The prevalence of thrombocytopenia at a primary care HIV clinic in South Africa - possible implications for neuraxial anaesthesia

Investigator

Carl Steadman MBChB (Stell) DA (SA)

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Mmed Anaesthesiology

University of Stellenbosch, Department of Anaesthetics and Critical Care

Promotor

Dr Marli Smit MBChB (Stell), Mmed Anaes (Stell), FCA (SA)

Statistician

Ms M Zunza, Biostatistics Unit, Faculty of Health Sciences, University of Stellenbosch

Declaration/Verklaring

Declaration

I, the undersigned, hereby declare that the work contained in this assignment is my original work and that I have not previously submitted it, in its entirety or in part, at any university for a degree.

Signature: CJ Steadman **Date: December 2017**

Verklaring:

Ek, die ondergetekende, verklaar hiermee dat die werkstuk vervat, my eie oorspronklikke werk is en dat dit nie van tevore in die geheel of gedeeltelik by enige universiteit te verkryging van ‘n graad voorgelê is nie.

Handtekening: CJ Steadman **Datum: Desember 2017**

Table of contents:

	Page:
Summary	4
Inleiding	6
Literature review	8
Methodology	16
Inclusion/exclusion criteria	17
Quality control	18
Primary and secondary outcomes	19
Ethical considerations and patient care	20
Budget	20
Data management and statistical analysis	21
Results	22
Discussion	24
Conclusion	26
Acknowledgements	27
Appendix	28
Bibliography	34

Summary

South Africa has a high incidence of Human Immunodeficiency Virus (HIV) infection, and many of these patients will require surgery during their lives. The exact prevalence of thrombocytopenia in South African, HIV-infected patients (naïve/non anti-retroviral therapy naïve) are unknown. The reported global prevalence of thrombocytopenia in HIV positive patients ranges between 5.5 to 50% [17], [18].

Neuraxial anaesthesia is contraindicated in patients with platelet counts of <75 x $10^{9/}$L, due to the risk of neuraxial haematoma.

The large variation in practice (in South Africa) in terms of preoperative special investigations (especially platelet count) in this patient population suggests that patients are either under investigated, or that unnecessary investigations are performed; with cost and time implications.

This wide range in thrombocytopenia prevalence, together with the anaesthetic implications secondary to thrombocytopenia was the motivation behind us conducting an audit to determine the prevalence of thrombocytopenia in HIV positive patients.

Our **primary outcome** was to determine the prevalence of thrombocytopenia in HIV-positive patients attending a primary care HIV clinic in the Western Cape, South Africa. **Secondary outcomes** were to:

1. Determine if there is any correlation between CD4 count and platelet count.
2. Determine what the influence of ART on platelet count is.
3. To make informal proposals regarding pre-operative special investigations (specifically platelet count) in the HIV positive patients.

Our study, consisting of 1,410 patients, provided the following important **results**:

The median CD4 count was 281 +/- 199 cells / mm^3. Thirty-one percent of patients had a CD4 count of < 200 cells / mm^3.

The median platelet count was 270 +/- 100x10^9/L. The platelet count was < 150 x 10^9/L (thrombocytopenia) in 6.5%, and < 75 x 10^9 /L (severe thrombocytopenia) in 0.7% of participants. Thrombocytopenia was more common in patients with a CD4 count < 200 cells/mm^3 ($p < 0.001$) and in ART naïve patients ($p = 0.02$). However, there was no connection between severe thrombocytopenia and a CD4 count of < 200 cells / mm^3 ($p = 0.36$) or ARV naivety ($p = 0.66$)

Infection and malignancy had no significant impact on thrombocytopenia ($p = 0.66$, Fischer's exact 0.3) nor severe thrombocytopenia ($p = 0.99$, Fischer's exact 0.5).

Conclusion:

In this descriptive study, we found that the prevalence of severe thrombocytopenia to be very low (0.7%).

We cannot make statistically supported deductions regarding this result because the prevalence of thrombocytopenia in the general population is unknown, and our study did not have a control group. However, we will propose that the following be kept in mind regarding preoperative special investigation decision making in HIV positive patients:

1. The incidence of neuraxial hematomas has not increased in conjunction with the increase in HIV positive patients,
2. The costs associated with special investigations.
3. The low prevalence of severe thrombocytopenia.
4. The lack of literature to support a safe cut-off for platelet count for neuraxial anaesthesia.

Inleiding

Suid-Afrika het 'n hoë insidensie van Menslike Immuniteitsgebreksvirus (MIV) infeksie, en baie van hierdie pasiënte sal tydens hulle lewe chirurgie benodig. Die presiese prevalensie van trombositopenie in Suid Afrikaanse, MIV geïnfekteerde pasiënte (met of sonder anti-retrovirale behandeling) is onbekend. Die gemelde prevalensie van trombositopenie in MIV positiewe pasiënte wêreldwyd wissel tussen 5.5 en 50%[17, 18]

Neuraksiale narkose is gekontraindikeerd in pasiënte met erge trombositopenie (75 x 10^9/L), as gevolg van ‘n neuraksiale hematoom risiko.

Daar is ‘n groot variasie in praktyk (in Suid Afrika) in terme van preoperatiewe spesiale ondersoeke (veral plaatjie telling) in díe pasiënt populasie. Dit impliseer dus dat pasiënte potensieël benadeel word of dat onnodige spesiale ondersoeke gedoen word – met koste en tyd implikasies.

Die groot reikwydte in die gerapoteerde prevalensie tesame met die kliniese narkose implikasies sekondêr tot trombositopenie, was die motivering om ‘n ouditstudie te doen wat die prevalensie van trombositopenie in MIV positiewe pasiënte beskryf.

Ons **primêre uitkoms** was om die prevalensie van thrombositopenie in MIV-positiewe pasiënte wat n primere HIV kliniek in Wes-Kaap, Suid-Afrika bywoon, te bepaal. Ons **sekondêre uitkomste** was -

1. Bepaal of daar enige korrolasie tussen CD4 telling en plaaitjie telling is.
2. Bepaal wat die invloed van ART op plaaitjie telling is.
3. ‘n Informele voorstel te maak in terme van pre-operatiewe spesiale ondersoeke (spesifiek plaaitjie telling) in HIV positiewe pasiënte.

Ons studie, wat uit 1410 pasiënte bestaan het, het die mees belangrike **resultate** getoon:

Die gemiddelde CD4 telling was 281 +/- 199 selle/mm^3. Agt en dertig persent van pasiënte het 'n CD4 telling < 200 selle/mm^3 gehad ($CI^{95\%}$ 0,36-0,41).

Die gemiddelde plaatjie telling was 270 +/- 100x10^9/L. Die plaatjie telling was <150 x 10^9/L (trombositopenie) in 6.5%, en < 75 x 10^9/L (erge trombositopenie) in 0.7% van die deelnemers. Trombositpenie was meer algemeen in pasiënte met 'n CD4 telling <200

selle/mm^3 (p <0.001) en in ART naïewe pasiënte (p = 0,02). Daar was egter geen verband tussen erge trombositopenie en ‘n CD4-telling van <200 selle/mm^3 (p = 0,36) en/of ARV naïwiteit (p=0,66) gevind nie.

Infeksie en maligniteit het geen beduidende impak op trombositopenie (p=0,66, Fischer se presiese 0,3) of erger trombositopenie (p=0,99, Fischer se presiese 0,5) gehad nie.

Gevolgtrekking:

In hierdie ouditstudie het ons gevind dat die prevalensie van erge trombositopenie baie laag (slegs 0,7%) was. Ons kan geen statisties ondersteunde afleidings maak na aanleiding hiervan nie aangesien die prevalensie van trombositopenie onbekend is in die algemene populasie, en ons studie nie ‘n kontrole groep gehad het nie.

Ons sal egter voorstel dat die volgende ingegagte gehou word tydens pre-operatiewe spesiale ondersoek besluitneming in die MIV positiewe pasiënte:

1. Die insidensie van neuraxiale hematome het nie toegeneem tesame met die toename in MIV positiewe pasiënte nie.
2. Die koste verbonde aan spesiale ondersoeke,
3. Die lae prevalensie van erge trombositopenie
4. Die gebrek aan literatuur ter ondersteuning vir die veilige afsny waarde van plaaitjie telling en neuraksiale narkose.

Literature review

HIV (human immunodeficiency virus) infection prevalence in South Africa in 2012 was estimated to be 12.2% of the population (a staggering 6.4 million people)[24]. The prevalence differs substantially between provinces, with the highest percentage found in Kwazulu-Natal (16.9%) and the lowest in the Western Cape (5%). Females are also more affected by the human immunodeficiency virus than males. Of these 6.4 million patients, 2.002 million are receiving antiretroviral therapy, with the exposure being highest in females; children and persons aged 50 years and older[24].The prevalence of HIV among antenatal women in South Africa in 2012 was found to be 29.5%[25]. This too varies between provinces, with the highest prevalence again being in KwaZulu-Natal and the lowest in the Western Cape.

What effect does HIV have on the platelet count and or platelet function?

Anaemia, neutropenia, and thrombocytopenia are commonly observed in HIV-infected patients[17]. Thrombocytopenia affects approximately 40% (range of 10-50%) of patients infected with HIV during the course of their illness, and low platelet counts may be the only haematological abnormality at initial presentation[18]. A study has demonstrated that the severity and incidence of thrombocytopenia is associated with the stage of disease[17].

The aetiology of thrombocytopenia in this population can be viewed as immune or non-immune.

Immune HIV-Related Thrombocytopenia includes:

1.Immune thrombocytopenia[19] (ITP, occurring in up to 30% of HIV patients),

2.Accelerated destruction of platelets due to the action of immune complexes

3.And the presence of anti-platelet and anti-HIV antibodies that cross-react with the platelet membrane.

During early HIV infection, thrombocytopenia is mainly mediated by peripheral platelet destruction, while in patients with advanced AIDS (acquired immunodeficiency syndrome) , thrombocytopenia occurs mainly due to decreased production of platelets and ineffective haematopoiesis due to direct HIV infection of the megakaryocyte[17].

Non-Immune HIV-Related Thrombocytopenia:

Myelosuppression is a common side effect of many chemotherapeutic agents used to treat HIV infection and its complications, such as Zidovudine, Acyclovir, Amphotericin B and Trimethoprim-sulfamethoxazole[17]. Opportunistic diseases are also important causes of myelosuppression in HIV infection. These may include Cryptococcus neoformans, Mycobacterium avium intracellulare and Mycobacterium tuberculosis, Pneumocystis jiroveci (PJP) and Histoplasma capsulatum. Malignancies, such as Non-Hodgkin's lymphoma, Hodgkin's disease and, rarely, Kaposi's sarcoma as well as concomitant HIV and hepatitis C virus infection have also been implicated[18]. Above mentioned drugs and opportunistic infections can therefore be viewed as the secondary causes of thrombocytopenia in HIV positive patients.

Platelet *function* in HIV has been assessed by means of platelet aggregometry[9, 20] and TEG[9] (thromboelastography). Through this, studies[9, 20] have shown that platelet responses in HIV infected individuals vary according to the agonist used. Haugaard et al[9] showed no influence of immunologic and virologic status on functional haemostatic variables. A study performed by Satchell et al[20] showed that platelet response to adrenaline was enhanced whereas response to other agonists (collagen, TRAP, ADP) was reduced in HIV positive when compared to HIV negative participants (in this study however only 20% of participants were not on antiretroviral therapy).

There are unfortunately few studies that have investigated the effect of the HIV on platelet function, and the results obtained in the above studies are conflicting. A further limitation of the study conducted by Satchell et al[20] was the small sample size (20 HIV positive patients, 20 HIV negative patients) and its cross sectional nature. Taken together, these studies suggested that the overall effect of HIV on platelet function is that of hypofunction. Neither study was able to conclude with a suggested prevalence of platelet dysfunction in HIV positive patients.

What effect does Antiretroviral Therapy potentially have on platelet count and/or function?

The prevalence of thrombocytopenia in HIV positive patientmb ranges from 5.5-23.5%[17] to between 10-50%[18] depending on the source quoted. In the era of HAART, the epidemiology of thrombocytopenia, including the frequency, severity and duration, is poorly characterized.[1]

Thrombocytopenia tends to occur in both early and late phases of HIV infection, as discussed earlier. In the late phase, one of the mechanisms proposed is that of decreased platelet production due to direct infection of megakaryocytes. This, together with the fact that HIV viral *replication* plays a pathogenic role in thrombocytopenia in HIV infected patients[3], would support the finding that severe thrombocytopenia is more common in patients with high viral loads. By lowering viral load, HAART should therefore theoretically mitigate thrombocytopenia.

Studies have shown that Zidovudine monotherapy improves platelet count[1, 2], and several case reports have proven that Didanosine is effective in treating HIV associated immune thrombocytopenia in both adults and children. Furthermore, the Swiss group for HIV studies found that following improvement of platelet count, switching from zidovudine to placebo resulted in a return of platelet count to baseline values[2]. These studies have shown that HAART induces a sustained positive platelet response in HIV associated severe thrombocytopenia.

Several clinical studies have suggested that HIV-infected patients have unexpectedly high rates of ischemic cardiovascular events, in particular coronary heart disease and myocardial infarction [5, 6]. This increased incidence of coronary heart disease has been attributed either to the chronic inflammation secondary to the (HIV) infection or to the detrimental effects of antiretroviral therapy on cardiovascular risk factors[6]. Data collection and Adverse events of anti-HIV Drugs study group (D: A: D) reported that current or recent (<6 months) exposure to Abacavir (ABC), was associated with a 1.9-fold higher relative risk of myocardial infarction (MI) compared with no use of the drug[6]. In contrast to ABC, Tenofovir (TDF) did not appear to have any substantial influence on the incidence of atherosclerotic cardiovascular events. This has prompted investigators to study the effects of ABC on platelet reactivity and aggregation. Platelet hyperactivity as well as increased platelet aggregation in patients on ABC has subsequently been confirmed in other studies[5, 6]. Furthermore, these effects were not seen in patients being treated with TDF when compared with ABC[6].

In June 2006, the FDA and Boehringer Ingelheim released new safety information for Aptivus® (Tipranavir) capsules, including a new black box warning that addresses reports of intracranial haemorrhage in patients taking Tipranavir in combination with ritonavir[29]. These intracranial haemorrhages occurred in 13 patients who were enrolled in a trial which was evaluating the combination of Tipranavir and ritonavir as part of an antiretroviral regime.

Tipranavir is not known to cause coagulopathies in humans; however, *in vitro* and animal testing has indicated that Tipranavir inhibits platelet aggregation and increases both Partial

Thromboplastin Time (PTT) and activated PTT (aPTT). These findings prompted research into the effects of Tipranavir on platelet aggregation, and they found there was a significant decrease in platelet aggregation in the presence of therapeutic Tipranavir concentrations in HIV patients on HAART[7]. The same publication showed that these detected effects were more distinct in the ex vivo–in vitro analysis of blood samples drawn from healthy volunteers than in the HIV-infected patients, which leads to the assumption that HIV-related cofactors may have attenuated the detected effects. More pertinent is the fact that Tipranavir is currently not included in the South African antiretroviral treatment guidelines[8].

Abacavir and Tipranavir have therefore been shown to have different effects on platelet reactivity and aggregation. These studies however only looked at the effects of these agents in isolation. The more clinically relevant question therefore remains; what effect would specific combinations of antiretroviral therapy have on platelet function?

A study[9] looking at the discrepant coagulation profile in HIV found that both untreated and treated HIV-infected individuals appeared hypocoagulable when investigated by functional haemostatic whole blood tests, TEG and platelet aggregation tests. The investigators stated further that their data did not support the hypothesis that coagulopathy in HIV infection is fully reversible with the initiation of combination ART.

From the evidence, it is clear that the HI virus causes thrombocytopenia by means of immune and non-immune mechanisms. HAART has been shown to be effective in improving the platelet count in some studies; however, other studies have not shown similar effects[1]. The question as to what the exact prevalence of thrombocytopenia in HIV positive patients (specifically in the South African setting) is, as well as the influence of ARV regimes/combinations on the prevalence of thrombocytopenia in this group of patients, remains unanswered. The analysis of the impact of HIV infection on platelet function has shown varied responses, and although the evidence is limited, the overall response is that of platelet hypofunction. Anti-retroviral therapy has also been shown to have a varying influence on platelet function and that it is dependent on the agent used.

Clinical relevance of thrombocytopenia, an anaesthetic perspective.

Thrombocytopenia and impaired platelet function may influence general as well as regional anaesthesia. Depending on the degree of derangement of these variables, the anaesthetic plan might require modification and the specific surgery might even be cancelled.

General anaesthesia

Platelet type bleeding may manifest as petechiae, mucous membrane purpura, or frank bleeding from mucosal surfaces resulting in vaginal, gastrointestinal or intracranial haemorrhage[34]. It is estimated that 7-8x10^9/L platelets are required to maintain vascular haemostasis[34]. For surgery however, the following are recommended[35]:

- For major surgery or invasive procedures the platelet is required to be above 50x10^9/L,
- For surgery to critical sites (eye surgery and neurosurgery) it is recommended that the platelet count be greater than 100x10^9/L.

Pre-operatively, the following may be used to guide platelet transfusion:

- In the bleeding patient with a platelet count below 50x109/L (symptomatic thrombocytopenia) and
- A platelet count of 75x10^9/L during massive transfusions.

A large observational study of more than 300000 non-cardiac surgical patients found that in patients with a platelet count of <100x10^9/L there is a 75% higher risk of receiving a blood transfusion, a 90% higher risk of 30 day mortality as well as an increased risk of pulmonary, renal, sepsis and wound complications when compared to patients with normal platelet counts[36].

It is clear that thrombocytopenia might influence the general anaesthetic plan, the extent of the surgical procedure, when surgery will be performed, the transfusion of blood products as well as the associated transfusion related complications.

Regional anaesthesia

Neuraxial anaesthesia is commonly performed for a wide variety of surgical procedures. It is however contraindicated in patients with a certain level of thrombocytopenia due to the risk of neuraxial hematoma[10]. In the general population, the incidence of neuraxial hematoma after epidural and spinal anaesthesia has been estimated at 1: 150,000, and 1: 220,000 respectively[10, 11, 12], while the incidence of spinal haematoma in obstetric patients is 1:200 000 [11].

An extensive review conducted by Kreppel et al (2003) [12] identified 613 case reports of spinal haematoma over a 170-year time span. They determined that most spinal hematomas have a multifactorial aetiology. No definite triggering factor for the spinal hematomas could

be identified in almost half of cases (43.6%), while thrombocytopenia was not specifically mentioned.

Between 1906 and 1994, Vandermeulen et al (1994)[13] reviewed 61 case reports of spinal haematoma following epidural or spinal anaesthesia. Of these 61 cases, 41 (68%) had evidence of abnormal haemostasis. The abnormal haemostasis was due to heparin in 30 cases and of the eleven remaining cases, only four had a reported thrombocytopenia, one of which also received heparin and the other was a chronic alcoholic[13]. In this study thrombocytopenia can thus be seen as a possible contributing factor to spinal haematomas in 6% of the cases reviewed.

No strong evidence is available to support the minimum allowable platelet count that is necessary to ensure the safe practice of regional anaesthesia. It has been suggested that a platelet count of 75 to 80 $\times 10^9.L^{-1}$ is safe for insertion of an epidural catheter (in the absence of other causes for abnormal coagulation) [10]. There is studies[11] suggesting that performing regional anaesthesia at platelet counts between 50 and $75x10^9/L^{-1}$ is safe, particularly in patients with ITP. A recently published report on parturients with platelet count of < 100 x 10^9/L who received neuraxial anaesthesia, cited the upper bound of the 95% CI for the risk of epidural haematoma for platelet count of 0-49 x 10^9/L as 11%, 50-69 x 10^9/L as 3 % and 70-100 x 10^9/L as 0.2%[34]. However, in patients with a rapidly falling platelet count, conditions associated with platelet dysfunction or coagulopathies, and in patients where a difficult or traumatic puncture is more likely (such as ankylosing spondylitis), more caution is required.[11] As always, the risk of a potential spinal haematoma should be weighed up against the benefit gained from the regional anaesthetic.

Another factor to keep in consideration is the aetiology of the thrombocytopenia. According to Warkentin et al[10], destructive thrombocytopenic disorders such as ITP (immune thrombocytopenic purpura) are associated with large, “hyper functional” platelets, suggestive of a lower bleeding risk at a given platelet count in comparison to for example a patient who is preeclamptic[10]. It is also important to recognize that certain platelet disorders (for example, gestational thrombocytopenia and ITP) are considered static, with stable platelet counts and preserved platelet function[10]. These conditions are therefore also viewed as having a lower risk of bleeding[10].

It is clear that HIV positive patients are prevalent in South Africa, especially in the pregnant population. We also established that the HI virus and HAART influence platelet count and function, and that there is a probable association between thrombocytopenia and neuraxial haematoma. The question now is: is there an increased risk for neuraxial haematoma in

HIV positive patients undergoing regional anaesthesia, do we therefore routinely need to exclude thrombocytopenia in this patient population?

A survey was distributed to all state employed members of the South African Society of Anaesthesiologists in order to determine what the current practice is in terms of pre-operative preparation of HIV positive patients requiring neuraxial anaesthesia. The results of the survey are displayed in graph 1 and table 1 (appendix A). Twenty one percent of respondents indicated that they perform a Full Blood Count (with emphasis on platelet count) prior to performing regional anaesthesia on HIV positive patients. This practice relates to the concern of quantitative (and qualitative) platelet defects associated with HIV infection and therefore, a potential increased risk of spinal haematoma following regional anaesthesia. In these hospitals this practice is applied to all HIV positive patients, irrespective of CD4 count or patients being treated with Highly Active Antiretroviral Therapy (HAART).

20-25% of HIV positive patients are likely to require surgery at any given time[28]. Currently, the cost of performing a Full Blood Count (FBC) as per the National Health Laboratory Service billing for public sector is R52,23 (a platelet count exclusively will cost R19.26). The financial implications of performing a pre-operative full blood count or platelet count on all HIV positive patients undergoing surgery is self-explanatory. Alternatively, if this investigation is necessary and it is not performed, serious complications might occur.

(There is also no evidence in the medical literature to suggest that neuraxial blockade is detrimental (from a neurological perspective) in HIV-infected patients[16]).

What is the point of care tests used to assess platelet function?

The automated *platelet count* is a fundamental component of laboratory testing, but it is neither a specific nor sensitive test of platelet function[21]. The *bleeding time* is a bedside test of platelet function, and it is not considered a reliable test due patient and operator variability[21]. The traditional (gold standard) for platelet function assessment is *platelet aggregometry*[21, 22].

Light transmission aggregometry (LTA) is the most frequently used test to identify and diagnose platelet function defects. The process involves the stirring of platelet rich plasma in a cuvette. The cuvette is placed between a light source and a detector. Following the addition of various agonists (ADP, collagen, thrombin, adrenaline, arachidonic acid), platelets aggregate and light transmission increases. The parameters that are measured include the slope of aggregation (%/min), the maximal amplitude (%) and the percentage of

aggregation after a fixed period of time[22]. Disadvantages of LTA include: platelet rich plasma is used instead of whole blood and in the absence of red/white blood cells together with low shear conditions; it does not accurately stimulate primary haemostasis. Other drawbacks include the need for a large sample volume and the fact that it is a time consuming, non-standardised process[22.] Nonetheless, LTA is still regarded as the gold standard for platelet function testing and is useful for diagnosing a wide variety of platelet defects and monitoring antiplatelet therapy[23].

Limitations associated with LTA prompted the development of other platelet function tests. The most widely used is the *Platelet function analyser (PFA) 100/200*. This machine aspirates blood through an aperture in a membrane. The membrane may be coated with collagen, ADP adrenaline or a combination of these. The end point of measurement is the "closure time" or cessation of flow through the aperture[21]. The advantages of this test include: ease of use, a high shear system and the use of whole blood. The closure time is however affected by haematocrit, platelet count and citrate concentration[21, 22]. Clinical applications include the identification of inherited and acquired platelet defects as well as the monitoring of antiplatelet therapy[23].

Flow cytometry is a popular and powerful laboratory technique used for the assessment of platelet function and activation[22, 23]. Its advantages include that a small volume of blood is required and results are independent of platelet count. Diluted anticoagulated whole blood is incubated with a variety of reagents, including dyes and antibodies. This technique allows for the analysis of individual platelet functional capability and the measurement of the expression of platelet activation markers on individual platelets. The most commonly used flow cytometry tests are the quantification of the basal platelet glycoprotein receptor status and the determination of the platelet granule composition[22]. Flow cytometric analysis of platelets is commonly used to measure the platelet count, determine the state of activation of platelets, to diagnose anomalies in number or function of platelet receptors, to monitor efficacy of antiplatelet drugs and to assess platelet turnover[23].

The *impact cone* was originally designed to monitor platelet adhesion to a polystyrene plate. The instrument contains a microscope and performs staining and image analysis of platelets that adhere and aggregate under a high shear rate. Results are reported as a percentage of the surface covered by platelets and the average size of adherent particles. This assay is fully automated, rapid and simple to use and requires a small volume of blood. It is suggested that the impact cone can detect numerous platelet defects, but the fully automated version has limited use and further studies have been suggested[22].

Methodology

This is a single-centered, retrospective and prospective descriptive study. The study population comprised of patients attending the Helderberg Hospital Infectious Disease Outpatient clinic in the Western Cape Province of South Africa.

Platelet count: Data was collected retrospectively by making use of the data bases which the above mentioned clinic maintains for all their attendees. For prospective data collection the platelet counts were obtained from blood specimens drawn from patients attending the clinic for the first time (ARV naïve patients). Other data collected were the patient's age, sex, use of Highly Active Antiretroviral therapy (as well as specific regime) and CD4 count, which was then correlated with platelet counts.

The definitions of thrombocytopenia in this study include a platelet count of $< 150x10^{9}.L^{-1}$, while a platelet count of $<75x10^{9}.L^{-1}$ will be considered as severe thrombocytopenia (and a contra-indication to performing neuraxial anaesthesia).

Data that was collected from each patient included (Appendix C):

- Age
- Sex
- The use of Highly Active Antiretroviral therapy, as well as the specific regime
- Platelet count
- CD4 count
- Results of platelet aggregometry
- Specific opportunistic infections or malignancies present

All already available data was captured prior to the collection of blood specimens and was noted on a predetermined data sheet (Appendix C). This data sheet was de-identified so that anonymity was preserved. The data was entered on a Microsoft Excel® spread sheet for processing, and thereafter was presented for statistical analysis.

Inclusion and exclusion criteria

Inclusion criteria

All HIV positive patients above the age of 18 years, irrespective of viral load, CD4 count and if they were on HAART or not.

Exclusion criteria

A) Thrombocytopenia[30]

1. Blood transfusion within the last 7-10 days;
2. Connective Tissue disorders: SLE;
3. Lymphoproliferative disorders: Leukaemia, lymphoma, myeloma, myelofibrosis and aplastic anaemia;
4. Drugs: Heparin, Glycoprotein IIb/IIIa receptor inhibitors (Abciximab, Tirofiban), Hydrochlorothiazide, Carbamazepine, Acetaminophen, Chlorpropamide, Ranitidine, Vancomycin;
5. Infections: Hepatitis C, Epstein Barr virus, Cytomegalovirus;
6. HELLP syndrome/gestational thrombocytopenia;
7. Immune thrombocytopenia: Idiopathic thrombocytopenic purpura; Non immune: thrombotic thrombocytopenic purpura, Haemolytic Uremic Syndrome; Folate/Vitamin B12 deficiency and thyroid disease.

   (Bactrim and Rifampicin were initially included as drugs used which would exclude admission into this study; however the routine use of Bactrim as prophylaxis against PJP infection would result in all patients with a CD4 count of less than 200 being excluded from this study. Furthermore, Rifampicin is a first line agent used in treatment of mycobacterium tuberculosis and patients with active tuberculosis were required to form part of the study population.)

Quality control

Specimen collection

Venipuncture:

The principal investigator, an experienced phlebotomist, drew blood using an atraumatic technique from the antecubital fossa, while using minimum tourniquet pressure. 19-21G needles and plastic syringes were used to collect the blood specimens. Three (3) mililitres (ml) of blood was collected and placed in EDTA tubes.

Anticoagulants:

EDTA tubes were used for collection of specimens for platelet count.

Specimen processing:

All specimens were maintained at room temperature. Immediately following blood collection, all tubes were gently mixed by inverting the tube 6 times. The tubes were not exposed to excessive agitation.

The instrument used for platelet count determination is calibrated using commercial controls, which are supplied by Siemens. The Tygerberg Hospital Haematology Laboratory is enrolled in the NHLS as well as the International External Quality Assurance program. The laboratory also has South African National Accreditation System accreditation.

Primary and secondary outcomes

Our **primary outcome** was to determine the prevalence of thrombocytopenia in HIV-positive patients attending a primary care HIV clinic in the Western Cape, South Africa.

The **secondary aims** were to:

Determine if there is any correlation between CD4 count and platelet count.

Determine what influence HAART might have on platelet count.

Suggest a guideline aimed at the perioperative preparation of HIV positive patients for regional anaesthesia that is both practical and cost effective.

Ethical considerations and patient care

There were minimal interventions and risks for the patient, blood samples comprised only 3 milliliters in total, the study population was fairly chosen and participants were able to withdraw from the study at any time.

From an ethical perspective, we are of the opinion that this study is sound. Patient autonomy was respected through proper consent prior to enrolment of all participants.

The aim of this study was to determine whether HIV infection affects platelet count and from these results, a conclusion can be drawn, aimed at the improvement of patient care.

This research study was submitted for approval by the Committee for Human Research at the University of Stellenbosch and was done according to internationally accepted ethical standards and guidelines.

A number was assigned to each patient. Data capture and presentation was performed using these numbers. The patients were de-identified and therefore patient identity and confidentiality was protected.

Budget

Two hundred platelet counts were drawn and submitted to the Haematology laboratory for analysis.

The cost of these investigations, together with the costs of the 5ml syringes, needles, paper and ink cartridges (required for the consent forms), amounted to R4500. A further R1140 was required in order to conduct the survey regarding the current practice in HIV positive patients requiring neuraxial anaesthesia. This funding was made available by the Department of Anaesthesiology and Critical care, Tygerberg Hospital, affiliated to the University of Stellenbosch. Any additional costs including travel expenses were at the chief investigators expense.

Data Management and Statistical Analysis

Ms Moleen Zunza, affiliated to the Biostatistics Unit at the Faculty of Health Sciences, University of Stellenbosch, was consulted for advice on the statistical handling of the data. She recommended that in order to establish a 40% prevalence of thrombocytopenia (with 95% power), 900 patients would be required for this study. Participants were grouped into strata according to gender, CD4 count (<200; > 200) the use of Antiretroviral therapy or not, and from these strata, participants were randomly selected. Associations between categorical values were tested using Pearson Chi squared and/or Fisher's exact tests.

Ms Zunza also assisted with the analyses of the data in the final study.

Results

Data are presented as median, mean +/- standard deviation (95% confidence interval of the mean [$CI^{95\%}$])

Demographics:

We enrolled 1420 subjects; their demographic data is presented in Table 2 and 3 (Appendix B). Platelet count was determined in 1410 participants, while a pilot study of 10 patients was performed to determine the prevalence of platelet dysfunction.

Antiretroviral therapy had and had not been initiated in 936 (66.4%) and 474 (33.6%) of subjects respectively. The different ARV regimens are presented in table 3. The majority (111 or 23%) of patients receiving ARVs were receiving the Stavudine/Lamivudine/Efavirenz regime (Appendix B, table 5).

For the entire study population, the mean CD4 count was 281+/- 199 cells/mm^3. 543 patients (38.51%) had a CD4 count of less than 200 ($CI^{95\%}$ 0.36-0.41)

Infection: 1319 (93.6%) study subjects had no opportunistic infections. 82 (5.8%) had been diagnosed as having Tuberculosis.

Malignancy: The most frequent malignancies encountered were cervical intraepithelial neoplasia (3/0.2%) and Kaposi sarcoma (4/0.3%)

Platelet counts:

For all individuals, including those prescribed and those not prescribed ARV's, the mean platelet count was 270±100 x 10^9/L (25^{th}-75^{th} percentiles 207-308). This data was not normally distributed (Shapiro-Francia W' test for normal data).
Platelet count was found to be less than 150 x 10^9/L in 91 (6.5%) of study participants (mean 116 x 10^9/L, standard deviation 32 x 10^9/L $CI^{95\%}$ 0.05-0.08) (Appendix B, table 3).
Platelet count was less than 75 x 10^9/L in 10 (0.7%) of the participants (mean 40 x 109/L, standard deviation 19x109/L, CI95% 0.003-0.01) (Appendix B, table 3).
If the CD4 count was less than 200 cells/mm^3, a greater proportion of participants had a platelet count of less than 150 x 10^9/l [53/1410 participants (9.8 %) vs. 38/1410 (4.4 %) p<0.001). A CD4 count of less than 200 cells/mm^3 was not associated with a greater proportion of patients having a platelet count of less than 75x10^9/ L [Four (0.3%) participants

had a platelet count of less than 75 X 10^9/L with a CD4 count of less than 200 cells/mm^3 (p=0.9)]

Platelet counts of less than 150 x 10^9/L occurred more frequently in patients who were ARV naïve than in those who had been commenced on ARVs. (7.9% vs. 3.6%) [p=0.02]

In participants in whom ARVs had been initiated, platelet counts of less than 75 x 10^9/L did not differ significantly from those in whom ARV therapy had not been initiated (0.4% vs. 0.9%) [p=0.36]

Infection and malignancy did not significantly increase the number of platelet counts of less than 150 x 10^9/L (p=0.66 Pearson Fischer's exact 0.3) nor on platelet counts of less than 75 x 10^9/L (p=0.99 Fischer's exact 0.5).

Discussion

Considering:

1. The high prevalence of people living with HIV/AIDS in South Africa (12.2%)[24] and
2. The fact that at least 20-25%[28] of these patients will require surgery at some stage in their lives;

It is highly likely that we (as anaesthesiologists) will encounter a HIV positive patient at some stage during our careers.

In terms of anaesthesia, there are a multitude of factors to consider when confronted with HIV positive patients[14], the incidence of platelet count and function abnormalities being among them. The lack of existing and consistent literature regarding this, in combination with the different practices in terms of pre-operative preparation in HIV positive patients prompted us to investigate the effect of HIV on platelet count.

In our study, the mean platelet count of the entire study population was 270±100 x 10^9/L. A platelet count of less than 150 x 10^9/L (thrombocytopenia) occurred in 6.5% of study participants; this is lower than the prevalence of 40% quoted in the literature[17, 18] as well as lower than that obtained in a retrospective study performed at our institution in 2009[31]. (This study looked at platelet counts in 302 pregnant HIV positive patients who delivered at Tygerberg Hospital. The prevalence of thrombocytopenia in the aforementioned study was as follows: 16.2% of patients had a platelet count of <150 x 10^9/L, while 3.3% of patients had a platelet count of <75 x 10^9/L).

In our study, less than 1% (0.7%) of the study population had a platelet count of less than 75 x 10^9/L (severe thrombocytopenia).
A platelet count of less than 150 x 10^9/L occurred more frequently in participants who had a CD4 count of less than 200 cells/mm^3, a trend reported in other studies[17], as well as in patients who were ARV naïve. In participants with platelet counts of less than 75 x 10^9/L however, neither CD4 count, nor ARV naivety were found to increase the frequency of this occurrence. Neither opportunistic co-infection nor co-malignancy had a significant impact on the number of platelet counts of less than 150 x10^9/L or platelet counts of less than 75 x 10^9/L.

Very little is known about the prevalence of thrombocytopenia in the general population[33]. A large observational study involving 12,517 inhabitants of ten villages in a Sardinia (Ogliastra)

did however demonstrate the prevalence of thrombocytopenia to be 3.9% (3.6%-4.3%) [33]. This is less than the prevalence obtained in our study. Of more significance (from an anaesthetic point of view) however is the prevalence of a platelet count of less than $75x10^9$/L, found to be less than one percent in our study.

Thrombocytopenia is not only induced by HIV, but also by a variety of medications, infections and malignancies. It may therefore be argued that it may well be necessary to perform platelet counts on HIV positive patients in whom these conditions may be present. Many of these conditions and medications were specifically chosen as exclusion criteria in our study, as we sought to establish what the effect of the HI virus was on platelet counts.

It is important to mention that this study has many strengths and limitations.

Strengths of this study include:

•The study population comprises solely South African patients and the data obtained was derived from and will be applied to patients in South Africa.
•This is a large study (1410 participants) with 95% power.
•The principal investigator was the sole individual involved in data collection and capture
•This is the first study looking at the prevalence of thrombocytopenia in HIV positive patients in South Africa.

Limitations of our study include

•Most of the data regarding platelet counts was collected retrospectively.
•There was no HIV negative control group for either platelet count or platelet function analysis.
•Only a pilot study was performed for platelet aggregometry.
•The prevalence of thrombocytopenia in the general population is unknown. We therefore do not have a control against which we can compare our results.
•Platelet function in HIV positive individuals may also affected by a variety of factors including Viral Load, CD4 count and anti-retroviral therapy.

Conclusion

Our clinical audit, consisting of 1410 patients, revealed the prevalence of thrombocytopenia and severe thrombocytopenia in the HIV positive population to be low (6,5%) and very low (<1%); both of which are much lower than the prevalence previously quoted in the literature[17, 18]. There was also no correlation between a specific ARV regime and CD4 count and severe thrombocytopenia (platelet count less than 75 x 10^9/L).

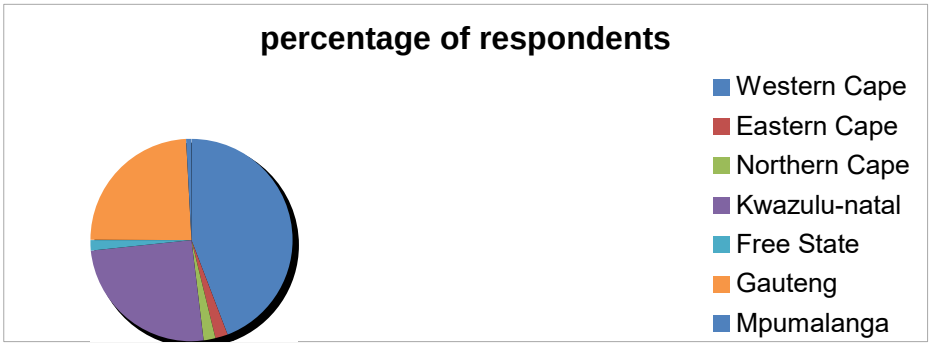
Even though this study wasn't designed nor conducted to produce new pre-operative neuraxial workup guidelines in HIV positive patients, the information obtained from the results are clear in terms of severe thrombocytopenia. Given this extremely low prevalence, we suggest that the routine performance of FBC/platelet count in HIV positive patients requiring neuraxial anaesthesia is not routinely indicated.

In terms of future research, we would recommend performing platelet counts in pre-operative HIV positive patients. This would make it possible to make formal recommendations in terms of pre-operative investigations in this patient population. It would also be insightful to perform a study to investigate the effect of both HIV infection and the different ARV regimes on platelet function.

Acknowledgement

I would like to acknowledge all staff members affiliated to the Department of Infectious Diseases at Helderberg Hospital; Mr. Wessel Kleinhans as well as Mrs. Marieta du Plessis as well as all the members of staff affiliated to the Haematology Laboratory at Tygerberg Hospital; Ms Zunza from the Biostatistics unit of the Faculty of Health Sciences, University of Stellenbosch as well as a special thanks to Dr Marli Smit for her invaluable advice, assistance and support.

Appendix A



Graph 1 demonstrating response rates to the survey of current practice throughout South African state hospitals regarding the perioperative preparation of HIV patients requiring neuraxial anaesthesia.

Table 1: Results from survey regarding the perioperative preparation of HIV patients requiring neuraxial anaesthesia.

	HIV+: no platelet count required	HIV+: platelet count required in all patients	Only require platelet count if CD4 <200	Only require platelet count if CD4 < 50	If on HAART no platelet count required
Western Cape	63	5	6	6	12
Kwazulu-Natal	26	7	12	5	6
Northern Cape	0	2	n/a	n/a	n/a
Gauteng	37	7	2	4	4
Free state	2	5	n/a	n/a	1
Mpumalanga	1	n/a	n/a	n/a	n/a
Eastern Cape	1	4	n/a	n/a	1
Limpopo	1	2	n/a	1	1

Appendix B

Table 2: Demographic data of participants enrolled in study.

	Number	%
Gender: Male	483	34.3
Gender: female	927	65.7
Infection: none	1319	93.6
Infection: TB	82	5.8
Malignancies	9	0.6
No ARV therapy	936	66.4
ARV therapy	474	33.6
CD4 >200	867	61.5
CD4<200	543	38.5
Platelets > 150	1319	93.6
Platelets < 150	91	6.4
Platelets < 75	10	0.7

Histogram of platelet counts

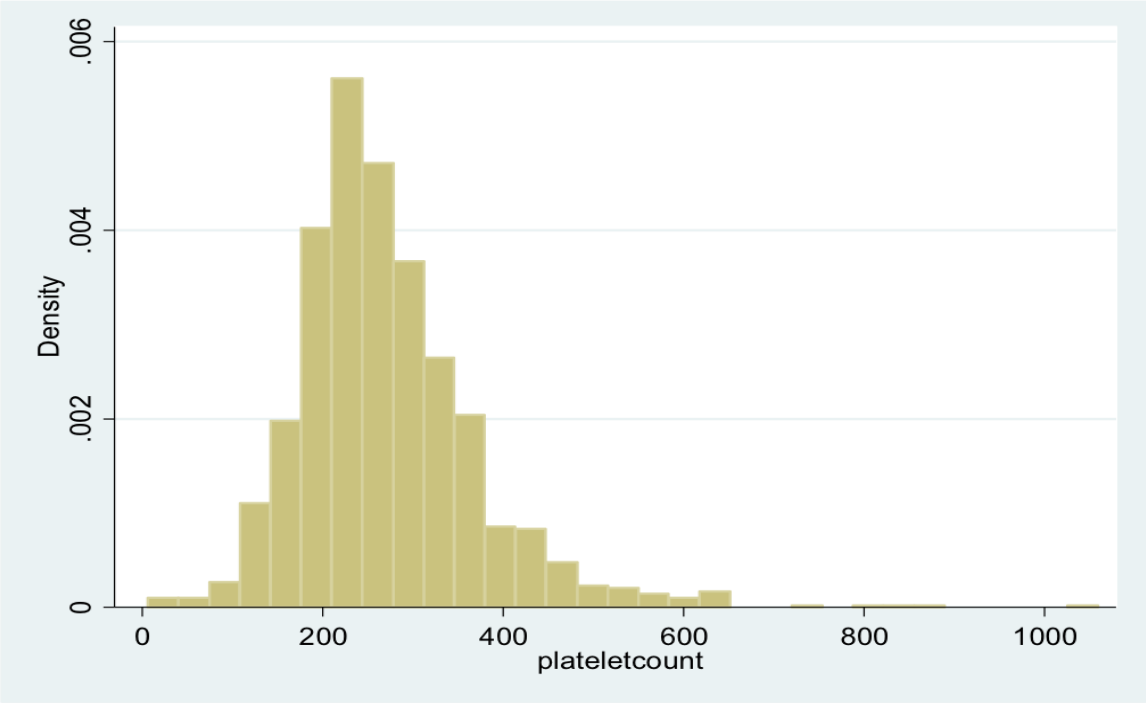


Table 3: Data regarding platelet counts

Platelet count 10^9/L	number	Proportion %	95% CI	average 10^9/L	SD	95% CI 10^9/L
>150	1319	93.6				
<150	91	6.5	5-7%	116	32	110-123
<75	10	0.7	0.3-1%	40	19	27-54

Table 4: Antiretroviral therapy regimes

Antiretroviral therapy regime	Number	Percentage
Tenofovir/Lamivudine/Efavirenz	59	12
Tenofovir/Lamivudine/Alluvia	12	2.5
Stavudine/Lamivudine/Nevirapine	94	20
Lamivudine/Nevirapine/Tenofovir	1	0.2
Stavudine/Lamivudine/Efavirenz	111	23
Tenofovir/Lamivudine/Nevirapine	26	5.5
Zidovudine/Didanosine/Alluvia	12	2.5
Zidovudine/Lamivudine/Efavirenz	35	7
Zidovudine/Lamivudine/Nevirapine	78	16
Tenofovir/Lamivudine/Alluvia	1	0.2
Zidovudine/Didanosine/Kaletra	3	0.6
Zidovudine/Lamivudine/Alluvia	12	2.5
Tenofovir/Emticitabine/Efavirenz	13	2.5
Zidovunie	10	2
Abacavir/Efavirenz/Lamivudine	1	0.2
Alluvia/Efavirenz	2	0.4
Stavudine/Lamivudine/Alluvia	2	0.4
Tenofovir/Lamivudine/Zidovudine	1	0.2
Zidovudine/Stavudine/Alluvia	1	0.2

Appendix C

Data sheet

Patient details/sticker **Study number allocated to patient**

Exclusion criteria excluded: Yes / No

Consent obtained: Yes / No

Antiretroviral therapy: Yes / No

Regime ----------

CD4 count	Platelet count

Secondary causes of HIV associated thrombocytopenia present (Encircle what is relevant)

Medication	Zidovudine, acyclovir, amphotericin B, Bactrim
Concurrent infections	Cryptococcus neoformans, Mycobacterium avium intracellulare and Tuberculosis, Pneumocystis carnii, Histoplsma capsulatum
Malignancies	Non-Hodgkin's lymphoma, Hodgkin's disease, Kaposi's sarcoma

Exclusion criteria

Thrombocytopenia.

1. Blood transfusion within the last 7-10 days
2. Connective Tissue disorders: SLE;
3. Lymphoproliferative disorders: Leukaemia, lymphoma, myeloma, myelofibrosis, aplastic anaemia;
4. Drugs: Heparin, Bactrim, Glycoprotein IIb/IIIa receptor inhibitors (Abciximab, Tirofiban), Hydrochlorothiazide, Carbamazepine, Acetaminophen, Chlorpropamide, Ranitidine, Rifampicin, Vancomycin;
5. Infections: Hepatitis C, Epstein Barr virus, Cytomegalovirus;
6. HELLP syndrome/gestational thrombocytopenia;
7. Immune thrombocytopenias: idiopathic thrombocytopenic purpura;
8. Non-immune: thrombotic thrombocytopenic purpura, Haemolytic Uremic Syndrome; Folate/Vitamin B12 deficiency and thyroid disease.

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