AUTOIMMUNE HAEMOLYTIC ANAEMIA AT CHRIS HANI BARAGWANANTH ACADEMIC HOSPITAL

A RETROSPECTIVE STUDY

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Master in Medicine

A research report submitted to the faculty of Health Sciences, University of Witwatersrand, in partial fulfillment for the degree of Master of Medicine (Internal Medicine)

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ETHICS COMMITTEE APPROVAL

This research was approved by the Ethics Committee for Research on Human subjects, University of the Witwatersrand - Clearance certificate number: M130627

DECLARATION

I, Mmuso Kgosi Mogwera declare that this research report is my own work. It is being submitted to the University of the Witwatersrand, Johannesburg, in partial fulfillment for the degree of Master of Medicine in the branch of Internal Medicine. It has not been submitted before for any degree at this University or any other University

Mmuso Kgosi Mogwera

Date:……..

DEDICATION

I dedicate this research report to my wife, Dr. Duduzile Msiza and my son

Motheo Mogwera

ABSTRACT

Background:

Autoimmune haemolytic anemia (AIHA) is a rare, acquired haemolytic anaemia, in which auto-antibodies are produced against the red blood cell surface antigens, leading to increased destruction of these antibody coated red blood cells

Aims of the study:

To describe the clinical presentation, laboratory features, treatment and outcome of patients with AIHA, seen at Chris Hani Baragwanath Academic Hospital (CHBAH), during the period 01/01/2000 to 31/12/2012 (i.e. 13 years)

Patients and Methods:

This is a retrospective study conducted at CHBAH. The demographic, clinical, laboratory, treatment and outcome data were captured and analyzed on all eligible patients with AIHA

Results:

Fifty one (51) patients with AIHA were reviewed during this 13 year period. There were 40 females and 11 males, with a female to male ratio of 3.63:1. The median age of the patients was 36 years (range 14 – 74 years).

Symptomatic anaemia was present in all the patients (100%). The mean haemoglobin at presentation was 4.84 g/dl range (1.5-10.3 g/dl) and the mean MCV (mean cell volume) was 108 fl (range 90.7-128 fl).

Jaundice was noted in 75% of the patients, while splenomegaly was evident in 29% of the patients. Fever was present in 12% of the patients.

A secondary cause was identified in 66% of the patients. The most common secondary cause was HIV (human immunodeficiency virus) infection. In the remaining 34% of the patients, the aetiology was unknown (idiopathic).

In addition to supportive measures (such as blood transfusion), corticosteroids formed the cornerstone of treatment. The increment of the haemoglobin at 3 weeks was a mean of 3 g/dl. A more rapid response was seen in primary compared to secondary AIHA.

Conclusion:

AIHA is a rare condition, which may be idiopathic (primary) or secondary. Approximately 4-5 new patients are seen each year. At CHBAH, AIHA presents at a younger age (approximately one decade earlier) with a marked female predominance. Secondary causes are more common than idiopathic AIHA, with HIV being the dominant secondary cause.

The management includes supportive measures (blood transfusion) and specific treatment modalities, such as immunosuppressives (with corticosteroids being the most commonly used agent), monoclonal antibodies and splenectomy.

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ABBREVIATIONS

AIHA: Autoimmune haemolytic anaemia

CAD: Cold agglutinin disease

cART: Combination anti-retroviral therapy

CLL: Chronic Lymphocytic Leukaemia

DAT: Direct Antiglobulin Test

Hb: Haemoglobin

HIV: Human immunodeficiency virus

LDH: Lactate dehydrogenase

MCD: Multicentric Castleman's disease

MCV: Mean cell volume

RPI: Reticulocyte production index

PRESENTATIONS

1. Mogwera M et al. Autoimmune Haemolytic Anaemia at Chris Hani Baragwanath Hospital. Oral presentation. Research day, 30th September 2015, Faculty of Health Sciences, University of the Witwatersrand.

CHAPTER 1: LITERATURE REVIEW

1. Literature review

1.1. Introduction and pathogenesis

1.1.1. Introduction

Autoimmune haemolytic anemia (AIHA) is a rare, acquired haemolytic anaemia, in which auto-antibodies are produced against the red blood cell surface antigens, leading to increased destruction of these antibody coated red blood cells. This process leads to a decreased life span of the red blood cells (from the normal life span of 120 days to as low as <15 days), with resultant anaemia [1].

The history of AIHA dates as far back as the year 1600. However, the term AIHA was first used in 1951, by Young and colleagues [2-4].

AIHA is a rare condition with an incidence of 1/100 000 per annum [1, 4,5]. There is a paucity of literature with regards to AIHA in South Africa and Africa as a whole. A study done in Nigeria suggests that AIHA is even rarer in Africans compared to other ethnic groups [6]. This may be related to the overall lower prevalence of autoimmune diseases in African populations. However, the Nigerian study concentrated on HIV associated AIHA, therefore the findings may not be a true reflection of AIHA in Africans.

Chronic autoimmune haemolytic anaemia is mainly a disease of adulthood. It is an acquired disease, without any specific predisposing factors [1]. The majority

of the patients are diagnosed in the 4th and 5th decades of life. The male to female ratio is estimated to be 1:1, 2 [1, 4-5].

1.1.2. Pathogenesis of AIHA

The mechanisms of haemolysis are different, depending on the type of antibody involved in the autoimmune process. The autoantibodies are either IgG or IgM antibodies [4, 5]. These antibodies attach to red blood cell surface antigens and form an antibody antigen complex, which can be lysed in the extra-vascular or intra-vascular space. IgG coated red blood cells are eliminated by phagocytes of the reticuloendothelial system, whereas IgM coated red blood cells are associated with both intravascular and extravascular haemolysis [3, 7].

The IgM antibodies activate the classical complement pathway, leading to c3b-mediated phagocytosis. Overwhelming complement activation is required to produce significant, clinically evident haemolysis. However, IgM coated red blood cell may also undergo extra-vascular haemolysis because the reticuloendothelial system has receptors for the C3b and iC3b complement component [1, 4]. This kind of haemolysis is driven by Kupffer cells in the liver and the degree of haemolysis depends on the type of antibody, its quantity, specificity and its ability to bind to tissue macrophages [3].

1.2. Aetiology of AIHA

AIHA is classified as idiopathic or secondary, depending on whether there is an identifiable related disease or not. Common causes of secondary AIHA are: (i) Lymphoproliferative disorders (e.g. chronic lymphocytic leukaemia/CLL), (ii) Infections (e.g. HIV), (iii) Autoimmune disease (e.g. systemic lupus erythematosus/SLE), and (iv) Drugs (e.g. alpha methyl dopa). There are other less common causes such as pregnancy. The prevalence of CLL associated AIHA ranges between 4,3% - 9% [8]. In a retrospective study done in India, it was found that 34% of patients had secondary AIHA, of which malignancy and autoimmune disease accounted for 7% and 8% respectively [5].

1.2.1. HIV associated AIHA

Anaemia is the most common haematological manifestation in HIV/AIDS patients. Anaemia is often multifactorial, and may arise from the direct or indirect effects of HIV on the haematopoietic system, the contribution of opportunistic infections such as tuberculosis, and from complicating malignancies such as lymphoma [9, 10]. Drugs used to treat HIV may also cause anaemia. Zidovudine (AZT), may cause bone marrow suppression, whereas Lamivudine (3TC) may cause pure red cell aplasia [11, 12]. Nutritional causes of anaemia are also common in HIV sero-positive patients [12]. Acute or chronic blood loss may also occur in relation to mucosal Kaposi's sarcoma and cytomegalovirus infection.

Anaemia of chronic disorder (ACD) is often contributory to the anaemia in patients living with HIV/AIDS. Other varieties of anaemia that complicate HIV include haemolytic anaemia (autoimmune and microangiopathic haemolytic anaemia), hypoplastic anaemia, pure red cell aplasia and secondary myelodysplasia.

Autoimmune haemolytic anaemia is rarely seen in HIV. It may be the presenting feature during the sero-conversion illness phase of the disease or it may manifest later in the course of the disease [13]. HIV associated AIHA occurs predominately in females between 25 and 40 years of age. This age and gender predilection may just be a reflection of the peak age of occurrence of HIV and the noticeable female predominance in HIV populations. Moreover, the causes of AIHA in HIV may be related to the HIV per se, or related to conditions that may complicate the HIV such as the lymphoproliferative disorders (non-Hodgkin lymphoma, Hodgkin lymphoma, multicentric Castleman's disease), or be coincidental, occurring with either another primary or secondary cause of AIHA [9-11, 13].

HIV sero-positive patients may have a positive Coomb's test without any other clinical or laboratory features of haemolysis, or the features of haemolysis may be overt. Rarely, these sero-positive individuals may present with severe life threatening episodes of AIHA [9]. Haemolysis is driven by an overabundance of non-specific immunoglobulins (polyclonal hypergammaglobulinaemia) and the presence of circulating immune complexes that are found in HIV infected

patients [9]. It may also be triggered by HIV associated malignancies that cause increased production of B-cells, such as lymphoma. The haemolysis that complicates HIV occurs mainly extravascularly, however, occasionally, the complement cascade maybe activated leading to intravascular hemolysis. Warm antibody, cold antibody and mixed warm and cold antibody types of AIHA have been described with HIV [9].

It is likely that HIV associated AIHA is under- diagnosed or misdiagnosed, as these patients may have other causes of anaemia co-existing with the AIHA [10]. An inadequate/inappropriate bone marrow response may result from such an interaction, or there may be reticulocytopenia, masking the typical hyperproliferative response seen and expected in AIHA.

The management of HIV associated AIHA poses a difficult challenge to clinicians because immunosuppressive drugs constitute the major therapeutic option used in AIHA. HIV/AIDS patients are already immunocompromised, and administration of immunosuppressive drugs may worsen/weaken their already suppressed immune system.

Despite the inherent risks already mentioned, corticosteroids remain the mainstay of treatment, with favourable responses seen in HIV associated AIHA. There needs to be a lower threshold and a higher index of suspicion with regard to infection in this group of doubly immunosuppressed patients, and appropriate prophylactic and therapeutic measures need to be instituted to significantly minimize this risk [9].

Use of intravenous immunoglobulin and splenectomy have also yielded good results [11]. Splenectomy is associated with a success rate of up to 75% in HIV infected patients. This favourable response was also associated with an increase in the CD4 count. However, the risk of overwhelming post splenectomy sepsis still remains an ongoing clinical concern [9, 11, 13].

1.2.2. Chronic lymphocytic leukaemia

Autoimmune cytopenias are a common feature of CLL, with AIHA being the commonest autoimmune cytopenia. About 5% of patients with CLL develop clinically relevant autoimmune haemolytic anaemia, although the Coomb's test may be positive in up to 20% of patients [15,16].

The haemolytic process is caused by loss of self-tolerance, resulting in the development of abnormal antibodies against red blood cells, although the exact mechanism is not well understood [16]. The AIHA in CLL may occur prior to the diagnosis, at the time of diagnosis or be related to the treatment used in CLL, specifically fludarabine [16]. Patients who present with AIHA complicating the CLL are more likely to have a progressive disease. The management of CLL associated AIHA is similar to that of idiopathic AIHA.

1.2.3. Systemic lupus erythematosus (SLE) associated AIHA

There are multifactorial causes for anaemia in SLE, ranging from ACD to blood loss (iron deficiency), renal disease, hypoplastic anaemia, PRCA and AIHA [17].

Fifty percent of patients with SLE will have anemia in the course of the disease, however, only 10% of SLE patients present with AIHA [18]. It is important to realize that a positive Coomb's test in SLE is not always associated with clinical features of a haemolytic anaemia, thus overestimating the diagnosis of AIHA [19].

The anti-red blood cell antibodies in SLE patients develop as a result of loss of self-tolerance. These antibodies are usually of the IgG subtype. Autoimmune haemolytic anaemia in SLE patients is associated with increased risk of the anti-phospholipid syndrome and its complications [18,19].

Treatment with steroids is effective. For those patients who present with refractory hemolysis, second line treatment should be considered. This includes immunosuppressant drugs such as azathioprine, cyclosporine, cyclophosphamide and more recently, monoclonal antibodies such as rituximab. Splenectomy should also be considered in patients with refractory SLE associated AIHA [18,19].

1.2.4. Multicentric Castleman's disease associated AIHA

Multicentric Castleman's disease (MCD) is a rare lymphoproliferative disorder, that is aetiologically linked to human herpes virus-8 (HHV-8) [20]. It is also associated with immunosuppression and is now being seen with increased frequency in association with HIV [21]. The common presenting features of

MCD include constitutional symptoms, fever, anaemia, lymphadenopathy and hepatosplenomegaly [21, 22].

Autoimmune haemolytic anaemia is an uncommon complication of MCD. However, in a recent review by Patel et al, 2015, in which 35 patients are described with MCD, AIHA was found to be present in 6 of the 35 patients [22].

Haemolysis associated with MCD may occur at any point during the course of the disease [24]. It may precede the diagnosis or be the presenting feature of MCD. The pathogenesis of this MCD associated AIHA is not clearly understood, but it is thought to be related to an increase in B-cell production (antibody production) that is found in MCD, that is related to increased levels of interleukin-6 [24]. The AIHA may also be associated with HIV or lymphoma that may complicate MCD [25].

The management of MCD associated AIHA is directed at controlling the primary disease as well as treating the haemolysis. Corticosteroids are used as first line treatment. Rituximab has been shown to confer favourable clinical responses, to both the haemolysis and MCD [26]. The anti-interleukin-6 antibody, tocilizumab has also proven to be effective in the management of both MCD and MCD associated AIHA , while splenectomy remains a treatment option for refractory AIHA [26, 27].

1.3. Diagnosis of AIHA

The diagnosis of autoimmune haemolytic anaemia requires a high index of suspicion. Commonly patients present with a triad of splenomegaly, anaemia and jaundice. However, the absence of any component of this triad does not exclude the possibility of haemolytic anaemia.

A positive Coomb's / direct antiglobulin test (DAT) is needed for confirmation of autoimmune haemolytic anaemia in a patient who presents with features of haemolysis [28]. This test detects antibody-coated red blood cells (RBC's) or complement proteins that are bound to the surface of the red cells – a blood sample is taken and the RBC's are washed and then incubated with Coomb's reagent (anti-human globulin). If agglutination occurs, this signals a positive direct Coomb's test, indicating that antibodies and/or complement are bound to the surface of RBC's.

A false negative direct antiglobulin test may occur in 10% of patients with autoimmune haemolytic anaemia [8]. These false negative tests are usually found in immunosuppressed patients, either as a result of suppression of antibodies by therapy or any other disorder that would lead to reduction in circulating immunoglobulins.

The Coomb's test may also be misleading, as it may be positive in patients who have no features of autoimmune haemolysis. This entity is common in patients

who have hyper-gammaglobulinemia. About 0.3% - 8% of hospitalised patients may have a positive Coomb's test without any features of haemolysis [8]. HIV infected patients may also have a positive Coomb's without having any symptoms or clinical signs suggestive of haemolysis.

The antibody titre of a Coomb's test cannot be used to predict the severity of haemolysis, as patients with low titres may present with severe haemolysis, whereas those with high titres may present with mild or moderate haemolysis.

A full blood count and peripheral blood smear are very important diagnostic tool when working up a patient for suspected autoimmune haemolytic anaemia. The haemoglobin is decreased, the reticulocyte count and the reticulocyte production index are increased and the mean cell volume is increased. The peripheral blood smear shows polychromasia, auto-agglutination and spherocytosis [8]. The platelet count and white cell count is usually normal.

Other supportive diagnostic tools are an increased unconjugated bilirubin, the presence of urobilinogen in the urine, a decreased haptoglobin level and an increased lactate dehygrogenase level.

1.4. Classification of AIHA

AIHA can be subdivided according to the type of antibody triggering the haemolysis. The different types are: (i) Warm antibody AIHA, (ii) Cold antibody AIHA, (iii) Mixed antibody AIHA, and (iv) Drug induced [8]. (See table 1.1)

Table 1.1 Classification of AIHA

1.Warm antibody AIHA a. Primary b. Secondary: SLE, drugs, lymphoproliferative disorders, infection
2. Cold antibody AIHA a. Cold agglutinin disease • Primary or Idiopathic • Secondary: post infectious, B cell lymphoproliferative disorders b. Paroxysmal cold haemoglobinuria
3. Mixed type AIHA a. Primary b. Secondary
4. Drug induced AIHA

1.4.1. Warm antibody AIHA

Warm antibody AIHA is characterised by the presence of IgG antibodies and accounts for approximately 70% of all the cases of AIHA [7]. The 'warm' antibodies bind to red blood cells at 37^0 Celsius (body temperature), and also have the ability to activate the compliment system. IgG antibodies are recognized by Fc receptors on various phagocytic cells (macrophages), and red blood cells that are coated with IgG antibodies, are attracted to the phagocytic cells at the Fc receptor site, opsonised and phagocytosed by these cells of the reticuloendothelial system [7].

Warm antibody AIHA may be idiopathic or may occur secondary to other conditions such as SLE, CLL, lymphoma, HIV and drugs (such as alpha methyl-dopa and fluderabine).

Drug induced warm antibody AIHA occurs due to the formation of antibodies, either against the drug itself or the red blood cell antigen. There are three mechanisms involved in drug induced AIHA: (i) drug adsorption (e.g. penicillin), (ii) immune complex formation (e.g. quinidine) and (iii) autoimmune induction (e.g. alpha methyldopa). Patients develop signs and symptoms of haemolysis post exposure to the offending drug. In drug adsorption, antibodies are directed against a drug bound to a red blood cell membrane, whereas in immune complex formation, there is formation of an immune complex between a drug and antigen antibody complex, which then causes haemolysis by attaching to the red blood cell. Some drugs induce antibody formation. The antibodies then react to the red blood cell membrane as an innocent bystander and not the offending drug. This mechanism is known as antibody/autoimmune induction.

The diagnosis of warm antibody AIHA is based on both clinical and laboratory evidence of haemolysis. The patient may present with symptomatic anaemia; fatigue, pallor, exertional dyspnoea and palpitations.

Laboratory investigations may show a macrocytic anaemia, reticulocytosis, raised unconjugated bilirubin, raised lactate dehydrogenase level and decreased haptoglobin. The Coomb's test is positive in approximately 80% of patients with warm antibody AIHA. IgG is detected in up to 66% of patients with warm antibody AIHA, and C3d accounts for only 14%. The 20% of individuals who present with a negative Coomb's test may be harbouring IgA antibodies or may have lower than detectable antibody levels [4].

Treatment of AIHA depends on the type of antibody that is present and on whether there is a detectable secondary cause or not. The management of secondary AIHA is the same as that of idiopathic AIHA, however, special attention should be given to the treatment of the underlying cause.

Treatment is aimed at achieving a haemoglobin level above 10g/dl and reducing blood product requirements.

Corticosteroids such as prednisone at a dose of 1mg/kg/day are used as first line treatment of warm antibody AIHA [17]. Corticosteroids prevent haemolysis by decreasing the synthesis of anti-RBC antibodies. They also decrease red blood cell sequestration by reducing the number of Fcy receptors on monocytes/macrophages [2, 8,17].

Approximately 80% of patients will show a response within the first 3 weeks of treatment. Those who do not show any response by the 3rd week are unlikely to respond to corticosteroids [2, 8, 18].

The dosage of corticosteroids should be slowly tapered/reduced (over a number of weeks), once the desired response is achieved. For patients who require >15mg/day of prednisone to maintain a Hb above 10g/dl (i.e. a partial response), compared to those who require <15mg/day (complete response), an alternative treatment should be considered [2, 8, 17].

Patients with HIV also show a favourable response to corticosteroids, however they should be monitored closely as they are at risk of opportunistic infections due to immunosuppression exacerbated by the therapy on the background of their compromised immune system.

Long-term use of corticosteroids is associated with side effects such as diabetes, hypertension, psychosis, osteoporosis, Cushing's syndrome and recurrent infections. Patients on long-term corticosteroids should be monitored for these complications. Calcium supplements, vitamin D and where necessary bisphosphonates should be given to reduce the risk of corticosteroid related osteoporosis. A protein pump inhibitor should also be given to minimize the risk of gastrointestinal bleeding that may occur as a complication of gastritis caused by corticosteroid use.

Splenectomy is another treatment option. It is recommended in those patients where a high dose of corticosteroids is required to maintain a response [2]. Splenectomy can be performed laparoscopically, provided that the spleen is not enlarged and there is no other surgical contraindication for this procedure. Splenectomy reduces haemolysis because the spleen is the primary site of extra-vascular red blood cell sequestration. It is effective in approximately 60% of patients with AIHA [2, 8]. Splenectomy has also been shown to be effective in patients with HIV associated AIHA, with a success rate of up to 75%. However,

these patients are at a higher risk of developing infections, particularly pneumococcal septicaemia [27].

The most feared complication of splenectomy is overwhelming post splenectomy sepsis (OPSI). This can be avoided by giving appropriate vaccination (pneumococcus, haemophilus influenza type B and meningococcus) at least 2 weeks prior to surgery, consideration of antibiotic prophylaxis in appropriate settings, and booster doses of vaccination [29].

Rituximab, an anti CD20 monoclonal antibody has been used for patients not responding to corticosteroids. It is effective in both refractory AIHA and cold antibody AIHA [30]. Rituximab acts against B cells that produce autoantibodies. The recommended dose is 375mg/m^2/week for 4 weeks [26, 30]. A study done by Barcellini et al, 2012, shows that low dose rituximab (100mg IV on day 7, 14, 21 and 28) together with a short course of prednisone is effective in AIHA, with a sustained response in 90% of patients [31].

Low dose cyclophosphamide is used for its cytotoxic effects in autoimmune haemolytic anaemia. The dose is 1,5mg -2mg/kg/day. Moyo et al, 2002, has demonstrated that high dose cyclophosphamide i.e. 50mg/kg/day for 4 days is effective and safe in refractory AIHA. Six out of the nine patients who were treated with high dosage cyclophosphamide achieved complete remission. All nine patients became transfusion independent [19].

Other modalities of treatment are plasma exchange, intravenous immunoglobulin and azathioprine [30, 33].

Supportive care is equally important. Blood transfusions are indicated in very severe (Hb <6.5 g/dl), symptomatic anaemia and other life threatening situations. Because of the significant antibody load, compatible blood may be difficult to obtain. In such situations, the least incompatible blood should be used, or washed red cells should be transfused or rarely an exchange blood transfusion may be necessary. Life threatening AIHA may be associated with mortality rates in the order of 4-11%, and use of blood transfusions in these situations is lifesaving and absolutely indicated [32].

Whole blood exchange transfusion has demonstrated rapid clinical response and reduction of mortality in patients presenting with severe life threatening AIHA. The mechanism responsible for this rapid improvement is that there is increased removal of free antibodies and IgG coated red blood cells during whole blood exchange transfusion [33].

Plasma exchange may offer short term improvement due to its ability to remove IgG or IgM plasma antibodies mediating haemolysis [33]. A single unit of plasma exchange removes approximately 60% of circulating antibodies, however, these benefits are short lived due to the continuous production of antibodies.

Intravenous immunoglobulin may also be used as a 2nd line therapy or as adjunctive therapy to corticosteroids. However, this therapy has been disappointing, with one study showing that only 14% of those treated with intravenous immunoglobulin had a haemoglobin increase of more than 2g/dl within 10 days of treatment [14]. Interestingly, intravenous immunoglobulin has shown a rapid haemoglobin improvement when used in patients with HIV associated AIHA [9].

1.4.2. Cold antibody AIHA

1.4.2.1. Cold agglutinin disease

Cold agglutinin disease is characterized by IgM antibodies, which attach to red blood cells when the patient is exposed to a cold environment. Agglutination is highest at 4^0 Celsius and is lowest at 37^0 Celsius. The binding of IgM antibodies to red blood cells activate the classical complement pathway, causing destruction of red blood cells and intravascular haemolysis in the cold extremities.

The complement is fixed through C3, and the C3 coated red cells are destroyed by macrophages in the liver. The antibody titers may range from 1:1000 to 1: 1million. Clinical features include acrocyanosis of the ears, nose and finger tips, pallor and jaundice. Laboratory features will reveal a mild to moderate anaemia with agglutination, which is more evident at lower temperatures. There is associated reticulocytosis and unconjugated hyperbilirubinaemia.

Cold agglutinin disease is also associated with mycoplasma pneumonia and infectious mononucleosis. Cold agglutinin disease is usually associated with blood type i/I, of which the blood type i is polyclonal and related to viral infections such as infectious mononucleosis. Haemolysis usually starts 2 weeks after the infection and it may last up to 6 weeks. Blood type I is related to mycoplasma pneumonia and lymphoproliferative disorders [1]. In elderly patients the most common cause of cold agglutinin disease is a B-cell lymphoproliferative disorder, accounting for approximately 90% of cases [34].

The management of patients with cold agglutinin disease starts with patient education, avoiding cold environments. The treatment of choice for these patients, in addition to appropriate supportive measures and treatment of the underlying cause, is rituximab. Seventy five percent (75%) of patients treated with rituximab achieve at least a partial remission, with complete remission being less common. A combination of rituximab and fludarabine is associated with a more favourable outcome, with approximately 21% of patients achieving complete remission [35]. Therapeutic plasma exchange may be helpful in patients presenting with severe life threatening haemolysis. If blood transfusion is needed, then blood warmers should be used to prevent precipitation of the haemolysed blood. Corticosteroids are generally ineffective in cold antibody AIHA [36].

1.4.2.2. Paroxysmal cold haemoglobinuria (PCH)

Paroxysmal cold haemoglobinuria was the first type of AIHA to be described by Johannes Acturius, in 1529. He described a condition in which the urine is "azure and livid as well as black", in patients complaining of loss of strength after exposure to a cold environment [3]. Paroxysmal cold haemoglobinuria is characterized by a biphasic antibody which attaches to red blood cells at low temperatures and causes haemolysis at 37^0 Celsius. It is usually transient.

Paroxysmal cold haemoglobinuria was typically seen in association with syphilis. However, PCH is now more common in paediatric patients than in adults, related to childhood viral infections. Patients present with fever, jaundice, pallor and haemoglobinuria. The haemolysis in PCH is usually intermittent [37]. These symptoms are usually preceded by an episode of a viral or upper respiratory tract infection. The haemolytic process in PCH is typically biphasic as it first requires the cold phase whereby antibody attaches to the red blood cell antigen and activates the complement cascade at temperatures lower than 20^0 Celsius, then the second phase (warm phase), whereby complement mediated lysis occurs [4].

Typically the highest temperature allowing antibody binding in PCH is usually 20^0 Celsius. However, in rare instances binding may occur at temperatures above 20^0 Celsius [37]. This type of PCH is considered to be monophasic in nature.

The diagnosis of PCH is based on the demonstration of the Donath-Landsteiner antibody, together with associated haemolysis. The Donath-Landsteiner antibody is a polyclonal antibody which is directed at the P- antigen of the red blood cell membrane. It activates the complement cascade and causes perforation of the red blood cell, leading to intra-vascular haemolysis [34]. Patients with PCH are usually stable and asymptomatic. They may experience intermittent episodes of severe haemolysis precipitated by exposure to cold environments or infection. Haemolysis usually occurs 4 to 10 hours post exposure to cold environments. Corticosteroids are not effective in cold antibody AIHA. The principles of management are similar to that of cold agglutinin disease.

1.4.3. Mixed or Combined AIHA

This type of AIHA consists of both cold and warm antibodies and may be related to lymphoproliferative disorders or infections such as HIV. The Coomb's test is positive for both IgG and C3b. The warm antibody is the usual cause of clinically evident disease. Patients with mixed AIHA present with severe haemolysis but show a favourable clinical response to corticosteroids and rituximab [36, 39].

1.5. Management of AIHA

Treatment of AIHA depends on the type of antibody involved and on whether there is a detectable secondary cause or not. The management of secondary AIHA is the same as that of idiopathic AIHA, however, special attention should

be given to the underlying cause. Treatment is aimed at achieving a haemoglobin level above 10g/dl and reducing blood product requirements. Corticosteroids such as prednisone at a dose of 1mg/kg/day are used as first line treatment of AIHA, particularly of warm and mixed antibody types [33]. Corticosteroids prevent haemolysis by decreasing the synthesis of anti-red blood cell antibodies. They also decrease red blood cell sequestration by reducing the number of Fcy receptors on monocytes/macrophages.

Approximately 80% of patients will show a favourable response within the first 3 weeks of treatment. Those who do not show any response by the 3rd week are unlikely to respond to corticosteroids [30].

The dosage of corticosteroids should be slowly reduced (over a number of weeks), once the desired response is achieved. For patients who require more than 15mg/day of prednisone to maintain a Hb above 10g/dl, an alternative treatment should be considered [33].

Second line treatments include rituximab, other immunosuppressive therapies such as cyclophosphamide, azathioprine, mycophenolate mofetil, danazol and splenectomy.

For cold antibody haemolytic anaemia, avoidance of cold environments, treatment of the underlying cause, and rituximab have proven to be beneficial.

Supportive therapy in the form of a blood transfusion should always be considered where appropriately indicated.

There is limited evidence on the role and effectiveness of haematopoeitic stem cell transplantion in the treatment of AIHA [30].

Table 1.2 Common side effects of drugs used in the treatment of AIHA

DRUG	Side effect
Prednisone	Acute psychosis Cushing's syndrome Osteopenia & osteoporosis Atrophic gastritis Hypertension Diabetes Increased risk of infections Hypokalemia
Rituximab	Fever and chills Anaphylactic reaction Pruritus, rash, arthralgia B-cell depletion, with increased risk of infections and activation of latent infection such as hepatitis viruses and JC polyoma virus
Azathioprine	Hepatotoxicity Bone marrow suppression Vomiting & diarrhoea Macrocytic anaemia
Danazol	Hirsutism Intracranial hypertension Thromboembolism Depression
Intravenous immunoglobulin	Generalised body pain Headache Aseptic meningitis

Chapter 2: Aims and objectives of the study

2. Problem statement / justification of the study

2.1. Introduction

AIHA is a chronic condition which requires long-term management. The current management is based on observational studies done mostly in developed countries.

There are no South African studies that describe the demographics and clinical presentation of patients with AIHA. It is likely that the profile of patients with AIHA is somewhat different in South Africa, and these potential differences need to be defined and better characterized. Therefore, a study of this nature is relevant and needs to be undertaken.

This current study is an attempt to fill this gap and serve as a pilot study from which other studies, particularly those of a prospective nature may be performed. The findings of this study will also help characterize AIHA in the local South African context and potential areas of relevant future research.

2.2. Aims of the study

The aim of the study is to determine the clinical profile of AIHA, as seen in adult patients who were diagnosed and treated at the Clinical Haematology unit, Department of Medicine, Chris Hani Baragwanath Academic Hospital (CHBAH).

2.2.1. Objectives of the study

1. To describe the demographic profile of patients presenting with AIHA at CHBAH.
2. To describe the clinical presentation of AIHA at CHBAH
3. To determine the causes of AIHA in adults at CHBAH.
4. To study and compare the treatment response, and the outcome in primary and secondary AIHA.

2.2.2. Study design

This was a retrospective study of all AIHA patients seen in the Clinical Haematology unit, Department of Medicine at Chris Hani Baragwanath Academic Hospital, during the period 01/01/2000 to 31/12/2012.

2.2.3. Population and setting

Chris Hani Baragwanath Academic hospital is situated in Soweto, south of Johannesburg, South Africa. It is a tertiary teaching hospital attached to the University of the Witwatersrand. The hospital serves as a referral centre for primary and secondary level health facilities in the central and southern parts of the Gauteng province. It also serves neighbouring provinces such as the North West province and parts of Mpumalanga.

The Clinical Haematology unit is a specialised division of the Department of Internal Medicine. During the study period, approximately 300 new patients were seen per annum with both benign and malignant disorders.

2.2.4. Sampling and sample size

The researcher used the patient statistic database of the division to identify potential patients with AIHA seen between the year 2000 and 2012. Sixty - two patient files with AIHA were identified. Six patients were excluded from the study as their records could not be found. A further 4 patients were also excluded due to insufficient records, and 1 patient was excluded because of an incorrect diagnosis. During the study period the Clinical haematology unit attended to 3100 new patients, with haematological diseases.

A diagnosis of AIHA was made, based on the relevant clinical and laboratory features detailed in the literature review.

2.2.5. Inclusion criteria:

- Adult patients, ≥ 14 years of age (definition based on the hospital criteria to admit patients to the adult wards)
- Confirmed AIHA (includes both primary and secondary AIHA), defined as: an acquired autoimmune disease characterised by production of auto antibodies against red cell surface antigens, leading to increased destruction of red blood cells.

2.2.6. Exclusion criteria:

- Patients with congenital haemolytic anaemia
- Coomb's negative haemolytic anaemia

- Coomb's positive patients without clinical and laboratory features of AIHA

2.3. Data collection

Data was collected retrospectively by the researcher. The researcher retrieved patients' folders from the Clinical Haematology unit using the list derived from the statistical database. Files of patients diagnosed with AIHA between 01/01/2000 and 31/12/2012 were reviewed. Permission was obtained from the head of the Clinical Haematology unit, Head of the Department of Internal Medicine and hospital management. A data sheet was used to collect information such as patient demographics, clinical presentation, investigations and management (see appendix A, p43).

2.4. Data analysis

Data was captured and stored in Microsoft excel. Data was then imported to a statistical package (STATA version 12). Different methods of data analysis were used. Categorical data were expressed in proportions and percentages. Mean and standard deviation was used for parametric data if normally distributed, however if the distribution was skewed, median and range was used. The multiple analysis of variance method was used to analyse the treatment response.

2.5. Ethics approval

The study proposal was approved by the Human Research Ethical Committee (HREC) of the University of Witwatersrand. Approval no: M130627

Chapter 3: Results

3.1 Demographics

Data was obtained from 51 eligible patients, diagnosed with AIHA, at the Clinical Haematology unit, CHBAH over a period of 13 years. The mean age of these patients was 36 years (range: 14-74 years). Forty patients (40/51 -78%) were female, and 11/51 (22%) were male. The female to male ratio was 3:1. (see figure 3.1 and table 3.1)

Table 3.1 Age at presentation of patients with AIHA

Descriptive Statistics						
	N	Minimum	Maximum	Mean	Std. Deviation	Median
Age	51	14	74	36.06	12.819	35

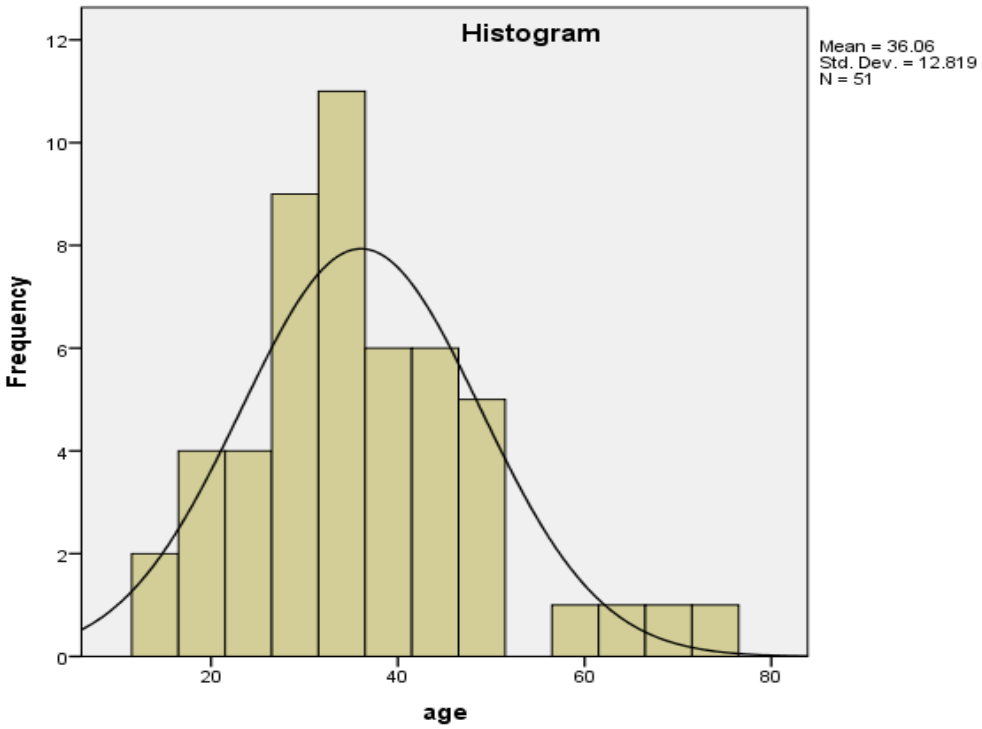

Figure 3.1 Histogram showing age distribution of patients with AIHA

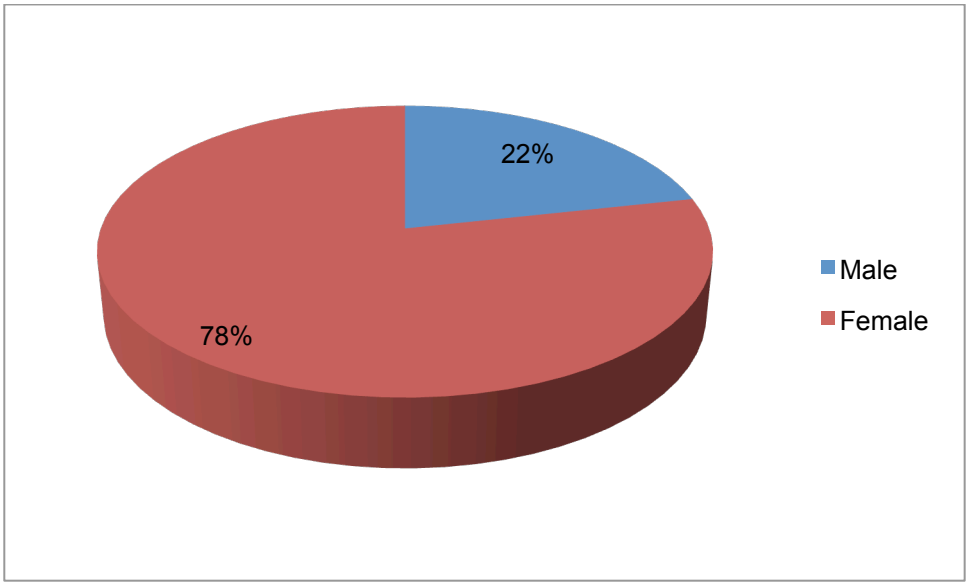

Figure 3.2 Gender distribution of patients with AIHA

3.2 Clinical presentation

All patients presented with symptomatic anaemia (100%). Ninety percent (90%) of patients were reported to have fatigue. Jaundice was noted in 75% of the patients. Splenomegaly was found on clinical examination and confirmed by ultrasonography in 29% of the patients, and 26% had hepatomegaly. Eighteen percent (18%) of the patients presented with bleeding manifestations (petechiae, purpura, ecchymoses or active mucosal bleeding). Fever was documented in 6 patients (12%).

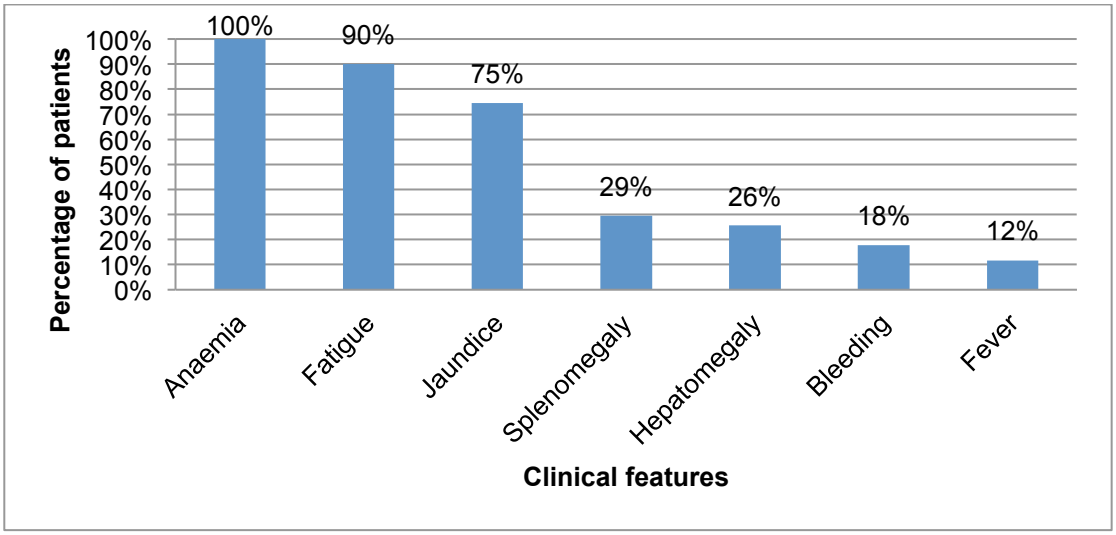

Figure 3.3 Clinical features of patients with AIHA

3.3 Laboratory features

The mean haemoglobin (Hb) at presentation was 4.8 g/dl (range: 1.5–10.3). The mean MCV was increased at 108 fl (range: 65–151). The mean total bilirubin was 54.5 umol/l (range: 8-151) and the mean unconjugated bilirubin was 38.3 umol/l (range: 2-123). The reticulocyte production index (RPI) was recorded in 41 patients. The mean RPI was 3.4 (range: 0-12.3). The mean LDH was 1538 U/L (range: 166–6140).

Table 3.2 Laboratory features of patients with AIHA

	N	Minimum	Maximum	Mean	Std. Deviation
Haemoglobin (Hb) at presentation	51	1.50	10.30	4.84	1.772
MCV	50	65	151.00	108.44	25.237
Platelets	51	10.90	515.00	249.19	142.526
Total bilirubin	46	8.00	151.00	54.54	34.747
Conjugated bilirubin	46	3.00	83.00	16.28	13.546
Unconjugated bilirubin	46	2.00	123.00	38.33	28.813
RPI	41	0	12.3	3.47	2.4
LDH	42	166.00	6140.00	1538.55	1420.247
Haptoglobin	36	0.10	3.90	0.44	0.714

Table 3.3 Coomb's test in patients with AIHA

Coomb's test (n=51)	Positive	51	100%
	Negative	0	0%

All patients had a positive Coomb's test. C3d, IgG and IgM were also recorded. Forty three of the 46 patients (93%) who had records for IgG were positive and 41/41 patients (100%) that had records for IgM were negative. Twenty five of the 44 patients (57%) had both C3d and IgG results, were positive for both C3d and IgG positivity. The results are shown in table 3.4 below.

Table 3.4 Antibody and complement subtypes in AIHA

	Results	Number	Percent
C3d only (n=44)	Positive	2	4.5%
Warm antibody,IgG (n=46)	Positive	43	93%
IgM (n=51)	Positive	0	0%
C3d & IgG (n=44)	Positive	25	57%

3.4 Aetiology and classification of AIHA

Thirty four percent (34% - 18/51) of the patients had primary or idiopathic AIHA, while sixty-six percent (66% -33/51) of all the patients had an underlying secondary cause of AIHA. Human immunodeficiency virus infection (HIV) was the most common secondary cause (29/33 - 88%), followed by SLE (2/33 – 6%), and CLL (1/33 – 3%) and Hodgkin lymphoma (1/33 -3%) (see figure 3.4 and table 3.5 below).

Two of the patients with HIV (7%) had Multicentric Castleman's Disease (MCD), while 5/29 (18%) had concomitant Tuberculosis, either pulmonary or disseminated and 2/29 (7%) had Kaposi Sarcoma (see table 3.6).

Evans syndrome (i.e. AIHA together with autoimmune thrombocytopenia) was present in (7/51 - 14%) of the patients.

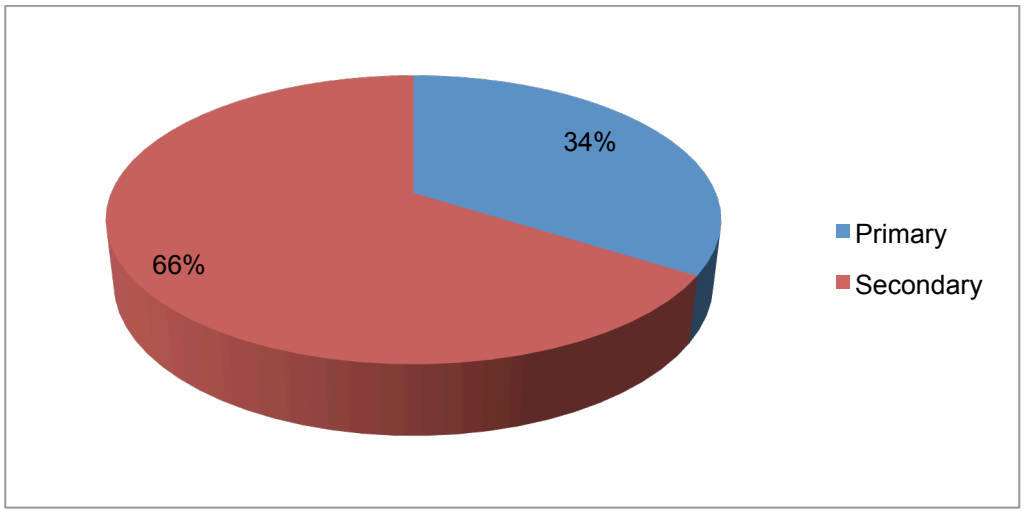

Figure 3.4 Causes of AIHA

Table 3.5 Secondary causes of AIHA

	Frequency	Percent
HIV (n=33)	29	88%
SLE (n=33)	2	6%
CLL (n=33)	1	3%
Hodgkin lymphoma (n=33)	1	3%

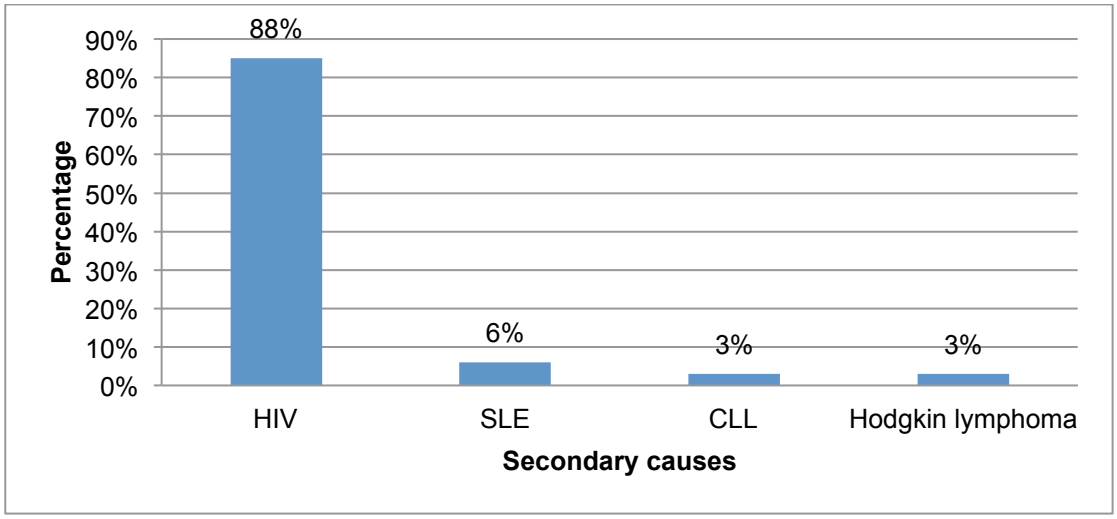

Figure 3.5 Secondary causes of AIHA

Table 3.6 HIV related AIHA

	Frequency (n=29)	Percentage
HIV only	20	69%
HIV &TB	5	17%
HIV& Kaposi Sarcoma	2	7%
HIV& Multicentric Castleman's Disease	2	7%

3.5 Treatment

All patients were treated with prednisone as first line treatment. Azathioprine was used on 13 patients (25%) who needed either prolonged use of high dose corticosteroids or who did not show any clinical response by the end of the 3rd week. Six patients (12%) received cyclophosphamide and 3 patients (6%) underwent a splenectomy.

Two patients (4%), received cyclosporine, and 1 patient (2%) was treated with rituximab for refractory AIHA.

Table 3.7 Treatment administered

(n=51)		Frequency	Percent
Prednisone	Yes	51	100%
Azathioprine	Yes	13	25%
Cyclophosphamide	Yes	6	12%
Splenectomy	Yes	3	6%
Cyclosporin	Yes	2	4%
Rituximab	Yes	1	2%
CHOEP	Yes	1	2%
ABVDP; SCT	Yes	1	2%
IVIG	No	0	0%

Forty one (41) of the fifty one (51) patients i.e. 80% had a presenting Hb of less than 65 g/dl and this was deemed clinically significant requiring blood transfusion. (see figure 3.6).

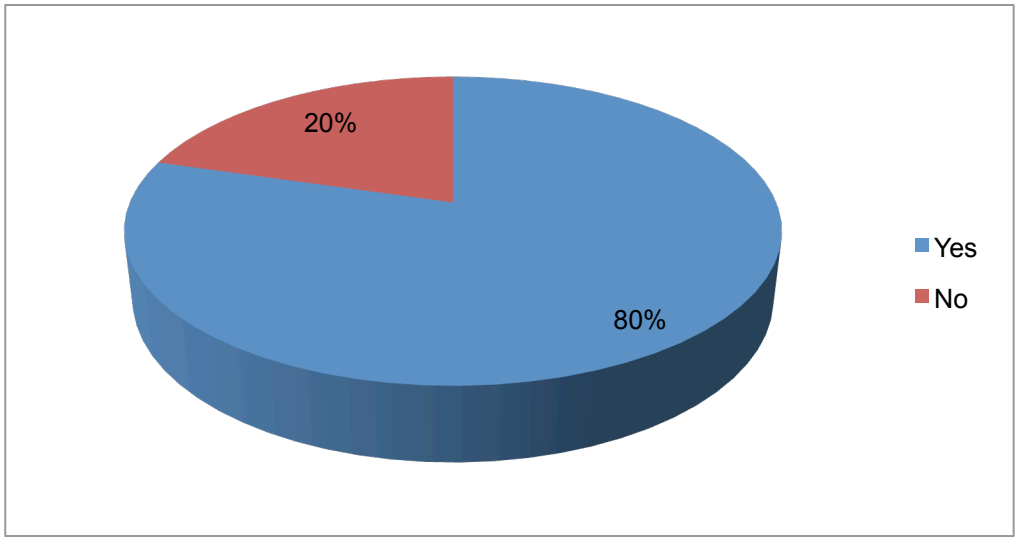

Figure 3.6 Percentage of patients with AIHA requiring blood transfusion

3.6 Treatment response

Haemoglobin was measured during in-patient care and at 3 weeks to assess the response to first line treatment. The mean Hb at 3 weeks was 8.6 g/dl, reflecting a mean increase in Hb of 3,7g/dl. Six patients (6/51 – 11%) had a haemoglobin above 10g/dl after 3 weeks of treatment.

Table 3.8 Time to remission in patients with AIHA

	Minimum	Maximum	Mean	Std. Deviation
Time to partial remission (wks)	2	104	14.43	26.428
Time to complete remission (wks)	4	364	44.78	72.107

The mean time to partial remission (Hb >10 g/dl and requiring >15 mg/day of prednisone) was 14.43 weeks (range: 2-104 weeks). The mean time to complete remission (Hb >10 g/dl and requiring <15 mg/day of prednisone) was 44.78 weeks (range: 4-364 weeks). The mean time to remission for patients with secondary AIHA was 52.55 weeks compared to 39.44 weeks for patients with primary AIHA. However, this difference was not statistically significant (p-value=0.652) (see figure 3.7).

Seventy-eight percent of the patients (40/51-78%) were recorded to be alive (based on the findings at the last documented clinic follow up). Twenty three patients (59%) were in partial remission and 16 (41%) were in complete remission.

Twelve percent of the patients (6/51 – 12%) were lost to follow up and 10 percent (5/51 -10%) died. The cause of death in the 5 patients included HIV associated opportunistic infections in 3 patients and it was unclear/unknown in the other 2 patients.

Table 3.9 Treatment outcomes

N=51	Frequency	Percent
Alive	40	78%
Partial remission	23	59%
Complete remission	16	41%
Lost to follow up	6	12%
Demised	5	10%

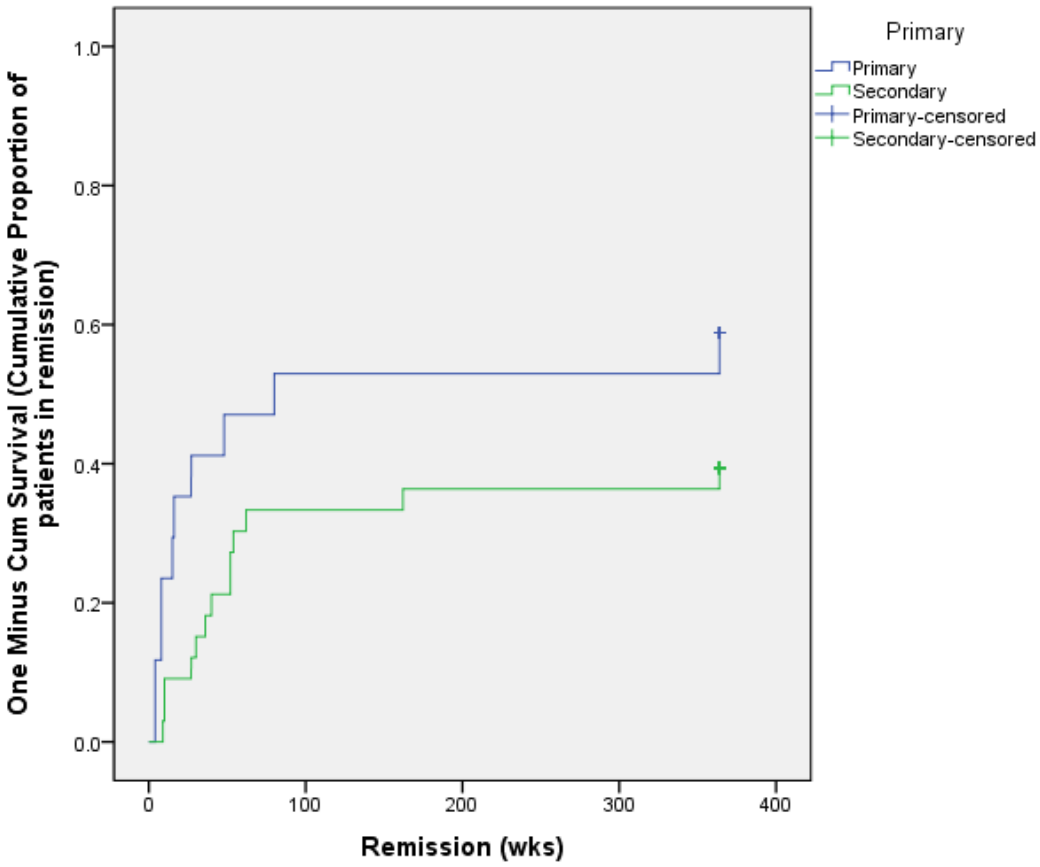

Figure 3.7 Time to remission in primary AIHA versus secondary AIHA

Chapter 4: Discussion

Autoimmune haemolytic anaemia is a rare condition in both the developing and developed world. A total of 62 adult patients were recorded with a diagnosis of AIHA at the Clinical Haematology unit, Department of Medicine, at CHBAH, over a 13 year period (01/01/2000 to 01/01/2012). This translates to an average of 4-5 new patients per year. Of these patients, only 51 were eligible for the study.

Furthermore, in this study, only patients with clinical evidence of AIHA were included, thus excluding a significant group of patients who were Coomb's positive, but without clinical evidence of AIHA.

The aetiology of AIHA in our patients was similar to that described in the literature [8]. However, in our study, secondary AIHA (66%) was more common than primary AIHA (34%) and the major contributor to secondary AIHA was HIV (88% of secondary AIHA).

Unlike in the Western world where CLL and autoimmune disease account for the majority of cases with secondary AIHA, HIV was the dominant cause of secondary AIHA in our patient population [8]. This is likely to be due to the high seroprevalence rate of HIV in our population impacting on the disease occurrence locally. The haemolysis in HIV is thought to be triggered by the increased concentration of immunoglobulin G antibodies which is part of the polyclonal hyperglobulinaemia seen in patients with this infection. These

antibodies react with red blood cell antigens, resulting in a positive Coomb's test in 20-40% of infected individuals. However, only a small number of patients develop clinically evident haemolysis [9].

The average age at presentation of AIHA is between 40 and 50 years, with a slight female predominance [4]. However, our study showed that the majority of patients were diagnosed during the 3rd and 4th decades of life, which is similar to the age distribution found in an Indian study by Naithani et al, 2006 [5]. The younger age and more marked female predominance is likely to reflect the major contribution made by HIV, which has a peak frequency in the 3rd and 4th decade and a female preponderance. Additionally, the age structure of patients in Africa is approximately a decade younger than in the Western world.

The clinical presentation of our patients was similar to that reported in the literature, with the classical triad of features of anaemia, jaundice and splenomegaly. Anaemia is the dominant feature, occurring in all the patients. However, the mean Hb of 4.84 g/dl was much lower than that which is generally encountered in AIHA, suggesting a more advanced stage at presentation, late presentations, or the contribution of multifactorial aetiologies to the anaemia.

The laboratory features of haemolysis are similar to that described in the literature. Of note is that there were no striking leucocyte abnormalities in the patients and a concomitant autoimmune thrombocytopenia (i.e. Evans syndrome) was present in 7/51 (14%) of the patients. Five of the seven patients

with Evans syndrome had HIV, 1 patient had SLE and in 1 patient the aetiology was unknown (idiopathic).

Human immunodeficiency virus infection was present in 29/33 (88%) of the patients with secondary AIHA. In the HIV positive subgroup of patients with AIHA, only 3 patients had a CD4 count above 350 cell/ml^3. However, there was no statistically significant correlation between the CD4 count and the severity of anaemia (p value=0.088). The mean CD4 count was 193.40 cells/ml^3. Eighty percent of the HIV seropositive individuals were already receiving cART at the time of the diagnosis of AIHA (with a mean of 10 months on therapy).

All the patients were treated with prednisone as a first line treatment. Treatment response was monitored by evaluating the haemoglobin level serially and importantly at 3 weeks. This evaluation revealed a mean haemoglobin increment of 3,7g/dl at 3 weeks. Even with this good incremental response at 3 weeks, only 6/51 patients (11%), had a haemoglobin above 10g/dl. This suggests that our patients present with much lower Hb levels, which may take longer than 3 weeks to show a rise above 10g/dl.

Azathioprine was used on 13 patients (25%) who needed either prolonged use of high dose corticosteroids or did not show any clinical response by the end of the 3rd week. Other immunosuppressives were less commonly used.

Three patients (6%) underwent a splenectomy. No post splenectomy complications were recorded. Rituximab was used in 1 patient (2%). One patient had an autologous stem cell transplant, which was not indicated for the AIHA, but rather for the primary disease (Hodgkin lymphoma).

The mean time to partial remission was 14 weeks, and primary AIHA patients had a shorter time to remission as compared to those with secondary AIHA. The mean time to remission for patients with primary AIHA was 39 weeks, and that of secondary AIHA was 52 weeks. However, this difference was not statistically significant (p value= 0.652).

Seventy-eight percent of the patients (40/51-78%) were recorded to be alive (based on the findings at the last documented clinic follow up). Twenty three patients (59%) were in partial remission and 16 (41%) were in complete remission.

Twelve percent of the patients (6/51 – 12%) were lost to follow up and 10 percent (5/51 -10%) died. The cause of death in the 5 patients included HIV associated opportunistic infections in 3 patients and it was unclear/unknown in the other 2 patients.

Chapter 5: Conclusion

5.1 Conclusion

Autoimmune haemolytic anaemia remains a rare condition. In our setting, AIHA is more commonly secondary rather than primary. The most common secondary cause is HIV. Autoimmune haemolytic anaemia is more common in females than males, and occurs at a younger age in our patient population.

The clinical and laboratory presentation is similar to that described in the literature, with anaemia, jaundice and splenomegaly being the dominant features.

Patients with AIHA show a favourable response to supportive treatment and immunosuppressive therapy. Corticosteroids (prednisone) is the mainstay of specific therapy, and was the initial treatment used in all our patients. In view of the very severe anaemia in our patients, the achievement of the Hb endpoints took longer. The time to remission was also longer in secondary compared to primary AIHA, however, the difference was not statistically significantly different.

5.2 Limitations of the study

- Due to the retrospective nature of the study, some of the data was missing, insufficient or incomplete
- Twelve percent of the patients were lost to follow up
- The study was limited to Chris Hani Baragwanath Academic Hospital. As such, the data may not be entirely representative of the findings

regarding AIHA in South Africa. This also has a bearing on the small number of patients in the study

- The study excluded patients who were Coomb's positive but had no clinical and other laboratory evidence of AIHA
- As this study only included patients with AIHA at their initial presentation, the diagnosis of AIHA may have been missed if it became apparent or occurred later in the course of the disease (as may be seen in CLL, other lymphoproliferative disorders and SLE)

Recommendations:

- Prospective study to evaluate the clinicopathological presentation and follow up of patients suspected of having an AIHA
- Prospective study to evaluate the percentage and risk of patients with a positive Coomb's test to develop other clinicopathological evidence of AIHA (specifically with regard to chronic disease such as HIV, SLE and CLL)
- Prospective, randomised control trials to evaluate the clinical and laboratory response to different treatment modalities, after incorporating patients from different treatment centres, in order to have a sufficient number of patients

Appendix A: Data collection sheet

Data sheet

Study allocation number:

Age:

Gender:

Date of first consult

Main complaint:

	yes	no	Duration	Details
Fatigue, dizziness, weakness etc.				
Bleeding				
Fever				
Weight loss				
Night sweats				
Jaundice				
Urine discolouration				
Other				

Other relevant medical history:

Drug history (name of drug, dose, duration, route of administration): mark with X

Penicillin/ampicillin		
Alpha methyldopa		
Rifampicin		
Fludarabine		
Quinidine		

Other:

Family history:

Clinical examination (if yes, provide details)

Pallor: yes/ no

Acrocyanosis: yes/no

Jaundice: yes/ no

Lymphadenopathy yes/ no

Petechiae/purpura yes/no

Hepatomegaly yes/ no

Splenomegaly yes/ no

Signs of CCF yes/ no

Haematuria

Other relevant features:

Diagnosis:

Treatment given: (name, dosage and duration)

Blood and blood product transfusion (type of product, number of units transfused, duration)

Surgery (e.g. splenectomy)

Response to treatment:

Complete or partial remission

Relapse: yes/no

Treatment of relapse disease:

Duration before relapse

Investigations

	Initial presentation	Best response after Rx	Last follow up
Hb			
MCV			
MCH			
WCC			
Platelets			
Neutrophil			

Lymphocytes			
Basophils			
Monocytes			
Eosinophils			
Peripheral smear			
Reticulocytes count - % and corrected reticulocyte count %			
RPI			
Total bilirubin			
Conjugated bilirubin			
Unconjugated bilirubin			
Albumin			
Total protein			
ALP			
GGT			
AST			
ALT			
Coomb's test (warm/cold/C3d)			
CD4 count			
Viral load			
Immunoglobulins			
LDH			
Antinuclear antibody			

Rheumatoid factor			
Sodium			
Potassium			
Urea			
Creatinine			
Vitamin B12			
Ferritin/iron studies			
Red cell folate			
Haptoglobin			
Urine(Haematuria/ haemoglubinuria/haemosiderinuria)			
Other			

Bone marrow aspirate and trephine report:

Imaging (where performed, e.g. CXR, abdominal sonar, CT scan):

Other relevant investigations (e.g. lymph node biopsy/ flow cytometry):

M130627

R14/49 Dr Mmuso Kgosi Mogwera

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M130627

NAME: (Principal Investigator) Dr Mmuso Kgosi Mogwera

DEPARTMENT: Internal Medicine
Faculty of Health Sciences, Wits

PROJECT TITLE: Autoimmune Haemolytic Anaemia at Chris Hani Baragwanath Academic Hospital

DATE CONSIDERED: 28/06/2013

DECISION: Approved unconditionally

CONDITIONS:

SUPERVISOR: Prof Moosa Patel

APPROVED BY:
Professor PE Cleaton-Jones, Chairperson, HREC (Medical)

DATE OF APPROVAL: 10/07/2013

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

DECLARATION OF INVESTIGATORS

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

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

Principal Investigator Signature Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

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