Review and Re-appraisal of Patients Treated with Splenectomy for Immune Thrombocytopenic Purpura at Five Years and Beyond

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Dissertation for the degree of Master of Medicine

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Declaration

I , Yunus Suleman Seth, declare that the work on which this thesis is based is original (except where acknowledgements indicate otherwise) and that , to the best of my knowledge, neither the whole work or any part of it, has been, is being or is to be submitted for any other degree at this or any other university.

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DEDICATION

To my mother, Rookaya , my wife Adrienne and my children Safiyah, Ahmed, Adam and Sarah for all their encouragement, love and support.

To the memory of my father, Suleman.

ACKNOWLEDGEMENTS

I would like to thank the following people for their help and support:

1. Professor Nicholas Novitzky - Professor and Head of the Department of Haematology, University of Cape Town Medical School - for his role as supervisor providing constant guidance and advice.
2. Rosalia Mothapi – for performing the pneumococcal antibody assay.
3. Mrs Adrienne Seth – for typing most of the manuscript and help with locating patients.
4. Professor Keith Klugman, former director of the South African Institute for Medical Research (SAIMR) in Johannesburg for agreeing to measure the pneumococcal antibody levels at the institutes' laboratory and his review of the results.
5. Mary Cornelius -for doing the full blood count/platelet counts and reviewing the blood smears.
6. Dr Kathy Taylor - for reviewing the splenic histology.
7. Sister Anelle Kotze - Practice sister at Stelkor Medical Doctors, Stellenbosch - for help with obtaining sera from control patients
8. Sister Vivienne Freeman - at the Sunset Pharmacy, Sea Point, for help with obtaining sera from control patients.
9. Secretarial staff, (especially, Mrs. Shameam McAleese and Mrs. Caroline Warburton) at The Department of Medicine/Care of the Elderly, Blackpool Victoria Hospital, Lancashire, England – for helping construct tables in the text and invaluable assistance with final formatting.
10. Dr.P.R.Kelsey, Consultant Haematologist and Clinical Director (Pathology Directorate) – Blackpool Victoria Hospital, Lancashire, England – for proof-reading and help with draft copies.
10. Dr. A.B.Ahmed – for help with statistical analysis and proof-reading.
11. Marilyn de Vries, Postgraduate Officer, University of Cape Town – for facilitating the submission of the dissertation.

ABBREVIATIONS

1. ANA	Antinuclear antibodies
2. ACIP	Advisory Committee on Immunization practice
3. AIDS	Autoimmune Deficiency Syndrome
4. ASH	American Society of Haematology
5. CD	Cluster of Differentiation
6. CPS	Capsular polysacchoride
7. EDTA	Edetic acid
8. ELISA	Enzyme linked immunosorbent assay
9. FcR	Fc receptor
10. GP	Glycoprotein
11. GMCSF	Granulocyte colony stimulating factor
12. HAART	Highly active anti retroviral therapy
13. Hib	Haemophilus influenza b
14. H.pylori	Helicobacter pylori
15. HIV	Human Immunodeficiency Virus
16. IL	Interleukin
17. ITP	Immune thrombocytopenic purpura
18. Ig	Immunoglobulin
19. IFN	Interferon
20. Kg	Kilogram
21. MHC	Major Histocompatibility complex
22. Mg	Milligram
23. PEG	Polyethylene glycol
24. PMP	Platelet micro-particles
25. SLE	Systemic lupus erythematosus
26. TPO (MK-CSF)	Thrombopoeitin
27. Th	T-helper
28. TNF	Tumour necrosis factor

ABSTRACT

Background. Idiopathic thrombocytopenic purpura (ITP) is an autoimmune disorder characterised by persistent thrombocytopenia (peripheral blood platelet count < 150 X 10^9/L) due to auto antibody binding to platelet antigen(s) causing their premature destruction by the reticuloendothelial system, in particular, the spleen.

Most of what we do with ITP patients is based on case series of selected patients whose course cannot be evaluated in the absence of a control group, and whose outcomes are evaluated by a surrogate marker, the platelet count.

Two studies from the University of Cape Town (49)(64) have contributed significantly towards establishing treatment protocols for ITP. Subsequently there have been more publications on understanding the pathogenesis and the management of ITP. Standard treatment for ITP is comprised of corticosteroids, intravenous immune globulin and splenectomy. Other forms of immunosuppression/experimental therapies are used for chronic refractory disease and acutely bleeding patients. The risk of pneumococcal infection post splenectomy is life long so all patients undergoing splenectomy are given the polyvalent (23-valent) unconjugated pneumococcal polysacchoride vaccine, pre-operatively.

The *aim* of this study was 1. to measure the success of splenectomy at a tertiary institution in South Africa at 5 years and beyond. 2. To review the incidence of complications peri-operatively and long term. 3. Review

the need for possible re-vaccination (as recommended in American and British guidelines) and/or prophylactic antibiotics and 4. perform a re-appraisal of patients found to be refractory to treatment.

Methods. A retrospective analysis of a chronologically defined cohort of adult patients - those who underwent splenectomy at Groote Schuur Hospital between 1987 and 1992 - were identified (n=52). Patients were seen for clinical review. Blood was obtained for estimation of full blood count, platelet count, serum immunoglobulin levels and serum for anti pneumococcal antibodies to serotypes 19F, 14, 6B, 5 and 1.

Results. Splenectomy for ITP was associated with low procedural morbidity and zero mortality in the cohort. 76.5% (n=36) had an immediate and sustained response to splenectomy. Of those who relapsed, post splenectomy an added 15% (n=7) responded to further immunosuppression, giving a total response to splenectomy of 91%.

Correlation analysis showed that patients, who, in the long term, continue to have a platelet count > $100x10^9$/L were younger (had splenectomy at a younger age) and took longer to get to splenectomy. Splenectomy was associated with a significantly higher mean corpuscular volume (MCV) and higher lymphocyte count, higher platelet count and lower immunoglobulin (Ig) M. ($p<0.05$).

Of the Ig G antibodies to five pneumococcal serotypes, patients treated with splenectomy for ITP had significantly lower levels of the 19F serotype antibody only at five years and beyond ($p<0.05$). One patient

died of overwhelming post splenectomy sepsis (OPSI) (the patient had not received the pneumococcal vaccine). Two patients (<4%) were identified as having chronic refractory ITP with platelet counts less than $30x10^9/L$.

Conclusions. 1.The management of ITP in patients who undergo splenectomy at our institution is both appropriate and effective. 2.Pneumococcal vaccination is essential pre-splenectomy. 3. At present there is not enough evidence to justify routine re-vaccination at (the recommended) five years post initial vaccination. 4. Prophylactic antibiotics appear to confer no added advantage for the prevention of post splenectomy sepsis which remains a lifelong unpredictable risk. 5. Patients with chronic refractory ITP (platelet counts < 30 X $10^9/L$) present a management problem as the goal of therapy may be to simply alleviate the consequences of thrombocytopenia rather than to attempt to normalise the platelet count.

CHAPTER ONE

1. INTRODUCTION

Thrombocytopenia is a substantial clinical problem. The most common mechanism is by consumption of platelets in the blood by disseminated intravascular coagulation or through immune mechanisms.

Thrombocytopenia may be suspected from bleeding symptoms or may be discovered by a routine blood count in an asymptomatic patient. Many large studies have shown that falsely low platelet counts may be due to platelet agglutination caused by the anticoagulant edetic acid (EDTA), used for blood count estimation, (in about one person in one thousand), irrespective of the presence or absence of any disease. Therefore the diagnosis of thrombocytopenia must always be confirmed by examination of the peripheral blood smear (1). Though rare, hereditary thrombocytopenias need to be distinguished from immune thrombocytopenic purpura (ITP) in order to avoid inappropriate treatment. Other disorders associated with thrombocytopenia, such as chronic liver disease with hypersplenism, and autoimmune, lymphoproliferative, and infectious diseases are generally accompanied by signs and symptoms suggesting systemic disease.

In acute ITP production of auto antibodies and immune complexes is probably linked to a transient anti-viral immune response, as clinically, the condition often occurs in previously healthy young children who are convalescing from an infectious disease (usually a viral upper respiratory tract infection). In most cases it is transient, requires no treatment and

spontaneously recovers. In the adult chronic form there is usually no obvious antecedent illness and most patients have chronic thrombocytopenia; spontaneous recovery is uncommon (2).

Adult chronic immune thrombocytopenic purpura is an autoimmune disorder with an estimated incidence of 58-66 new cases per million persons per year (3). The reported prevalence being 1-13 per 100 000 persons and the mortality rate associated with this condition is about four percent (4). The condition was recognised over a century ago and credit for the suggestion of splenectomy as a cure for ITP is usually given to Kaznelson of Prague. The first splenectomy in this disease was performed in November 1916 (5).

This study deals exclusively with adult patients although nearly 10% of the study population were diagnosed initially in childhood and subsequently splenectomized in adulthood. (See table 4).

2. PATHOGENESIS

2.1 Immune mechanisms

Autoimmune disorders are multifactorial. It is likely that loss of tolerance to a self-antigen alone is insufficient to generate the autoimmune disorder (6). Instead, patients probably require the presence of at least three determinants in an individual at one particular time, viz. (a) a specific set of genetic determinants [e.g. polymorphisms within major histocompatibility complex (MHC), cytotoxic T-lymphocyte antigen 4 (CTLA4) or other genes];(b) dysregulation of the immune response (involving dendritic cells, T or B cells, or all three);(c) an environmental 'trigger'(7).

2.1.1 Platelet proteins and the characteristics of the antibody in ITP

The resting circulating platelet formed by cytoplasmic fragmentation from megakaryocytes in bone marrow or lung is discoid in shape with a diameter of 3-4 microns and a thickness of approximately 1-micron.

At the surface membrane, the platelet possesses a substantial exterior coat or glycocalyx. This dense layer is rich in glycoproteins of at least ten different types. Of these glycoprotein 1b (GP1b) which exists as a complex of the disulphide linked GP1b alpha and GP1b beta sub units with GP1X being the most prominent, whilst the GP11b-111a complex is the most abundant (8).

The hypothesis that ITP is caused by one or more platelet autoantibodies usually directed at the IIb/IIIa complex or other platelet membrane antigen is supported by early observations that infusions of plasma from patients with ITP caused acute thrombocytopenia in normal subjects. (9). Although most autoantibodies recognise extracellular regions of platelet membrane glycoprotein complexes, some appear to be specific for the cytoplasmic (C terminal) portion of GPIIIa.

In ITP platelets are coated by antibodies, predominantly immunoglobulin (Ig) G, but sometimes of the Ig M class. Antibody coated platelets will then bind to macrophage Fc receptors in the spleen and to some extent in the liver and be destroyed in a manner probably analogous to that of erythrocytes in auto immune haemolytic anaemia. In this model, IgG coated blood cells, are preferentially sequestered in the spleen (10). Platelet associated IgG can be quantified by direct assays but its diagnostic value in ITP is limited as platelet associated IgG is elevated in

many other clinical situations, including malignancy, sepsis, toxaemia of pregnancy and immune complex disease (11). Where quantitative measurements of platelet IgG were developed, and high values were noted, it was assumed that all platelet IgG was located on the platelet surface and was antiplatelet antibody (12). These assumptions were later shown to be untrue. The great majority of platelet IgG is in equilibrium with plasma, increasing predictably in patients with increased plasma IgG concentrations of any cause (13). In several studies, the sensitivity of platelet associated IgG in ITP is as high as 90% but the specificity is only in the order of 50% (14).

2.1.2 The Role of T Lymphocytes and Cytokines

The initial stimulation for the production of platelet auto-antibodies is unknown but the involvement of T-helper (Th) lymphocytes and antigen presenting cells in a process driven and regulated by complex cellular and soluble mechanisms is hypothesised (15).

Several abnormalities within T cell populations have been described in patients with ITP (16). The T cell subsets can be identified by their secreted cytokine products and are subdivided on this basis to Th1 ; Th2 and Th0 responses (this is a CD4+ [helper] sub population). Data suggest that the T cell responses in chronic ITP are associated with Th0 and Th1 activation patterns. Th1 response causes the release of predominantly, interleukin 2 (IL-2), interferon(IFN) gamma , granulocyte macrophage colony stimulating factor (GMCSF) and tumour necrosis factor (TNF).

The trigger factor and regulation of T cell responses in ITP is still unknown. Signalling defects in the T lymphocytes of patients with ITP have also been described (17). In addition to Th1 activation, macrophages

exposed to immune stimuli release haemopoietic growth factors which may contribute to the immunopathology in chronic ITP.

2.1.3 The Role of Complement

Apart from the role played by T-cells (and their products) and macrophages, there is some evidence that the complement system is also involved in the pathogenesis of ITP.

The complement system consists of 18 plasma proteins that, when activated, can give rise to inflammatory and immunoregulatory mediators. In a manner similar to the coagulation cascade, the complement system is activated through a cascade of proteolytic cleavage of its early components and can occur via two convergent pathways. The classical pathway is initiated by complement fixing immune complexes (Ig G and Ig M) while the second, alternative pathway is triggered by a variety of substances including Ig A aggregates, endotoxin and polysaccharide components of bacterial cell walls.

The various components of the complement system have cytolytic as well as specific and potent pro-inflammatory activities.

Platelet microparticles (PMP) are vesicles derived from platelet membranes measuring less than 0.5 microns. The membrane attack complex (factors C5b to 9) generated during complement activation is one important factor that can induce PMP. The phenomenon has been described as a self-protection against complement attack by releasing C5b to9 - enriched membrane vesicles.

In vitro experiments using monoclonal antiplatelet antibodies showed a marked variation in resistance to complement mediated lysis in platelets from different individuals. Platelets from female subjects were significantly more sensitive to lysis and this might explain the higher incidence of ITP in women (14).

2.2 Other Immune and Non-immune Mechanisms

2.2.1 The Role of Drugs

Many drugs when present in solution, are able to induce a remarkable class of antibodies that bind to platelet glycoproteins. A subset of these patients, also produce true antibodies that bind to platelet glycoproteins in the absence of the drug.

Some of these autoantibodies persist and even without further exposure to the drug thrombocytopenia is perpetuated. Hence while some patients' thrombocytopenia responds to drug withdrawal, other patients may have persistent thrombocytopenia which often cannot be temporally related to the ingestion of a drug.

2.2.2 The Role of Infection

It has always been postulated that ITP may also be triggered by exposure to exogenous substances such as bacteria or viruses. Many viruses interact directly with megakaryocytes and suppress platelet production (18). By definition, this is not immune thrombocytopenia. In varicella and infectious mononucleosis, it is believed that megakaryocytes are affected and platelet production is suppressed and a clinical and bone marrow picture consistent with ITP is sometimes seen (19).

Recently, some studies have reported the presence of helicobacter pylori (H. pylori) in patients with ITP. Antibiotic therapy aimed at eradication of h. pylori resulted in the resolution of ITP (20) (21). Other studies have generated conflicting data (22).

Nevertheless, as serological assays and breath tests for detecting H.pylori are readily available, this would be recommended in patients' refractory to therapy.

2.2.3 Human Immunodeficiency Virus (HIV) and ITP

During the time that this study has been in preparation, HIV related thrombocytopenia has become the most common cause of ITP in South Africa. An Italian review quotes a prevalence of HIV related thrombocytopenia of between five to fifteen percent (23). Of these a proportion between six and twenty four percent have severe thrombocytopenia. The pathophysiology is similar to non-HIV related ITP. There is accelerated peripheral platelet destruction and decreased or ineffective production of platelets from the infected megakaryocytes. HIV related thrombocytopenia responds to anti retroviral therapy but may recur once the therapy is stopped. Most studies have evaluated zidovudine but combination therapy (HAART) is equally efficacious. When anti retroviral agents fail to maintain a safe platelet count; treatment used in classic ITP can be employed (24).

In Scaradavou's review (25) of HIV related thrombocytopenia, the possibility of treating the thrombocytopenia with pharmacological hyperstimulation of megakaryocytopoiesis with thrombopoietin is considered. (See below for discussion of thromboietin as prime regulator of thrombopoiesis). Evidence is cited in the review of the role of the chemokine receptor, CXCR4, which is a co receptor for the cellular entry

of lymphotropic HIV strains, being expressed on megakaryocytes; as a result the development of chemokine receptor antagonists may modify the course of the disease.

There is evidence in the literature that splenectomy for HIV related thrombocytopenia is as effective as splenectomy for non-HIV related ITP (26) (27). The effectiveness of the therapy again suggests that the predominant mechanism of thrombocytopenia in HIV infected patients is increased destruction of platelets because of platelet associated immunoproteins.

Splenectomy during the asymptomatic phase of HIV disease may also result in slower time to autoimmune deficiency syndrome (AIDS) as this is associated with a temporary reduction of plasma viraemia and increase in absolute CD4 and CD8 lymphocyte counts (28).

There is no data available on the HIV status of the patients in this study, but given their clinical findings at review, it would be unlikely that any one would have had HIV infection at the time of diagnosis of ITP and at splenectomy.

3. MEGAKARYOCYOTOPOIESIS, THROMBOCYTOPOIESIS AND THROMBOKINETICS.

Diploid pluripotential stem cells in the bone marrow give rise to megakaryocytes. The platelet progenitors, under the influence of humoral regulation, go through a process of maturation, proliferate and then fragment their cytoplasm to produce circulating platelets.

Principally 2 processes regulate Megakaryocytopoiesis:
Megakaryocyte colony stimulating factor (MK- CSF) and haemopoietic cytokine growth factors - mainly granulocyte macrophage colony stimulating factor (GM -CSF) Interleukin -3 (IL-3); IL-6 and IL-11. T cells, monocytes, endothelium, marrow stroma and other cell types produce the growth factors that promote megakaryopoiesis (19).

IL-11 has been shown to promote megakaryocytopoiesis at several points in the maturation pathway. Recombinant IL-11 has been used with some success in chemotherapy induced thrombocytopenia (29).

Thrombopoietin is now established to be the prime regulator of thrombopoiesis. It is known to regulate megakaryocyte maturation and hastens platelet release. Thrombopoietin (TPO, also called MK-CSF) is a polypeptide of 353 amino acids encoded by a single gene located on chromosome band 3q 27-28. It functions both early and late in megakaryocytopoiesis as a lineage specific promoter of megakaryocyte maturation and platelet production (30).

It was only in 1994 that this polypeptide was cloned and characterised and made available for clinical investigations.
Two forms of recombinant TPO have been developed. One called "recombinant human thrombopoietin" is a full length polypeptide; the other, a truncated protein containing only the receptor binding region, which has been chemically modified by the addition of polyethylene glycol (PEG) is termed "PEG - conjugated recombinant human megakaryocyte growth and development factor."

In the absence of TPO or it's receptor Mpl, on target cell membranes, megakaryocytes in the bone marrow are reduced and platelets are produced at only 15% of the normal rate of production (31).

In patients with ITP, TPO levels are normal or slightly elevated.
Pre and post treatment serum TPO concentration was measured in 35 patients with ITP in a Dutch study (32). In patients with very low platelet counts (less than 20 x 10^9/L), pre-treatment TPO was significantly higher than in patients with higher counts. These findings suggest that although no absolute deficiency of TPO has been demonstrated, relative endogenous TPO deficiency could play a role in the pathogenesis of ITP. Therefore, exogenous TPO, perhaps administered at high pharmacological doses would be efficacious in ITP patients with a decreased platelet turnover rate.
TPO holds promise as a useful therapeutic agent in the prevention and treatment of thrombocytopenia in cancer patients and other disorders (33). Clinical experience thus far indicates that TPO has been remarkably well tolerated and has a favourable toxicity profile.
However, the role of TPO in the treatment of ITP is yet to be defined.

3.1 Thrombokinetics

Normal marrow contains about six million megakaryocytes per kilogram of body weight. Each megakaryocyte releases several thousand platelets. The complete sequence of megakaryocyte maturation is completed in 4 to 5 days. The normal daily rate of platelet production is equivalent to about 35000 ± 4300 platelets per microlitre of blood. Platelet synthesis by marrow can be increased about eight folds. About two thirds of the

platelets are to be found in the systemic circulation while the balance (± 30%) constitutes an exchangeable splenic pool. The life span of normal platelets is 9 ± 1 days (34). Platelets in ITP are large, young platelets with increased levels of surface associated IgG (35). These platelets are so called stress platelets produced at a time of increased megakaryocyte turnover. In severe ITP few platelets survive long enough to die of old age.

Mechanisms implicated in the low platelet counts of ITP include disorders of production, distribution or destruction, or a combination of these factors.

In an important study from the Blood Platelet research unit, S.A. Medical Research Council and University of (Orange) Free State by Prof. Anthon du P. Heyns et al "Platelet turnover and kinetics in Immune Thrombocytopenia" was investigated (36). Radionucleotide isotopes ^{51}Cr and ^{111}In were used as platelet labels and mean platelet survival and turnover as well as platelet distribution were studied. The thrombocytopenia of ITP is primarily a consequence of peripheral destruction of platelets released from the marrow at a normal or above normal rate. The estimate of the extent of the increase in platelet turnover varies from a mean of 4.9 times normal to 2.3 times normal (37) (38).

Previous studies have shown that the antibody implicated in the pathogenesis of ITP binds not only to the platelets but to the megakaryocytes as well, thereby causing platelet production to be impaired at the level of the mature megakaryocyte (39).

Heyns, Badenhorst et al were able to conclude in their study that "platelet turnover is not always normal or increased in ITP but is low in severe disease." A relationship between blood platelet count and the sequestration pattern was observed. Splenic sequestration was seen in patients with a relatively high platelet count, whereas diffuse reticulo-endothelial system sequestration occurred in those with low platelet counts. The pattern of platelet sequestration, being related to the severity of the disease.

Flow cytometry using thiazole orange staining, measures platelet RNA which is helpful in assessing platelet maturity. Reticulated platelets are significantly increased in children with ITP, reflecting increased platelet production, compared with normal children and with other causes of thrombocytopenia, such as acute leukaemia, aplastic anaemia, etc. (40). Measuring reticulated platelets may be useful in assessing thrombocytopenic patients with clinical features atypical for ITP.

3.2 The Spleen

The spleen has a pivotal role in the pathogenesis of ITP. It is both source of antibody and the major site of platelet removal. Splenectomy is therefore effective because the principal organ for platelet destruction and antibody production is removed. Karpatkin et al demonstrated synthesis of specific IgG antiplatelet antibody by the spleen. This amounted to between 0.6% to 5% of total IgG produced. (41).

Immunohistochemical studies on spleens of patients with ITP help us to better understand immunologic and pathophysiologic changes in the organ.

Hyperplastic follicles in the white pulp or foamy macrophages in the marginal zone (or both) are major pathologic features in most spleens in ITP (42). Foamy macrophages are the result of the destruction and phagocytosis of platelets. Although hyperplastic follicles and foamy macrophages are the characteristic morphologic features of the spleen in most cases of ITP, these features are not specific for ITP.

In the study by Winde G.; Schmid K.W.et al (43) it was concluded that "the correlation of megakaryocytopoeisis and the site of thrombocytolysis to the stages of remission was significant. Patients with hyperplasia of splenic follicles had significantly higher platelet counts 2 years after operation than did those without hyperplastic splenic follicles".

Splenic histology of patients in this study showed the characteristic morphologic features of the spleen in ITP with no distinctive features.

4. CLINICAL ASPECTS

4.1 Diagnosis

The platelet count serves as a surrogate marker of activity in ITP. Diagnosis is based upon history, clinical examination and isolated thrombocytopenia. Patients are usually well, except for symptoms and signs of bleeding. Asymptomatic petechiae are noted in dependent regions and purpura may be evident. Epistaxis, gingival bleeding and menorrhagia (in women) are common presentations, while gastrointestinal bleeding and haematuria are less common. Bleeding symptoms are rare unless the

thrombocytopenia is severe (less than 10 X 10^9/L).

The incidence of the disorder is increasing especially among older adults, with the routine reporting of platelet counts; currently 30-40% of adult patients are symptom free and the diagnosis is made incidentally (44).

Full blood count shows a low platelet count with otherwise normal results, and peripheral blood smear in a patient with no clinically apparent associated conditions or factors that can cause thrombocytopenia (i.e. HIV infection, systemic lupus erythematosis (SLE), lymphoproliferative disorders, myelodysplasia, aggamaglobulinaemia, alloimmune thrombocytopenia, congenital or hereditary thrombocytopenia and drug induced thrombocytopenia).

Patients with isolated abnormalities on serologic tests (antinuclear or antiphospholipid antibodies) but without a clinically evident disorder (such as SLE) are considered to have typical ITP because positive serologic tests are commonly encountered and do not appear to affect the clinical course. Antinuclear antibodies (ANA) are a heterogeneous group of immunoglobulins which bind to nuclear antigens. The test can be positive in 5-30% of normal subjects even in high titres.
Anti-phospholipid antibodies include lupus anti-coagulant, anti-cardiolipin antibodies and others. These are a heterogenous population of antibodies directed at complexes of phopholipids with different plasma proteins. They have been found in normal subjects, usually women, and in a variety of conditions (45).

In one study 44% of 66 ITP patients had abnormal ANA titres and in

another study 40% of 109 patients had raised anticardiolipin antibodies. (46)(47).

Bone marrow aspiration is indicated in patients more than sixty years of age to exclude myelodysplastic syndromes - a group of conditions in which there is blood cytopenia as well as evidence of disordered maturation (dysplasia) in one or more of the myeloid cell lineages. Blood cell production from dysplastic lineages is ineffective and consequently, may lead to anaemia, thrombocytopenia or neutropenia.

When, in 1996, the American Society of Hematology (ASH) issued their practice guidelines on ITP, the panel of experts were sharply divided on the question of whether it is necessary or appropriate to perform a bone marrow aspiration/biopsy to establish the diagnosis of ITP in all adult patients at presentation (2).

However, it is common practice for all patients at Groote Schuur Hospital, in whom a diagnosis of ITP is being considered, to undergo a bone marrow examination. This is due to the fact that no specific criteria establish the diagnosis of ITP and the diagnosis is made by exclusion of other causes of thrombocytopenia. Furthermore there is a high incidence of collagen vascular diseases and other serious diseases such as tuberculosis and HIV (that affect the bone marrow) in the Western Cape population.

4.2 Clinical Course

As this study deals with adult patients, the clinical course in childhood ITP will only be mentioned briefly.

4.2.1 Childhood ITP

Traditionally childhood ITP is divided into acute and chronic forms - persistence of ITP for greater than six months is generally accepted as the criterion for chronicity.

In 80-90% of children, the disease is self-limiting and belongs to the acute form. Males and females are equally affected. Older age, a more insidious onset and female gender appear to be associated with the chronic form of ITP.

4.2.2 Adult ITP

Adult ITP is usually chronic and has a gradual onset but can also present acutely. Females are affected more often than males, spontaneous recovery is rare and haemorrhagic deaths are more common in the untreated elderly patient.

The natural history of this condition is not established because of previous uncertain diagnoses and more recent intervention with splenectomy.

With the routine use of automated platelet counts, the clinical spectrum of ITP has widened to include patients with previously undiscovered, asymptomatic thrombocytopenia. Available evidence suggests that only about 5% of adults with chronic ITP have spontaneous remission (48).

The degree of thrombocytopenia required for haemorrhage varies between $10 \times 10^9/L$ and $30 \times 10^9/L$.

The bleeding is predominantly dermal and mucosal and bleeding into the skin with pinpoint haemorrhage (petechiae) particularly in dependent areas where there is increased capillary pressure is common. Easy bruising or inappropriate bleeding were the features at presentation in 90% of the cases in the study by Jacobs, Wood and Dent (49).

Purpura, menorrhagia, epistaxis and gingival bleeding are common while gastrointestinal bleeding and haematuria are less commonly encountered.

The most serious complication is spontaneous bleeding into the central nervous system resulting in death. While this occurs too rarely for an incidence to be calculated (2) it is the most common cause of death and may occur acutely or at any time during a prolonged course (50).

The diagnostic margins between primary autoimmune thrombocytopenic purpura and thrombocytopenia secondary to (or part of) an autoimmune disorder can be blurred. In the series of 148 patients in the study by Jacobs Wood and Dent (49), eighteen patients had additional diseases - eight with SLE. Their data also suggest that the prevalence of autoimmune disorders in the population of mixed ancestry in the Western Cape is higher than the prevalence within Black or Caucasian backgrounds. The fact that there is another university hospital (Tygerberg Hospital/Stellenbosch University) close by would suggest that the scope of the problem is twice as big.
The need for treatment varies from patient to patient and is determined by their clinical status.

In a review by Portielje et al (51), the natural history of 152 adult

patients with ITP consistently managed and followed up over a ten-year period was studied. Ninety three percent of patients ultimately achieving a platelet count of greater than 30 X 10^9/L did so within two years. Some eighty five percent achieved platelet counts above 30 X 10^9/L off treatment and had a long term mortality identical to that of the general population; nine percent of patients with severe ITP (platelet count less than 30 X 10^9/L) had refractory ITP with an associated mortality risk of 4.2 (bleeding and infection contributing equally). Six percent of patients had a platelet count greater than 30 X 10^9/L while on maintenance therapy: the mortality in this group was only slightly above that of the general population. The study concludes that most adult patients with ITP have a good outcome with few in-patient admissions and no excess mortality.

These findings would tend to favour a policy of using therapy only when absolutely required, thereby minimising the risks of infection through immunosuppression, and reserving treatment for those patients with severe symptomatic ITP.

4.3 Therapeutic Options

Therapeutic options for ITP include the following:

<u>Standard treatment</u>

Glucocorticoids

Intravenous immune globulin

Splenectomy

Refractory disease

Oral dexamethasone

Intravenous methylprednisolone

Danazol

High-dose Intravenous immune globulin

Anti-D Immunoglobulin

Dapsone

Azathioprine

Vinca alkaloids

Intravenous cyclophosphamide

Combination chemotherapy

Cyclosporin A

Experimental Therapies

Inteferon alpha

Rituximab

Campath-1H

Mycophenolate mofetil

Anti-CD40 ligand

Protein A immunoadsorption columns

Stem cell transplantation (7)

What constitutes best treatment is not really evidence based but there appears to be agreement globally that standard therapy comprises: oral corticosteroids, intravenous immune globulin and splenectomy. The efficacy of available therapies can be unpredictable and may be transient. Standard therapy is discussed below, followed by all other therapies in no order of preference. Treatment must be individualised.

4.3.1 Corticosteroids and Dexamethasone

Good evidence in the literature supports the use of corticosteroids as primary therapy. Steroids are given to patients whose platelet counts are less than 50 X 10^9/L and are symptomatic (purpura, easy bruising etc.).

Treatment is commenced with a dose of one milligram per kilogram (1mg/kg) and this high dose is maintained until there is a significant increment in the platelet count, after which time the dose is tapered down to a minimum amount required to maintain a safe platelet count. Both lower (0.5 mg/kg/day) and higher (2mg/kg/day) doses are equally efficacious (52).

Corticosteroids prevent sequestration of antibody coated platelets and also impair antibody production and/or binding to platelets (53). Steroids paralyse macrophages reducing the clearance rate of platelets. Most studies quote effective responses from corticosteroids at between 36-44% (platelet count improved to greater than 100 X 10^9/L). Furthermore, the duration of corticosteroid treatment does not affect the therapeutic response in the end.

"The use of corticosteroids for more than 30-45 days demonstrated no additional favourable results" (54).

When patients are deemed to have chronic ITP, those with platelet counts greater than 30 X 10^9/L and who are asymptomatic do not routinely require treatment unless they are undergoing any procedure likely to induce blood loss, including surgery, dental extraction or delivery (55).

Patients who fail to achieve platelet counts of greater than 30 X 10^9/L after six to twelve weeks and remain symptomatic, those requiring greater than 0.25 mg/kg/day or those relapsing with a gradual reduction in steroid dose proceed to splenectomy.

Alteration of steroid regimens may also be useful in treating apparently resistant ITP.

High dose dexamethasone given in six cycles of limited duration (40mg per day for four days every 28 days) has been found to be effective in a small group of patients that was resistant to at least two and up to five different therapeutic regimens (56).

While this study was based on the results of only ten patients with refractory ITP, the results suggest that familiar agents employed in novel dose schedules may be effective in treating apparently resistant ITP. Pulsed high dose dexamethasone provides a cost effective and well-tolerated alternative to splenectomy and to more toxic medical treatments for this condition.

4.3.2 Intravenous Immune Globulin

Immunologic reactions can be modified, often dramatically, by the intravenous administration of large amounts of immune globulin. Immunoglobulins (Ig) are the products of mature B cells or plasma cells. Of the five classes of immunoglobulin, Ig G is the main one. Ig products for intravenous use are preparations of mainly Ig G. It is obtained from plasma collected from healthy donors and prepared from pooled material from at least 1000 donors (57).

immune globulin in pregnancy but there are no good comparative studies and the decision must take into account maternal clinical factors in addition to expense, availability and remote risk of microbial transmission by intravenous immune globulin (2).

The usual dosage regimen is 400 mg per kg body weight given by intravenous infusion on days one through five for adult patients.

Response, defined as a platelet count greater than 50 X 10^9/L can be expected within 21 days.

The mechanism of action of immune globulin in ITP is not clearly understood. It is postulated that the inhibition action of intravenous immune globulin is mediated at least in part by the variable region of antibodies, probably by an anti-idiotypic mechanism (62).

Furthermore, if the receptors for the Fc portion of IgG on reticulo endothelial cells were saturated by intravenous immune globulin then cells such as platelets, vulnerable because they are coated with auto-antibody would be less likely to be attacked and destroyed. Blockade of the Fc receptor (FcR) can be demonstrated after such treatment (63).

This does not explain the long-term benefits produced by intravenous immune globulin and other important mechanisms must be involved in long term remissions.

Jacobs, Wood and Novitzky (64) showed in their study at Groote Schuur Hospital that "intravenous gammaglobulin has no advantages over oral corticosteroids as primary therapy for adults with immune thrombocytopenia." Theirs was a prospective randomised clinical trial comprising three groups of clinically and haematologically well matched

patients. Group 1 was given oral prednisone at a dose of 1 mg/kg/day (n = 17). Group 2 was given high dose intravenous gammaglobulin 400mg/kg on days 1 through to 5 (n =13). Group 3 was given a combination of both agents on the same schedule (n = 13).

The study concluded that in previously untreated adults with symptomatic immune thrombocytopenia, gammaglobulin offered no advantage over conventional corticosteroid administration as the primary form of therapy. Furthermore the use of both agents combined offered no added benefit. Response, defined as a platelet count greater than 50 X 10^9/L was achieved in 82%, 54% and 92% of patients in groups 1,2 and 3 respectively, but was only significantly different between groups 2 and 3 (p = 0.0365).

Although there was a trend in favour of the steroid administered groups, relapse was not significantly different which occurred at a median of 184, 32 and 76 days respectively nor was the average time to splenectomy different at 339, 59 and 98 days, respectively.

Gammaglobulin has a place in the treatment of chronic refractory ITP. Two patients included in this study had a satisfactory response to the use of intravenous immune globulin after splenectomy failed to restore platelet counts to safe levels. Both patients have maintained normal platelet counts at 5 years and beyond.

This treatment is expensive and inconvenient but it is sometimes the only effective option (65).

4.3.3(i) Splenectomy

Splenectomy is the most successful therapeutic approach in ITP. Splenectomy was the first effective treatment for ITP (5). It was more than twenty years later (1950) that glucocorticosteroid therapy was introduced.

The earlier studies reported better long-term results, as there was a tendency to combine the results of children and adults.
Most studies quoted in the literature suggest that about two thirds of patients achieve and sustain a normal platelet count after splenectomy and require no additional therapy (2).

The study by Jacobs, Wood and Dent (49) reviewed 148 adults with ITP. Of these, 102 went on to have a splenectomy. While about 40% achieved complete remission immediately post splenectomy, an overall response rate of 93% is possible. The prospective study from the University of Muenster, Germany (43), included 72 patients who had undergone splenectomy for ITP. The results once again showed an overall remission rate of about 93% (complete remission 72%; partial remission 15%; partial remission affording further medical support 6% and no remission 4%).

4.3.3(ii) Pneumococcal vaccination

All patients undergoing splenectomy are given a pneumococcal vaccination two weeks prior to surgery. Preferably two other vaccines should be added, viz. Haemophilus influenza b (Hib) and quadrivalent meningococcal vaccines.

Splenic macrophages have an important filtering and phagocytic role in removing bacteria and parasitised red blood cells from the circulation (66).

Fulminant, potentially life-threatening infection is a major long-term risk after splenectomy - described by King and Shumaker in 1952 (67).
Later studies have shown the increased risk of sepsis to be life long even though most infections occur within the first two years after splenectomy (68).

Streptococcus pneumoniae afflicts large numbers of susceptible patients and is a major cause of morbidity and mortality in many countries. Of the estimated 150 000 to 570 000 cases of pneumococcal pneumonia which occur annually in the USA, approximately five percent are fatal (Immunisation Practices Advisory Committee 1984).*

The pneumococcal vaccine consists of purified capsular polysaccharide antigens of the 23 most prevalent or invasive serotypes of streptococcus pneumoniae. The efficacy of the vaccine, defined by the reduction in invasive pneumococcal infections is on average estimated at between 65% and 90%. Raised antibody levels have been demonstrated 5 years after immunisation but the duration of protection is unknown (69). Cases of fatal post splenectomy pneumococcal sepsis despite prophylaxis with penicillin and pneumococcal vaccine have been described.

Apart from asplenic individuals who have been shown to benefit from pneumococcal immunisation, it is also beneficial in other groups of patients who are considered to be at risk of infection with encapsulated

* CDC Pneumococcal polysaccharide vaccine usage, United States. MMWR 1984:33;273-6,281

organisms - for example, patients with chronic lymphocytic leukaemia, Hodgkin's disease, myeloma, HIV related disease or other immunodeficiency states and chronic lung disease.

A retrospective cohort study showed that pneumococcal vaccination of elderly people with chronic lung disease was associated with reduced hospital admissions for pneumonia. (70).

More recently, conclusions from a three-year prospective study conducted in Stockholm County, Sweden, confirmed that the incidence of hospital treatment was lower in the vaccinated than in the unvaccinated cohort for influenza, pneumonia, pneumococcal pneumonia and invasive pneumococcal disease. Total mortality was 57% lower in the vaccinated than in unvaccinated individuals (71).

Though most infections occur within the first two years post splenectomy, up to a third may by manifested at least five years later. British guidelines recommend lifelong prophylactic antibiotics. This is not recommended practice in South Africa or in Germany. Antibiotic prophylaxis may not prevent sepsis. Furthermore, the emergence of antibiotic resistant bacterial strains must also be considered. Compliance is unpredictable and likely to be poor (72) (73).

Guidelines for the prevention and treatment of infection in patients with an absent or dysfunctional spleen issued by the working party of the British Committee for standards in Haematology Clinical Haematology Task force, recommended re-immunisation of asplenic patients every five to ten years (72).

There is some evidence to show that following pneumococcal vaccination, serotype specific antibody levels decline after five to ten years and decrease more rapidly in some groups than others (74).
Data concerning serological correlates of protection are inconclusive, which limits the ability to precisely define indications for re-vaccination based on serologic data alone.

Recommendations by the Advisory Committee on Immunisation Practices (USA) in 1997 recommend revaccination once in those persons aged 2 years or older with functional or anatomic asplenia (sickle cell disease, splenectomy) and those with conditions associated with rapid antibody decline after initial vaccination (nephrotic syndrome, renal transplantation, HIV infection, leukaemia, lymphoma, Hodgkin's disease, multiple myeloma, generalised malignancy and organ or bone marrow transplantation) (75).

The need for subsequent doses of pneumococcal vaccination after the first revaccination is, (according to American recommendations) unclear and can only be re-assessed when additional data become available.
The existing guidance on the use of polysaccharide pneumococcal vaccines was revisited in a British Medical Journal editorial recently (76). The authors noted the apparent conflict between evidence of effectiveness of pneumococcal polysaccharide vaccines and existing recommendations for their use.

4.3.4 Other Immunosuppression

In the majority of cases initial treatment of ITP with corticosteroids, followed, if necessary, by splenectomy results in normal or "safe" platelet

counts in more than 70% of patients.

In circumstances where patients were unwilling to undergo splenectomy or have failed splenectomy, other immunosuppression is used.

Evidence base for the use of these agents is small and experience is limited.

4.3.4(i) Anti-D Immunoglobulins

Anti-D immunoglobulins are used to prevent the production of anti-D antibodies in rhesus-negative individuals following exposure to rhesus positive red blood cells. Its main application is in the prevention of rhesus haemolytic disease of the newborn.

The benefit of anti-D in ITP patients was first reported by Salama et al (77). Most reports relate to patients with chronic ITP.

A randomised trial of intravenous Ig G: intravenous anti - D, and oral prednisone in childhood acute ITP (78) showed that a single dose of 800mg/kg iv Ig G offers the fastest recovery for the least treatment.

In a single arm open-label study of anti-D, including 261 patients and in which all subgroups were included (i.e. children, adults, HIV positive patients, HIV negative patients, patients with acute ITP and patients with chronic ITP) 46% of patients showed an increase in platelet count of more than 50 X 10^9/L. (79). Eleven splenectomized patients were treated as a separate group. Results indicate that anti-D treatment is suitable for Rhesus (D)-positive patients who have not undergone splenectomy.

The treatment is not recommended for refractory patients post splenectomy. Most benefit was noted in children with persistent ITP and in patients with HIV related thrombocytopenia.

4.3.4(ii) Vincristine

Vincristine, a vinca alkaloid, is given at a dose of 1-2 mg per week intravenously. The expected response time is 7-10 days. In view of the potential side effects, especially peripheral neuropathy, treatment should be discontinued if a permanent response is not achieved after 4 - 6 doses. (80).

4.3.4(iii) Danazol .

Danazol, an attenuated androgen, acts primarily to inhibit pituitary gonadotrophins. Its mechanism of action in improving the platelet count in ITP is not fully understood. It is given at a dose of 200mg orally four times daily for at least six months because responses can be slow. As it is hepatotoxic liver function should be checked at regular intervals. When a response occurs doses should be continued for at least a year and then tapered by 200mg per day every four months (81).

4.3.4(iv) Colchicine.

Colchicine is administered at a dose of 0.6 mg orally, three times daily for at least 2 months (82). Diarrhoea may necessitate a reduction in dose. If a response occurs the dose can be reduced to the lowest possible level to maintain a safe platelet count.

4.3.4(v) Dapsone.

This anti-leprotic drug has been used in refractory ITP at doses of 75 mg daily orally. Patients must be screened for erythrocyte glucose - 6 - phosphate dehydrogenase deficiency as those who are found deficient can have serious life threatening haemolysis if given dapsone (83). In a series of 66 adults with chronic ITP, responses were noted in 33%, with a median duration of treatment required for a response being 21 days. Sustained responses were observed in 19 patients (84).

4.3.4(vi) Staphylococcal -A- Immuno adsorption.

The role of Staphylococcal -A- Immuno adsorption is not clear and the side effects may outweigh the benefits. Best results occur in patients with relatively higher platelet counts and more recent disease which makes its use outside of a trial setting very infrequent (85). Patients are treated 3 times per week for two weeks, according to manufacturers instructions, require good venous access and the cost of this treatment is about four times that of a course of gammaglobulin.

4.3.4(vii) Cyclophosphamide

Cyclophosphamide is a cytotoxic drug widely used in the treatment of chronic lymphocytic leukaemia, lymphomas and solid tumours. In addition to the side effects common to many cytotoxic drugs there are two problems associated with prolonged usage. Firstly, gametogenesis is severely affected and secondly it is associated with a marked increase in secondary malignancies. In view of the above, dosage should begin with 150mg/day orally and should be adjusted with the aim of maintaining mild neutropenia (86). Responses usually occur within 8 weeks and treatment should continue for 3 more months and then stopped. If relapse occurs,

such long-term risks as secondary malignancy must be weighed against the benefits of resuming therapy. Patients need to be adequately rehydrated and are urged to drink at least 2 litres of fluid daily to prevent haemorrhagic cystitis which may be caused by a urinary metabolite of cyclophosphamide, acrolein.

High dose cyclophosphamide treatment is reserved for patients who have life-threatening symptoms; extremely low platelet counts, and are refractory to other less toxic forms of therapy. The recommended dose is 1 - 1.5g/m^2 of body surface area intravenously and should be repeated at 4 week intervals. If, after two courses, no response is seen then therapy should be discontinued. For those who do respond, at least three courses are suggested. The precautions listed above need to be adhered to. In addition patients could be primed with intravenous re-hydration and given mesna, which reacts specifically with the toxic metabolite acrolein in the urinary tract thus preventing toxicity. Mesna is given intravenously initially and then orally.

4.3.4(viii) Azathioprine

Azathioprine is an antiproliferative immunosuppressant widely used for transplant recipients and in the treatment of autoimmune conditions usually in combination with steroids. Its main toxic effect is myelosuppression although hepatic toxicity is also well recognised. In ITP responses to azathioprine therapy occur slowly over 3-6 months and in many instances treatment is stopped before giving it a fair trial. Recommendations are that dosage should commence at 150mg/d orally and should be adjusted to result in mild neutropenia. If a response occurs (at 3-6 months) then the full dose should be continued for 18

months and therapy should be discontinued thereafter. The decision to continue therapy for long term is determined by evaluating the risk-benefit ratio. Serial blood counts should be monitored for neutropenia. (87).

4.3.4(ix) Interferon.

Treatment with interferon is controversial. A dose of 3 million units subcutaneously 3 times per week for four weeks has been used. Interferon may suppress platelet production and a case report (88) has highlighted this problem indicating that this contributed to a patients death. Currently, interferon is not likely to have a role in the management of ITP.

4.3.4(x) Vinblastine

Vinblastine belongs to the vinca alkaloid group of cytotoxic agents which are usually used to treat the acute leukaemias, lymphomas and some solid tumours. In patients who respond to vinca alkaloids vinblastine 5-10mg intravenously every one to four weeks can sometimes be used effectively for long intervals (65).

4.3.4(xi) Cyclosporine

Cyclosporine, a calcineurin inhibitor is a potent immunosuppressant which is virtually non-myelotoxic but markedly nephrotoxic. The suggested dose in ITP is 1.25 to 2.5mg per kg orally twice daily. Cyclosporin levels must be measured regularly and renal function closely monitored (65).

4.3.4(xii) Combination chemotherapy

Robert McMillan from the Scripps research institute recently reported their experience and follow up of patients treated for refractory ITP with combination chemotherapy. All twelve patients had severe disease with periodic mucosal bleeding following failed splenectomy and failed an average of five other therapies. Five achieved complete remission. One had a partial remission but succumbed to fulminant hepatitis C acquired from a transfusion. One patient who was given additional therapy after relapse following combination chemotherapy died of a disseminated fungal infection. (consequent upon immunosuppression). Yet another patient given this "rescue" therapy went into remission. The remaining 3 patients had no response and died from central nervous system bleeding.

This group of patients highlights the difficulties encountered in treating patients with refractory ITP.

Combinations of chemotherapy included:

CMOPP (cyclophosphamide, vincristine, prednisone and procarbazine.

CEP (cyclophosphamide, vincristine and etoposide)

CVP (cyclophosphamide, vincristine and prednisolone) (89).

4.3.4(xiii) Rituximab (Anti-CD20 antibody)

Rituximab (chimeric anti-CD20 monoclonal antibody) used in a dosage of 375 milligrams per kilogram weekly for four weeks was shown to be useful in a single study (90). Five patients responded completely and a partial response was seen in five more. In seven patients the response was sustained beyond six months. Younger patients appeared to have better response rates.

4.3.4(xiv) Campath-1H

Campath-1H is a humanized IgG monoclonal antibody which targets the CD52 antigen, present on mature human lymphocytes and monocytes. In a study by Willis et al (91), Campath-1H was used in 21 patients with autoimmune cytopenias. Fifteen of these patients responded with a sustained response in six patients. For patients with ITP this therapy can only be given consideration when all other therapies have failed and one is faced with a patient deemed to be at serious risk of bleeding (7).

4.3.4(xv) Mycophenolate mofetil

Mycophenolate mofetil is an immunosuppressive agent originally used to prevent acute rejection in solid organ transplants. Its use as a second line agent in autoimmune conditions appears promising. In a British study (92), published this year, four patients with auto immune haemolytic anaemia and six patients with ITP were treated with mycophenolate mofetil. Five of the six patients with ITP showed a complete or good partial response to the treatment. The dose used was 500 mg twice daily, increased to one gram twice daily after a fortnight. The drug was well tolerated and may be used alone, as a steroid sparing agent or alongside other second line immunosuppressive therapy.

4.3.5 Therapeutic Options in refractory chronic ITP

Refractory chronic ITP is by convention defined as those in whom treatment with standard dose corticosteroids and splenectomy fails and who require further treatment because of unsafe platelet counts or clinical bleeding. This group responds poorly to subsequent treatment, has significant morbidity from the disease and its treatment, and has a mortality rate of about 16% (4).

Chronicity is defined empirically as severe thrombocytopenia with platelet counts of less than 20 to 30 X 10^9/L for more than six months in adult patients.

McMillan R (93) described a structured approach to the treatment of refractory chronic immune thrombocytopenic purpura based on "a literature review and personal experience." Treatments described were as follows:

(i) Level One Therapy - moderate risk

Corticosteroids

Vinca Alkaloids

Danazol

Colchicine

Dapsone

(ii) Level Two Therapy - greater toxicity

Staphylococcal - A Immunoadsorption

Cyclophosphamide

Azathioprine

(iii) Level Three Therapy - reserved for patients refractory to the first two levels and who have life threatening symptoms or dangerously low platelet counts.

High dose cyclophosphamide

Combination chemotherapy

(iv) Level Four Therapy - when all else has failed.

Interferon

Gammaglobulin

Vincristine

Cyclosporine

This particular structured approach is clearly debatable as it is not entirely evidence-based and clinical practice does vary in different centres.

More recently, a management algorithm, representing a synthesis of the published literature, expert opinion, American Society of Hematology guidelines and the authors experience was published. This also incorporated the use of experimental agents - antibodies against CD 20, antibody against CD 154, bone marrow transplantation and thrombopoietin (94).

A further review in The British Journal of Haematology (7) has stated that terms such as "first-line" and "second-line" are confusing and unhelpful and that it may be better to adopt the terms 'standard', 'refractory' and 'experimental' treatments, (as listed in section 4.3 above).

4.3.6 Defining Response to Therapy

The platelet count serves as a surrogate marker of success in the treatment of ITP. Many patients receive treatment on the basis of a low platelet count rather than clinical need. This may lead to the perception that an increase in the platelet count is as a result of the treatment and not just the natural variability of the disease.

There is some evidence to show that a "safe" platelet count is greater than 30 X 10^9/L. Patients who maintain their platelet counts above this level have a long term mortality risk equal to the general population (51).

There are no standardised criteria defining patterns of response to splenectomy. In the study by Jacobs, Wood and Dent (49), complete remission was defined as a platelet count constantly greater than 200 X 10^9/L; continuous complete remission as maintenance of normal counts without treatment; incomplete remission when values remained between 100 and 200 X 10^9/L; partial remission between 50 and 100 X 10^9/L; and failed treatment when platelet counts remained constantly under 50 X 10^9/L. Other studies have also defined patterns of response using the platelet count in a similar way, albeit with different limits. Portielje et al (51), identified patients with an initial platelet count between 30 X 10^9/L and 100 X 10^9/L as 'moderate thrombocytopenia' and those with a platelet count at presentation of less than 30 X 10^9/L as 'severe thrombocytopenia'. Complete response was defined as a platelet count of more than 100 X 10^9/L without therapy for at least two months. Partial response was defined as a platelet count of more than 30 X 10^9/L, without therapy for at least two months. Response to maintenance therapy was defined as a platelet count of more than 30 X 10^9/L during drug therapy. No response was defined as a platelet count of fewer than 30 X 10^9/L with or without therapy.

5. PREGNANCY AND ITP

Thrombocytopenia, observed in about 7% of pregnancies, may be related to previously acquired or inherited diseases or to pregnancy related complications, such as pre-eclampsia, sepsis or obstetrical disseminated intravascular coagulation (95). In 75% of these cases the cause of the thrombocytopenia is considered to be gestational thrombocytopenia,

generally assumed to be secondary to an increased platelet consumption within the placental circulation and/or to hormonal inhibition of megakaryocytopoiesis (95)..

ITP cannot be distinguished from gestational thrombocytopenia with certainty because the diagnosis of both conditions is based on the observation of thrombocytopenia with no other apparent cause (2).

The clinical importance of this distinction between ITP and gestational thrombocytopenia is important to the foetus as ITP with thrombocytopenia may harm the foetus, if it is severe, whereas gestational thrombocytopenia does not (96).

The study by Ajzenberg N et al (95) found biological features of an auto-immune disorder in 48% of the women and chronic thrombocytopenia in 55%. It must, however be emphasised that these results are from a highly selected population of women referred because of thrombocytopenia detected during pregnancy. This study used the threshold of 70 X 10^9/L to differentiate between mild and severe thrombocytopenia.

Foetal and/or neonatal thrombocytopenia occurs in between 4 to 13% of cases of gestational thrombocytopenia (97). In neonates born to mothers with ITP the risk of a new-born having a platelet count less than 50 X 10^9/L ranged from 0 - 60%, with a weighted mean of 13% (96).

The treatment of ITP in pregnant women must take into account the potential adverse effects of therapy on the course of pregnancy and foetal development.

The two main treatment modalities in pregnant women are corticosteroids and intravenous immune globulin, While consensus has not been reached in the international arena, local practice favours the use of corticosteroids as first line therapy. Glucocorticoids are safe in terms of potential teratogenicity but high systemic doses may produce foetal and neonatal adrenal suppression and risk of intra-uterine growth retardation. In the mother, they may exacerbate gestational diabetes mellitus and postpartum psychiatric disorders (98). Cytotoxic agents are avoided during pregnancy, as is splenectomy. However, in patients in whom the disease is refractory a splenectomy may be undertaken, but the benefits need to be weighted against increased foetal mortality.

Caesarean section as the preferred method of delivery is only indicated if the maternal platelet count is less than 50 X 10^9/L. This is a contentious issue and some clinicians may not recommend caesarean section even at lower platelet levels, supporting the view that the mode of delivery in women with ITP should be decided by purely obstetric indications. A maternal platelet count greater than 50 X 10^9/L is considered sufficient to prevent complications from excessive maternal bleeding at vaginal delivery or caesarean section (2).

In this study eighteen patients had a total of 29 pregnancies of which 23 resulted in a successful outcome. The outcome of pregnancy post splenectomy appears to be generally uncomplicated.

CHAPTER TWO

1. AIM OF THE STUDY

The aims of the study are as follows:

1.1 Aim 1

To measure/confirm the success of splenectomy at five years and beyond.

Splenic histology was analysed by a pathologist to determine if histologically defined patterns influenced patient outcome.
Apart from the study by Jacobs, Wood and Dent (49), there is no other study done locally to confirm the success of splenectomy and its effectiveness and appropriateness at our institution.

In the literature, there is evidence from other centres (where population demographics are different) that splenectomy for ITP is a successful procedure, with low morbidity and mortality (99), (100), (101), (102), and (103).

1.2 Aim 2.

To review the incidence of complications

-Perioperatively.

-Long term.

Mortality associated with the procedure (splenectomy) is less than 2%, most centres, however, quoting a zero percent mortality. Morbidity figures are about 16%.

1.3 Aim 3.

To review the status of pneumococcal vaccination and the role (if any) of prophylactic antibiotics. Currently local practice does not recommend re-vaccination with pneumococcal vaccine.

Serum pneumococcal antibody levels for five serotypes (19F; 14; 6B; 5 and 1) will be measured against a cohort of control patients who have intact spleens and received the pneumococcal vaccine for other indications.

As prophylactic antibiotics are not given locally, an appraisal of the literature will be performed and the relevant recommendations made regarding the use of prophylactic antibiotics.

1.4 Aim 4.

To perform a re-appraisal of patients found to be refractory to treatment.

2. STUDY DESIGN

The study is a retrospective analysis of a chronologically defined cohort of adult patients who underwent splenectomy as a treatment for ITP between 1987 and 1992 at Groote Schuur Hospital, Cape Town.

CHAPTER THREE

1.MATERIALS AND METHODS

1.1 Records

Records kept at the Department of Haematology at Groote Schuur Hospital were accessed. Details of patients who were identified as having had a splenectomy between the time period 1987 and 1992 were selected. In order that all patients were identified, cross referencing from the departments of Pathology, Surgery and Medical records was undertaken. Patients whose indication for splenectomy was for the treatment of ITP were then contacted and invited to come in to the hospital for a clinical review and blood tests. Informed consent was obtained (appendix E)). Those patients who were not contactable initially were traced and home visits were undertaken.

1.2 Clinical examination

A clinical history was obtained from the patients, paying particular attention to features associated with ITP and other auto immune disorders as well as instances of possible infection requiring antibiotic treatment following splenectomy. Patients were given a routine physical examination.

1.3 Blood Tests

The following blood tests were done on all the patients:

1.3(i) Full blood count/platelet count.

The blood counters used at the Haematology laboratory at Groote Schuur Hospital were the Technicon analysers H1 and H2.These automated haematology analysers measure a number of traditional haematological variables. Information of diagnostic relevance is obtained not only from the numerical data produced by the instrument but also from inspection of the graphical output. The graphical output, together with instrument flags, are also important in alerting the technician to abnormal samples and anomalous test results. A senior laboratory technician reviewed the blood smears. Features of hyposplenism (mean platelet volume, platelet distribution width and Howell Jolly bodies) were specifically noted.

1.3(ii) Immunoglobulin levels

These levels were measured in the Chemical Pathology Laboratory at Groote Schuur Hospital. The analytical technique used was nephelometry, which is the direct measurement of light scattered by a suspension of particles. The particles are made up of immune complexes (of variable number and sizes) formed by the reaction of antigen with antibody and is dependent on the relative concentrations of the reactants. The scattered light (also named Tyndall light) is measured at right angles to the incident light. This gives the Tyndall ratio, which is the ratio of the Tyndall intensity to that of the incident radiation. The Tyndall intensity is compared with that of a standard suspension of known concentration. Even though the kinetics of immune complex formation measured by nephelometry can be sufficiently different in the three phases - antibody excess, equivalence, and antigen excess - there are several automated instruments commercially available which overcome this problem and are able to perform a wide variety of specific protein analysis with accuracy.

Furthermore these analyses take as little time as running a glucose test and with similar simplicity.

1.3(iii) Serum for anti pneumococcal antibody levels

The ideal study design for evaluation of the efficacy of the pneumococcal vaccine would have been the determination of the incidence of pneumococcal disease in a vaccinated group against the incidence in a non vaccinated control group, but such trials in patients with ITP requiring splenectomy are not likely to meet with ethical approval.

Serum for anti pneumococcal antibody levels was taken from all patients who were clinically evaluated for the study.

As the pneumococcal vaccine was not given to any other category of adult patients in the state hospital services, control samples were obtained from 16 patients who were given the pneumococcal vaccine in the private sector. These control patients had an intact spleen and were not on corticosteroid therapy. They were vaccinated for primary health care reasons - age greater than 65 years, history of asthma, bronchitis or previous bronchopneumonia.

Immunity to serious pneumococcal infections in adults is related to serotype -specific anti-capsular polysaccharide antibodies (CPS)(104). Children and adults have, as a rule, natural anti-CPS antibodies in the serum. The pneumococcal capsular polysaccharides used as antigens for measuring type specific antibodies have been shown to contain CPS (105).

The polyvalent pneumococcal capsular polysaccharide vaccine (Pneumovax) contains CPS but because this gives rise to only weak (less than 2-fold anti-CPS responses), these are expected to contribute little to the "type

specific" antibody response measured (106).

The specificity of the immune response to the 23-valent pneumococcal polysaccharide vaccine in healthy adults can be estimated by measuring immunoglobulin G (IgG) antibody titres by enzyme linked immunosorbent assay (ELISA) or the opsonophagocytosis assay. (Both the pneumococcal polysaccharide and conjugate vaccines induce type specific opsonic antibodies). Opsonophagocytosis assays are labour intensive and difficult to perform with large numbers of samples (107).

Serum was sent to The South African Institute for Medical Research (Diagnostic Serology) for analysis. Levels of IgG antibodies against serotypes 19F, 14, 6B, 5 and 1 were evaluated. The method employed is described below.

ELISA for anti-pneumococcal PS antibodies.

The ELISA used for the measurement of IgG antibodies against pneumococcal polysaccharide antigen was based on the method originally described by Koskela (108) and subsequently modified by Kayhty et al (109). An addition of absorption step, described by Concepcion and Frasch for increased specificity was incorporated (107).

Microtiter plates (MaxiSorp, Nunc, Roskilde, Denmark) were coated with the respective PS (ATCC, Rockville, MD) in pyrogen-free PBS (1, 19F and 6B, 10ug/ml; 14and 5, 5ug/ml) by incubating for 5 hours at 37 degrees Celsius (°C). Plates were stored at 4°C for up to 4 weeks. After four washes with PBS containing 0.05% Tween 20 (T-PBS), plates were post coated with 10% of foetal calf serum in PBS (F-PBS).

Sera were diluted 1:100 in F-PBS containing10ug/ml of pneumococcal C polysaccharide (CPS) plus 10ug/ml of pneumococcal polysaccharide type 22F absorbent (CPS was obtained from State Serum Institute, Copenhagen, Denmark. And PS type 22F from ATCC) to neutralise CPS antibodies. After 30 minutes of incubation with the absorbent at 37°C, 3-fold dilutions were made in F-PBS. Serum dilutions were incubated for two hours at 37°C, after which plates were washed as above. Alkaline phosphatase-conjugated AffiniPure Goat anti-human IgG (Jackson ImmunoResearch Laboratories, INC.) was diluted in F-PBS added to the plates and incubated for 2 hours at 37°C. Plates were washed three times with T-PBS and twice with demonised water; then substrate, p-nitro phenyl phosphate (Calbiochem an affiliate of Merck KGaA Darmstadt, Germany), was added and incubated for 30min at 37°C.

The A405 was read on ELISA reader (Multiskan RC, Lab systems, Helsinki). Anti-pneumococcal PS concentrations were calculated by comparing the titres of the samples to titre of the reference serum 89-SF3 received from the US Food and Drug Administration (Bethesda, MD). The results are given as micrograms per millilitre calculated on the basis of the assigned IgG values of the 89-SF3-reference serum (110).

1.3(iv) Megakaryocyte Derived Growth Factor Assay

Serum was obtained for estimation of megakaryocyte derived growth factor – this is to be done at a later stage as part of a separate study.

1.4 Splenic Histology

With the assistance of a histopathologist, splenic histology was reviewed on all available specimens.

2. DEFINING RESPONSE TO THERAPY IN THIS STUDY

There is no standard definition for response to therapy in the literature (See above).

For the purposes of this study, which looked specifically at the response to splenectomy for ITP, the following definitions were used:

1. Complete remission - When a platelet count of greater than or equal to 150 X 10^9/L was achieved.

2. Partial remission - a platelet count of 50 - 149 X 10^9/L or an increase of greater than 30 X 10^9/L with respect to the baseline value. (Chronic ITP).

3. Failed response - was a platelet count less than 50 X 10^9/L or an increase less than 30 X 10^9/L with respect to the baseline value. (Chronic refractory ITP).

There was a subgroup of patients who responded to splenectomy, with a return of the platelet count to the normal laboratory range, initially, followed by a relapse, requiring further immunosuppressive therapy. Immunosuppression was later withdrawn and the platelet count was maintained in the normal laboratory range. (see Table 5).

CHAPTER FOUR

RESULTS

Results are provided in this section in a series of tables and figures. The full laboratory investigations and statistical analyses are included as 'Appendix A, B and C. Further clinical details are provided in the discussion.

1. Demographic data (tables 1 and 2)

The total number of patients' records reviewed for the period 1987-1992 was sixty-three. This accounted for all patients diagnosed as having ITP and requiring therapeutic splenectomy during that period at Groote Schuur Hospital in Cape Town. The majority of patients had failed immunosuppression with steroids while a few had failed other second line therapies as well.

Four (6.3%) were no longer living in the Western Cape but were confirmed to be alive and well (personal communication).
Seven (11%) could not be traced.
Five (8%) had died.

Twelve patients (19%) were Caucasian; two (3%) were Black African and forty-nine (78%) were Coloured (mixed ancestry).

TABLE 1: Racial characteristics and gender distribution of patients who had undergone splenectomy for immune thrombocytopenia between 1987 - 1992 at Groote Schuur Hospital, Cape Town.

PATIENT	Number n=63	(%)
CAUCASIAN	12	19%
BLACK AFRICAN	2	3%
COLOURED (MIXED ANCESTRY)	49	78%
GENDER (MALE:FEMALE)	12:51	19:81

Forty-seven patients were reviewed clinically, for the purposes of this study and the five deceased patients' records were included in the analysis of the results (n=52).

At the time of splenectomy, 75% (n=39) had not responded to treatment with corticosteroids alone; 19.2% (n=11) of the patients had a combination of corticosteroids and intravenous immune globulin given when one or the other had failed to restore the platelet counts to 150 X 10^9/L or higher; 5.8% (n=3) were receiving azathioprine;

TABLE 2: (n=52) Characteristics of patients reviewed at 5 yrs and beyond post splenectomy for ITP.

PATIENTS REVIEWED IN THE STUDY	
TOTAL NUMBER	52
NUMBER REVIEWED CLINICALLY	47
NUMBER DECEASED	5
GENDER: MALE: FEMALE	10 : 42
AGE AT DIAGNOSIS (YRS)	31.5 ± 14.7
AGE AT SPLENECTOMY (YRS)	32.8 ± 14.0
TIME FROM DIAGNOSIS TO SPLENECTOMY (WEEKS)	35.7 ± 64.3
AGE AT REVIEW (YRS)	38.7 ± 10.8
AGE AT DEATH (YRS)	62.8 ± 20.2

*Plus-minus values are means ± SD (standard deviation)

2. Clinical Review

2.1 Patients diagnosed initially in Childhood. (n = 5) (Table 4).

Five patients were seen initially as cases of paediatric ITP who did not proceed in the usual way of childhood ITP towards spontaneous resolution.

Mean age at diagnosis was 11 ± 2.1 years and mean age at splenectomy was 21 ± 6.0 years.

TABLE 3: (n=5) Characteristic of patients who were diagnosed with autoimmune thrombocytopenic purpura in childhood and treated with splenectomy as adults.

PATIENT NO	MALE/FEMALE	AGE AT DIAGNOSIS (YRS)	AGE AT SPLENECTOMY (YRS)	RESPONSE TO SPLENECTOMY
1	F	13	30	YES *
2	F	11	23	YES - SUSTAINED
3	F	10	13	YES - SUSTAINED
4	M	13	19	YES - SUSTAINED
5	M	8	21	YES - SUSTAINED

Initial response with relapse at 5 months but responded promptly to further immunosuppression.

2.2 Patients diagnosed and treated in adulthood. (n = 42)

2.2.1 Immediate post operative complications. (Table 4)

Eight patients (15%) experienced some morbidity as a result of their splenectomy. Two patients had both poor wound healing and incisional hernias. Mortality related to the procedure was zero.

TABLE 4: n = 52 (15.4%) Number of patients with immediate post operative complications

COMPLICATION/MORBIDTY n = 52	NO. OF PATIENTS AFFECTED	(%)
1. SUB-PHRENIC ABSCESS	3	(5.8)
2. POOR WOUND HEALING	3	(5.8)
3. INCISIONAL HERNIA	2	(3.8)

2.2.2 Patients who relapsed post splenectomy but recovered spontaneously or with further immunosuppression (n = 6) (Table 5). These patients required immunusuppression for varying lengths of time but at review were noted to be in complete remission, off all therapy. Results are shown in table 5.

The response to splenectomy is usually immediate and indeed this was noted in all but one patient.

TABLE 5: (n = 6) Characteristics of patients requiring further immunosuppression post-splenectomy and resulting in complete remission

PATIENT GENDER	TIME TO RELAPSE IN MONTHS	TYPES OF IMMUNO-SUPPRESSION USED	TIME TO RESPONSE AND WITHDRAWAL OF IMMUNO-SUPPRESSION (MONTHS)	OTHER MEDICAL CONDITIONS/ COMPLICATIONS
FEMALE	0	VINCRISTINE AZATHIOPRINE PREDNISOLONE HIGH DOSE DECADRON	72	AZATHIOPRINE INDUCED HEPATITIS VINCRISTINE RELATED PERIPHERAL NEUROPATHY LISTERIA MONOCYTOGENES SEPTCAEMIA
FEMALE	60	PREDNISOLONE CYCLOPHOSPHAMIDE	6	THYROTOXICOSIS REQUIRING I^{131} TREATMENT 4 YRS LATER
FEMALE	2	AZATHIOPRINE	22	EPILEPSY SLE MITRAL VALVE REPLACEMENT MEMBRANOUS NEPHRITIS
FEMALE	7	NIL	11	HYPERTENSION DEPRESSION
FEMALE	0	PREDNISOLONE IV. GAMMAGLOBULIN PREDNISOLONE	6	3 ATTACKS OF TONSILLITIS
MALE	1	PREDNISOLONE AZATHIOPRINE CYCLOPHOSPHAMIDE	72	NIL

2.2.3 Patients diagnosed as having chronic ITP. (n = 4) (Table 6)

Unlike the patients described in Table 5, (who, upon review, have had a complete response, these four patients are considered to have chronic ITP. One patient maintains a normal platelet count on maintenance azathioprine and another patient has safe platelet counts (range 76 - 115) on no treatment.

Two of the four patients in this category would be considered to have chronic refractory ITP as their platelet counts are persistently less than $30 \times 10^9/L$.

TABLE 6: (n=4) Characteristics of patients who have chronic / chronic refractory ITP following splenectomy.

PATIENT GENDER	AGE AT DIAGNOSES (YEARS)	TREATMENTS USED	OTHER MEDICAL CONDITIONS COMPLICATIONS	CURRENT STATUS
FEMALE	48	PREDNISOLONE IV.GAMMAGLOBULIN SPLENECTOMY AZATHIOPRINE CYCLOPHOSPHAMIDE	INSULIN REQUIRING DIABETES HYPERTENSION OBESITY DRUG (STEROID) INDUCED HEPATITIS ↑CHOLESTEROL	CHRONIC ITP MAINTAINS NORMAL PLATELE COUNTS ON AZATHIOPRINE 150 MG/ DAY
MALE	38	PREDNISOLONE SPLENECTOMY	NIL	CHRONIC ITP MAINTAINS SAFE PLATELET COUNT ON NO TREATMENT
FEMALE	50	SPLENECTOMY DANAZOL PREDNISOLONE ASCORBIC ACID AZATHIOPRINE	CHRONIC VENOUS STASIS ULCERS HYPERTENSION CHRONIC SMOKING RELATED OBSTRUCTIVE PULMONARY DISEASE	CHRONIC REFRACTORY ITP ON NO THERAPY PLATELET <30
MALE	28	PREDNISOLONE SPLENECTOMY IV.GAMMAGLOBULIN VINCRISTINE VINBLASTINE	NIL	CHRONIC REFRACTORY ITP ON NO THERAPY PLATELET <30

3. Other complications/medical problems noted at review

One patient developed thrombocytosis post splenectomy. The platelet count was 907 X 10^9/L post splenectomy and nearly a year later was 594 X 10^9/L. She was also noted to be hypertensive and suffered a minor cerebrovascular accident two years post splenectomy.

Two patients were diagnosed as Type 2 diabetes (one of them steroid related) and two more were noted to be hypertensive at review.

The son of one patient developed childhood ITP aged 7 years and recovered spontaneously.

4. Pregnancy and ITP.

Eighteen patients had a total of twenty-nine pregnancies with twenty-three live births and six abortions. The diagnosis of ITP was made in the peripartum period of five (28%) of the eighteen patients. The method of delivery was normal vaginal delivery in sixteen (70%) and by caesarean section in seven (30%).

5. Deceased patients. (n = 5) (Table 3)

Five patients died during the study period. Four of these patients had other associated serious disorders including breast carcinoma, head and neck carcinoma, high grade non-Hodgkin's lymphoma and systemic lupus erythematosis.

Table 7 shows the characteristics of patients who died following splenectomy for ITP. Mean age at death was 62.8 ± 20.2(SD).

TABLE 7: Characteristics of patients who died following splenectomy for ITP. (n=5)

PATIENT GENDER	AGE AT DIAGNOSIS OF ITP	RESPONSE TO SPLENECTOMY	COMPLICATIONS	DIAGNOSIS LEADING TO DEATH	AGE AT DEATH	TIME FROM SPLENECTOMY TO DEATH
FEMALE	54	NO	UNKNOWN	UNKNOWN	62	8 YRS
FEMALE	40	YES#	CRYPTOCOCCAL MENINGITIS STAPHYLOCOCCAL SEPTICAEMIA MYELOPATHY	HIGH GRADE NON-HODGKINS LYMPHOMA	47	7 YRS
FEMALE	78	NO	GASTRO-INTESTINAL BLEEDING	HEART FAILURE BREAST CANCER	85	7 YRS
FEMALE	36	NO*	SUBPHRENIC ABSCESS E. COLI SEPTICAEMIA SYSTEMIC LUPUS ERYTHEMATOSIS PNEUMOCOCCAL SEPTICAEMIA WITH WATERHOUSE FRIDERICHSEN SYNDROME	OVERWHELMING POST SPLENECTOMY SEPSIS (OPSI)	39	3 YRS
FEMALE	72	YES	NIL	ANAPLASTIC CARCINOMA (CERVICAL NODE)	82	10 YRS

* Partial response to immunosuppression post splenectomy

Subsequent relapse

6. Laboratory Findings

The mean platelet count at diagnosis, prior to splenectomy, was 20 X 10^9/L ± 13.

The median time to splenectomy was only 13 weeks in this study (range 1 week – 313 weeks).

The median time to peak platelet count post splenectomy was 10 days (range 1-90).

Splenectomy was associated with a significantly higher MCV, higher lymphocyte count, higher platelet count and lower IgM ($p < 0.05$). (Table 8 and fig 1).

Correlation analysis showed that patients who, in the long term, continue to have a platelet count greater than 100 X 10^9/L, were younger (had splenectomy at a younger age) and took longer to get to splenectomy. Their presentation MCV was higher while the platelet count and platelet distribution width was lower. (Appendix B (iv)).

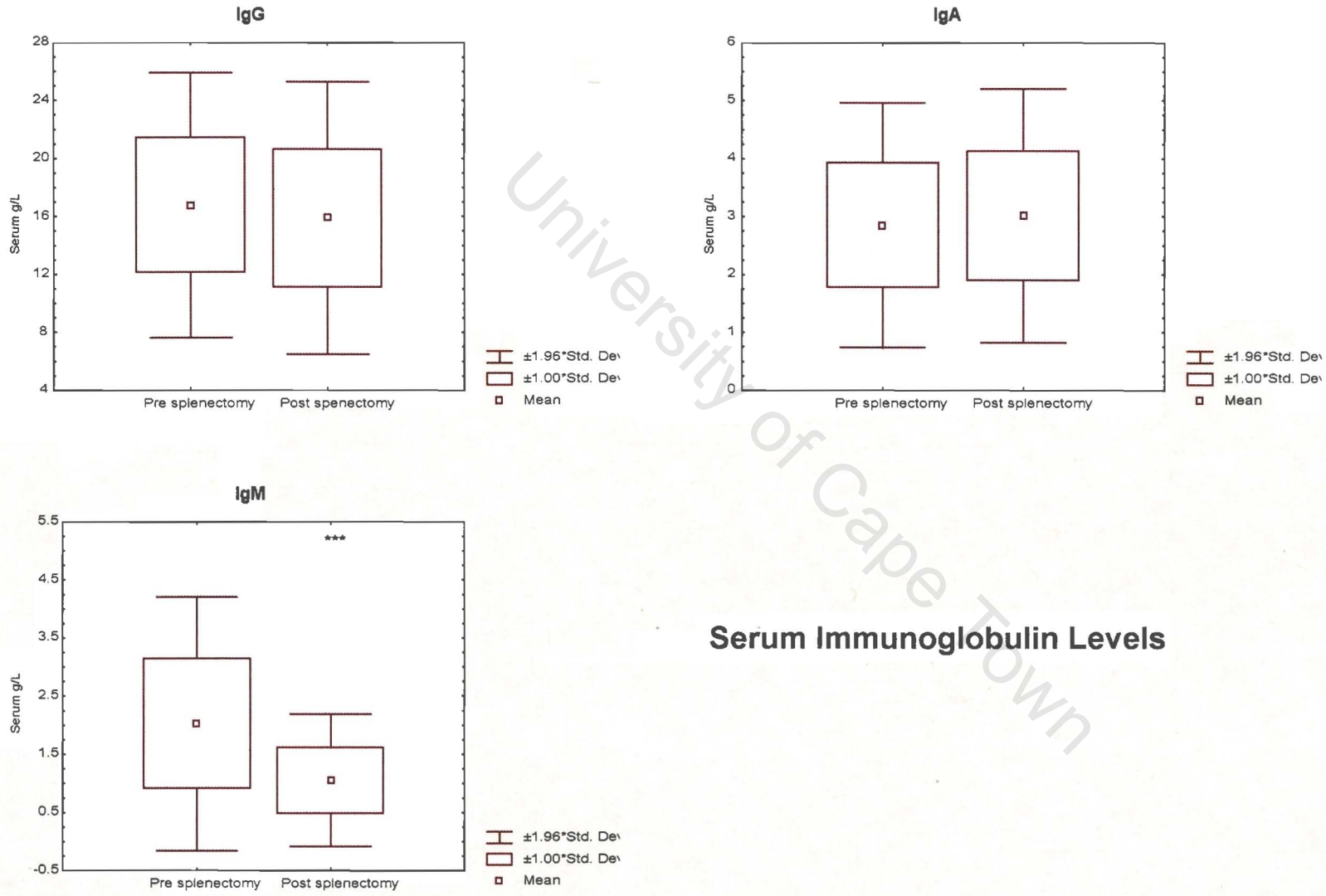
TABLE 8: Laboratory values in patients pre and post splenectomy for ITP.

LABORATORY VALUE	PRE SPLENECTOMY (n=52)	POST SPLENECTOMY (n = 47)	P VALUE
HAEMOGLOBIN Hb (g/dl)	12.7 ± 2.1	13.1 ± 1.3	
MEAN CORPUSCULAR VOLUME MCV (fl)	85.8 ± 8.0	91.4 ± 7.3	< 0.05
WHITE BLOOD COUNT WBC ($\times 10^9$ /L)	8.1 ± 3.9	8.6 ± 2.6	
LYMPHOCYTE COUNT ($\times 10^9$/L)	2.2 ± 1.1	3.1 ± 1.2	< 0.05
PLATELETS ($\times 10^9$/L)	20.1 ± 13.0	311.5 ± 111.1	< 0.05
PLATELET DISTRIBUTION WIDTH (PDW) (%)	NA	46.2 ± 6.6	
MEAN PLATELET VOLUME (MPV) (0-20 fL)	NA	8.7 ± 1.1	
IMMUNOGLOBULIN G (g/l)	16.8 ± 4.7	15.7 ± 4.8	
IMMUNOGLOBULIN A (g/l)	2.9 ± 1.1	3.0 ± 1.1	
IMMUNOGLOBULIN M (g/l)	2.1 ± 1.1	1.1 ± 0.6	< 0.05

NA = not available

*Plus-minus values are means ± SD (standard deviation)

Figure 1



Serum Immunoglobulin Levels

7. Histology of the spleen.

Forty-nine histological specimens were available for review. All but one had hyperplastic follicles of variable size. Germinal centres were not identified in twenty-seven (55%), were small in sixteen (33%) and were considered to be active and numerous in six (12%). Histiocytes were seen in all, but foamy macrophages were identified in thirty-four (69%).

8. Serum anti-pneumoccal antibody levels.

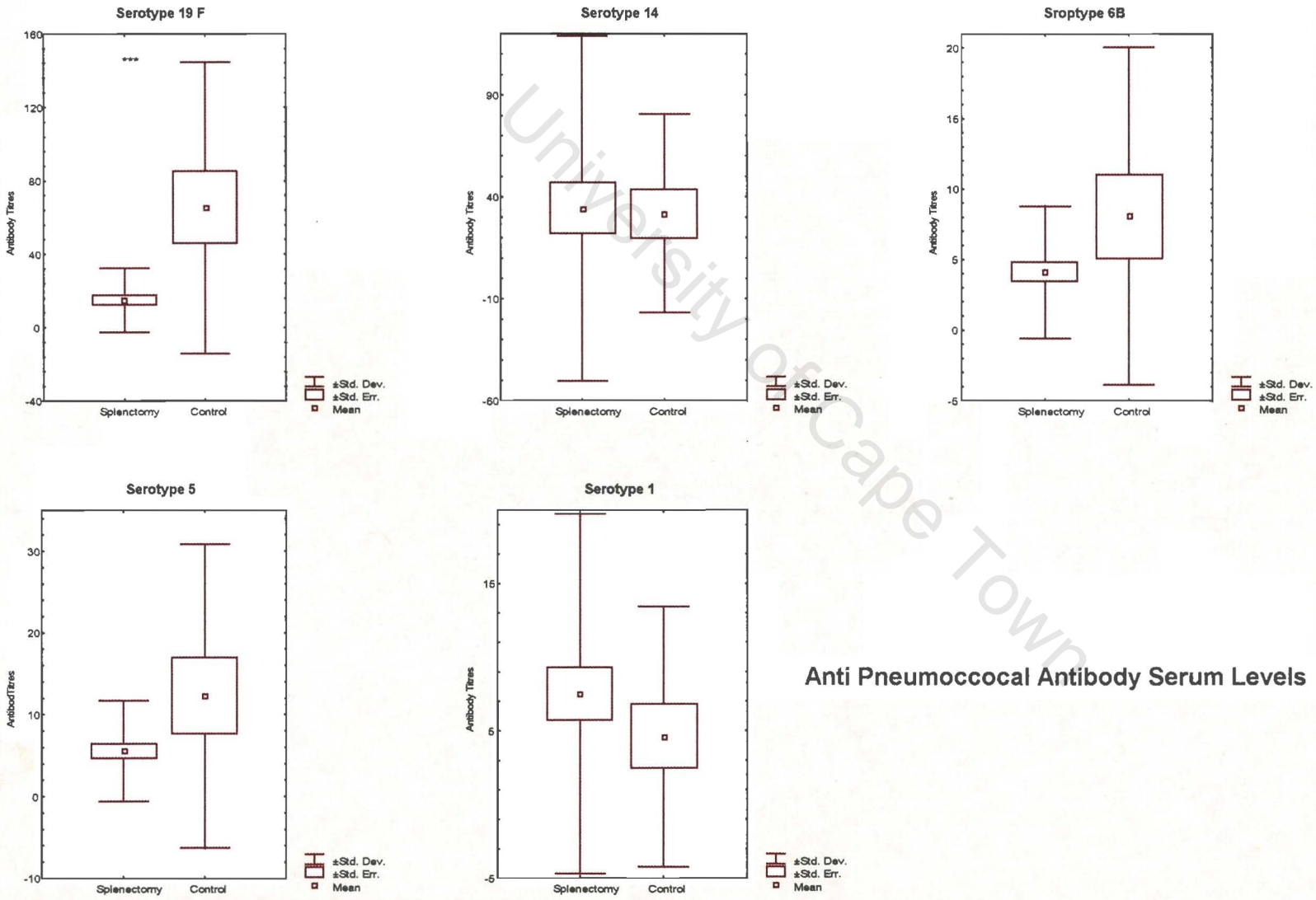
IgG antibodies to five pneumococcal serotypes (19F; 14; 6B; 5; 1) were measured. Patients treated with splenectomy for ITP had significantly lower levels of the 19F serotype antibody only ($p < 0.05$) compared with the control patients (15.07 ± 17.47 vs 65.26 ± 79.35). Table 9 and Figure 2

TABLE 9: Mean antibody levels to 5 pneumococcal serotypes comparing patients who underwent splenectomy for ITP with control patients (intact spleens). Titres expressed as micrograms per millilitres.

PNEUMOCOCCAL SEROTYPES	SPLENECTOMIZED PATIENTS (n = 46)	CONTROL PATIENTS (n = 16)
19 F	15.07 ± 17.47	65.26 ± 79.35
14	34.37 ± 84.47	32.00 ± 48.54
6 B	4.11 ± 4.68	8.10 ± 11.96
5	7.53 ± 12.20	4.60 ± 8.81
1	5.56 ± 6.14	12.29 ± 18.55

***Plus-minus values are means ± SD (standard deviation).**

Figure 2



The other four serotypes showed no significant differences in antibody titres between post splenectomy patients and control patients.

The mean age at vaccination of patients who underwent splenectomy was significantly less than that of the controls (30.5 ± 11.2 years vs 49.1 ± 14.4 years).

The time interval between vaccination and antibody testing was much shorter in the control patients (98.0 ± 24.4 months vs 1.9 ± 1.0 months) (see tables 9 and 10).

TABLE 10: *Age in years of patients tested for pneumococcal antibody levels and time interval in months between vaccination and antibody testing.*

	SPLENECTOMIZED PATIENTS	CONTROLS
AGE (YRS) PNEUMOCOCCAL VACCINE GIVEN	30.5 ± 11.2	49.1 ± 14.4
TIME INTERVAL BETWEEN VACCINATION AND ANTIBODY TESTING (MONTHS)	98.0 ± 24.4	1.9 ± 1.0

*Plus-minus values are means ± SD (standard deviation)

Other autoimmune conditions. While only patients who had ITP at the time of splenectomy were included in the study four patients were subsequently diagnosed with other autoimmune disorders (all female).

Patient no.1 (table 3), who developed ITP at age 13 years had a strong family history of autoimmune disease (mother had systemic lupus erythematosis, sister had scleroderma and a brother had polyarteritis nodosa requiring renal transplantation). She had a good response to steroid therapy at diagnosis and went into remission for 17 years. The patient re-presented with epistaxis and petechial skin haemorrhage aged 30 years, underwent splenectomy which resulted in normalisation of her platelet count, but relapsed five months post splenectomy.
Intravenous gammaglobulin was then administered followed by corticosteroids for a few months, once again resulting in complete remission. Five years later the patient was noted to have autoimmune haemolytic anaemia and required further immunosupression (cyclophosphamide and prednisolone). At the time of review she was off all immunosuppressants and had a platelet count well within the normal range.

Patient no. 3 (table 3), diagnosed at age 10 years and splenectomised age 13 years was subsequently found to have Evans syndrome (immune haemolysis of red blood cells and thrombocytopenia).

In retrospect one patient had already fulfilled criteria for SLE when the diagnosis of ITP was made. She also had other medical problems viz.:

epilepsy, hypertension, membranous nephropathy and mitral valve replacement. She required 22 months of azathioprine, at a maintenance dose of 150mg/day, post splenectomy before achieving complete remission.

Consequences of failure to provide pneumococcal vaccine pre-splenectomy. The fourth patient (included in Table 7) was diagnosed as having SLE soon after the diagnosis of ITP was made. Splenectomy was undertaken approximately one month after being diagnosed with ITP after corticosteroid therapy failed to improve her platelet count. Splenectomy did not normalise her platelet count and further immunosuppression was required.

Two months post splenectomy she presented with a subphrenic abscess with intrathoracic extension. She developed an E.coli septicaemia but responded to antibiotic therapy. She was subsequently maintained on azathioprine 100mg /d, Danazol 200mg Q.I.D and prednisolone in a variable dose. She maintained a 'safe' platelet count (range 98 - 128 X 10^9/L) for two and a half years before presenting to hospital with a two-day history of diarrhoea and vomiting and collapsed and died suddenly. Blood cultures (taken ante-mortem) confirmed streptococcus pneumoniae septicaemia and at post mortem she had features in keeping with acute adrenocortical insufficiency with bilateral adrenal haemorrhage (Waterhouse - Friderichsen syndrome). This was the only patient at review whose records showed no evidence of her having had the pneumococcal vaccine and she died of overwhelming post splenectomy sepsis (OPSI).

In the paper by Deodhar, Marshall & Barnes (112), a review of post mortem records of all patients who had had a splenectomy and died in Cornwall between 1981 & 1992 was undertaken. Of the sixty-three patients, four (6%) were diagnosed as having pneumococcal septicaemia. Six patients who died were identified as not having had pneumococcal vaccination. Time from splenectomy to death ranged from 3 years to 24 years. It was once thought that the main risk of OPSI occurred during the first few years following a splenectomy but it has been demonstrated that the risk is life long (113). A recent report in the Lancet outlines the series of unfortunate events leading to the death of a patient thirteen years post partial splenectomy, who did not receive pneumococcal vaccination (114).

Patients undergoing splenectomy have a 12.6 - fold increased risk of developing late septicaemia compared with the general population; however the incidence of this complication as well as OPSI is low (115). The use of pneumococcal vaccine, re-vaccination and prophylactic antibiotics is discussed later.

Deceased patients. Apart from the above-mentioned patient who died from OPSI the remaining four patients' deaths could not be directly attributed to ITP. One patient was diagnosed with high-grade non-Hodgkin's lymphoma six months after splenectomy and low platelet counts pre-splenectomy were more likely to be secondary to the non-Hodgkin's lymphoma. Yet another patient was diagnosed with ITP when she was having a mastectomy for carcinoma of the breast (aged 78 years) raising the possibility of thrombocytopenia secondary to a malignant process once again.

A third patient was diagnosed aged 72 years but also had a persistent mild lymphocytosis and monocytosis at the time. Repeated search for possible leukaemia/lymphoma or a collagen vascular disease proved fruitless. Three years post splenectomy the patient was discharged from follow up with a normal platelet count. Six years later she presented with an enlarged cervical node, which at biopsy showed features of an anaplastic carcinoma - she died soon thereafter. The remaining patient was diagnosed at 54 years of age and died eight years post splenectomy - cause unknown.

Interpretation of laboratory findings. The significantly higher MCV post splenectomy is unlikely to be due to drug therapy, viz. azathioprine as only five patients received the drug post splenectomy. Pre-splenectomy occult iron deficiency is also unlikely to provide a satisfactory explanation for the significantly higher MCV post splenectomy.

Higher lymphocyte counts post splenectomy may be due to loss of the reservoir function of the spleen. An analysis of the T cell and B cell subsets would have been important but as this was not done pre-splenectomy it would be difficult at this stage to interpret such an analysis. Evaluation done in HIV positive patients with ITP has shown the total blood lymphocyte count to be increased, with rises in both the CD4 and especially CD8 count post splenectomy (116)(117).

The removal of the spleen, which is an IgM producing/secreting site, explains the significantly lower IgM post splenectomy.

The role of the spleen in IgM production is evident with pneumococcal vaccination. Vaccination induces a significant IgM pneumococcal antibody response but this response is smaller in splenectomized groups (vaccine given after splenectomy) than it is in non-splenectomized individuals (118).

Conversely, the two most reliable parameters to assess the presence of functional splenic tissue are the absence of Howell Jolly bodies and normal IgM blood levels (119).

Of the 4 patients deemed to have chronic/chronic refractory ITP, one patient was shown not to have Howell Jolly bodies but the patients IgM level was low normal at 0.6 grams/L (0.5 - 3.5 grams/litre). The patient has a normal platelet count on maintenance azathioprine.

Accessory splenic tissue. The presence of accessory splenic tissue can result in a failure to respond, provide only a partial response to splenectomy or cause a delayed recurrence of thrombocytopenia. A variety of techniques can be used to identify accessory splenic tissue. Computed tomography (CT) was helpful in the past so magnetic resonance imaging (MRI) would be equally useful. Abdominal scintigraphy with autologous erythrocytes labelled with Tc -99m and opsonized with anti-D IgG (radioimmune method) has also proved to be useful in the past identifying accessory splenic tissue in twelve out of twenty eight patients (120).

Conclusions on patients who underwent splenectomy/ and required further immunusuppression. This study has confirmed that, in patients who fail to respond to splenectomy, therapy must be individualised. Search for accessory spleens has not been done previously, in patients relapsing or found to be refractory to treatment and this issue needs to be addressed for the appropriate patient(s).

Patients in the long term, who continue to have a platelet count greater than 100 X 10^{9}/L- had splenectomy at a younger age and took longer to get to splenectomy. However, mean time to splenectomy was 35.7(± 64.3) weeks in this study. Other studies quote a mean interval of about three years between the diagnosis of ITP and splenectomy (43) (121).

Splenectomy is usually prescribed by many haematologists after three to six months of follow up for unsuccessful ITP therapy. Decisions about splenectomy depend on the severity of the disease, tolerance of corticosteroids and patients preferences with regards to surgery (94). All splenectomies performed on the study population were open laparotomies. Laparoscopic splenectomy has become available in some centres worldwide and a French series quoted 120 such procedures at a single institution with no mortality and good results (122).

The value of treating asymptomatic patients is unproved (51)

As the median time to splenectomy was only 13 weeks in this study (range 1 week - 313 weeks), it is likely that a small percentage of patients may not have required splenectomy for resolution of their thrombocytopenia. Most responses to corticosteroids do, however, occur within the lst 3 weeks (123).

No correlation could be obtained between the variation in splenic histology and the clinical or laboratory feature reviewed previous observations of hyperplastic follicles in the white pulp and/or foamy macrophages in the marginal zone were confirmed (42).

Interpretation of results of pneumococcal antibody levels. Results of antibody levels to five pneumococcal serotypes showed no significant differences in antibody titres between post splenectomy patients and control patients in four serotypes, the exception being serotype 19F.

Given the fact that the common infecting serotypes are 1;2;7;12;6 and 18, serotype antibody levels can only be considered to be surrogate for immunity. A true test of immunity would require a randomised trial in vaccinated vs control patients.

It remains unclear as to what concentration of serotype specific antibodies indicates protection against pneumococcal disease. Opsonic activity and antibody avidity may be even more important determinants of protection than antibody concentration. (124).

Pneumococcal vaccination/re-vaccination. The use of pneumococcal vaccination pre-splenectomy for ITP is well established (72)(75).

Re-vaccination has been recommended by both British and American guidelines for those who have received the vaccine more than 5 years previously. Evidence for the effectiveness and need for re-vaccination is lacking and the findings in this study argue against the re-vaccination of patients splenectomized for ITP, even though they are considered to be high risk (anatomic asplenia + immunosuppression).

The guidelines recommend re-vaccination for individuals who are at risk for serious pneumococcal infection and those who are likely to have a rapid decline in pneumococcal antibody levels.

The accuracy of radioimmunoassay for determination of pneumococcal antibody levels has been questioned as the assay detects antibody directed against both capsular and cell wall polysaccharides and the latter does not appear to be protective against pneumococcal infection. (125)(126).

A meta-analysis of randomised controlled trials on the efficacy of pneumococcal vaccination (127) concluded that, "pneumococcal vaccination appears efficacious in reducing bacteraemic pneumococcal pneumonia in low risk adults. However evidence from randomised controlled trials fails to demonstrate vaccine efficacy for pneumococcal infection - related or other medical outcomes in the heterogeneous group of subjects currently labelled at high risk."

The evidence base for re-vaccination is weak. Furthermore, re-vaccination may not provide additional protection. (128).

Antibiotic prophylaxis. Our current practice is not to provide life-long antibiotic therapy. Results prove this to be a sensible policy. British recommendations are to provide life-long penicillin prophylaxis. This has proven to be impractical and there has been no clear data in adult patients of its effectiveness. Compliance is poor. Furthermore, the emergence of penicillin resistant strains of pneumococci further reduces the effectiveness of the prophylaxis.

A review in the German medical literature (129) concluded that long-term antibiotic prophylaxis is not advised in adults.

Conclusions on re-vaccination and antibiotic prophylaxis. Both re-vaccination and long-term prophylactic antibiotic therapy has not been proven to be beneficial and our current practice in South Africa, which is - not to provide both, life long antibiotics and routine re-vaccination - is justified. There is however a need for us to educate patients about the potential risk of sepsis and advise patients' general practitioners about this life long risk and the need for prompt antibiotic therapy (and referral to hospital) in asplenic patients.

CONCLUSION

Splenectomy for ITP was associated with a low procedure related morbidity and zero mortality in this series of patients.

It is the treatment of choice after relapse following immunosuppression with corticosteroids. Decisions about splenectomy depend on the severity of the disease, tolerance of corticosteroids, and the patient's preferences with regard to surgery and should be offered to patients within three to six months if more than twenty to thirty milligrams of prednisolone is required to maintain a platelet count above $30 \times 10^9/L$.

Younger patients do better than older patients in general.

This study confirms the success of splenectomy (as treatment of ITP) with over 90% of patients in complete remission, at 5 years and beyond.

A proportion of patients who initially do not respond to splenectomy, following corticosteroids, respond to other immunosuppression post splenectomy.

There was no single predictive factor for the success of splenectomy as treatment. Previous studies, as well as this one, indicate that younger patients who had an initial response with corticosteroids or gamma globulin, have shorter disease duration, (and display predominantly splenic sequestration of platelets), are more likely to have a successful outcome from splenectomy.

Treatment must be individualised.

Pneumococcal vaccination is essential pre splenectomy. At present there is not enough evidence to justify routine re-vaccination at five years post initial vaccination.

Prophylactic antibiotics appear to confer no added advantage for the prevention of post splenectomy sepsis, which remains an unpredictable lifelong risk.

The management of ITP in patients who undergo splenectomy at our institution is both appropriate and effective.

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APPENDIX A(i)

	SEX F or M	AGE @ DIAGNOSIS	AGE @ SPLENECTOMY	DATE OF DIAGNOSIS	DATE OF SPLENECTOMY	TIME IN WEEKS	AGE@ PRESENT	DEATH
1	F	25	26	05/02/1987	24/09/1987	33	37	
2	F	48	50	12/07/1990	13/10/1992	13	56	
3	F	36	36	21/04/1993	12/10/1993	25	41	
4	M	18	19	01/07/1985	29/04/1987	95	30	
5	F	31	31	21/05/1991	03/12/1991	28	38	
6	F	72	72	20/07/1987	31/08/1987	6		82
7	F	13	30	1974-13/8/91	24/09/1991	6	37	
8	F	11	17	1984-2/4/90	24/07/1990	3	35	
9	F	54	54	15/09/1990	16/10/1990	5		62
10	F	19	19	24/05/1987	07/10/1987	19	30	
11	F	38	40	28/05/1990	12/11/1991	24	47	
12	F	24	25	31/1989	20/06/1989	20	34	
13	F	30	30	18/12/1991	21/04/1992	18	37	
14	F	26	29	23/09/1987	21/08/1990	152	37	
15	F	40	40	15/04/1987	03/06/1987	7		47
16	M	21	22	1/12/87-13/5/88	28/06/1988	6	32	
17	F	40	45	19/09/1987	22/06/1992	248	51	
18	M	28	28	30/03/1990	03/07/1990	14	36	
19	F	27	27	21/08/1989	08/09/1989	3	36	
20	F	10	13	1986-18/10/89	14/11/1989	3	23	
21	F	19	19	12/02/1993	15/09/1993	31	24	
22	F	26	28	25/11/1986	29/08/1988	92	38	
23	M	32	33	30/01/1988	22/03/1988	7	43	
24	F	29	29	28/11/1990	10/12/1990	2	38	
25	F	21	22	16/01/1991	12/02/1991	4	29	
26	F	26	30	14/10/1989	20/04/1993	183	35	
27	F	19	19	05/11/1991	11/02/1992	10	26	
28	F	29	29	17/04/1989	22/05/1989	5	39	
29	F	22	22	08/06/1991	09/07/1991	4	29	
30	F	24	24	14/10/1993	30/11/1993	7	29	
31	F	78	78		09/09/1987			84
32	F	25	25	06/12/1991	31/03/1992	16	32	
33	F	51	51	04/10/1989	07/11/1989	5	60	
34	F	52	53	21/10/1991	09/06/1992	33	59	
35	F	36	36	01/07/1988	01/08/1988	4		39
36	F	33	34	10/11/1992	02/03/1993	16	39	
37	F	23	23	14/05/1990	26/06/1990	4	31	
38	F	20	20	08/09/1992	14/09/1992	1	28	
39	F	33	34	20/07/1989	24/10/1990	14	42	
40	F	27	28	16/07/1986	31/03/1987	37	39	
41	F	33	37	27/09/1983	14/10/1987	159	48	
42	M	24	24	11/02/1993	01/06/1993	15	29	
43	M	56	56	07/10/1989	07/11/1989	4	65	
44	M	56	57	05/12/1988	14/11/1989	45	65	
45	F	50	50	02/09/1987	22/09/1987	3	61	
46	M	23	24	02/11/1987	13/01/1988	10	34	
47	F	39	39	18/04/1990	03/07/1990	11	47	
48	M	9	21	1979-23/1/85	08/01/1991	313	28	
49	F	20	20	29/03/1988	14/06/1988	11	30	
50	M	16	16	22/03/1988	19/07/1988	17	26	
51	F	41	41	09/08/1993	14/12/1993	18	46	
52	F	33	33	02/04/1991	18/06/1991	11	40	

APPENDIX A(ii)

ULL BLOOD COUNT AT DIAGNOSIS

MAXIMAL PLATELET COUNT POST SPLENECTOMY

	HB	WCC	MCV	LYMPH	PLATE	
1	11.1	4.9	80	4.29	26	10/7- 447
2	12.7	7.3	85	1.46	26	6/7-228
3	14.6	9.4	92	3.57	11	8/7-800
4	14.7	12.2	85	2.56	21	22/7-456
5	13	13.5	97	4.18	16	8/7-711
6					24	Dead
7	8.3	2	70	1.28	17	15/7-423
8	11.7	6	75	1.86	8	14/7-592
9	11.8	25.4	82	4.31	43	4/7-69
10	12.1	5.1	83	2.55	46	15/7-763
11	12.7	7.8	94	2.88	48	14/7-707
12	11.3	5.9	89	2.12	3	10/7-702
13	13.9	5.5	85	1.54	29	9/7-198
14	13.9	3.9	89	1.17	17	17/7-494
15	12.8	11.3	86	0.45	17	15/7-610
16	14.3	6.8	83	0.47	12	3/7-455
17	13	5.7	89	2.16	9	23/7-224
18	14.3	4.17	81	1.87	13	3/7-206
19					4	1/7-275
20	8.6	5.9	106	1.23	16	10/7-736
21	12.4	8.6	88	1.29	10	7/7-618
22	13.6	10.3	93	3.39	8	9/7-345
23	15.9	11.5	93	1.84	24	3/12-484
24	9.2	9.5	94	2.09	9	10/7-130
25	10.8	8.8	81	0.79	10	2/7-294
26	7.9	5.2	97	1.64	8	13/7-907
27	11.6	8.8	85	3.25	24	15/7-526
28	15.5	10.3	93	2.47	10	7/7768
29	11.7	13.3	88	1.99	9	21/7-56
30	11.4	6.3	81	2.2	40	14/7-230
31						Relapse&Recovered
32	13	4.5	90	1.21	31	8/7-1058
33	15.5	4.4	87	2.11	36	15/7-492
34	13.9	9.8	85	1.66	10	15/7-699
35					37	6/52-55
36	12.8	5.8	74	1.33	48	10/7-578
37	15.2	14.2	80	2.84	6	25/7-648
38	11.4	13.5	94	3.1	10	1/7-408
39	12.4	6.85	92	1.84	44	9/7-552
40	13.2	8.7	82	4.08	15	1/12-550
41	11.5	12.8	88	3.2	47	8/7-455
42	17.5	7.2	90	2.16	10	15/7-533
43	13.1	6.9	60	2.07	9	15/7-348
44	15.9	6.3	89	2.58	30	2/7-167
45	7.7	11.2	71	4.59	23	4/7-164
46	15.5	4.3	96	1.12	22	7/7-143
47	12.6	8.6	80	4.1	13	8/7-485
48	14.4	4.3	90	0.52	8	12/7-576
49	12.8	7.3	80	2.26	8	14/7-535
50	12.6	4.7	86	1.27	17	8/7-650
51	11.8	4.5	78	1.8	14	8/7-383
52	14	7.8	81	2.1	29	8/7-520

APPENDIX A(iii)

FULL BLOOD COUNT AT REVIEW

	PLATE	MPV	PDW	HJB	HB	WCC	MCV	LYMPH
1	153	10.1	41.6		11.9	9.6	84	4.93
2	257	9.8	44.5		11	7.7	91	2.47
3	381	7.1	50		13.4	10.9	109	4.6
4	328	7.9	46.8		13.3	8.2	92	2.72
5	227	10.2	40.6		13.6	11.8	100	5.44
6	452	Dead	Dead	Dead	Dead	Dead	Dead	
7	345	9.1	42.9		10.8	4.6	92	0.71
8	435	8.7	43.8		13	8	92	2.32
9	Dead	Dead	Dead	Dead	Dead	Dead	Dead	
10	351	9.2	41.5		11.9	7.2	90	3.61
11	86	10.1	45.3		13.7	8.9	96	4.32
12	360	8.9	43.6		11.9	9.2	95	2.24
13	244	8.7	44.5		12.8	7	89	2.19
14	369	8.4	44.1		13.3	7.8	95	2.32
15	Dead	Dead	Dead	Dead	Dead	Dead	Dead	
16	349	7	51		14.1	11.7	92	3.35
17	293	9.7	42.8		15.7	10.6	88	4.22
18	25	8.9	59.5		13.6	7.3	84	2.64
19	341	8.1	46.3		14.5	7.6	93	1.95
20	437	7.9	45.5		11.9	10.4	93	3.1
21	341	7.9	46.6		14	6.3	96	2.32
22	326	9.2	42.2		12.1	8.9	92	3.17
23	281	9.8	41.5		16	9.5	95	3.65
24	248	8.4	49.7		10.8	7.5	93	1.87
25	441	9.3	42.7		12.6	5.4	92	2.09
26	509	6.5	50.6		13.4	7.6	81	3.12
27	300	7.6	47.2		11.1	6.6	91	4.14
28	498	6.4	52		13.6	12.4	87	3.58
29	300	9.1	44		14.8	7.3	94	3.76
30	147	8.9	46.8		12	7.6	88	2.7
31								
32	393	8.6	45.7		13.2	8.4	95	2.37
33	402	8.7	45.8		14.5	4.7	96	1.98
34	341	7.4	49.7		13	12.9	96	5.69
35	Dead	Dead	Dead	Dead	Dead	Dead	Dead	
36	286	8.9	45.1		13.3	6.1	85	2.19
37	436	7.3	47.5		12.9	14.8	89	3.79
38	287	10.2	41.9		12.7	7.9	93	1.8
39	278	9.8	42		11.5	8.1	96	2.65
40	410	8.6	43.4		13.5	13.3	84	4.94
41	242	9.3	47		11.7	8.8	104	4.58
42	279	10.5	37.9		14.2	12.3	91	5.22
43	475	9.3	44.2		11.1	6.8	62	1.49
44	221	10	42.4		15.1	6.3	95	2.26
45	11	7.7	81.1		13.7	10.2	95	2.42
46	332	9.4	41.5		13.9	4.4	104	2.05
47	256	10.4	39.4		12.3	14.6	79	5.9
48	291	8.7	44.4		15.2	4.4	89	1.63
49	259				13.4	9.1	85	4.13
50	336	7.4	51.5		13.9	6.2	100	1.69
51	166	9.1	49		13.1	7.1	86	3.23
52	430	7.7	49.3		11.8	11.1	90	3.82

APPENDIX A(iv)

	PRE SPLENECTOMY			POST SPLENECTOMY		
	IgG	IgA	IgM	IgG	IgA	IgM
1	18.7	3.48	0.88	17.7	4.1	0.7
2	12.7	1.95	0.58	13.3	1.3	0.6
3				13.3	2.2	0.4
4	10.98	3.04	1.31	13.2	3.5	0.7
5	12.8	2.52	2.67	14.9	1.7	1.5
6				Dead	Dead	Dead
7	24.1	4.92	1.4	17.8	3.6	0.4
8	18.7	2.63	2.34	13.6	1.9	1.2
9	15	6.75	4.17	Dead	Dead	Dead
10	16.3	3.63	1.86	18.9	6.3	0.6
11	13.2	3.11	5.21	17.8	3.6	2.4
12	20.9	2.45	2.47	17.5	2.6	1.9
13	17.7	3.47	3.36	14.8	3.3	2.3
14	24.7	3.54	0.94	19.5	3.3	0.4
15				Dead	Dead	Dead
16	12.5	1.93	1.94	17.3	3.9	0.8
17	14.2	2.37	1.01	17.2	4.3	0.7
18	15.3	2.24	1.05	17.1	2.8	0.6
19				15.9	3.2	1.8
20				10.6	1.2	0.8
21				13.1	2.8	1
22	15.9	1.22	<0,35	11.3	3.2	0.2
23				16.7	5.7	0.8
24	22.4	3.56	3.43	23.8	3.7	1.7
25	8.3	2.02	1.5	16.8	3.2	0.9
26	24.9	2.69	2.26	20.4	3.4	1.1
27	20.4	2.49	3.98	33.2	1.7	1.7
28	16.9	3.7	0.84	14.1	4.1	0.7
29	13.8	2.39	2.31	13.9	2.4	0.6
30				15.8	2.8	1.8
31						
32	13.9	1.28	2.3	13.1	1.5	1
33	10.5	2.2	1.94	9	2.4	0.7
34	28.6	3.38	1.33	22.5	3.9	0.7
35				Dead	Dead	Dead
36	19.7	3.94	2.38	15.2	3.4	1.1
37	13.9	2.72	1.46	10.3	2.8	1.9
38				12.7	1.6	1.1
39	16.2	2.18	1.3	15.4	2	0.6
40	13.2	1.69	0.71	12.9	1.8	<0.2
41	18.1	3.19	2.81	14.6	3.2	1
42	19.9	2.18	3.33	17.7	2.2	1.1
43				12.7	3.9	1.2
44				8.8	3.3	0.3
45				13	3.9	0.8
46	21.3	2.57	2.52	26.1	2.2	1.3
47				13.6	2.7	2.4
48				13.2	3.1	1.4
49	13.1	2.29	1.79	8.8	1.4	0.6
50	17.6	4.36	2.21	21.6	5.2	1.2
51				25.8	3.7	1.9
52	11.2	1.8	0.95	11.5	1.9	0.7

APPENDIX B(i)

Descriptive Statistics YY

	Valid N	Mean	Confid. -95.000%	Confid. 95.000	Sum	Minimum	Maximum	Range	Variance	Std.Dev.	Standard Error	Std.Err. Skewness	Skewness	Std.Err. Kurtosis	Kurtosis
AGE	45	29.1556	25.1926	33.1185	1312.00	9.0000	72.000	63.000	174.00	13.1908	1.96637	1.186494	.353732	1.65890	.694544
AGESx	45	30.7111	26.9398	34.4824	1382.00	13.0000	72.000	59.000	157.57	12.5528	1.87127	1.338935	.353732	1.74508	.694544
TIMESx	45	39.1778	18.8507	59.5049	1763.00	1.0000	313.000	312.000	4577.79	67.6593	10.08606	2.700751	.353732	7.18442	.694544
DEATH	1	101.0000			101.00	101.0000	101.000								
HB	43	12.8442	12.2036	13.4848	552.30	7.9000	17.500	9.600	4.33	2.0815	.31742	-.334235	.361358	.34148	.709035
WCC	43	7.6547	6.7164	8.5929	329.15	2.0000	14.200	12.200	9.29	3.0487	.46492	.599720	.361358	-.44186	.709035
MCV	43	86.1163	83.6467	88.5858	3703.00	60.0000	106.000	46.000	64.39	8.0244	1.22371	-.631959	.361358	2.03161	.709035
LYMPH	43	2.1567	1.8567	2.4568	92.74	.4700	4.290	3.820	.95	.9748	.14866	.591862	.361358	-.16605	.709035
PLATL	45	18.7556	15.0432	22.4679	844.00	3.0000	48.000	45.000	152.69	12.3567	1.84203	1.019459	.353732	.09920	.694544
MPV	43	8.7256	8.3995	9.0517	375.20	6.4000	10.500	4.100	1.12	1.0597	.16160	-.374282	.361358	-.54195	.709035
PDW	43	45.1279	44.0711	46.1847	1940.50	37.9000	52.000	14.100	11.79	3.4339	.52366	.260353	.361358	-.58777	.709035
POSTSx	44	491.8636	425.6876	558.0397	21642.00	56.0000	1058.000	1002.000	47377.84	217.6645	32.81416	.152910	.357484	-.00212	.701676
iGg	32	17.0025	15.2672	18.7378	544.08	8.3000	28.600	20.300	23.17	4.8131	.85084	.442099	.414457	-.28160	.809371
Iga	32	2.7431	2.4294	3.0568	87.78	1.2200	4.920	3.700	.76	.8701	.15382	.479815	.414457	-.04173	.809371
IgM	32	5.0034	-1.2543	11.2612	160.11	.5800	100.000	99.420	301.26	17.3567	3.06827	5.634152	.414457	31.82307	.809371
PLATNOW	45	329.6222	303.1228	356.1217	14833.00	147.0000	509.000	362.000	7779.97	88.2041	13.14869	.028961	.353732	-.38884	.694544

Descriptive Statistics NN

	Valid N	Mean	Confid. -95.000%	Confid. 95.000	Sum	Minimum	Maximum	Range	Variance	Std.Dev.	Standard Error	Std.Err. Skewness	Skewness	Std.Err. Kurtosis	Kurtosis
AGE	6	41.0000	31.0000	51.0000	246.000	28.0000	54.0000	26.0000	90.80	9.5289	3.8902	.19972	.845154	-.78305	1.740777
AGESx	6	41.3333	31.4293	51.2374	248.000	28.0000	54.0000	26.0000	89.07	9.4375	3.8528	.05584	.845154	-.61987	1.740777
TIMESx	6	9.5000	.9808	18.0192	57.000	3.0000	24.0000	21.0000	65.90	8.1179	3.3141	1.45354	.845154	1.44895	1.740777
DEATH	3	101.0000			303.000	101.0000	101.0000	0.0000	0.00	0.0000	0.0000				
HB	5	11.8600	8.7650	14.9550	59.300	7.7000	14.3000	6.6000	6.21	2.4926	1.1147	-1.50781	.912871	2.88717	2.000000
WCC	5	11.9740	1.9704	21.9776	59.870	4.1700	25.4000	21.2300	64.91	8.0566	3.6030	1.49105	.912871	2.83093	2.000000
MCV	5	82.8000	72.4338	93.1662	414.000	71.0000	94.0000	23.0000	69.70	8.3487	3.7336	-.15157	.912871	1.06702	2.000000
LYMPH	5	2.8200	.6805	4.9595	14.100	.4500	4.5900	4.1400	2.97	1.7231	.7706	-.43297	.912871	-1.31988	2.000000
PLATL	6	30.1667	14.9674	45.3660	181.000	13.0000	48.0000	35.0000	209.77	14.4833	5.9128	.03896	.845154	-2.27318	1.740777
MPV	3	8.9000	5.9190	11.8810	26.700	7.7000	10.1000	2.4000	1.44	1.2000	.6928	.00000	1.224745		
PDW	3	61.9667	17.1851	106.7483	185.900	45.3000	81.1000	35.8000	324.97	18.0270	10.4079	.60421	1.224745		
POSTSx	6	301.8333	4.1016	599.5650	1811.000	55.0000	707.0000	652.0000	80489.37	283.7065	115.8227	.84892	.845154	-1.59704	1.740777
IgG	3	14.5000	11.6786	17.3214	43.500	13.2000	15.3000	2.1000	1.29	1.1358	.6557	-1.59710	1.224745		
IgA	3	4.0333	-1.9102	9.9768	12.100	2.2400	6.7500	4.5100	5.72	2.3926	1.3814	1.47798	1.224745		
IgM	3	3.4767	-1.9013	8.8547	10.430	1.0500	5.2100	4.1600	4.69	2.1649	1.2499	-1.29334	1.224745		
VAR17	3	40.6667	-58.3982	139.7316	122.000	11.0000	86.0000	75.0000	1590.33	39.8790	23.0241	1.49501	1.224745		

Mann-Whitney U Test (multivariate2.sta)

Group 1: 100-Y Group 2: 101-N

	Rank Sum N	Rank Sum U	Z	p-level	adjusted	Z p-level	Y	Valid N N	Valid N exact p	2*1sided
AGE	1088.000	238.0000	53.0000	-2.39729	.016522	-2.39898	.016447	45	6	.014297
AGESx	1093.000	233.0000	58.0000	-2.25112	.024385	-2.25300	.024266	45	6	.022484
TIMESx	1216.000	110.0000	89.0000	1.34482	.178692	1.34638	.178191	45	6	.188424
DEATH			0.0000	0.00000	1.000000	0.00000	1.000000	1	3	0.000000
HB	1074.500	101.5000	86.5000	.70875	.478485	.70921	.478198	43	5	.490001
WCC	1018.500	157.5000	72.5000	-1.18125	.237513	-1.18157	.237385	43	5	.245850
MCV	1077.500	98.5000	83.5000	.81000	.417947	.81156	.417048	43	5	.429462
LYMPHS	1022.000	154.0000	76.0000	-1.06312	.287734	-1.06318	.287708	43	5	.305438
PLTL	1102.500	223.5000	67.5000	-1.97338	.048461	-1.97714	.048034	45	6	.046555
MPV	1005.500	75.5000	59.5000	-.22244	.823971	-.22274	.823741	43	3	.834124
PDW	965.000	116.0000	19.0000	-2.02422	.042956	-2.02466	.042911	43	3	.041370
POSTSX	1176.000	99.0000	78.0000	1.61212	.106946	1.61216	.106937	44	6	.111778
iGg	589.500	40.5000	34.5000	.79550	.426331	.79566	.426234	32	3	.445531
IgA	561.000	69.0000	33.0000	-.88388	.376766	-.88395	.376732	32	3	.411306
IgM	555.000	75.0000	27.0000	-1.23744	.215934	-1.23752	.215902	32	3	.239878
VAR17	1170.000	6.0000	0.0000	2.87494	.004044	2.87533	.004039	45	3	.000116

APPENDIX B(ii)Descriptive Statistics (results2.sta) Pre splenectomy Group 1

	Valid N	Mean	Confid. -95.000%	Confid. 95.000	Sum	Minimum	Maximum	Range	Variance	Standard Std.Dev.	Std.Err. Error	Skewness	Std.Err. Skewness	Kurtosis	Kurtosis
Hb	48	12.74167	12.12620	13.35713	611.600	7.70000	17.5000	9.8000	4.4927	2.11960	.305938	-.442732	.343149	.45546	.674397
WCC	48	8.10458	6.95743	9.25174	389.020	2.00000	25.4000	23.4000	15.6078	3.95067	.570230	1.917185	.343149	6.51662	.674397
MCV	48	85.77083		83.43844		88.10322	4117.000	60.00000		106.0000	46.0000	64.5208	8.03249	1.159390	-.561169
Lymphs	48	2.22583	1.91529	2.53638	106.840	.45000	4.5900	4.1400	1.1438	1.06949	.154368	.554858	.343149	-.31961	.674397
Platelets	51	20.09804		16.44032	23.75576		1025.000	3.00000	48.0000	45.0000	169.1302	13.00501	1.821065	.869772	.333464
MPV	46	8.73696	8.42353	9.05038	401.900	6.40000	10.5000	4.1000	1.1139	1.05543	.155615	-.356145	.350096	-.57701	.687628
PDW	46	46.22609		44.27608		48.17610	2126.400	37.90000		81.1000	43.2000	43.1189	6.56650	.968177	3.568047
IgG	35	16.78800	15.18771	18.38829	587.580	8.30000	28.6000	20.3000	21.7026	4.65860	.787447	.567648	.397694	-.06782	.777794
IgA	35	2.85371	2.48352	3.22390	99.880	1.22000	6.7500	5.5300	1.1614	1.07766	.182158	1.502744	.397694	3.90191	.777794
IgM	35	4.90114	-.85474	10.65703	171.540	.58000	101.0000	100.4200	280.7631	16.75599	2.832279		5.877586		.397694

Post-splenectomy Group 2

	Valid N	Mean	Confid. -95.000%	Confid. 95.000	Sum	Minimum	Maximum	Range	Variance	Standard Std.Dev.	Std.Err. Error	Skewness	Std.Err. Skewness	Kurtosis	Kurtosis
Hb	47	13.0809	12.7058	13.4559	614.80	10.80000	16.0000	5.2000	1.63	1.2775	.18634	.11313	.346570	-.370463	.680915
WCC	47	8.6191	7.8562	9.3821	405.10	4.40000	14.8000	10.4000	6.75	2.5986	.37905	.57310	.346570	-.131502	.680915
MCV	47	91.4468	89.3068	93.5868	4298.00	62.00000		109.0000	47.0000	53.12	7.2885	1.06314	-1.13583	.346570	5.225716
Lymphs	47	3.1353	2.7726	3.4981	147.36	.71000	5.9000	5.1900	1.53	1.2355	.18022	.50149	.346570	-.519332	.680915
Platelets	48	311.5625	279.2969		343.828	114955.00	11.00000	509.0000		498.0000		12347.44	111.1190	16.03865	-.68793
MPV	0														
PDW	0														
IgG	47	15.9149	14.5072	17.3226	748.00	8.80000	33.2000	24.4000	22.99	4.7943	.69933	1.33924	.346570	2.790145	.680915
IgA	47	3.0191	2.6916	3.3467	141.90	1.20000	6.3000	5.1000	1.24	1.1156	.16273	.64433	.346570	.773119	.680915
IgM	47	1.0532	.8831	1.2233	49.50	.20000	2.4000	2.2000	.34	.5793	.08450	.78655	.346570	-.153735	.680915
Plat post Sx50		469.0600	403.2141	534.9059	23453.00	55.00000	1058.000	1003.000	53680.87	231.6913	32.76610	.06713	.336601	-.354576	.661908

Statistical differences

Mann-Whitney U Test (results2.sta)

By variable VAR11

Group 1: 1 Group 2: 2

	Rank Sum Group 1	Rank Sum Group 2	U	Z	p-level	adjusted	p-level	Z	Valid N Group 1	Valid N Group 2	exact p	2*1sided
Hb	2199.000	2361.000	1023.000	-.78158	.434466	-.78191	.434276		48	47	.438403	
WCC	2093.500		2466.500		917.500	-1.56689	.117151	-1.56715	.117090	48	47	.117463
MCV	1798.500	2761.500	622.500	-3.76276	.000168	-3.76830	.000165		48	47	.000125	
Lymphs	1809.000	2751.000	633.000	-3.68460	.000229	-3.68477	.000229		48	47	.000178	
Platelets	1373.500	3576.500		47.500	-8.23715	.000000	-8.23944	.000000	51	48	.000000	
MPV			0.000	0.00000	1.000000	0.00000	1.000000		46	0	0.000000	
PDW			0.000	0.00000	1.000000	0.00000	1.000000		46	0	0.000000	
IgG	1555.000	1848.000	720.000	.96093	.336594	.96110	.336507		35	47	.340873	
IgA	1357.000	2046.000		727.000	-.89531	.370630	-.89558	.370484	35	47	.375146	
Plats now	1924.000	1479.000	351.000	4.420286		.000010	4.423561	.000010	35	47	.000005	

APPENDIX B(iii)

Serotypes

Descriptive Statistics (pneumoccocal.sta)
by VAR8: G_1:1

	Valid N	Mean	Confid. -95.000%	Confid. 95.000	Sum	Minimum	Maximum	Range	Variance	Standard Std.Dev.	Std.Err. Error	Skewness	Std.Err. Skewness	Kurtosis	Std.Err. Kurtosis	
19F	47	15.07674	9.946115	20.20737	708.607	1.178000	88.5650	87.3870	305.349	17.47424	2.54888	2.203696	.346570	6.03067	.680915	
14	47	34.37060	9.568648		59.17255	1615.418	.050000	480.0810	480.0310	7135.543	84.47214	12.32153	4.453846	.346570	20.51607	.680915
6B	47	4.11274	2.737542	5.48795	193.299	.127000	24.6690	24.5420	21.938	4.68376	.68320	2.371192	.346570	7.42033	.680915	
5	47	7.53113	3.946706	11.11555	353.963	.050000	59.2430	59.1930	149.037	12.20806	1.78073	2.758954	.346570	7.68116	.680915	
1	47	5.56680	3.762323	7.37127	261.639	.139000	26.8270	26.6880	37.771	6.14580	.89646	1.611011	.346570	2.37478	.680915	

Descriptive Statistics (pneumoccocal.sta)
by VAR8: G_2:2

	Valid N	Mean	Confid. -95.000%	Confid. 95.000	Sum	Minimum	Maximum	Range	Variance	Standard Std.Dev.	Std.Err. Error	Skewness	Std.Err. Skewness	Kurtosis	Std.Err. Kurtosis	
19F	16	65.26166	22.97592	107.5474	1044.187	2.782000		226.0760		223.2940		6297.346		79.35582		19.83896
1.289671	.564308	.16764	1.090774													
14	16	32.00697	6.14081	57.8731	512.112	.050000	150.8550	150.8050	2356.318	48.54192	12.13548	1.758085	.564308	1.96359	1.090774	
6B	16	8.10044	1.72491	14.4760	129.607	.040000	44.2630	44.2230	143.153	11.96467	2.99117	2.201562	.564308	5.07320	1.090774	
5	16	4.60606	-.09208	9.3042	73.697	.050000	36.5540	36.5040	77.736	8.81680	2.20420	3.571518	.564308	13.50509	1.090774	
1	16	12.29456	2.40582	22.1833	196.713	.257000	59.0450	58.7880	344.391	18.55778	4.63945	1.829753	.564308	2.12731	1.090774	

Mann-Whitney U Test (pneumoccocal2.sta)
By variable VAR8
Group 1: 1 Group 2: 2

	Rank Sum Group 1	Rank Sum Group 2	U	Z	p-level	adjusted p-level	Z Group 1	Z Group 2	Valid N Group 1	Valid N Group 2	2*1sided exact p
19F	1316.000	700.0000	188.0000	-2.96859	.002994	-2.96859	.002994	47	16	.002473	
14	1543.500	472.5000	336.5000	.62372	.532817	.62373	.532812	47	16	.536497	
6B	1476.000	540.0000	348.0000	-.44213	.658398	-.44213	.658398	47	16	.667161	
5	1588.500	427.5000	291.5000	1.33428	.182120	1.33430	.182115	47	16	.183986	
1	1443.000	573.0000	315.0000	-.96321	.335449	-.96321	.335449	47	16	.342920	

APPENDIX B(iv)

Gamma Correlations (multivariate2.sta)

MD pairwise deleted

		Valid N	Gamma	Z	p-level
VAR18	**&AGE**	**51**	**.61194**	**2.92225**	**.003475**
VAR18	**&AGESx**	**51**	**.57895**	**2.74846**	**.005988**
VAR18	&TIMESx	51	-.35659	-1.6452	.099920
VAR18	&DEATH	4			
VAR18	&Hb	48	- .20000	- .86273	.388286
VAR18	&WCC	48	.32710	1.43144	.152304
VAR18	&MCV	48	- .23077	- .99729	.318622
VAR18	&LYMPH	48	.29302	1.28371	.199243
VAR18	**&PLATEL**	**51**	**.50943**	**2.42596**	**.015268**
VAR18	&MPV	46	.08065	.27230	.785393
VAR18	**&PDW**	**46**	**.70543**	**2.44762**	**.014380**
VAR18	**&POSTSx**	**50**	**-.40909**	**-1.9468**	**.051556**
VAR18	&piGg	35	- .28421	-.95701	.338562
VAR18	&iGa	35	.31250	1.06155	.288439
VAR18	&iGm	35	.43750	1.48617	.137233
VAR18	&VAR17	48	-1.00000	-3.47455	.000512

APPENDIX C(i)

PNEUMOCOCCAL ANTIBODY TITRES-PATIENTS

	19F	14	6B	5	1
1	1.178	15.055	7.284	6.953	5.95
2	3.656	26.9665	6.95	14.466	0.65
3	5.4425	3.87	3.481	3.1	1.734
4	1.702	6.515	1.017	<0.05	0.139
5	44.585	14.0575	4.5515	36.26	16.382
6	2.99	64.047	1.185	2.018	0.707
7	52.927	56.45	3.741	3.93	5.66
8	4.537	8.5131	8.915	8.915	0.962
9	8.2895	5.5315	3.295	2.042	2.027
10	24.509	14.808	10.962	1.355	4.3855
11	17.671	7.224	1.764	1.682	1.383
12	6.373	9.186	24.669	6.786	15.453
13	8.382	4.679	0.483	32.474	4.13
14	2.613	3.631	0.684	59.243	0.3125
15	1.279	9.052	10.2145	1.577	1.577
16	2.5885	6.8605	0.95	7.653	2.1115
17	88.565	110.978	0.426	0.789	0.439
18	18.7	33.572	4.328	3.159	3.233
19	4.3265	17.6935	3.304	1.7765	1.377
20	14.503	344.428	15.498	11.914	7.048
21	26.3795	480.081	4.5	1.634	16.385
22	24.9161	11.5037	1.0125	3.785	1.3315
23	32.975	5.905	2.717	5.32	1.166
24	16.6326	18.174	4.213	3.483	6.556
25	7.366	7.555	0.835	0.636	2.91
26	29.068	22.18	0.991	0.911	0.502
27	1.712	3.94	1.066	5.834	8.134
28	12.985	12.363	0.306	1.5365	8.863
29	4.515	3.469	6.014	10.723	7.942
30	11.003	72.639	4.888	8.639	1.216
31	47.042	3.433	2.315	0.286	8.917
32	2.353	32.611	0.295	1.648	5.658
33	16.897	51.317	4.442	30.807	20.373
34	3.273	12.707	11.467	1.497	0.769
35	1.495	4.478	1.27	3.414	1.852
36	17.488	10.2485	0.918	1.54	0.739
37	9.4	13.81	1.217	0.6935	8.9055
38	5.002	6.763	6.229	0.663	26.827
39	8.456	3.4002	3.588	3.14	17.4405
40	6.002	<0.05	0.329	1.7205	11.32
41	47.415	15.888	2.2155	6.942	1.7435
42	1.218	2.883	0.127	2.091	11.3865
43	4.663	3.86	0.314	1.313	0.964
44	1.658	9.775	1.113	3.431	7.69
45	25.624	18.645	4.159	40.704	2.535
46	19.916	13.746	10.05	2.309	1.131
Mean	15.2233	35.635	4.136	7.795	5.628
Median	8.336	11.504	3.006	3.14	2.723
Range	87.387	477.192	24.54	58.457	26.688
SD	17.63	86.13	4.73	12.41	6.19

APPENDIX C(ii)

PNEUMOCOCCAL ANTIBODY TITRES - CONTROLS

CONTROL SAMPLES

	19F	14	6B	5	1
1	18.4945	19.923	1.384	1.937	59.045
2	15.166	90.07	3.963	36.554	11.438
3	209.871	0.747	16.631	1.352	1.0705
4	11.129	3.28	16.034	0.478	48.174
5	202.75	131.668	44.263	1.7845	37.455
6	226.076	7.31	0.93	4.918	3.99
7	8.802	7.647	0.975	4.439	11.63
8	2.782	5.306	8.056	0.203	0.463
9	43.561	17.793	3.44	1.1115	6.649
10	99.689	1.316	3.597	4.792	2.222
11	100.489	36.504	24.116	8.098	2.5125
12	7.611	<0.05	0.321	4	0.257
13	16.24	150.855	2.275	0.683	3.076
14	17.674	0.727	2.765	0.117	2.271
15	53.773	2.536	0.817	3.18	3.043
16	10.079	36.3795	<0.05	<0.05	3.417
Mean	65.26	34.13	8.637	4.909	12.2946
Median	18.08	7.647	3.44	1.937	3.2465
Range	223.294	150.12	43.942	36.47	58.788
SD	79.35	49.46	12.18	9.03	18.5578

APPENDIX D

PROTOCOL FOR PNEUMOCOCCAL ANTIBODY TITRE EVALUATION IN PATIENTS GIVEN PNEUMOCOCCAL VACCINE.

INTRODUCTION

Pneumococcal vaccine is recommended for:

1. Immunocompetent individuals.

2. Those who are at increased risk for pneumococcal disease or its complications because of chronic diseases e.g. Cardiovascular or pulmonary diseases, diabetes mellitus, alcoholism, liver cirrhosis, cerebrospinal fluid leaks.

2. Immunocompromised adults.

   Those who are at increased risk for pneumococcal disease or its complications e.g. Those with splenic dysfunction or anatomic asplenia, Hodgkins disease, lymphoma, leukaemia, multiple myeloma, nephrotic syndrome, chronic renal failure, organ transplantation, HIV infectionn (controversial at present), adults 65 years of age or older.

THE VACCINE AND IT'S USES.

Polyvalent pneumococcal vaccine is a sterile solution containing antigenic capsular polysaccharides extracted from streptococcus pneumoniae. Currently available polyvalent pneumococcal vaccine affords protection against the twenty-three most prevalent of invasive capsular types of streptococcus pneumoniae which reportedly account for at least 90% of all pneumococcal isolates.

The usual dose is 0.5ml and each dose contains 25 micrograms of each of the 23-polysaccharide antigens. Most healthy adults show a twofold or greater rise in antibody titre 2-3 weeks after vaccination. A theoretical protective titre of 300 micrograms/ml is obtained in more than 80% of patients.

REVACCINATION

The US Public Health Service Advisory Committee on Immunisation Practices (ACIP) does not currently recommend revaccination or routine administration of booster doses. However revaccination is to be considered for adults at highest risk who received the 23 - valent vaccine six or more years ago and for those who have exhibited a rapid decline in pneumococcal antibody levels.

British guidelines reported in the British Medical Journal (17/2/96 vol. 312 p430-434) for patients with operative splenectomy and functional hyposplenism recommend re-immunisation of asplenic patients every 5-10 years.

Furthermore lifelong prophylactic antibiotics are offered to all splenectomised patients.

USE OF THE VACCINE IN SOUTH AFRICA

Among adult patients the prime indication for the administration of pneumococcal vaccine is for those patients who are undergoing splenectomy. The vaccine has been used infrequently for any of the

other indications listed in the literature.

More recently the vaccine has been marketed in the private health care sector and patients targeted for vaccination include elderly persons greater than 65 years of age, asthmatics and those with a history of bronchitis or previous pneumonia.

PATIENTS UNDERGOING ELECTIVE SPLENECTOMY FOR IMMUNE THROMBOCYTOPENIA

This group constitutes the largest proportion of patients receiving pneumococcal vaccination (usually two weeks prior to the operation).

Revaccination is not routinely practised or recommended. Furthermore waning antibody levels, findings of varying efficacy and vaccine failure have been reported in the literature.

Limited data suggest that clinical efficacy may decline six or more years after vaccination.

RETROSPECTIVE STUDY AT THE DEPARTMENT OF HAEMATOLOGY AT GROOTE SCHUUR HOSPITAL

A retrospective study reviewing adult patients who required splenectomy for ITP is looking at (among other parameters and outcomes) the incidence of serious infection (especially life threatening pneumococcal infection). Patients included in the study were splenectomized between 1987 to 1992.

In effect these 47 patients (55 included in the study: 5 deceased) would

have received their pneumococcal vaccine more than six years ago, affording us the opportunity to check the pneumococcal antibody titres to some of the serotypes against which vaccination was provided.

METHODS

Adult patients who had splenectomy for ITP were identified from the records at the Dept. of Haematology at Groote Schuur Hospital. The fixed time period 1987 - 1992 was chosen and those who were residing in and around Cape Town were contacted. Each patient was interviewed by the attending physician, examined and had blood samples taken for full blood count, immunoglobulin levels, pneumococcal antibody titres and thrombopoietin levels.

As the pneumococcal vaccine is rarely given to any other category of adult patients in the state hospital services, control patients were obtained via the private sector. All control patients had an intact spleen and were not on long term steroid therapy.

The ITP group had a mean age of 30.5 years ± 11.2 at the time of vaccination while the control group had a mean age of 49.1 years ± 14.4. While the ITP group had received their vaccine more than six years ago the control group had received their vaccination less than months ago.

CONCLUSION

For reasons indicated above, the two groups (ITP vs control) could not be matched for age and for duration since the vaccine was given.

Nevertheless the researcher hopes to be able to conclude

(a) that the vaccine is efficacious

(b) that antibody levels may decline after six years and that revaccination may be necessary

(c) that indications for revaccination may differ in South Africa in comparison with the USA and Europe.

Appendix E

REVIEW AND RE-APPRAISAL OF PATIENTS TREATED WITH SPLENECTOMY FOR IMMUNE THROMBOCYTOPENIC PURPURA AT FIVE YEARS POST OPERATIVELY AND BEYOND.

CONSENT FORM

You are a patient who is currently attending or previously attended The Dept. of Haematology at Groote Schuur Hospital for treatment of a condition called immune thrombocytopenic purpura (ITP). As part of the treatment of this condition, you had your spleen removed, i.e. a splenectomy.

This study is reviewing patients who have had a splenectomy for ITP.

The benefits to you are that you will have a medical review to ensure that all is well or to identify any problems that you have or may have had as a consequence of your treatment. At the first visit some routine blood tests will be done and you will undergo a physical examination. Should no problems be uncovered then there will be no further follow up for the purposes of this study and you can be reassured that all is well. On the other hand, should any problems be identified you will be notified and followed up.

As previously explained it is anticipated that you will be required to attend the clinic on only one occasion. In the extremely unlikely event that there is a technical problem beyond our control you may be required to undergo the blood tests again.

If you have any questions with regard to this study please ask Dr. Seth who is the attending physician.

....

Name Date

....

Witness (1)

....

Witness (2)

Appendix F

CONSENT FORM (CONTROL PATIENTS)

We, at the Dept. of Haematology, Groote Schuur Hospital are currently busy with a study reviewing patients who have had their spleens removed as a treatment for a medical condition.

All of these patients had "Pneumovac" prior to this procedure.

You have received "Pneumovac" (in the same way that a flu vaccine is given) to help prevent pneumococcal infection. Part of our interest is to see if patients given the vaccine have adequate antibody levels (i.e. the vaccine is doing its job).

We would therefore be extremely grateful if you would oblige with a small blood sample for us to do our testing for pneumococcal antibody levels.

Should you have any queries please do not hesitate to discuss it with the attending physician, Dr.Y.S. Seth.
Thank you for your co-operation.

.....
Patient

.....
Witness

.....
Date