe epidural analgesia in thirty parturients with platelet counts between 69,000 and 98,000 mm(-3). Anesth Analg 1997;85:385-8.63.

63. Wandt H, Schäfer-Eckart K, Greinacher A. Platelet transfusion in hematology, oncology and surgery. Dtsch Arztebl Int 2014;111:809-15.

64.Squires JE. Indications for platelet transfusion in patients with thrombocytopenia. Blood Transfus 2015;13:221-6.

65.Gaydos LA, Freireich EJ, Mantel N. The quantitative relation between platelet count and hemorrhage in patients with acute leukemia. N Engl J Med 1962 ;266:905-9.

66.Schiffer CA, Anderson KC, Bennett CL, et al. Platelet transfusion for patients with cancer: clinical practice guidelines of the American Society of Clinical Oncology. J Clin Oncol. 2001;19(5):1519-38.

67. Bishop JF, Schiffer CA, Aisner J, et al. Surgery in acute leukemia: a review of 167 operations in thrombocytopenic patients. Am J Hematol. 1987; 26(2):147-55.

68.McVay PA, Toy PT. Lack of increased bleeding after liver biopsy in patients with mild hemostatic abnormalities. Am J Clin Pathol. 1990;94(6):747-53

69.Tanaka M, Balki M, McLeod A, Carvalho JC. Regional Anesthesia and Non-Preeclamptic Thrombocytopenia: Time to Re-Think the Safe Platelet Count. Rev Bras Anestesio 2009; 2:142-153.

70.Burger J. Abdominal incisions: techniques and postoperative complications. Review. Scand J Surg 2002; 91:315–321.

71.Haram K, Svendsen E, Abildgaard U. The HELLP syndrome: clinical issues and management. A Review. BMC Pregnancy Childbirth 2009 9:8.

Appendix A: Patient information sheet

Wits University

Information Sheet

Good day, my name is Dr Mama-Asu Koranteng and I am a medical doctor in the obstetrics and gynaecology department. I am doing research as part of my qualification to be a specialist in obstetrics and gynaecology. High blood pressure is a very common problem in our hospitals. I am doing research on high blood pressure in pregnancy and how it affects the platelet count. Platelets are a part of the blood that stops you from bleeding to death if you cut your self. At the moment we do not know what will happen to the platelet count when you come into hospital – does it go down, does it go up, or does it stay the same. By observing patients such as yourself, we may be able to learn what happens with the platelet count. I am asking for your permission to let me look through your hospital file to see why you were admitted, I will then take down your hospital number and check all your blood results on the computer until you are discharged.

I am not one of the doctors treating you – I only want to take information about blood pressure from your file and follow up the platelet count results. So, what I am doing will not affect your treatment at all. Collecting this information is anonymous; your name will not be used. You do not have to decide right now if you are willing to participate. I can answer any questions you may have now or later. You do not have to take part in this research if you do

not wish to do so and refusing to participate will not affect your treatment. You can change your mind about participating at any time. This information will be shared only with doctors and other health care professionals so that we may learn more about high blood pressure in pregnancy and the platelets.

Name of Principal Investigator: Dr Mama- Asu Koranteng

Cell phone: 0726372648

Email address makoranteng@gmail.com

Ethics committee at Wits: (011) 7171234

Supervisor: Prof E Buchmann

Appendix B:

Informed Consent form for pregnant women with high blood pressure in pregnancy (adopted from WHO guidelines)

I have read the information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily to participate in this research.

Signature of Participant _____ Date _____

Or

Thumb print of participant 

Signature of witness _____ Date _____

Appendix C:Data sheet

Hospital	
CHBH	
CMJAH	
RHM	

Study number			

Age			Gravidity		Parity		Gestational age booked		

HIV	-	+	CD4			ARVs	Y	N	HIV WHO staging	

Diabetes	Y	N

Systolic blood pressure at booking (mmHg)		Diastolic blood pressure at booking (mmHg)		Proteinuria at booking	

Aspirin use in antenatal period	Y	N	Aldomet use in antenatal period	Y	N	Nifedipine use in antenatal period	Y	N

Date of admission		Gestational age at admission	

Highest SBP (mmHg)		Highest DBP (mmHg)		Quantity of proteinuria on admission	

Peripheral smear		LDH		Haptoglobin	

Pulmonary oedema	Y	N	Imminent eclampsia	Y	N	Symptoms:	HELLP syndrome	Y	N

Dopplers (resistance index)	

MgSO4 administered	Y	N	Steroids administered	none	
				Full course not completed by second platelet count	
				Full course completed by second platelet count	

1st result	Date	Time of specimen recorded by the laboratory	Recording
Platelet count			
White cell count (WCC)			
Haemoglobin (Hb)			
Haematocrit (Hct)			
Aspartate transaminase (AST)			
Creatinine			

2nd result	Date	Time of specimen recorded by the laboratory	Recording
Platelet count			
WCC			
Hb			
Hct			
AST			
Creatinine			

3rd result	Date	Time of specimen recorded by the laboratory	Recording
Platelet count			
WCC			
Hb			
Hct			
AST			
Creatinine			

4th result	Date	Time of specimen recorded by the laboratory	Recording
Platelets			
WCC			
Hb			
Hct			
AST			
Creatinine			

Date of delivery	
Time of delivery	
Mode of delivery	
Birth weight/gestational age	
Outcome (alive/stillbirth)	
Appropriateness for gestational age	

Appendix D:Ethics approval

R14/49 Dr Mama-Asu Koranteng

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M130737

NAME: (Principal Investigator)	Dr Mama-Asu Koranteng
DEPARTMENT:	Obstetrics and Gynaecology Chris Hani Baragwanath Academic Hospital,Rahima Moosa Hospital,Charlotte Maxeke Johanneburg Academic Hospital
PROJECT TITLE:	Platelet Count Trends in Pregnant Women who have Pre-Eclampsia with Thrombocytopenia
DATE CONSIDERED:	26/07/2013
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof E Buchmann
APPROVED BY:	Professor PE Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	16/08/2013

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Secretary in Room 10004, 10th floor, Senate House, University.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES