Splenectomy for Immune Thrombocytopenia: Our 11-year experience

Thesis submitted to the University of Cape Town in fulfillment of the requirements for the degree of Master of Medicine (MMed)

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July 2013

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Acknowledgments

1. Professor Nicholas Novitzky

*HOD Haematology Groote Schuur Hospital*

Supervisor for this dissertation

2. Professor Eugenio Panieri

*HOD Endocrine surgery, Groote Schuur Hospital*

For access to surgical records

3. Dr Henri Carreri

*Department of Public Health, University of Cape Town*

For statistical support

4. Maia Lesotsky

*Department of Internal Medicine*

For statistical support
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2. I have used the Vancouver convention for citation and referencing. Each contribution to, and quotation in this report from the work(s) of other people has been attributed, and has been cited and referenced.

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_____________________________
Dr Katherine Antel
Dissertation Abstract

Background

Splenectomy has been practiced for the treatment of ITP for the past few decades. Currently it is utilised when a patient is either dependent or resistant to steroid treatment and the platelet count remains less than 30×10⁹/L. Recently new agents have been added to the armamentarium used to treat ITP, including immunosuppressants such as rituximab and the new thrombopoetin-receptor agonists. This has brought into question the role of surgery for the treatment of ITP, and the need to compare the response and complication rates of splenectomy to these newer agents.

Historic studies done on splenectomy for the treatment of ITP have been performed in the setting of low HIV prevalence. There is a relative paucity of data on the response rate in HIV-associated thrombocytopenia to splenectomy and the durability of response to splenectomy is unclear in this patient population.

Methods

We retrospectively analysed 73 consecutive patients who underwent splenectomy for ITP from 2001 to 2011. The primary objective was to determine the rate of complete response, this was defined as a platelet count greater than 100×10⁹/L at one year post splenectomy. Results were compared between HIV positive and HIV negative patients. The secondary objectives were: to evaluate the intra-operative and post-operative complications and mortality in the HIV positive and HIV negative groups, and to investigate for associations between co-morbidities, pre-operative treatment and response to splenectomy.

Results

HIV status was known for 58 of the 73 patients included in the study: 12 were HIV positive (21%), and 46 were HIV negative (79%). All patients had been treated with oral prednisone at a dose of 1-2mg/kg/day. Median age at
spleenectomy was 33 years (range: 16-70 years).

Median time to spleenectomy from diagnosis of ITP was 14 months (range 1-167 months). Splenectomies were performed by open laparotomy in 26 patients (38%) and were laparoscopic in 43 (62%). The rate of complete response was 80% (CI 69-91%) - these patients maintained platelet counts of greater than 100×10⁹/L at one year and did not require further immunosuppressive therapy. At one year there was no statistically significant difference in the platelet count between HIV positive and negative patients.

There were 11 post-operative complications in 10 patients (16% complication rate). The complication rate in the HIV positive patients was not statistically significantly different to the HIV negative patients (18% and 16% respectively). The median post-operative discharge day was day 3 (range: 2-8); with no statistically significant difference in the HIV positive and HIV negative patient groups. The 90-day mortality rate was 1.38%; one of the 72 patients died at 10 weeks post-splenectomy. Seven patients (six HIV negative and one HIV positive) died during the follow up period. One patient died from overwhelming pneumococcal sepsis.

**Conclusion**

The rate of complete response was in keeping with other studies; the complete response rate to splenectomy appears higher than that reported for rituximab or the new thrombopoetin agonists.

The results show that splenectomy is effective and safe in the HIV positive and negative population and supports the recommendation that splenectomy remains the second-line treatment of choice, with the newer therapies reserved for patients who cannot have surgery or for whom splenectomy fails.
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**Abbreviations used**

**AIDS** – Acquired immunodeficiency syndrome

**ANA** - Anti-nuclear antibody

**ARV** - Anti-retroviral

**CMV** - Cytomegalovirus

**CSF** - Cerebrospinal fluid

**CVA** - Cerebrovascular accident

**EPO** - Erythropoetin

**GSH** - Groote Schuur Hospital

**CART** – Combination Antiretroviral Therapy

**HIV** - Human immunodeficiency virus

**H. pylori** – Helicobacter pylori

**IgG** – Immunoglobulin G

**IgM** - Immunoglobulin M

**ITP** - Immune thrombocytopenia

**IVIG** - Intravenous immunoglobulin

**NHLS** - National Health Laboratory System

**OPSI** – Overwhelming post-splenectomy sepsis

**PE** - Pulmonary embolism

**TB** - Tuberculosis

**TPO** – Thrombopoetin

**UCT** - University of Cape Town

**RPR** – Rapid plasma reagin

**SLE** – Systemic lupus erythematosus
PART A: STUDY PROTOCOL

Protocol submitted for approval in 2012: please note that there was an amendment to the protocol – the dates for data collection were expanded to include 2011 data, this has been approved by the UCT ethics board (see appendix 1) and the UCT Internal Medicine M. Med committee.

The protocol contains a brief review of the literature as an introduction and background for the study protocol; to avoid repetition it is suggested that the examiner preferentially reads Part B (Literature review).
I. PROTOCOL

1. Introduction

Immune thrombocytopenia (ITP) is a condition of excessive platelet destruction (due to autoantibodies) with an inadequate or absent compensatory bone marrow response, which results in a low platelet count. ITP can be acute (most common in childhood, and usually post-viral infection) or chronic. Chronic ITP can be idiopathic or secondary to: autoimmune disorders (such as SLE, Evans syndrome, thyroid disease); infections (HIV infection, hepatitis and h. pylori); drugs; and lymphoproliferative disease. Most important in our context is the association with HIV infection.

In HIV infection thrombocytopenia is the second most common haematological abnormality (after anaemia) and the causes are multifactorial including: autoimmune destruction of platelets and marrow suppression and megakaryocyte dysfunction (from direct HIV infection, cytokine storm, drugs and co-morbid conditions such as TB and CMV). The current best medical practice to manage ITP in HIV is with antiretroviral therapy and glucocorticoids. But there is a paucity of data on the quality of the response to steroids, the relapse rate and in particular, the longer-term outcome of these patients (1). There have been a few studies that have demonstrated that splenectomy as a treatment for HIV-ITP is safe and effective (to be discussed in more detail below) and after initial concerns in the pre-cART era that splenectomy caused a more rapid progression to AIDS were dispelled (9), it has been a more commonly used strategy. There is however no data of outcomes from the South African context on the effectiveness of splenectomy in the HIV positive population; this would be important and relevant to obtain because of the high rate of other co-morbidities in HIV thrombocytopenia (i.e. TB) and because there are other medical treatments that could potentially be employed prior to a surgical procedure.
The prevalence of thrombocytopenia in HIV shows a bimodal distribution with a peak in early HIV and then again in advanced HIV, but may occur at any time during the course of the infection. The reported prevalence varies depending on the source, but stated to occur in as many as 40% of HIV infected individuals (2); according to another source chronic thrombocytopenia occurs in about 10% of HIV-infected patients, and up to a third of patients with AIDS(3). The prevalence of platelet count less than 50×10⁹/L is seen in 1-5% of cases (3), with one large study reporting the incidence at 3.2% in the cART era (4).

There are no South African data on prevalence of HIV-ITP, but it is likely to be affected by the local occurrence of conditions associated with HIV-ITP such as: co-infection with Hepatitis B and C (4,5); prevalence of drug use (thrombocytopenia has been shown to be more severe in HIV-infected drug users) (6); and nutritional status as HIV-ITP has also been associated with poor nutritional status (specifically selenium deficiency)(6).

Thrombocytopenia is an important problem in HIV, both because it is common and because it is associated with significant morbidity and mortality. Thrombocytopenia has been associated with rapid progression to AIDS, this is thought in part to be due to the association of thrombocytopenia with a high viral load (2, 6, 7). It is also associated with an increased risk of bleeding and haemorrhagic complications.

The mechanisms by which HIV infection can cause thrombocytopenia are multiple and vary depending on the stage of HIV infection. These mechanisms include: direct infection of the platelets and megakaryocytes with HIV through the CD4 and CXCR4 receptors (6), increased peripheral platelet destruction, and myelosuppression secondary to increased susceptibility to opportunistic infections such as CMV and TB and antiretroviral agents.

Early in HIV the predominant mechanism is increased peripheral consumption. Multiple auto-antibodies and cross-reactive antibodies have been identified which ultimately result in increased platelet destruction via Fcy-mediated phagocytosis by macrophages in the spleen or complement-independent platelet fragmentation by the generation of peroxide (5). These antibodies are
multiple, but include: anti-glycoprotein (GP)IIb and/or GPIIIa (however, cross-reactive antibody between HIV gp160/120 and platelet GPIIb/IIIa has also been demonstrated)(1) and more recently an anti- talin antibody that attacks the cytoskeleton of the platelet has been identified (7). Talin cross-links actin filaments and cytoplasmic tails of beta integrins at focal adhesion points within platelets.

Late in HIV, the predominant cause of thrombocytopenia is inadequate thrombopoiesis – secondary to ‘cytokine storm’ in the bone marrow (unmitigated cytokine release) and direct infection of the megakaryocytes (1). Megakaryocytes bear CD4+ receptors capable of binding HIV-1 as well as the co-receptor molecule CXCR4 (13) which enable HIV-1 viral entry into the megakaryocyte and interfere with normal platelet production. Late in HIV there is also increased susceptibility to intercurrent opportunistic infections that may cause bone marrow suppression (in our setting, TB being the most important condition to exclude). But this is likely an over-simplification in terms of the pathogenesis at early and late HIV; with an imbalance of marrow production, shorted platelet lifespan and immune destruction present in variable degrees throughout the course of HIV.

Patients with HIV-ITP have increased megakaryocyte mass as seen on bone marrow biopsy, as well as a six-fold increase in thrombopoetin levels (13) but this is inadequate to compensate for the amplified peripheral consumption as well as reduced rate and ineffective production of platelets.

**Treatment:**
Treatment guidelines for HIV-associated thrombocytopenia have not yet been formalised because of a lack of high-quality clinical trials. Treatment is therefore mainly based on expert opinion, and has followed the treatment of ITP in HIV-ve patients (2). Destruction and production of platelets are mediated at many different levels and therefore the various treatment strategies aim to target these levels.
Medical Treatment

Glucocorticoids work by inhibiting the expression of splenic macrophage Fc receptors, and thereby decreasing the splenic destruction of platelets. Steroids also inhibit the lymphoid clone that produces the auto antibody leading to improvement of platelet counts in patients who display adequate marrow compensation. However glucocorticoids at high doses have high risks in HIV positive patients: steroids can directly up-regulate HIV-1 replication, as well as increase the risk of opportunistic infections (2). Prednisone can produce an increase of the platelet count to over 100×10⁹/L in over half of patients treated, but only a minority of patients will maintain platelets above 50 after cessation of therapy (2).

Danazol (an attenuated androgen) has been used with mixed results – its overall efficacy has been shown to be quite unsatisfactory in HIV positive patients (2).

Very small studies have tested dapson to treat HIV-associated thrombocytopenia, and they have shown some success. It is hypothesized that it acts by a competitive effect on red cell clearance by the reticuloendothelial system (14).

Intravenous immune globulin produces significant improvement in the majority of patients with HIV associated thrombocytopenia (over 90% reported in a review of cases), and the response rate is typically very rapid, usually after one dose. But its use is limited because the response is typically transient, and so multiple infusions may be required, at a high cost (14). It is thought to work by saturating the Fc receptors in the spleen, thereby preventing the removal of platelets.

Anti-Rhesus immunoglobulin is a solution of IgG anti-D (anti-RhD) antibodies, it has shown to have a similar response rate to IVIG, at less cost, but the concern is that it can lead to haemolysis and for this reason it can only be given to patients with an adequate haemoglobin, and who are Rhesus positive (2). It works by the competitive binding of Fc receptors in the spleen (Rh- antigen binds to the red
cell membrane, the sensitised RBCs are cleared by the spleen) and consequent reduction in platelet clearance.

Anti-retroviral agents were initially thought to raise platelet count by decreasing the HIV viral load burden; this is now thought to likely not be the full mechanism, since certain antiretroviral agents are better than others at raising platelet count. In the years before cART; numerous studies demonstrated that zidovudine (AZT) mono therapy was shown to be efficacious in increasing the platelet count (10). In one study comparing cART to AZT mono therapy; response rates to cART have been found to be similar. cART was found to increase the platelet count to above $50 \times 10^9/L$ in 73% of patients, with a complete response (increase of platelets $> 100 \times 10^9/L$) in 53% of patients; this response occurred within the third month of treatment and was sustained throughout the treatment period (10). A limitation of AZT is that it can directly cause marrow suppression leading to leukopenia and anaemia. Antiretroviral drugs will decrease the viral load and number of infected cells in the bone marrow, and decrease the ‘cytokine storm’ in the bone marrow but they may not modify the formation of anti-platelet antibodies.

Recently rituximab has been added to the armamentarium to treat ITP, but it has not been studied systematically in HIV associated thrombocytopenia. Currently it has been used in heavily treated patients, in the setting where splenectomy has either failed or the patient has contraindications to splenectomy. Rituximab is a monoclonal antibody against CD20+ B cells – its mechanism of action is thought to be due to B cell depletion (however B cell depletion occurs in all patients whereas a clinical response does not, and the time to clinical response with an increase in platelet count is often irrespective of immunoglobulin levels), and so other mechanisms have been proposed including Fc receptor saturation in the spleen with resultant incompetence to bind platelets (11) and increased activity of T cell regulatory cells (12). In a recent meta-analysis the rate of long-term complete response was 20%, with an overall response rate of 57% (increase in platelet count to $>100$, but the mean duration of response was 43 weeks) (11). Rituximab is generally safe – however it depletes antibody producing lymphocytes and further immunosuppression
needs to be considered in the HIV positive population. Its use is limited by high cost.

**Surgical Treatment**

Splenectomy is generally reserved for those patients who have failed medical therapy. In our setting, it is used after patients have failed steroid therapy. Splenectomy has been shown to be both relatively safe in HIV, as well as efficacious (5). In a systematic review published in 2004 by Kojouri et al complete response (defined as achievement and maintenance of a normal platelet count (150×10⁹/L) for all measurements 30 days or longer after splenectomy, and with no additional treatment for ITP) following splenectomy was reported to reach 60% (8). In a different article, Oksenhendler et al reported long-term experience in a cohort of 185 patients with HIV-ITP (9). Splenectomy was performed in 68 of these patients, at an average of 13 months from initial diagnosis of HIV-ITP. A response was seen in 92% of patients and maintenance of the elevated platelet count for longer than 6 months was documented in 82%. However in some studies, as many as a third of the HIV positive patients who underwent splenectomy displayed an incomplete response to the treatment (2). A problem when reviewing these articles is that ‘complete response’ is defined differently, and the studies often use platelet count at one month to determine response but we know that patients can later relapse, we therefore are still unsure about the durability of splenectomy.

In the review by Kojouri, the mortality rate of splenectomy was reported 0.2% and 1.0% for laparoscopy and open laparotomy, respectively (8). Mortality was primarily due to bleeding, sepsis (pneumonias and sub diaphragmatic abscesses) and venous thromboembolism. Late complications were not reviewed. In the Oksenhendler series 5.8% of patients undergoing splenectomy experienced fulminant infection, consisting of Streptococcus pneumoniae meningitis in two and Haemophilus influenzae sepsis in one individual(9). Splenectomy is therefore not a definitive or curative treatment for many patients and it carries a risk of increased susceptibility to infections as well as operative risks and is therefore only used after medical treatment has failed.
2. **Justification**

We want to compare the outcome in HIV positive and HIV negative patients (in terms of response to splenectomy and survival), and report on the complication rate and rate of opportunistic infections in these two groups. The justification for carrying out this research is that we will be able to define better the population of patients requiring splenectomy for the management of ITP. We may be able to ascertain indicators that can predict failure to steroid treatment. Finally, as splenectomy is an irreversible procedure and there is alternative medical treatment available for HIV-ITP, we need to question whether it is still appropriate to offer splenectomy after the failure of steroids in the HIV positive population.

3. **Hypothesis**

We postulate that in HIV positive patients the response rate to splenectomy is lower since the pathogenesis involves decreased production as well as increased consumption of platelets. In addition we advance that in this patient group there is a higher rate of post-operative as well as long term complications. However, including only patients that underwent splenectomy selects out the most symptomatic cases of ITP and in the literature the response rates for both groups is around 60%.

II. METHODS

1. **Study Design**

The study design will be a retrospective cross-sectional descriptive and analytical study of patients who underwent splenectomy for ITP. The sequential names of patients who underwent splenectomy cases will be retrieved from the NHLS laboratory information system and their outcomes will be studied from the clinical charts.

2. **Subject Identification**

The patients chosen will be the patients who have undergone splenectomy for ITP in the period 2000-2010. This population is the group of patients with ITP as defined by low platelet count and bone marrow findings showing increased
megakaryocyte mass who have relapsed after steroid and or other medical therapy.

3. Measurement

i. A data capture sheet will be used for record review (taken from the folders of selected individuals). The data capture sheet will include:
   - Clinical information
     i. Patient demographic details
     ii. Current co morbidities (TB, malignancy, etc)
     iii. Concomitant medication, type of cART
   - Laboratory data
     i. Bone marrow finding compatible with ITP – yes or no
     ii. HIV status
     iii. Viral load, CD4 counts
   - Other co-morbidities
     i. Platelet count at diagnosis / three month / six month / 1 yr and pre-splenectomy
     ii. Platelet count 6 months post splenectomy and 1 year post splenectomy
   - Treatment given:
     i. Steroids – duration and dosage per kg if available
       1. Quality of response
       2. Recurrence rate
     ii. Other treatment given prior to splenectomy
     iii. Length of time on ARVs
   - Post splenectomy immunosuppressive therapies
   - Rate of infections, particularly OPSI
   - Peri-operative morbidity and mortality
   - Pre-operative management
     i. Plasmapheresis
     ii. Serum immunoglobulins
     iii. Platelet transfusions

ii. Variables:
• Gender
• HIV status (binary variable)

iii. Quality Control:
• The patients will be identified using NHLS records of spleen histology results for ITP, and double checked with the surgical notebooks.
• The data taken from the folders will include treatment, patient demographic details, co-morbidities and outcome.

III. ANALYSIS and STATISTICS
The data will be collated onto a single page summary table. These data will be entered into MS Excel. The rate of treatment outcome after splenectomy will be correlated with HIV status, as well as the rate of short and long term complications, including OPSIs. An odds ratio will be calculated to determine the likelihood of treatment success in the two groups. The length of treatment time prior to splenectomy will be compared in the HIV negative versus HIV positive groups.

IV. ETHICS
The study will be submitted to the Ethics committee of the UCT for approval. The names of the patients used in the study will be kept confidential. Since the study involves a folder review and no intervention I do not think that the patients included into the study need to be contacted for informed consent. I have privileged access to both the NSHL and GSH records by virtue of being employed as a medical doctor at GSH.

V. LOGISTICS
1. Timing
Data collection and entry onto excel: October 2012
Synthesis and analysis of data: April 2013 (during M. Med rotation). Writing up M. Med: April 2013

2. Budget
The study will not require external funding.
VI. REFERENCES

PART B: LITERATURE REVIEW

Word count: 3959 words
The objectives of this literature review were to appraise published evidence addressing:

1) The mechanism of disease in immune thrombocytopenia (ITP) and in Human Immunodeficiency Virus (HIV)-associated thrombocytopenia
2) The response rates and side-effects of current medical therapy for ITP
3) The published data on splenectomy: response rates, rates of relapse, long-term outcomes, and complications

The following databases were searched for relevant literature via the University of Cape Town EZproxy system: PubMed, Google Scholar, EBSCOHost, and the Cochrane database using the keywords: HIV and ITP; HIV and thrombocytopenia; Splenectomy for ITP; rituximab for ITP; thrombopoietin agonists for ITP; South Africa ITP; HAART (highly active antiretroviral therapy) for ITP. Articles published from 1985-2013 were included. Sixty-six relevant studies were identified. These studies included: eight on mechanism of disease in ITP, two on natural history and mortality in ITP, eight on thrombocytopenia in HIV, seven on splenectomy for ITP (including one review article), four on response to steroids, two studies on rituximab in ITP and four studies on thrombopoietin agonists (eltrombopag and romiplostim).

1.) Mechanism of disease in ITP and HIV associated thrombocytopenia

Platelets are cell fragments (without a nucleus) that play an essential role in haemostasis by forming an haemostatic plug and activating the clotting cascade to form a thrombus. Platelets are formed predominately in the bone marrow from megakaryocytes (about 7-15% of thrombopoiesis is extramedullary). Approximately $2 \times 10^{11}$ platelets are produced every day. Under conditions of increased platelet consumption this can increase eight times and is regulated by thrombopoietin. Platelets have a life span of 7-10 days and are cleared by the cells of the reticuloendothelial system including the spleen.\(^1\)
Immune thrombocytopenia (ITP) is an autoimmune disorder characterized by immunologic destruction of platelets, as well as an inadequate bone marrow response to low platelets. ITP can be primary or secondary to: autoimmune disorders (such as SLE, Evans syndrome, thyroid disease), infections (HIV, viral hepatitis and H. pylori), drugs and lymphoproliferative disease. The overall incidence of ITP (using a platelet count of < 150×10⁹/L) in adults has been estimated at 3.9 per 100,000 person-years (2) with a prevalence of 50.29/100,000 (3). These data are from recent large studies in Europe; there are no data available on prevalence in South Africa.

**Pathophysiology**

Thrombocytopenia in ITP is complex and predominately due to platelet-reactive autoantibody production, along with T-cell abnormalities and immune-mediated megakaryocyte injury and dysfunction that results in relative platelet underproduction (4-8).

Historic studies by Harrington and Holingsworth in the 1950's (9) demonstrated that the transfer of plasma from ITP patients induced thrombocytopenia in healthy recipients. Subsequent studies by Shulman *et al* identified the plasma substance as IgG antibodies against platelet glycoproteins (10,11). Auto-antibodies in ITP are primarily directed against the glycoproteins GPIIb/IIIa or GPIb/IX. The formation of these auto-antibodies is most often idiopathic but is sometimes due to cross-reactive antibody production after an infection (e.g. HIV, hepatitis C and H. pylori) (4).

Opsonised platelets are removed from the circulation by splenic macrophages via low-affinity Fc receptors. The peptide fragments expressed within MHC class II on the macrophages stimulate helper T cells, which results in the activation of B cells to continue to produce autoantibodies (4). The T helper cell response is abnormal in ITP, with studies showing an imbalance in Th1/Th2 ratios (6). A decrease in the number of regulatory T cells (Treg) may partly be responsible for this heightened Th1 response – it was recently demonstrated that in mice that were Treg-deficient, 50% became thrombocytopenic (8). Auto-
reactive T cell clones against GPIIb/IIIa epitopes in ITP patients have been identified, which adds further complexity to the pathogenesis of ITP as well as a possible new treatment target(7).

Despite the increase in platelet destruction in ITP, there is a sub-maximal response to increase platelet production. Morphological abnormalities of megakaryocytes in ITP were first demonstrated in the 1940’s(12) and platelet kinetic studies have shown platelet production remains unchanged or is decreased in patients with ITP(13). Megakaryocytes at the maturation phase contain glycoproteins GPIIb/IIIa or GPIIb/IX(14) and it is thought that the autoantibodies probably also affect platelet production by immune destruction of megakaryocytes. Further supporting evidence for this hypothesis is that in vitro studies have shown suppression of megakaryocyte growth and maturation by the IgG fraction of ITP plasma(15). See Figure one for an illustration of the combination of immune system abnormalities which contributes to increased platelet destruction and decreased platelet production.

![Figure 1](image_url)

**Fig 1.** Demonstrating the pathophysiology of ITP:
1. Autoreactive antibodies bind to platelets and facilitate macrophage destruction in the spleen; peptide fragments expressed on the surface of the macrophages stimulate the Th cells which further activates B cells to produce more antibody (and Treg cells fail to suppress this).

2. The autoantibodies are depicted in the bone marrow, affecting megakaryocyte function.

3. Cytotoxic T cells are thought to play a role in platelet and megakaryocyte destruction.

(Figure reproduced with permission from Kashiwagi, Tomiyana. *Pathophysiology and Management of Primary Immune Thrombocytopenia*, published in Int J of Hematology 2013 May 24. See appendix 2 for permission)

The primary problem in ITP is the risk of haemorrhage – most significantly spontaneous haemorrhage that can lead to intracerebral bleeds or other catastrophic bleeding. The risk of fatal bleeding is 0.02 to 0.04 cases per 1000 patient-years\(^{(16)}\) and ITP has been associated with a risk of death that is four times greater than that of the general population due to infection, haematological malignancies and bleeding\(^{(17)}\). Quality of life is lower than that of the general population, predominately because of fatigue that is independent of platelet count and not fully understood\(^{(17)}\). A platelet count of greater than 30×10⁹/L is the goal of treatment because patients with this platelet count have been shown to have a long-term mortality rate equal to that of the general population\(^{(17)}\).

**HIV associated thrombocytopenia**

Thrombocytopenia is a common haematological abnormality in HIV and the cause is multifactorial, including autoimmune destruction of platelets, reduced platelet lifespan, and megakaryocyte dysfunction resulting in impaired thrombopoiesis. Thrombocytopenia is an important condition in HIV because of the risk of haemorrhage and it has been shown to be associated with more rapid disease progression to AIDS. Platelets engulf HIV virions and therefore are thought to play a role in the immune response to HIV,\(^{(18)}\) which partly explains the association of thrombocytopenia with a high viral load\(^{(18,19,20)}\).
The prevalence of thrombocytopenia in HIV shows a bimodal distribution with a peak in early HIV and then again in advanced HIV, but may occur at any time during the course of the infection. Prior to the use of combined antiretroviral therapy (cART), prevalence of ITP (platelet count < 150×10^9/L) in HIV positive patients was approximately 11-23%\(^{(21,22,23)}\). Thrombocytopenia in the era of cART is much lower, with one large study (of 2298 outpatients) reporting the incidence at 3.2% \(^{(20)}\).

In South Africa the prevalence of thrombocytopenia at enrolment for cART is 4\%\(^{(24)}\). It is interesting that in this study South Africa had a lower rate of thrombocytopenia than India, Malawi, Brazil or the US (at enrolment for cART). Factors that have been shown to be associated with thrombocytopenia in HIV include: co-infection with Hepatitis B and C \(^{(21,20)}\); prevalence of drug use (thrombocytopenia has been shown to be more severe in HIV-infected drug users)\(^{(19)}\); and poor nutritional status (specifically selenium deficiency)\(^{(19)}\).

Early in the course of HIV infection, the predominant mechanism of ITP is increased peripheral consumption due to autoantibodies. Multiple cross-reactive antibodies have been identified which ultimately result in increased platelet destruction via Fcγ-mediated phagocytosis by macrophages in the reticuloendothelial tissues (especially the spleen), or complement-independent platelet fragmentation by the generation of hydrogen peroxide\(^{(25)}\). These antibodies are directed at multiple epitopes, but include antibodies to HIV glycoprotein120 and the P24 antigen that then cross-react with glycoprotein (GP)IIb and/or GPIIIa on platelets\(^{(18,26)}\). More recently, an anti-talin antibody that attacks the cytoskeleton of the platelet has been identified in patients with HIV and it has been proposed that this is the predominant antibody responsible for platelet depletion \(^{(27)}\). Talin cross-links actin filaments and cytoplasmic tails of beta integrins at focal adhesion points within platelets. Talin can be cleaved during platelet activation and by HIV-1 protease and thus in HIV infection becomes more readily exposed to the immune system.

Late in HIV, the predominant cause of thrombocytopenia is inadequate
thrombopoiesis secondary to unmitigated cytokine release in the bone marrow and direct infection of the megakaryocytes\(^{(28)}\). Megakaryocytes bear CD4+ receptors capable of binding HIV-1 as well as the co-receptor molecule CXCR4\(^{(29,30)}\) that enables HIV-1 entry into the megakaryocyte and results in interference with normal platelet production. Patients with HIV-associated ITP have increased megakaryocyte mass as seen on bone marrow biopsy\(^{(31)}\) as well as an increase in thrombopoetin levels,\(^{(32)}\) but this is inadequate to compensate for the amplified peripheral consumption from an immune dysregulation and dysmegakaryopoiesis. On histological examination, megakaryocytes are dysmorphic, with naked nuclei (apoptotic megakaryocytes), hypolobation of the nuclei, tendency to form discrete nuclear lobules, and clustering of the megakaryocytes\(^{(31)}\).

Late in HIV, there is increased susceptibility to intercurrent opportunistic infections that may cause bone marrow suppression (in our setting TB is the most important condition to exclude).

It must be noted that the above discussion is likely an over-simplification of the pathogenesis in early and late HIV infection, since an imbalance of marrow production, shortened platelet lifespan and immune destruction are present in variable degrees throughout the course of HIV.

2.) Treatment of ITP

Current practice is to initially treat ITP with high dose prednisone (1 to 2mg per kg per day orally) for 2 to 4 weeks followed by tapering over a few weeks if the platelet count responds. If the platelet count fails to respond to this, the patient is given other immunosuppressive therapy including dexamethasone, azathioprine and cyclophosphamide. If the platelet count is refractory to these drugs and the patient urgently needs treatment (for example prior to surgery or if the patient is bleeding), the patients are treated with plasmapheresis, IVIG (intravenous immunoglobulins) and platelet transfusion\(^{(33)}\).
If the platelet count does not respond to steroid treatment or if the ITP relapses after weaning steroid therapy (steroid dependence), the patient should be considered for splenectomy, possibly with a trial of an immunosuppressant such as azathioprine before surgery\(^{33}\).

Current practice for the management of ITP in HIV is antiretroviral therapy and glucocorticoids. However, there is a paucity of data on the quality of the response to steroids, the relapse rate and in particular, the longer-term outcome of these patients\(^{21}\). There have been a few studies that have demonstrated that splenectomy as a treatment for HIV-ITP refractory to medical therapy is safe and effective (to be discussed in more detail below) and after initial concerns in the pre-cART era that splenectomy caused a more rapid progression to AIDS were dispelled\(^{34}\), it has been a more commonly used strategy. Treatment guidelines for HIV-associated thrombocytopenia have not yet been formalised because of a lack of high-quality clinical trials. These are therefore based on expert opinion, and have followed the treatment of ITP in HIV negative patients\(^{35}\).

**Glucocorticoids**

Corticosteroids are effective first line treatment in ITP and have several mechanisms of action. They work by inhibiting the expression of splenic macrophage Fc receptors, and thereby decrease the splenic destruction of platelets. Steroids also inhibit the lymphoid clone that produces the auto-antibody directed against platelet membrane epitopes. However, glucocorticoids at high doses have risks. In particular they increase the risk of infection, especially tuberculosis in our patient population.

Conventionally prednisone is used at a dose of 1-2mg/kg/day and can produce an initial increase in the platelet count to over \(100 \times 10^9/L\) in about 80% of patients\(^{36}\), but the long term response is much lower and only a minority of patients will maintain platelets above \(100 \times 10^9/L\) after cessation of therapy\(^{36,37,38}\). There were no randomised controlled trials of steroids versus placebo in the literature (likely because steroids have an established benefit).
Three large cohort studies with heterogenous definitions of ‘complete response’ were reviewed; the results are included in Table 1 below. It should be noted that ‘Complete response’ by the definitions given is not the only goal of therapy, as a platelet count of >30×10⁹/L does not need further treatment (33).

<table>
<thead>
<tr>
<th>Study</th>
<th>Total no pts</th>
<th>No of pts on steroids</th>
<th>CR definition</th>
<th>CR rate</th>
<th>Mean follow-up (months)</th>
<th>Relapse rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pamuk et al</td>
<td>321</td>
<td>129</td>
<td>&gt;100×10⁹ at 3 mo</td>
<td>52%</td>
<td>33</td>
<td>58%</td>
</tr>
<tr>
<td>Li et al</td>
<td>1791</td>
<td>689</td>
<td>&gt;100×10⁹ (no time period given)</td>
<td>30%</td>
<td>36</td>
<td>Not included</td>
</tr>
<tr>
<td>Zimmer et al</td>
<td>201</td>
<td>118</td>
<td>&gt;150×10⁹ at 6 mo</td>
<td>22%</td>
<td>Not included</td>
<td>78%</td>
</tr>
</tbody>
</table>

Table 1. Response rate to glucocorticoid therapy. CR – complete response.

High dose dexamethasone was shown to be effective in one study that included 125 patients with ITP; almost 40% had a sustained response that lasted 2-5 years (39).

**Intravenous immunoglobulin and anti-D immunoglobulin**

Intravenous immunoglobulin produces a significant improvement in the majority of patients with idiopathic and HIV associated thrombocytopenia (40,41). The response is rapid, usually after one dose, however it is typically transient, and so multiple costly infusions may be required, limiting its use. The mechanism of action is saturation of Fc receptors in the spleen, thereby preventing the removal of platelets. Its primary use is for emergency management of ITP in settings of life-threatening bleeding, or prior to surgery.

Intravenous anti-rhesus immunoglobulin is a solution of IgG anti-D (anti-RhD) antibodies, it has a similar response rate to IVIG (42), at less cost. However the concern is that it can lead to haemolysis and for this reason it can only be given to patients with an adequate haemoglobin level, and who are Rhesus positive. It increases platelet counts in the short term but does not have a durable effect (43). It works by the competitive binding of Fc receptors in the spleen: Rh- antigen binds to the red cell membrane and these sensitised RBCs are cleared by the
spleen with consequent reduction in platelet clearance. In South Africa only the intramuscular form of anti-D is available.

**Azathioprine and Cyclophosphamide**

In their consensus document, Provan *et al* recommended azathioprine, cyclophosphamide and other immunosuppressive therapies (mycophenolate mofetil, cyclosporin, vincristine) to be used as second-line agents prior to splenectomy\(^{(44)}\). However, the most recent guidelines from the American Society of Hematology have not recommended included these drugs because of considerable variability in individual responses and toxicity, including immune suppression, hepatic toxicity and secondary malignancies\(^{(33)}\).

**Danazol and Dapsone**

Danazol (an attenuated androgen) has been used to treat ITP with mixed results – its overall efficacy has been shown to be quite unsatisfactory in HIV positive patients\(^{(45)}\).

Dapsone is thought to act by a competitive effect on red cell clearance by the reticuloendothelial system. Very small studies have tested dapsone's efficacy in ITP\(^{(46,47)}\), and they have shown some success.

**Antiretroviral agents for HIV associated thrombocytopenia**

Antiretroviral agents work to raise the platelet count primarily by decreasing the HIV viral load burden, but this is unlikely the comprehensive mechanism since certain antiretrovirals are more effective than others. In the years before cART numerous studies demonstrated that zidovudine monotherapy was efficacious in increasing the platelet count \(^{(48-52)}\).

In one study comparing cART to AZT monotherapy response rates to cART were found to be similar. Combination antiretroviral therapy was found to increase the platelet count to above 50 \(x10^9/L\) in 73% of patients, with a complete
response (increase of platelets > 100×10⁹/L) in 53% of patients; this response occurred within the first three months of treatment and was sustained throughout the treatment period (53).

In practice it is preferable to use cART over AZT monotherapy because of the risk of HIV resistant strains being selected with monotherapy. Thrombocytopenia is therefore an indication to start cART, and based on the study above(53) the regime need not contain AZT (a drug that is associated with significant side effects including bone marrow suppression and a high frequency of gastrointestinal complaints).

Rituximab

Rituximab is a monoclonal antibody against B-cells that targets CD20. Rituximab is used for the treatment of B-cell lymphoid malignancies and autoimmune conditions such as rheumatoid arthritis. Recently, rituximab has been added to the armamentarium to treat ITP, but it has not been studied systematically in HIV-associated thrombocytopenia. In ITP its mechanism of action is thought to be due to B cell depletion, however B cell depletion occurs in all patients whereas a clinical response does not. Furthermore, the time to clinical response (an increase in platelet count) is often irrespective of immunoglobulin levels. Therefore, other mechanisms have been proposed including Fc receptor saturation in the spleen with resultant inability to bind platelets(54) and an increase in activity of T-cell regulatory cells(55). In a recent meta-analysis the rate of long-term complete response was 20%, with an overall response rate of 57% (increase in platelet count to >100×10⁹/L, but with a mean duration of response of 43 weeks)(55). Currently it is being utilised in heavily treated patients, in the setting where either splenectomy has failed or the patient has a contraindication to splenectomy.

Rituximab is generally safe, however it depletes antibody-producing lymphocytes therefore making patients more susceptible to infection, and its use is limited by high cost. A rare reported side effect is progressive multifocal leukoencephalopathy caused by reactivation of latent JC virus, but the strength
of association is weak as there are only a few case reports. The 2011 American Society of Hematology (ASH) treatment guidelines gave rituximab a weak recommendation (grade 2C) for patients who have failed steroids, IVIG and splenectomy(33).

**Thrombopoetin receptor agonists**

Thrombopoetin (TPO) is the primary cytokine that regulates platelet production and is produced by the liver and kidneys. TPO binds to the c-Mpl (myeloproliferative leukaemia virus oncogene) receptor to activate the JAK2 tyrosine kinase pathway dependent genes. This stimulates megakaryopoiesis which increases the number of platelets produced. The production of thrombopoetin is fairly constant and yet the levels vary according to the platelet count. The mechanism of this is thought to be that platelets and megakaryocytes bind free TPO and degrade it, so the higher the platelet mass the lower the level of thrombopoetin.

TPO receptor agonists work by stimulating the c-Mpl receptor. The first TPO receptor agonists caused a cross-reactive autoantibody and resulted in severe thrombocytopenia in healthy study subjects. Second generation TPO receptor agonists (such as romiplostim and eltrombopag) have no homology to endogenous TPO and do not have this effect. These drugs have demonstrated durable response rates (platelet count more than 100×10^9/L at 6 to 8 weeks) in 50 to 60% of patients as long as treatment is maintained(56,57) and were associated with fewer treatment failures, fewer splenectomies, less bleeding and a better quality of life(58). However, in a large Cochrane systematic review neither TPO agonist improved the rate of severe, life-threatening or fatal bleeding(59). In addition, eltrombopag has shown to increase alanine aminotransferase and indirect bilirubin concentrations and these levels need to be monitored on treatment.
3.) Splenectomy for ITP

The spleen has a major role to play in immunity: it is largely responsible for the effective clearance of encapsulated bacteria (opsonised bacteria are removed by splenic macrophages) and production of antibody (IgM memory B cells originate in the splenic marginal zone in response to antigen and activated T cells). It also has an anti-inflammatory function, having been shown to dampen down the cytokine response\(^{63,64}\), which when unchecked has deleterious effects in sepsis.

In the management of ITP splenectomy is reserved for those patients who have failed medical therapy. In our setting, it is utilised for patients who are either resistant to steroids (platelet count does not increase to >50×10\(^9\)/L despite prednisone at a dose of 1-2mg/kg) or in patients who are steroid-dependent (relapse off treatment).

Splenectomy has been shown to be both relatively safe in ITP, as well as effective\(^{60}\). In a large systematic review published in 2004, complete response following splenectomy was reported to reach 60%\(^{60}\). Here, complete response was defined as achievement and maintenance of a normal platelet count (150×10\(^9\)/L) for all measurements for 30 days or longer after splenectomy with no additional treatment for ITP. However, a difficulty when reviewing these articles is that ‘complete response’ is defined differently: some studies use platelet count at one month to determine response, but we know that patients can relapse later therefore uncertainty remains as to the durability of splenectomy for ITP.

The mortality rate of splenectomy has been reported to be 0.2% and 1.0% for laparoscopy and open laparotomy, respectively\(^{60}\). Mortality was primarily due to bleeding, sepsis (pneumonias and subdiaphragmatic or splenic bed abscesses) and venous thromboembolism. Complication rates were 12.9% (318 of 2465 patients) with laparotomy and 9.6% (88 of 921 patients) with
laparoscopic splenectomy. A well recognised complication of splenectomy is postsplenectomy sepsis (PSS) or overwhelming post-splenectomy infection (OPSI). OPSI occurs at an estimated incidence of 0.23–0.42% per year, with a lifetime risk of 5%\(^{61}\). It has a short prodrome with low-grade fever, chills, pharyngitis, muscle aches and often vomiting or diarrhoea. Deterioration is abrupt, often within hours, and the mortality rate is 50-70% despite antibiotics and intensive support\(^{62}\). In a large review of 12514 patients, pneumococcus was the leading cause of sepsis (in 50-90% of cases), followed by Haemophilus influenzae, then meningococcus\(^{62}\).

There are recommendations for reducing the risk of PSS including immunization against common or dangerous organisms, and possibly prophylactic antibiotics. It is recommended that all patients receive the pneumococcal vaccine two weeks prior to splenectomy. In HIV negative patients either the 23- or the 7-valent vaccine is recommended\(^{62}\). The 7-valent vaccine is more immunogenic and does not require a booster. In HIV positive patients the 23 valent vaccine (Pneumovax) has been shown to have an increased rate of pneumonic events\(^{65}\), so it is to be avoided in HIV, while the 7-valent vaccine is protective against pneumococcus \(^{66}\). Other recommended vaccines include an annual influenza vaccine, a single Haemophilus influenzae vaccine (considered immunogenic in asplenic individuals) and the meningococcal vaccine. Although meningococcus does not play a large role in OPSI, given the severity of infection if it does occur it is recommended to give the vaccine.

There is no clear consensus regarding the use of prophylactic antibiotics as the potential benefit is balanced by poor adherence to treatment as well as the potential to select out resistant strains. It is not recommended following splenectomy for ITP and no research has been done to look at this in HIV-infected individuals. The practice at Groote Schuur Hospital over the study period was not to use prophylactic antibiotics.

Initial concerns that splenectomy hastened the development of AIDS have been dispelled, and splenectomy in HIV-ITP has been shown to be both safe and effective\(^{34}\). Oksenhendler et al reported long-term experience in a cohort of
185 patients with HIV-ITP\(^{(34)}\). Splenectomy was performed in 68 of these patients, at an average of 13 months from initial diagnosis of HIV-ITP. A response was seen in 92% of patients and maintenance of the elevated platelet count for longer than 6 months was documented in 82% of splenectomised patients.

In the Oksenhendler series (in HIV) 5.8% of patients undergoing splenectomy experienced fulminant infection, consisting of Streptococcus pneumoniae meningitis in two and Haemophilus influenzae sepsis in one individual\(^{(34)}\).

Splenectomy is therefore efficacious and safe for the majority of patients but carries a risk of increased susceptibility to infections as well as operative risks, and is therefore only used after medical treatment has failed.

**LITERATURE REVIEW CONCLUSIONS**

After a review of the literature, it is evident that there is a wealth of information on the pathogenesis and pathophysiology of ITP, and treatment of ITP. The areas that have not been well studied are: the long-term outcomes of splenectomy for ITP (with most studies only including a month of follow-up time\(^{(60)}\)), and larger studies are needed on splenectomy for ITP in HIV particularly in a third-world setting.

There are new agents that have been proposed as second-line therapy for ITP (namely ritixumab and TPO agonists) and it has therefore become more relevant to quantify the risks of splenectomy as well as the treatment response to be able to guide clinical decision making.
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(31) Zucker-Franklin D, Termin CS, Cooer MC. Structural changes in the megakaryocytes of patients infected with the human immune deficiency virus (HIV-1). Am J Pathol 1989; 134: 1295-1303


PART C: JOURNAL-READY MANUSCRIPT

SPLENECTOMY FOR IMMUNE THROMBOCYTOPENIA: OUR 11-YEAR EXPERIENCE

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Manuscript prepared for the British Journal of Haematology: journal submission guidelines appendix 3

Word count: 4749 words

Abstract: 191 words

No conflict of interest to declare
Summary:

Background

Splenectomy is indicated in immune thrombocytopenic purpura (ITP) when a patient is either dependent or resistant to steroid treatment (platelet count <30×10⁹/L). New agents are being developed to treat ITP, and have brought into question the relevance of splenectomy. Of particular interest in our context is the role of splenectomy in ITP patients with HIV infection.

Methods

In this retrospective study, consecutive patients who had undergone splenectomy in the period 2001-2011 were identified. Follow-up period platelet counts and outcomes were reviewed, and compared in HIV negative and positive patients.

Results

Seventy-three patients were included in the study, 12 were HIV positive. Splenectomy was laparoscopic in 43 (62%) and by laparotomy in 27 patients (38%) with an overall 16% complication rate. Complete response rate was 80% (CI 69-91%). The 90-day mortality rate was 1.38%. Seven patients died in the follow up period. There was no statistically significant difference in response rates or proportion of complications in the HIV positive patients.

Conclusion

Splenectomy is effective and safe irrespective of HIV status and remains an appropriate second-line treatment, with the newer therapies reserved for cases refractory to splenectomy or when surgery is contraindicated.

Keywords:

Splenectomy; ITP (immune thrombocytopenia); HIV (human immunodeficiency virus); Platelets; South Africa
Introduction

Splenectomy has been practiced as treatment of ITP for the past few decades; currently it is utilised when a patient is either dependent or resistant to steroid treatment and the platelet count remains $<30 \times 10^9/L$\(^{(1)}\). Recently, new agents have been added to the current armamentarium used to treat ITP; including immunosuppressants such as rituximab and the new thrombopoetin-receptor agonists. This has brought into question the role of surgery for the treatment of ITP, and the need to compare the response and complication rates of splenectomy to these newer agents. As a result, treatment of patients with ITP who fail first-line therapy is controversial\(^{(2)}\), and the decision of whether to try further medical therapy or splenectomy should be based on efficacy and side-effect profile of the agents (including the operative morbidity and mortality), patient fitness for surgery and co-morbidities as well as patient preference and cost. Splenectomy has perioperative and long-term risks, including mortality\(^{(3,4)}\), but it offers the possibility of cure in the majority of patients\(^{(3,5,6)}\). However, newer treatments may potentially allow splenectomy to be deferred for prolonged periods, as well as providing alternative treatment options for patients who fail splenectomy.

Historic studies done on patients undergoing splenectomy for the treatment of ITP have been performed in setting of low prevalence of HIV. There is a relative paucity of data on the response rate in HIV-associated thrombocytopenia with splenectomy and the durability of response to splenectomy has yet to be established in this patient population.

Methods

Ethical Approval

Approval was obtained by the UCT ethics committee (approval no. 437/2012); see ethics approval attached as appendix 1.
**Patient Selection**

Seventy-three consecutive patients who had undergone splenectomy for ITP in the time period of 2001 – 2011, were retrospectively identified. ITP was confirmed as the diagnosis using the American Society of Haematology Guidelines\(^1\): patients with an isolated thrombocytopenia (platelets <100 ×10\(^9\)/L (anaemia only if significant bleeding related to thrombocytopenia), platelets on smear of normal to large in size while the bone marrow biopsy was not required for diagnosis. Patients with primary ITP, as well as patients with secondary ITP due to HIV, Hepatitis and connective tissue disease were included. Patients with lymphoproliferative disease were not included.

These patients were identified using both the histology records (all patients who undergo splenectomy for medical indications have the spleen sent for histology at Groote Schuur) at the National Health Laboratory System (NHLS) and the surgical records (the operative notes) held by the department of surgery at Groote Schuur Hospital. Although the protocol required that the excised spleens from patients undergoing splenectomy was referred for histological examination (except in trauma cases), this may not always have been done which may have created selection bias.

**Clinical Information**

Patients’ folders were obtained both from the haematology records (the haematology ‘buffs’), and the Groote Schuur folders. If necessary, the microfilmed folders were also reviewed.

The medical records of these patients were reviewed for the clinical and laboratory information regarding their diagnosis and management:

1. Initial treatment: patients included in the study were often either treated at peripheral hospitals with prednisone and referred to GSH after unsuccessful weaning or failing therapy outright, or were attending the GSH Haematology clinic from diagnosis. After patients were confirmed as being steroid dependent or steroid resistant at GSH they were evaluated for splenectomy as second line management.

2. Co-morbidities.

3. Laboratory results for HIV, anti-nuclear antibody, hepatitis B surface antigen, renal function and spleen histology results. These results were obtained from the NHLS’ online system which includes laboratory records from any public hospital in the Western Cape.
were not available from the NHLS (prior to 2003) the information was obtained from the folders.

4. Specific pre-operative medical management including platelet transfusions, plasmapheresis or IVIG.

5. Operative notes: type of splenectomy performed (open versus laparoscopic), intra-operative difficulties or complications. Patients either underwent laparoscopic or open splenectomy - the choice of technique depended predominately on the time period during which the operation was performed. More patients had open surgery early in the study period (before 2004) and the reverse in the latter period. Certain patients were precluded from laparoscopic surgery on the basis of expected difficulties, for example obesity or previous laparotomy with possible adhesions. The reasons for open splenectomy were recorded.

6. Post-operative details: complications, length of hospital stay

7. Post-operative clinic follow-up: later complications

8. In the case of the HIV positive patients: CD4 count at diagnosis of ITP or at referral to GSH and anti-retroviral treatment received.

9. The platelet counts at 1 month, 6 months and 1 year were recorded (within 2 weeks of the exact date). If the platelet count was not available for one year exactly, but there was a count from after one year, then the platelet count nearest this date was recorded.

10. The entire set of platelet counts was reviewed following splenectomy for the duration of patient follow-up, and if there was a platelet count of less than 100 ×10⁹/L this result was recorded and the medical management of the relapse was reviewed.

11. For survival data, the patient’s last GSH visit and latest blood test results were considered. Whichever of these was most recent was taken to be the date the patient was last definitely known to have been alive.

The relevant blood and histology results on these patients were obtained from the NHLS, using both the online system, which can access results of blood tests taken at any public hospital in the Western Cape, and the Groote Schuur blood results. Where the blood results were not available from either of these two systems, the information was obtained from the folder.
Definitions of response to medical treatment

All patients were initially treated with oral corticosteroids and were recorded as being either steroid dependent or steroid-resistant.

1. **Steroid dependence** was defined as a platelet count dropping to less than $10^9/L$ within 6 months from weaning off steroids.

2. **Steroid resistance** was defined as a platelet count never recorded at greater than $10^9/L$ despite corticosteroids at 2mg/kg/day.

Definitions of response to splenectomy

Platelet counts post-splenectomy were recorded at one month, six months, and one year but all available results were examined and if there was a result <100×$10^9/L$ this was specifically recorded. Due to heterogeneity in study definitions of response in the literature, as well as the longer duration of our study, we defined response rates as follows:

1. **Complete response** was defined as a platelet count greater than $10^9/L$, maintained for at least a year as the latest follow-up, or until the most recent if contact was maintained for longer than a year, without the patient receiving further immunosuppression (other than being weaned off steroids in 1 month to 6 weeks post-operative period).

2. **No response**: the platelet count never increased to >30×$10^9/L$ in the first year post splenectomy.

3. **Partial response** was defined as patients who achieved a platelet count of 30-100×$10^9/L$ in the year post splenectomy.

Patients were offered immunisation against pneumococcal and haemophilus influenza infections 2 weeks prior to splenectomy; this was not specifically audited except if a patient developed post-splenectomy sepsis.

Definition of Hepatitis B positive and ANA positive

Patients were regarded as having chronic Hepatitis B if the surface antigen was positive or if there was a recorded viral load on the NHLS system. Over the time period of the study, the laboratory reference range for anti-nuclear antibody (ANA) changed and so the patient was reported as positive if the result was above the reference range given.
Statistics

Microsoft Excel 2010 (Microsoft Corporation, Washington, USA) was used for
database and data input. Statistical analyses were performed using STATA statistical
software, version 12.0 (STATA Corporation, College Station, Texas, USA).
Descriptive characteristics of the patients were analysed, mean ± standard deviation
was used for normally distributed data and median plus interquartile ranges for non-
normally distributed data. Survival analysis was based on the Kaplan-Meier estimate
and the log-rank test was used for survival comparisons between the HIV negative
and HIV positive groups. To assess the association between HIV status and
complete response the prevalence ratio was calculated (since the prevalence of the
‘complete response’ was >10% and in this situation odds ratios overestimate the
magnitude of risk). Continuous and categorical variables were compared using chi2,
and when the expected frequencies in any cell of the contingency tables was <5 the
Fishers exact test was used. All P-values were considered significant at P ≤ 0.05. To
compare means if the data was normally distributed (tested by doing a Shapiro Wilk
test) then a t-test was performed; if the data were not normally distributed then a
Wilconxon-Mann-Whitney test was performed.

Results

1. Patient characteristics

The median age at splenectomy was 33 years (range: 16-70 years). There was
a female preponderance with a ratio of 3:1, 56 patients (77%) were female 17
patients were male (23%). Of the 58 patients for whom the HIV status was
known, 12 were HIV positive (21%), and 46 were HIV negative (79%). Of the
36 patients for whom the Hepatitis B status were known; 5 were positive (14%)
and 31 were negative (86%). Of the 39 patients for whom the ANA status was
known, 19 patients (49%) were ANA positive and 20 (51%) were negative.
Eight (11%) of the patients had diabetes, 9 (12%) had Systemic Lupus
Erythematosis (SLE) and 10 (14%) had Tuberculosis (TB) which was diagnosed
within 6 months before or after the diagnosis of ITP. One patient had proven
Helicobacter pylori gastritis at the time of diagnosis of ITP, three had
hypothyroidism and two likely had syphilis (positive RPR titres). Two patients
were pregnant at the time of diagnosis of ITP, and one patient underwent
splenectomy while pregnant. See Table 1 for patient characteristics at diagnosis.

<table>
<thead>
<tr>
<th>Table 1. Patient characteristics at diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variables</td>
</tr>
<tr>
<td><strong>Age (yrs), median (range)</strong></td>
</tr>
<tr>
<td><strong>Gender</strong></td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td><strong>HIV status</strong></td>
</tr>
<tr>
<td>Positive</td>
</tr>
<tr>
<td>Negative</td>
</tr>
<tr>
<td>Unknown</td>
</tr>
<tr>
<td><strong>Hepatitis B status</strong></td>
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</tr>
<tr>
<td>Negative</td>
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</tr>
<tr>
<td><strong>ANA</strong></td>
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<tr>
<td>Positive</td>
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<tr>
<td>Negative</td>
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</tr>
<tr>
<td><strong>Co-morbidities</strong></td>
</tr>
<tr>
<td>Diabetes</td>
</tr>
<tr>
<td>SLE</td>
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<tr>
<td>TB</td>
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</tbody>
</table>

2. **Medical treatment prior to splenectomy**

Of the patients for whom details regarding treatment prior to splenectomy were available (n=69); 37 (54%) were steroid-dependent, and 32 (46%) were steroid-resistant. Corticosteroids (oral prednisone) were given to all patients at a dose of 1-2mg/kg/day. Azathioprine was given to 23 patients (33%); the less frequently used medical treatments were: polyvalent human immunoglobulin ('Polygam': Natal Bio Products) (n=6); intravenous dexamethasone to (n=5), plasmapheresis (n=5); cyclophosphamide (n=2); dapsone (n=1); danazol (n=1); splenic radiation (n=1). See table 2 for initial treatment.

<table>
<thead>
<tr>
<th>Table 2. Response to initial therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Response to oral steroids (n=69)</strong></td>
</tr>
<tr>
<td>Steroid dependent</td>
</tr>
<tr>
<td>Steroid resistant</td>
</tr>
<tr>
<td><strong>Treatment (n=69)</strong></td>
</tr>
<tr>
<td>Prednisone</td>
</tr>
<tr>
<td>Azathioprine</td>
</tr>
<tr>
<td>Polygam</td>
</tr>
<tr>
<td>IVI dexamethasone</td>
</tr>
<tr>
<td>Plasmapheresis</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
</tr>
<tr>
<td>Dapsone</td>
</tr>
<tr>
<td>Danazol</td>
</tr>
</tbody>
</table>
3. HIV positive population

Of the 12 patients who were HIV positive, 7 were female and 5 male. In the HIV positive population the median age was 36 (range: 26-55 years). The median CD4 count (in cells/mm³) at diagnosis of ITP was 253 (range 52-1798): 7 (70%) of patients had CD4 counts less than 300 cells/mm³, 2 had CD4 counts greater than 700 cells/mm³, and 1 patient had a CD4 count between 300-700 cells/mm³. There are two HIV positive patients for whom CD4 counts and details of antiretroviral treatment details could not be traced. Combination antiretroviral therapy (cART) was given to 11 patients prior to splenectomy. The patient who did not receive cART was given AZT monotherapy post-operatively - she was pregnant at the time of splenectomy and AZT was initiated at 28 weeks gestational age. One patient was given AZT monotherapy for the treatment of ITP for 2 years before the initiation of cART and splenectomy. See Table 3 for details of anti-retroviral treatment. Three patients were already on cART when they developed ITP and the other 9 patients developed ITP and were subsequently put onto cART.

Table 3. HIV positive patients: CD4 counts and ARVs

<table>
<thead>
<tr>
<th>CD4 (cells/mm³) at diagnosis (n=10)</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;100</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>100-300</td>
<td>6</td>
<td>60</td>
</tr>
<tr>
<td>301-700</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>&gt;700</td>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td>ARVs* prior to splenectomy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AZT, 3TC, EFV</td>
<td>4</td>
<td>33</td>
</tr>
<tr>
<td>D4T, 3TC, EFV</td>
<td>3</td>
<td>25</td>
</tr>
<tr>
<td>TDF, 3TC, EFV</td>
<td>2</td>
<td>17</td>
</tr>
<tr>
<td>AZT, 3TC, NVP</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>D4T, 3TC, NVP</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>NONE**</td>
<td>1</td>
<td>8</td>
</tr>
</tbody>
</table>

*ARVs = antiretrovirals: AZT = zidovudine; 3TC = lamivudine; EFV = efavirenz; NVP = nevirapine; TDF = tenofovir; ** The patient not given ARVs was given AZT after splenectomy

4. Operative information

Of the 69 patients for whom operative details could be traced, splenectomies were by open laparotomy in 26 patients (38%) and by laparoscopy in 43 (62%). See figure 1 for a depiction of open versus laparoscopic splenectomy by year. The median platelet count at splenectomy was $187 \times 10^9/L$ (range: 6-615 $\times 10^9/L$).
This is a surprisingly high number and reflects that many of these patients were responsive to steroids, but were steroid dependent at the time of splenectomy.

Median time to splenectomy from diagnosis of ITP was 14 months (range 1-167 months); in the HIV positive population it was 8.5 months (range: 1-42), and in the HIV negative population 30 months (range: 1-167 months); this was a non statistically significant difference (z=1.55 and p=0.12 using the Wilcoxon Rank test).

The median discharge was on the 3rd post-operative day (range: day 2-8): for HIV positive patients it was day 2.5 (range: 2-6) and for HIV negative patients day 3 (range: 2-8). This was a non-statistically significant difference (p=0.5 with Fisher’s exact test).

The intra-operative complication rate for splenectomy was low (10%) and complications included conversion to open splenectomy for bleeding (n=2), and adhesions (n=3). There was no intra-operative mortality.

There were 11 post-operative complications in 10 patients (16% complication rate). The most common post-operative complication was infection. Five patients developed intra-abdominal sepsis: one had a splenic bed abscess and one developed a subphrenic abscess (both were drained percutaneously); three required re-look laparotomies on post-operative days 2, 4 and 11 respectively. One patient required intensive care post-operatively but did well after the re-look laparotomy. Two patients developed wound infections that resolved with oral antibiotics and did not require readmission.

Two patients developed post-operative thrombosis: one patient presented 8 days post-surgery with a pulmonary embolism (PE) and the other patient developed mesenteric vein thrombosis also had wound sepsis, she was noted at splenectomy to be morbidly obese (BMI=62). Neither of these patients had thrombocytosis at the time of presentation with the thrombosis. The patient with the PE had a platelet
count of $302 \times 10^9/L$ and the patient with mesenteric vein thrombosis had a platelet count of $210 \times 10^9/L$.

One HIV positive patient developed drip-site sepsis, and one HIV positive patient had sub acute small bowel obstruction that resolved with conservative management. The complication rate in the HIV positive patients was non statistically significantly different to the HIV negative patients (18% and 16% respectively; Fisher’s exact test $p=0.59$).

There was 1 post-operative death at 10 weeks post-surgery giving a 90 day mortality rate of 1.38%. This patient was HIV positive, had had an uncomplicated splenectomy and was discharged on day 2 post-operation. She presented again ten weeks later in septic shock (with an unidentified organism) and required inotropes and dialysis; her platelet count remained $<30 \times 10^9/L$. She died within 24 hours of admission. This patient had received the 23-valent pneumococcal vaccine as an inpatient one day prior to her surgery.

The mean discharge day was day 3 (range: 2-8); with no statistically significant difference in the HIV positive (mean: 2.5; range: 2-6) and HIV negative patient groups (mean: 2; range: 2-8); with a $p$ value of 0.5 with Fisher’s exact test. See table 4 for operative information.

<table>
<thead>
<tr>
<th>Table 4. Operative information</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Type of splenectomy</strong></td>
</tr>
<tr>
<td>Open laparotomy</td>
</tr>
<tr>
<td>Laparoscopic</td>
</tr>
<tr>
<td><strong>Intra-operative complications</strong></td>
</tr>
<tr>
<td>Mortality</td>
</tr>
<tr>
<td>Adhesions (converted to open)</td>
</tr>
<tr>
<td>Bleeding (converted to open)</td>
</tr>
<tr>
<td>Convert to open reason not found</td>
</tr>
<tr>
<td><strong>Post-operative complications</strong></td>
</tr>
<tr>
<td>Death</td>
</tr>
<tr>
<td>Intra-abdominal sepsis</td>
</tr>
<tr>
<td>Wound infection</td>
</tr>
<tr>
<td>Thromboembolic*</td>
</tr>
<tr>
<td>Drip site sepsis</td>
</tr>
<tr>
<td>Sub-acute small bowel obstruction</td>
</tr>
</tbody>
</table>

*one pulmonary embolism, one mesenteric vein thrombosis
5. Response to splenectomy

Patients were followed up for a mean of 48 months (range 2-138 months). Of the 55 patients for whom either the follow-up was at least a year, 44 (80%) had a complete response (CI 69-91%). At the one year platelet count there was no statistically significant difference between HIV positive and negative patients: t test = 0.39, 42 degrees of freedom, p value 0.69. See figure 2 for a box plot depicting platelet count at one year for HIV positive and negative patients.

At one month 89% of patients had a platelet count >100×10⁹/L; at six months 82% and at one year 94% (this is different to the rate of ‘complete response’ because 5 patients required further immunosuppression to maintain this platelet count).

Eleven patients did not have a complete response. Of these 11 patients: 7 had a partial response and 4 had no response to splenectomy. All 11 patients were re-treated with steroids, and 4 were treated with azathioprine. Of the 7 patients with a partial response, 6 of the 7 patients later achieved platelet counts of >100×10⁹/L.

At one month 7% of patients had platelet count <30×10⁹/L; 6% at 6 months
and 4% at one year. The reason for this decrease in percentage is partially attributable to patient mortality since a greater proportion of patients with platelets $<30 \times 10^9$/L than patients with platelets $>30 \times 10^9$/L died. The patients who had no response to splenectomy fared poorly – with a 100% mortality rate in the study follow-up period. Compared to the morality rate of 4.5% in the complete responders this is a statistically significant different ($p=0.01$). See table 5 for platelet count post-splenectomy. Figure 2 depicts platelet count at one year by HIV status.

### Table 5. Platelet counts post splenectomy

<table>
<thead>
<tr>
<th>Platelet count</th>
<th>1 month (N=59)</th>
<th>6 months (N=52)</th>
<th>12 months (N=52)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$&lt;30$</td>
<td>4 (7%)</td>
<td>3 (6%)</td>
<td>2 (4%)</td>
</tr>
<tr>
<td>30-99</td>
<td>3 (5%)</td>
<td>3 (6%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>$\geq 100$</td>
<td>52 (88%)</td>
<td>46 (89%)</td>
<td>49 (94%)</td>
</tr>
</tbody>
</table>

### Figure 2

Comparison of platelet count at one yr by HIV status

6. **Predictors of response**

For an association between variables and complete response, a prevalence ratio was calculated (see Table 6). The variables associated with a lower rate of complete response have a value less than one, and the conditions with a higher rate of complete response a value greater than one.

The variables that yielded prevalence ratios of close to 1 and therefore showed neither a favourable nor unfavourable outcome were: gender, HIV status, and
Four conditions were associated with a lower response rate to splenectomy, but due to low patient numbers only two were two statistically significant: patients pre-treated with azathioprine and those with diabetes. The prevalence of complete response was lower in both patients who were Hepatitis B positive and in those that had positive ANA titres, although neither was statistically significant.

There was no significant difference in the outcome among those who had TB or SLE. It is interesting that SLE patients had a (non significant) higher rate of complete response but ANA positivity was associated with a worse outcome. All 8 patients with SLE were ANA positive; but 10 were ANA positive and did not have SLE. In these patients the rate of complete response was 66% (CI 0.28-1.05) and if ANA positive and the patient had SLE the complete response rate was 88% (CI 0.58-1.17) (p=0.58).
### Table 6. Variables and rate of complete response (N%CR); with calculated prevalence ratios.

<table>
<thead>
<tr>
<th>Variables</th>
<th>N</th>
<th>N%CR</th>
<th>Prevalence ratio</th>
<th>CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>11</td>
<td>9 (82)</td>
<td></td>
<td>0.97</td>
<td>0.71-1.33</td>
</tr>
<tr>
<td>Female</td>
<td>44</td>
<td>35 (80)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>HIV status</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>9</td>
<td>7 (78)</td>
<td></td>
<td>0.99</td>
<td>0.67-1.45</td>
</tr>
<tr>
<td>Negative</td>
<td>38</td>
<td>30 (79)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Hepatitis B</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>3</td>
<td>2 (66)</td>
<td></td>
<td>0.83</td>
<td>0.37-1.9</td>
</tr>
<tr>
<td>Negative</td>
<td>25</td>
<td>20 (80)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>ANA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>18</td>
<td>14 (78)</td>
<td></td>
<td>0.84</td>
<td>0.63-1.12</td>
</tr>
<tr>
<td>Negative</td>
<td>14</td>
<td>13 (93)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Diabetes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>5</td>
<td>2 (40)</td>
<td></td>
<td>0.47</td>
<td>0.83-1.51</td>
</tr>
<tr>
<td>No</td>
<td>48</td>
<td>41 (85)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>TB</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>6</td>
<td>5 (83)</td>
<td></td>
<td>1.25</td>
<td>0.16-9.64</td>
</tr>
<tr>
<td>Negative</td>
<td>49</td>
<td>39 (80)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Azathioprine</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>21</td>
<td>13 (62)</td>
<td></td>
<td>0.62</td>
<td>0.45-0.87</td>
</tr>
<tr>
<td>No</td>
<td>27</td>
<td>27 (100)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Steroid resistant</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>22</td>
<td>17 (77)</td>
<td></td>
<td>0.92</td>
<td>0.7-1.21</td>
</tr>
<tr>
<td>No</td>
<td>31</td>
<td>26 (84)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>SLE</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>8</td>
<td>7 (88)</td>
<td></td>
<td>1.12</td>
<td>0.83-1.51</td>
</tr>
<tr>
<td>No</td>
<td>48</td>
<td>36 (78)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

N= number with variable and for whom complete response rate known. P value calculated with Fisher's exact test.

### 7. Mortality and Morbidity

Seven patients died in the follow up period. Six patients with recorded deaths were HIV negative; 4 were female and 2 male and one was an HIV positive female. Four of the seven deaths occurred in patients who had no response to splenectomy.

Four deaths may have been related to the splenectomy: one was a young male (age 24) who died from overwhelming pneumococcal sepsis proven on blood and CSF culture - he had received the pneumococcal vaccine; but died at 18 months post splenectomy. The second was a female age 50 with diabetes who died from a cerebrovascular event 6 months post splenectomy, of note is...
that her platelet count at the time of CVA was $1214 \times 10^9/L$ which may have caused the CVA. The third was an HIV positive female who died from an unidentified source of sepsis at 10 weeks post op; she presented in septic shock and renal failure requiring dialysis. The fourth death was in a patient who had a partial response to splenectomy who died from a ruptured appendix and sepsis at 68 months post-splenectomy.

The other three deaths were likely unrelated to splenectomy, but all three of the deaths were in patients with no response to splenectomy: One patient died from an intra-cerebral haemorrhage at 91 months post-splenectomy with a platelet count $<10 \times 10^9/L$; one died from an unknown cause at 52 months post splenectomy with a platelet count of $26 \times 10^9/L$; one died from adenocarcinoma of the prostate at 6 months post-splenectomy with a platelet count of $17 \times 10^9/L$.

See Table 7 for all-cause mortality by gender and HIV status as well as the corresponding Kaplan-Meier survival estimates, only patients for whom HIV status was known were included in the analysis (Figure 3 and 4). Male gender was associated with a non-statistically significant higher mortality rate (despite fewer deaths, because of the relatively fewer months of follow-up). HIV was not associated with a statistically significant difference in mortality.
Figure 4: Kaplan-Meier survival estimates by HIV status

Figure 4: Kaplan-Meier survival estimates by gender
Table 7. All-cause mortality by patient-time (months), (p value calculated by Fisher’s exact test)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Person time (months)</th>
<th>Deaths</th>
<th>Rate (per 1000 person month)</th>
<th>95% CI</th>
<th>P=</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>854</td>
<td>1</td>
<td>1.17</td>
<td>0.16-8.31</td>
<td>1.00</td>
</tr>
<tr>
<td>Negative</td>
<td>5246</td>
<td>6</td>
<td>1.14</td>
<td>0.51-2.54</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>1087</td>
<td>2</td>
<td>1.83</td>
<td>0.46-7.35</td>
<td>0.66</td>
</tr>
<tr>
<td>Female</td>
<td>5933</td>
<td>5</td>
<td>0.84</td>
<td>0.35-2.02</td>
<td></td>
</tr>
</tbody>
</table>

Conclusions and Recommendations

Splenectomy remains an effective method of managing ITP in both HIV positive and negative patients. The complete treatment response in this study of 80% is within the range previously reported in the literature of 60-80% (3,5,6). One of the strengths of this study is the long follow-up time; a mean follow-up of 48 months is significantly longer than most studies of splenectomy for ITP (3,5,6). The complete response rate to splenectomy appears higher than that reported for rituximab (8) or the new thrombopoetin agonists (10).

The patients included in this study were a heavily pre-treated group (100% given oral corticosteroids at a dose of 1-2mg/kg/d and 33% given azathioprine), which is typical of a study showing outcomes of second-line therapy. It is interesting to note that in the patients who had no response there was a 100% (n=4) mortality; however in the patients with a partial response to splenectomy, six of the seven patients later had a good response with platelet counts of >100×10⁹/L, maintained for the duration of follow-up. This is also in keeping with the literature which has shown that patients with refractory ITP after splenectomy have a good response to additional treatment (3). The poorer response (although not statistically significant in this study) of patients with ANA positivity has been previously described in a large cohort study by Li et al (11). This requires further investigation, but this may be a sub-
group of patients in whom further medical management is indicated prior to splenectomy.

Although our HIV positive cohort was small (n=12), the treatment response in this group was not significantly different to the HIV negative cohort. This is also in keeping with the literature where the response rate to splenectomy in the HIV positive population was 92% and a long-term (6 month response) was seen in 82% of cases\(^\text{5}\). The CD4 count at diagnosis of ITP well demonstrated the typical bimodal distribution of ITP in HIV - showing ITP early in HIV with CD4 counts higher than 700/mm\(^3\), and late in HIV with CD4 counts less than 300/mm\(^3\). A limitation in our study was the small number of HIV positive patients, which led to results not being statistically significant.

The complications, morbidity and mortality associated with splenectomy were not statistically significantly higher in the HIV positive population than in the negative population (18% and 16% respectively; Fisher’s exact test p=0.59). Our complication rates were slightly higher than reported\(^\text{3}\), but this is difficult to interpret since the investigators did not make it clear what they considered to be ‘complications’ and we also included minor complications such as drip site sepsis. Our patient population is mostly of a low socio-economic status, as we treat patients who generally have no medical insurance, and this might have influenced the post-operative complication rate (due to poor sanitation, being without access to running water, poor nutrition, overcrowding, and more difficulty in accessing healthcare as possible factors).

Within the current treatment milieu with a range of options now available to offer to patients who fail steroid therapy, one needs to compare the outcomes and complication rates of splenectomy with those of the newer agents. Patients need to be informed that although the reluctance to undergo surgery is understandable, splenectomy is an operation with low morbidity and extremely low mortality which may be safer than the new medical treatments available as well as more effective.

If we compare the outcome of splenectomy with the best data for rituximab (a
meta-analysis), rituximab showed a complete response rate of 41% (95% CI: 0.33–0.51) for 346 patients (7); and the author’s conclusion was that rituximab should be used earlier in non-splenectomised patients; in this large review however they did not look at side-effects and rate of complications or cost associated with rituximab therapy before making the recommendation that it should be offered before splenectomy. In a separate article, of 35 patients there were two deaths following rituximab; one of which, a pneumonia at 13 weeks, was possibly related to rituximab mediated immunosupression(9). It is therefore not an innocuous treatment and patients need to be informed of the risks prior to treatment. In our setting response rates, complication rates, as well as costs are considered and with the current body of evidence it is reasonable to consider rituximab only after splenectomy.

Considering the TPO agonists romiplostim and eltrombopag, these drugs have demonstrated durable response rates (platelet count more than 100 x10⁹/L at 6 to 8 weeks) in 50 to 60% of patients as long as treatment is maintained(12;13). Complications on treatment with eltrombopag include an increase in alanine aminotransferase and indirect bilirubin and an increase in thrombotic events. More studies are needed to investigate the role of this treatment; presently it remains that these agents could be used in the setting of failure of splenectomy and other second-line agents.

Before considering splenectomy in any patient one has to weigh up the risk of bleeding and compare that to the risks of splenectomy; the risk of bleeding in ITP is only higher than the general population at a platelet count of <30×10⁹/L(14) and the risk of fatal bleeding is 0.02 to 0.04 cases per patient-year(15). The platelet count at which the risks of splenectomy are less than the risk of bleeding is likely to be even lower than 30×10⁹/L. In our study the median platelet count prior to splenectomy was high, but these values were only maintained prior to surgery by continuous high doses of prednisone as well as other immunosuppressives.

The results from this study are promising and suggest that splenectomy should remain second-line treatment for ITP as it is relatively safe as well as
effective for the treatment of ITP, including ITP secondary to HIV. The complication and success rates following splenectomy in this study is similar to the international literature, indicating that our level of operative expertise and pre and post-operative care is of an international standard.
References:


(14) Cohen YC, Djulbegovic B, Shamai-Lubovitz O, Mozes B. The bleeding risk and natural
Appendix 1: Ethics approval

UNIVERSITY OF CAPE TOWN

Faculty of Health Sciences
Human Research Ethics Committee
Room E52-24 Groote Schuur Hospital Old Main Building
Observatory 7925
Ms S Auffenberg - Tel: (021)4689492 • Fax (021)4666411
email: sumayah.auffenberg@uct.ac.za

27 August 2012

HREC REF: 427/2012

Dr K Auffenberg,
Internal Medicine

Dear Dr Auffenberg,

PROJECT TITLE: A REVIEW OF PATIENTS REQUIRING SPLENECTOMY FOR ITP: OUR TEN YEAR EXPERIENCE

Thank you for submitting your research proposal to the Faculty of Health Sciences Human Research Ethics Committee. It is a pleasure to inform you that the Ethics Committee has formally approved the above-mentioned study.

Approval is granted until 28 August 2013

Please submit an annual progress report (HES015) if the research continues beyond the expiry date. Please submit a brief summary of findings if you complete the study within the approval period so that we can close our file (HES010).

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please quote the HREC REF in all your correspondence.

Yours sincerely,

PROFESSOR MARC BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS

Federal Wide Assurance Number: FWA00003137
Institutional Review Board (IRB) number: IRB00001005

The notice is to confirm that the University of Cape Town Human Research Ethics Committee complies with the Ethics Standards for Clinical Research with a novel drug in patients, based on the Medical Research Council (MRC-USA), Food and Drug Administration (FDA-USA), International Convention on Harmonisation Good Clinical Practice (ICH-GCP) and Declaration of Helsinki guidelines.

The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidance E2B: Note for Guidance on Good Clinical Practice (CPMP/ICH/343/98) and FDA Code Federal Regulations Part 312, 811 and 312.
FACULTY OF HEALTH SCIENCES
Research Ethics Committee

Form FHS006: Protocol Amendment

HREC office use only (FWA00012577, HREC001935)

☐ Approved  ☐ Type of review requested  ☐ Full commission

This serves as notification that all changes and documentation described below are approved.

Signatures Chairperson of the HREC

Date

(1.1. All amendments should include a table specifying the changes for the amendment (note: was
already dated 23 April 2012)

Principal investigator to complete the following:

1. Protocol Information

Date: 27 August 2012


Protocol title: A review of patients returning for splenectomy for SFP. one year after operation.

Protocol number (if applicable):

Principal Investigator: Katherine Arkel
Email: katherinarke@gmail.com

1.1. Does this protocol receive US Federal funding? ☐ Yes ☐ No
1.2. Is this a major or minor amendment? ☐ Major ☐ Minor

2. List of Proposed Amendments with Revised Version Numbers and Dates

Changes to the protocol:

- Data collection dates expanded to include splenectomies performed in 2011 (Jan - Dec)

RESEARCH ETHICS COMMITTEE

2013-07-15

HEALTH SCIENCES FACULTY
UNIVERSITY OF CAPE TOWN
Appendix 2: Thompson’s article on prevalence ratio

Permission to use diagram

LHJISH <ljh@aii.so.or.jp>
To: Katherine Antel <katherineantel@gmail.com>
Cc: klimura@hhs.u-tokyo.ac.jp

Re: Figure 1 from "Pathophysiology and management of primary Immune Thrombocytopenia, by Drs. Hironori Kashiyagi and Yoshiaki Tomiyama, Int. J. Hematol. 2013;98: 24-33."

Dear Dr. Antel,

The Japanese Society of Hematology hereby grants you to reproduce this material for non-commercial use subject to the conditions below.

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- No modification of figures is permitted

With my best regards,
Makiko Osumi-Lewin wrote for
Toshio Klimura, M.D.
Editor-in-Chief, LH

From: Katherine Antel [katherineantel@gmail.com]
Sent: Wednesday, December 04, 2013 4:16 AM
To: LHJISH
Subject: Re: Permission to use diagram

[Attachment]

38,1,24_Antel_12942013.pdf
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Hirokazu Kashiwagi <kashi@hp-blood.med.osaka-u.ac.jp>  
To: Katherine Antel <katherinentel@gmail.com>  

Wed, Oct 30, 2013 at 7:45 AM

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Sincerely,

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Appendix 3: Thompson’s article on prevalence ratio

Prevalence odds ratio or prevalence ratio in the analysis of cross sectional data: what is to be done?

Mary Lou Thompson, J H Myers, D Kreibul

Abstract

Objective—To review the appropriateness of the prevalence odds ratio (POR) and the prevalence ratio (PR) as effect measures in the analysis of cross sectional data and to evaluate different models for the multivariate estimation of the PR.

Methods—A system of linear differential equations corresponding to a dynamic model of a cohort with a chronic disease was developed. At any point in time, a cross sectional analysis of the people then in the cohort provided a prevalence based measure of the effect of exposure on disease. This formed the basis for exploring the relations between the POR, the PR, and the incidence rate ratio (IRR). Example illustrative solutions for various IRRs, prevalences, and differential cubic rates. Multivariate point and interval estimation of the PR by logistic regression is illustrated and compared with the results from proportional hazards regression (PH) and generalised linear modelling (GLM).

Results—The POR is difficult to interpret without making restrictive assumptions and the POR and PR may lead to different conclusions with regard to confounding and effect modification. The PR is always conservative relative to the IRR and, if POR > 1, the POR is always > PR. In a fixed cohort and with an adverse exposure, the POR is always > IRR but in a dynamic cohort with sufficient underlying follow up the POR may overestimate or underestimate the IRR, depending on the duration of follow up. Logistic regression models provide point and interval estimates of the PH (and POR) but may be inadmissible in the presence of many covariates. Proportional hazards and generalised linear models provide statistical methods directed specifically at the PH, but the interval estimation in the case of PH is conservative and the GLM procedure may require constrained estimation.

Conclusions—The PR is conservative, consistent, and interclass related to the IRR and should be used in preference to the POR. Multivariate estimation of the PR should be enforced by means of generalised linear models or, conservatively, by proportional hazards regression.


Keywords: prevalence, cross sectional study, logistic regression

In the analysis of data from cross sectional studies, two ratio measures of effect suggest themselves, the prevalence odds ratio (POR) and the prevalence ratio (PR), sometimes incorrectly called the prevalence rate ratio. The choice between these two has been the source of ongoing debate in the epidemiological literature in the past few years. Although it is not shown that the PR and POR will be similar for a rare disease, they may be very different for a common disease, and common diseases are the focus of cross sectional studies. A recent paper has illustrated the divergence of the POR and PR for different underlying disease prevalences.

The debate recognizes these differences and has had two main themes: firstly, discussion of which of the two effect measures is the more appropriate; and secondly, disagreement on the appropriate model with which to construct multivariate estimates of the PR and its standard error.

We present an analysis which clarifies the relation (between PR, POR, and the incidence rate ratio (IRR) in cross sectional studies of chronic disease, and use this to make recommendations about the appropriate rate measure of effect. We also derive an expression for the variance of the log of the estimated PR from logistic regression which involves standard results for the variance and covariance of the logistic regression coefficients, thus enabling use of unstable unstable logistic regression packages to carry out PR analysis.

Measures of effect in cross sectional studies

Cross sectional studies are conducted with many objectives, sometimes the interest is primarily descriptive, but increasingly these studies are used, despite their well known limitations, to seek aetiological information. Interest may also be in drawing causal conclusions from cross sectional studies of prevalent conditions because of, or in the difficulty of the collection of data for, other examples, in developing countries where public health and demographic data may be sparse, there may be little information on people with prevalence surveys, at least in preliminary studies. In occupational studies (and other conditions—such as respiratory symptoms)—there are rarely alternative appropriate reference groups. But these studies are not necessarily seen as purely descriptive programmes. More publications suggest that respiratory symptoms can be studied qualitatively and harmfully by counts of chronic conditions. This may be useful in identifying hazardous exposures instead of...
Appendix 4: British Journal of Haematology submission guidelines

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Instructions to Authors

Research papers
The majority of papers published in the Journal report original research into scientific and clinical haematology. All papers are subject to review and authors are urged to be brief; long papers with many tables and figures may require shortening if they are to be accepted for publication.

Preparation of manuscripts
Manuscripts should be formatted with wide margins and bear the title of the paper with the name and address of the author(s), together with the name of the hospital, laboratory or institution where the work has been carried out. Authorship should be restricted to individuals who have made a significant contribution to the study. The name, full postal address and e-mail address of the author to whom readers should address correspondence and reprint requests should be given on the first page; this will appear as a footnote in the journal and the publishers will send proofs to this author at the given address unless contrary instructions are written on the manuscript. Correspondence during the peer-review process will be with the author indicated during submission. A running short title of not more than 60 characters and spaces should be included. An informative summary of not more than 200 words must be included at the beginning of the paper and supplied when prompted during the online submission process. Papers should normally be divided into summary, introduction, methods (and/or materials), results, discussion, acknowledgements and references. SI units should be used throughout. Keywords
Five keywords must be supplied after the summary.

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Illustrations
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back with the name(s) of the author(s) and the title of the paper. Where there is doubt as to the orientation of an illustration the top should be marked with an arrow. Photographs and photomicrographs should be unmounted glossy prints and should not be retouched.

Where printed, diagrams should be on separate sheets. Lines should be of sufficient thickness to stand reduction. Each illustration should be accompanied by a legend clearly describing it. In the full-text online edition of the journal, figure legends may be truncated in abbreviated links to the full-screen version. Therefore the first 100 characters of any legend should inform the reader of key aspects of the figure.

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Tables should be as few as possible and should include only essential data; they should be printed on separate sheets and should be given Roman numerals.

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