APLASTIC ANAEMIA AT CHRIS HANI BARAGWANATH ACADEMIC HOSPITAL

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ETHICS COMMITTEE APPROVAL

This research was approved by the Human Research Ethics Committee (Medical), University of the Witwatersrand (Protocol Ref no: M130867).

DECLARATION

I declare that this dissertation is my own unaided work. It is being submitted for the degree of Master of Medicine to the University of the Witwatersrand. It has not been submitted before for any degree or examination at this or any other University.

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Muhammed Faadil Waja

DEDICATION

To my parents, brothers and family

ABSTRACT

Aplastic (hypoplastic) anaemia (AA) is a rare condition that is characterised by pancytopenia in the peripheral blood, a hypocellular/acellular bone marrow and the absence of an abnormal infiltrate. Aplastic anaemia is classified as congenital/inherited and acquired. Acquired AA can be subdivided into idiopathic (primary) which is the most common form and secondary, depending on whether there is an identifiable cause. Inherited bone marrow failure syndromes are rare.

Clinical and laboratory data implicate immune-mediated mechanisms in the pathogenesis of AA. Clinically AA manifests with features of bone marrow failure. Allogeneic haematopoietic stem cell transplantion is a potentially curable treatment in patients with severe AA who are candidates for transplantation. Immunosuppressive therapy with ATG as the backbone is effective in 60-80% of patients.

The aim of this study was to document the demographic profile, clinical features, treatment modalities and outcome of patients diagnosed with AA who presented to the Clinical Haematology Unit at Chris Hani Baragwanath Academic Hospital and to compare it with studies done nationally and internationally. There is a paucity of studies on aplastic anaemia in South Africa.

The data of patients diagnosed with aplastic/hypoplastic anaemia at the Clinical Haematology Unit of Chris Hani Baragwanath Academic Hospital were retrospectively reviewed and analysed.

The majority of patients had idiopathic acquired aplastic anaemia (82%). The median age at presentation was 24.5 years and although a second peak occurring after 60 years is reported in the literature, there was no clear second peak in our patients. The male-to-female ratio was

1.7:1. The clinical presentation is similar to that reported in the literature with the most common presenting features being anaemia and bleeding. Infection was less common.

The majority (69.9%) of patients had severe aplastic anaemia. The most common secondary cause was HIV accounting for 12% of patients. The clinical presentation was similar in both the HIV seropositive and HIV seronegative patients.

Inherited bone marrow failure syndromes are rare. Two patients (2%) were confirmed genotypically to have Fanconi Anaemia.

Allogeneic HLA-identical sibling stem cell transplantation is associated with a 75-90% probability of long-term cure. Most patients are candidates for a stem cell transplant but are excluded based on a lack of HLA compatibility. In this cohort, 2 patients underwent HLA-identical haemopoietic stem cell transplantation with both achieving a complete response.

Immunosuppressive therapy was given to the majority of patients. The overall response rate was 60.6% which largely reflects the response to immunosuppressive therapy as only 2 patients underwent a stem cell transplant, and is similar to that reported in the literature. Less severe aplasia was associated with a better response. There were 3 (3%) patients who transformed to acute myeloid leukemia, 5 (5%) who developed haemolytic paroxysmal nocturnal haemoglobinuria and none who transformed to a solid malignancy. A significant number of patients (45%) were lost to long-term follow up.

The 5 year survival probability was 69.2%. In studies from outside of South Africa, survival is reported to be 75-80% following immunosuppressive therapy. In the HIV positive patients, the 5-year survival probability was statistically significantly worse (37.5%) as compared to the HIV negative population (78.5%). There was a trend towards an inferior response (less

than partial or no response versus partial or complete response) in the HIV positive patients but this was not statistically significant.

Survival was significantly associated with response to treatment. Patients who responded to treatment had a 5-year survival probability of 88.9% compared to 17.3% non-responders (p=0.0001). The leading cause of mortality was sepsis.

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To the patients whose data has been used in this study.

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1. AML	Acute myeloid leukemia
2. ANF	Antinuclear factor
3. ATG	Antithymocyte globulin
4. AA	Aplastic anaemia
5. BRCA	Breast cancer
6. CD	Cluster of differentiation
7. CAMT	Congenital amegakaryocytic thrombocytopenia
8. CMV	Cytomegalovirus
9. DNA	Deoxyribonucleic acid
10. DIC	Disseminated intravascular coagulation
11. DC	Dyskeratosis congenita
12. EBV	Epstein Barr virus
13. FA	Fanconi anaemia
14. GPI	Glycophosphatidylinositol
15. GVHD	Graft versus host disease
16. G-CSF	Granulocyte colony stimulating factor
17. HSCT	Haematopoietic stem cell transplant
18. Hb	Haemoglobin
19. HIV	Human immunodeficiency virus
20.HLA	Human leukocyte antigen
21. IST	Immunosuppressive therapy
22. IBMFS	Inherited bone marrow failure syndromes

23. INF	Interferon
24. LRTI	Lower respiratory tract infection
25. MMF	Mycophenolate mofetil
26. MDS	Myelodysplastic syndrome
27. NHP2	Gene name encoding H/ACA ribonucleoprotein subunit 2
28. NOP10	Gene name for the H/ACA ribonucleoprotein complex subunit Nop10
29. PNH	Paroxysmal nocturnal haemoglobinuria
30. PIGA	Phosphatidylinositol glycan class A
31. RPI	Reticulocyte production index
32. SDS	Shwachman- Diamond syndrome
33. SCT	Stem cell transplant
34. SLE	Systemic lupus erythematosus
35. TERC	Telomerase RNA component
36. TERT	Telomerase reverse transcriptase
37. TB	Tuberculosis
38. TNF	Tumour necrosis factor
39.WHO	World Health Organisation

CHAPTER 1

LITERATURE REVIEW

Aplastic anaemia (AA) is defined as a pancytopenia with a hypocellular bone marrow in the absence of an abnormal infiltrate or marrow fibrosis. The diagnosis of aplastic anaemia requires at least two of the following: (a) haemoglobin <10g/dl, (b) neutrophil count of < $1.5x10^9$/l and (c) platelet count < $100x10^9$/l. The severity of AA is assessed by the full blood count and bone marrow parameters according to the modified Camitta criteria (Camitta et al, 1975).

The incidence of AA is 2-3/ million population per year in Europe, but is higher in East Asia. There is a biphasic distribution with the first peak at 10-25 years and the second over 60 years. There is no significant difference in incidence between males and females (Marsh et al, 2009; Montane et al, 2008; Young et al 2008; Issaragrisil et al, 2006).

Aplastic anaemia is classified as: i) primary- congenital (inherited) and acquired, idiopathic and ii) secondary (to a number of known causes). The majority (70 – 80%) of patients have acquired idiopathic AA (Marsh et al, 2009).

The inherited bone marrow failure syndromes (IBMFS) account for up to 15 – 20% of AA, the commonest being Fanconi anaemia (FA). Other IBMFS include Dyskeratosis congenita (DC), Shwachman-Diamond syndrome (SDS) and Congenital amegakaryocytic thrombocytopenia (CAMT). Secondary causes of bone marrow hypoplasia include: post viral infections (e.g. viral hepatitis, cytomegalovirus (CMV), Epstein-Barr virus (EBV), human immunodeficiency virus (HIV), parvovirus B19, drug induced (e.g. chloramphenicol, anti-epileptics, anti-thyroid drugs, chemotherapy, etc.), radiation, toxins (e.g. solvents, benzene, pesticides, etc.), autoimmune disorders (e.g. SLE), paroxysmal nocturnal haemoglobinuria

(PNH), pregnancy and malignancies such as AML and hypocellular myelodysplastic syndrome (MDS) (Issaragrissil et al, 2006; Parker et al, 2005; Brown et al, 1997).

The pathogenesis of acquired aplastic anaemia is unclear, although a number of potential mechanisms have been postulated. Bone marrow failure results from an insult to the haematopoietic cell compartment. On morphology, the bone marrow is replaced by fat. There is a significant reduction in CD34 positive cells and in vitro assays have suggested that the stem cell pool is greatly diminished in severe disease. Qualitative abnormalities such as shortened telomere length and a limited number of functional stem cells have also been implicated. Intrinsic stem cell defects are present in IBMFS. Aplastic anaemia does not appear to result from a defect in the bone marrow microenvironment as it can be cured by haematopietic stem cell transplantation suggesting that the defect is in the stem cell compartment (the "seed" rather than the "soil") (Young, 2005).

The most widely accepted aetiopathogenetic mechanism of AA is that of an immune phenomenon in which cytotoxic T-cells are implicated. The evidence for this is that some patients with AA who were being prepared for stem cell transplantation with antithymocyte globulin (ATG) showed recovery in their marrow function (Young et al, 2006). Today ATG forms the backbone of immunosuppressive therapy for AA. Laboratory data also support an important role for the immune system in AA. The removal of T-cells from the bone marrow of patients with AA was associated with improved colony formation in vitro. Numerous cytotoxic T cells are found in AA and immunosuppressive therapy results in their reduction (Young, 2005). Cytotoxic T-cells produce cytokines like TNF alpha and INF gamma that targets self and inhibit the differentiation of the haemopoietic stem cells, inducing apoptosis. This ultimately results in critical loss of quantity and quality of the self-renewing haematopoietic multipotent stem cell compartment leading to bone marrow failure (Young et al, 2006).

Patients with aplastic anaemia most commonly present with features of bone marrow failure: symptoms and signs of anaemia, bleeding and infection. There is no lymphadenopathy or hepatosplenomegaly (Gordan-Smith et al, 1991). Bleeding is the most common presenting symptom. Patients present with easy bruising, gum bleeding, epistaxis, menorrhagia, petechiae, purpura and ecchymosis. There is also a risk of intracranial haemorrhage and retinal bleeds due to the thrombocytopenia. Symptoms of anaemia are frequent, and include fatigue, weakness, dyspnoea and palpitations. Infection is less commonly the presenting feature. Clinical findings are confined to the haematologic system unless there is a secondary cause for the AA such as systemic lupus erythematosis, HIV, etc.

The clinical evaluation of aplastic anaemia has to include looking for features of IBMFS. This is essential for the appropriate management, education and genetic counselling. A family history of blood disorders, consanguinity, malignancy or congenital abnormalities may indicate the presence of an inherited bone marrow failure syndrome.

The IBMFS have features unique to each syndrome. The physical findings in FA include short stature, pigmentary skin changes (hyper/hypo pigmentation), Fanconi "facies" including microcephaly, small eyes, epicanthal folds and abnormal positioning of the ears, and skeletal abnormalities mainly affecting the thumb and radius. Cardiac and renal anomalies (pelvic and horseshoe kidneys) also occur. Some patients with FA may have no apparent clinical abnormalities. FA is inherited in an autosomal recessive/X-linked recessive manner and mutations have been found in at least 15 genes. There is an increased risk of MDS and acute myeloid leukemia (AML), as well as hepatic tumors and squamous cell carcinoma of the head and neck in these patients. The median age is 6.5 years with an equal male to female ratio (Shimamura et al, 2010). Chromosomes in FA are particularly susceptible to DNA cross-linking agents which forms the basis for a diagnostic assay. The FA genes play a role in the cellular response to DNA damage. Mutations in the FA genes leads to impairment in the

Fanconi anaemia- breast cancer (FA-BRCA) pathway, abnormal handling of oxidative stress, aberrant activation of mitogen activated protein kinases and defective telomere maintenance. The net impact of this is increased genomic instability and altered cell survival.

A major cause of mortality in FA is bone marrow failure. Androgens such as oxymetholone and corticosteroids like prednisone improve haematopoietic function. Oxymetholone produces haematological responses in 50-70% of patients but its effects decrease over time. Side effects of oxymetholone include virilisation, liver dysfunction, hepatic tumors and peliosis hepatis. Haematopoietic stem cell transplant (HSCT) from a Human leucocyte antigen (HLA) -identical sibling donor is the treatment of choice. Reduced intensity conditioning regimens are used due to the hypersensitivity of FA cells. Long-term follow up of patients surviving HSCT show a high incidence of malignancies. The reasons for this include the inherent susceptibility to develop malignancy in FA patients and the use of radiotherapy as part of the conditioning. Hence conditioning regimens without radiotherapy are preferred. Gene therapy for the treatment of FA is still being explored (Dokal, 2011).

Dyskeratosis congenita has the diagnostic triad of nail dystrophy, reticular skin pigmentation and oral leucoplakia. These features may remain absent in a subset of patients. Its inheritance is autosomal dominant, autosomal recessive and X-linked. Like FA, there is an increased propensity to develop MDS/AML and solid tumors. Patients with DC are also predisposed to the development of pulmonary fibrosis. The median age is 14 years (Shimamura et al, 2010). Telomeres are very short in patients with dyskeratosis congenita due to germline mutations in telomere biology genes. In the majority of patients the telomere lengths are below the first percentile for age (Blanche et al, 2015). Telomerase is an RNA-protein complex that is important in maintaining telomere length after cell division. Some of the components of this complex include dyskerin, TERC, TERT, NHP2, NOP10. Mutations occurring in the

components of the telomerase complex and shelterin complex, as occurs in different subtypes of DC, result in telomere shortening.

Improvement in haematopoietic function can be achieved in some patients with oxymetholone and growth factors (granulocyte colony stimulating factor [G-CSF] and erythropoietin). Their effectiveness is transient and the main treatment is allogeneic HSCT, which is the only curative treatment. As there is a predisposition to develop pulmonary fibrosis, drugs that are toxic to the pulmonary system should be avoided (Dokal, 2011).

Shwachman Diamond Syndrome is characterised by neutropenia, pancytopenia, and exocrine pancreatic failure. It is inherited as an autosomal recessive disorder. There is an equal male to female ratio and an increased propensity to develop MDS/AML (Shimamura et al, 2010). Somatic abnormalities associated with SDS include short stature, protuberant abdomen, ichthyotic skin rash, hypertelorism, syndactyly, cleft palate and skin pigmentation. Neutropenia is the commonest cytopenia. The SBDS gene mutation is present in > 90% of patients with SDS. It is thought to play a role in ribosomal biogenesis and ribonucleic acid metabolism.

Oral pancreatic enzymes are used to treat the malabsorption as a result of the pancreatic insufficiency. G-CSF may produce an improvement in the neutrophil count and the anaemia and thrombocytopenia may respond to oxymetholone. Allogeneic HSCT is also a treatment option (Dokal, 2011).

The diagnosis of AA can only be confirmed by special investigations. The diagnostic work-up of AA includes the exclusion of other possible causes of pancytopenia with a hypocellular bone marrow, exclusion of inherited AA, screening for an underlying cause of AA and the documentation of a co-existing abnormal cytogenetic or PNH clone. The Full blood count characteristically shows a pancytopenia. A normocytic or macrocytic anaemia with a

reticulocytopenia is present. A microcytic anaemia is uncommon, and its presence should lead one to suspect PNH. The bone marrow is hypocellular with increased fat spaces. Erythropoiesis, granulopoiesis and megakaryopoiesis is reduced to absent and macrophages, plasma cells and mast cells appear prominent. Dyserythropoiesis is common, but dysplastic megakaryopoiesis and granulopoiesis are not a feature of AA. There is no infiltrate or fibrosis on the trephine biopsy (Marsh et al, 2009).

Screening for inherited disorders should be performed especially in children and in particular for the commoner FA and DC. A diagnosis of FA can be made by demonstrating increased chromosomal breakage following exposure of peripheral blood lymphocytes or skin fibroblasts to mitomicin C or diepoxybutane. Mutation analysis can also be undertaken for both FA and DC. Testing for the other IBMFS depends on clinical suspicion.

Many drugs and chemicals have been implicated in the aetiology of aplastic anaemia, but for most drugs it may be difficult to prove causality (Issaragrisil et al, 1997). Drug classes that are associated with AA include: cytotoxics, antibiotics, anti-inflammatories, anti-convulsants, anti-thyroids, anti-depressants, and anti-malarials (Baumelou et al, 1993). Many chemotherapeutic drugs such as alkylating agents, antimetabolites, antimitotics cause bone marrow suppression as part of its toxic effects; these are dose-dependent, occur in most patients and are generally reversible. However, a number of drugs cause idiosyncratic reactions leading to AA without a clear dose-response relationship. Idiosyncratic drug reactions resulting in AA are rare and difficult to study (Young et al, 2006). Not all associations necessarily reflect causation as some drugs may have been used to treat symptoms of aplastic anaemia. In principle, these drugs should be withdrawn and the patient monitored for improvement in the blood counts.

Epidemiologic data link benzene to AA (Smith et al, 1996). Benzene is also a cause of acute leukemia. In this regard an occupational history is important especially in industries where benzene is used as a solvent. Industries now regulate benzene exposure. It is noteworthy that benzene metabolites occur in some lead-free gasoline. Agricultural pesticides such as organochlorines, organophosphates as well as cutting oils and lubricating agents have also been associated with AA (Muir et al, 2003).

Radiation can cause marrow aplasia as it damages DNA. The bone marrow is particularly susceptible as it has a high cell turnover. Radiotherapy can result in marrow aplasia especially where the radiation field involves large areas of bone marrow. It is dose-dependent. Accidental exposure can occur in power plant workers, employees of hospitals, laboratories and industries such as food sterilization and metal radiography. There is an increased risk of haematologic and non-haematologic malignancies after radiation exposure (Young, 2005).

Liver function tests are performed to detect hepatitis. Viral studies for hepatitis A, hepatitis B, hepatitis C, EBV, CMV, parvovirus B19 and HIV are performed to exclude a viral infection as a cause for the pancytopenia. Hepatitis is the commonest preceding infection accounting for up to 5% of patients. The marrow aplasia manifests 1-2 months after liver inflammation and usually in young men. The hepatitis is seronegative and is thought to be due to an as yet undiscovered virus. Parvovirus B19 is more commonly a cause of pure red cell aplasia rather than generalised bone marrow failure.

Vitamin B12 and folate levels are measured as severe megaloblastic anaemia can present with pancytopenia. The anti-nuclear antibody test is done to screen for autoimmune diseases.

AA can occur in systemic lupus erythematosis and eosinophilic fasciitis. Another immunologic disease which can result in aplasia is transfusion associated graft versus host

disease which may occur after transfusion of non-irradiated blood products to an immunodeficient recipient.

Paroxysmal nocturnal haemoglobinuria (PNH) consists of a triad of features, viz. haemolytic anaemia, thrombosis and pancytopenia. It is an acquired condition due to a hematopoietic stem cell mutation defect. The defect in the phosphatidylinositol glycan class A (PIGA) gene results in an inability to synthesize glycosyl- phosphatidylinositol anchor that binds proteins to cell membranes. This results in cells deficient in complement-regulating surface proteins such as CD55 and CD59 which renders cells susceptible to intravascular haemolysis by complement proteins. Patients with PNH can develop aplastic anaemia and patients with aplastic anaemia can develop PNH. The Ham test and sucrose lysis tests have been used previously to detect PNH clones but have now been replaced by flow cytometry which is sensitive and quantitative. PNH clones have been detected in up to 50% of AA patients (Dunn et al, 1999). They are also seen in MDS. These clones may remain stable, regress or increase and some patients may develop haemolysis. Functional studies of bone marrow from patients with PNH, even those with predominantly haemolytic manifestations show defective haematopoiesis. One explanation for the aplastic anaemia/PNH syndrome is that the PNH clones are protected from immune-mediated destruction as they lack a GPI-anchored protein which may act as an autoantigen.

Cytogenetic analysis of the bone marrow may be difficult as insufficient metaphases are obtained. Abnormalities of chromosomes 5 and 7 may suggest MDS. Abnormal cytogenetic clones may be present in 12% of cases with aplastic anaemia (Gupta et al, 2006).

Patients with aplastic anaemia should be managed in a tertiary care centre. Management includes supportive care as well as specific therapy for the disease. Supportive care is essential to ensure that the patient survives to benefit from specific therapy. Support with red

cell and platelet transfusions is essential in patients with aplastic anaemia to maintain a safe blood count. Leucodepleted blood products should be used to reduce the risk of alloimmunisation and platelets should be single donor/ apharesis and irradiated (Marsh et al, 2009; Killick et al, 1997). Alloimmunisation can result in an increased risk of graft rejection after allogeneic stem cell transplantation (Kaminsky et al, 1990). Cytomegalovirus (CMV) negative blood products should be given until the patients CMV status is known (Pamphilon et al, 1999). Repeated transfusions cause iron overload and iron chelation may be given to reduce the harmful effects of iron overload. It is usually indicated when the ferritin is >1000µg/l or there is evidence of organ dysfunction related to excess iron. Deferiprone is associated with agranulocytosis and is thus better avoided. Desferioxamine or the oral iron chelator deferasirox are suitable iron chelators. Platelet transfusions are given when the platelet count is $<10x10^9$/l or $<20x10^9$/l if the patient has pyrexia, sepsis, DIC or when the patient is bleeding. In patients with platelet refractoriness due to alloimmunisation, HLA compatible platelets are usually effective. Leucodepleted red blood cells are given when there is symptomatic anaemia (usually < 8g/dl) and to maintain a safe haemoglobin level. In patients with cardiac or pulmonary disease, a higher haemoglobin level may be required.

As patients with AA are neutropenic, there is an increased risk of infection which is determined by the neutrophil count and the individual, with some patients having recurrent infections and others very few. Neutropenic patients are at an increased risk of bacterial and fungal infections (Ljungman 2000). Patients with severe neutropenia are given prophylactic antibiotics against gram negative bacteria and anti- fungal prophylaxis. Anti-viral prophylaxis and pneumocystis jirovecii prophylaxis are given in some centres during immunosuppressive therapy and post bone marrow transplant. For patients who have not undergone specific treatment, prophylactic antibiotics are considered for severe neutropenia and for patients who have frequent and/or severe infections. Active infection in the presence of neutropenia must

be aggressively treated empirically with parenteral, broad spectrum antibiotics. Specific areas of infection should be looked for on physical examination and radiographically in the neutropenic patient including oropharyngeal infections and anorectal abscesses, pneumonia, sinusitis and typhlitis (necrotizing enterocolitis). With contaminated indwelling catheters, gram positive cover with a drug such as Vancomycin should be added. Neutropenic patients are also susceptible to fungal infections (commonly Candida and Aspergillus) and a persistent fever on adequate antibacterial cover implies fungal disease. Timely initiation of antifungals should be undertaken.

Haemopoietic growth factors alone do not form part of the specific treatment of severe aplastic anaemia as they are ineffective (Marsh et al, 2007). However, G-CSF may be used in patients with neutropenic sepsis not responding to antibiotics and antifungal agents as it may cause a temporary rise in the neutrophil count in patients with residual granulocytic activity.

The standard treatment for a patient diagnosed with severe aplastic anaemia is either allogeneic stem cell transplantation from an HLA-identical sibling donor or immunosuppressive therapy (IST) with ATG and cyclosporin. Haematopoietic stem cell transplant from an HLA-identical sibling donor is the best treatment for the young patient. Transfusions of blood products from family members should be avoided to prevent sensitization to HLA antigens. In general, transfusions should be minimised as alloimmunisation may result in a higher risk of graft failure. In this regard, the search for a donor should be done as soon as possible and the transplantation should not be unnecessarily delayed. Patients with aplastic anaemia are followed up indefinitely as they may relapse or develop clonal disorders such as MDS, leukemia, PNH and solid tumors. The risk of clonal disease is 8% for MDS/leukemia (AML), 10% for haemolytic PNH and 11% for solid tumors at 11 years (Frickhofen et al, 2003). Repeat bone marrow examination is indicated if a clonal disease is suspected.

The outcome of HLA-identical sibling transplantation is a 75-90% probability of long term cure (Myers et al, 2009; Champlin et al, 2007; Kahl et al, 2005). Adverse factors that affect outcome after an allogeneic HSCT in AA include age > 40 years, prior failed immunosuppressive therapy, long interval between diagnosis and transplantation, a large transfusion burden and active infection prior to HSCT (Marsh et al, 2011). Graft failure occurs in 4-14%. Acute graft versus host disease (GVHD) is reported in 12-30% and chronic GVHD occurs in 30-40% of patients (Marsh et al, 2009b).

Allogeneic HLA-identical sibling donor stem cell transplantion is indicated in patients with severe or very severe aplastic anaemia, who are younger than 40 years of age and have an identical sibling match. Children with non-severe AA who are transfusion dependent and who have an HLA-identical sibling donor are also candidates for allogeneic HSCT. The cut-off of 40 years is generally used as an older age is associated with a poorer outcome. However some centres transplant patients who are over 40 years of age (<55 years). The dose of haematopoietic stem cells for bone marrow transplantation is at least 3 x 10^8 nucleated marrow cells/kilogram and for peripheral blood at least 2 x 10^6 CD34+ cells/kg. Lower doses increase the risk of graft failure (Niederwieser et al, 1998; Russell et al, 1998).

For patients with acquired aplastic anaemia, bone marrow transplants compared to peripheral blood stem cell transplants have been associated with less graft versus host disease (Schrezenmeier et al, 2007). The conditioning regimen recommended for patients <30 years of age is high dose cyclophosphamide 50mg/kg for 4 days and antithymocyte globulin (ATG) 1.5 vials/10kg for 3 days if using Thymoglobuline, Genzyme together with methylprednisolone 2mg/kg for 3 days. Ciclosporin and methotrexate are used for GVHD prophylaxis. Allemtuzumab used as part of the conditioning regimen results in less GVHD. For patients > 30 years of age cyclophosphamide 1200mg/m^2, fludarabine 120mg/m^2 and either ATG or allemtuzumab is considered (Maury et al, 2007). Ciclosporin is usually tapered

after approximately one year to reduce the risk of late graft rejection which is associated with early discontinuation. Progressive mixed chimerism also predicts graft rejection and ideally chimerism should be monitored during tapering. If there is an increasing proportion of recipient cells, tapering must be delayed (Marsh et al, 2009b).

GVHD is a serious complication as it adversely impacts on quality of life and survival, especially in AA where there is no benefit of having a graft- versus- disease effect. Risk factors for chronic GVHD include: acute GVHD, high marrow cell dose and use of peripheral blood stem cells (Schrezenmeier et al, 2007), older age, the use of ATG relative to an allemtuzumab-based conditioning regimen (Gupta et al, 2004) and complete donor chimerism.

Risk factors for graft rejection include: alloimmunisation from multiple blood transfusions, low dose of donor haematopoietic stem cells, pretransplant and post transplant immunosuppression, T-cell depletion of donor marrow, donor –recipient gender mismatching and progressive mixed chimerism (Mcann et al, 2007; Stern et al, 2006). The treatment of graft rejection may include a second HSCT or if late graft failure occurred after withdrawal of ciclosporin, its reintroduction at therapeutic doses may result in a response. Complete recovery of autologous haemopoiesis may occur after graft rejection in some instances.

Matched unrelated donor HSCT is an option for patients without a matched sibling donor. Outcomes have improved and have been attributed to the use of leucodepleted blood products, improved tissue typing and conditioning regimens (Maury et al, 2007).

Umbilical cord blood is also a source of stem cells. It is associated with a lower risk of GVHD. However, due to the low number of haemopoiteic cells that is obtained from a donation, graft rejection is a major issue.

The long-term effects of HSCT may include infertility and an increased frequency of secondary solid malignancies when irradiation is used. Radiation has also been implicated in impaired growth and development in children (Socie et al, 2003; Socie et al, 1993). However, increasingly radiation-free regimens are being used. Fertility is usually preserved when using high dose cyclophosphamide and it is not necessary for sperm or oocyte cryopreservation pre-transplantation (Sanders et al, 1996). For patients using a fludarabine-based regimen there is currently insufficient data on fertility post transplant.

For patients who are not eligible for a bone marrow transplant, immunosuppressive therapy using a combination of antithymocyte globulin (ATG) and ciclosporin is indicated. These include patients with non-severe aplastic anaemia who are transfusion dependent or have a neutrophil count of $<0.5x10^9$/l; patients with severe or very severe AA who are older than 40 years of age and younger patients who have no matched sibling donor.

ATG forms the backbone of immunosuppressive therapy. Both a horse and a rabbit preparation are available for use. The horse preparation (Lymphoglobuline) was generally the standard preparation used and the rabbit ATG (Thymoglobuline) was used if a second course of ATG was required (Scheinberg et al, 2006a). However, due to availability issues, usage of the rabbit ATG is increasing. The possible mechanisms of action of ATG include: T-cell depletion by complement-mediated lysis; destruction of activated cytotoxic T lymphocytes by Fas-mediated apoptosis and antibody-dependent cellular cytotoxicity; reduced apoptosis and Fas expression on AA CD34+ bone marrow cells and direct stimulation of T-regulatory cells (Marsh et al, 2011).

Side effects of ATG include allergic reactions such as fever and rigors, rash, and fluid retention. Anaphylaxis is possible and hence a test dose is usually given. Worsening thrombocytopenia requiring platelet transfusions often occurs due to the anti-platelet activity

of ATG. Serum sickness generally occurs 7 to 14 days after initiation of ATG. Steroids like methylprednisolone and prednisone, paracetamol and antihistamines are used for prophylaxis and alleviation of these manifestations. Ciclosporin can be started concomitantly with the ATG or after ATG. The dosage is adjusted to maintain adequate trough levels (150-200ng/ml). There is a risk of relapse with rapid tapering of ciclosporin (Saracco et al, 2008). Side effects of ciclosporin include hypertension, renal dysfunction, gum hypertrophy and neurotoxicity.

Responses to immunosuppressive therapy takes 3-4 months to manifest and during this period supportive care has to continue. A second course of ATG can be given if there is no response to the first course or if there is a relapse after initial response to treatment.

The use of ATG alone induces a partial response in 50% of patients. The response rate of ATG and ciclosporin is 60-80% with 5 year survival rates of 75-85% (Locasciulli et al, 2007; Bacigalupo et al, 2000a). Combined treatment is thus the standard for severe and very severe AA. Most patients receiving immunosuppressive therapy achieve a partial response (independence from transfusion and a neutrophil count adequate to prevent infection). Haematologic response strongly correlates with survival (Young, 2005). Relapse is common after IST, reported to be around 30% (Schrezenmeier et al, 1993). Relapse often occurs as ciclosporin is discontinued; some patients respond to reinstitution of ciclosporin and some are dependent on continuous use of ciclosporin.There is a 30-60% response rate to a second course IST (Tichelli et al, 1997). Patients are also at a risk of developing PNH, MDS and AML. Factors associated with a higher risk of developing clonal disorders include repeated courses of ATG, older age, high doses, use of G-CSF with ATG and ciclosporin, and shortened telomeres (Marsh et al, 2011). Mycophenolate mofetil (MMF), an immunosuppressive agent, has been used in patients refractory to ATG and cicolosporin, but has not shown good responses. The addition of MMF to ATG and ciclosporin has also not

resulted in better responses or a reduced relapse rate in AA (Schrezenmeier et al, 2003). The addition of sirolimus to ATG and ciclosporin has also not resulted in a significant difference in response.

The use of immunosuppressive therapy in older patients is associated with a less favourable response and survival compared to younger patients (Tichelli et al, 1999). There is an increased risk of mortality from bleeding and infection in this population and there may also be an increased susceptibility to the side effects of ATG and ciclosporin. Co-morbid diseases are more prevalent in the older population as well.

In patients with aplastic anaemia, small PNH clones are common (Socie et al, 2000). These clones may remain stable, increase or decrease in size. The patients with PNH clones are treated similarly to patients without a PNH clone. However, in haemolytic PNH, folate and iron supplementation may be required and prednisone and eculizumab are effective in reducing the haemolysis and thrombosis (Hill et al, 2005).

Aplastic anaemia can present in pregnancy. The disease may remit spontaneously after termination of the pregnancy or after delivery. In patients who have responded to IST there is a significant chance of relapse (Tichelli et al, 2002). However, after successful allogeneic bone marrow transplant, relapse in pregnancy is uncommon (Kahl et al, 2005). Supportive care is the mainstay of treatment and ciclosporin is safe to use in pregnancy.

The prognosis of AA initially was poor with rapid deterioration and death. The use of blood products and antibiotics provided some benefit but spontaneous recovery occurred in only a few patients. The prognosis of severe disease was worse than non-severe disease. Current treatment has significantly improved the outcome. Long-term survival is good with HLA-matched sibling HSCT and IST. However, successful transplantation cures marrow failure whereas patients who receive IST remain at risk of relapse and malignant transformation.

Outcomes for matched unrelated donor HSCT is improving and it may become an important treatment modality in the future. The advancement in gene sequencing technologies may also make it easier to detect the IBMFS and may give us more insight into the pathogenesis of acquired AA.

CHAPTER 2

PATIENTS AND METHODS

The population studied consisted of patients diagnosed with aplastic / hypoplastic anaemia who presented to the Clinical Haematology Unit, Department of Medicine at Chris Hani Baragwanath Academic Hospital during the period 1 January 1995 to 31 December 2012.

The inclusion criteria were:

All patients with a confirmed diagnosis aplastic / hypoplastic anaemia using clinical, laboratory and bone marrow assessments.

The exclusion criteria were:

Patients not fulfilling the definition of aplastic / hypoplastic anaemia (ie. a peripheral pancytopenia together with a hypocellular / acellular bone marrow in the absence of an abnormal infiltrate or marrow fibrosis).

The collection of data was done retrospectively from the patient files kept at the Haematology Unit at Chris Hani Baragwanath Academic Hospital. Laboratory data not found in the patient files were obtained from the hospital laboratory results electronic database.

A data sheet (see appendix A and B) was used to obtain the relevant data for the study. This included the following information: demographics, clinical presentation, laboratory investigations, treatment, outcome, and follow up of patients.

The computer programme Redcap was used to facilitate electronic data capture and for analysis. The data was subsequently exported from Redcap to Microsoft Excel where further data analysis was performed. GraphPad Prism version 6 was also used for some of the data analysis.

For the purposes of the study the following definitions were applicable:

Definition of severity of Aplastic Anaemia (AA)

Severe AA	Bone marrow cellularity < 25%, or 25-50% with <30%
(Camitta et al,1975)	residual haemopoietic cells.
	2/3 of the following:
	Neutrophil count $<0.5x10^9/l$
	Platelet count $<20x10^9/l$
	Reticulocyte count $<20x10^9/l$ or corrected count <1%
Very severe AA	As for severe AA but neutrophils $<0.2x10^9/l$
Non severe AA	Patients not fulfilling the criteria for severe or very severe AA

The reticulocyte count was corrected for haemoglobin using the following formula:

Corrected reticulocyte count = % reticulocytes x patients Hb/normal Hb

The normal haemoglobin for a male was taken to be 14-18g/dl and for a female 12-16g/dl.

The reticulocyte production index (RPI) was calculated as follows:

RPI = % reticulocytes/maturation time x patients haematocrit/normal haematocrit.

The grading of the cytopenias was based on the WHO haematological toxicity scale demonstrated in the following table (Provan et al, 2009).

Table 2.1 WHO haematological toxicity scale

Parameter	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Haemoglobin	≥ 11.0	9.5-10.9	8.0-9.4	6.5-7.9	<6.5
Leucocytes	≥ 4.0	3.0-3.9	2.0-2.9	1.0-1.9	<1.0
Neutrophils	≥ 2.0	1.5-1.9	1.0-1.4	0.5-0.9	<0.5
Platelets	≥ 100	75-99	50-74	25-49	<25

Criteria for response to therapy in aplastic anaemia

Less than a partial response / None: Not meeting the criteria below

Partial response: Transfusion independent

No longer meeting criteria for severe AA

Complete response: Haemoglobin > 10g/dl

Neutrophil count > $1.0x10^9$/l

Platelet count > $100x10^9$/l

Definition of relapse

Loss of a complete or partial response.

CHAPTER 3

RESULTS

The records of patients with AA who presented to the Clinical Haematology Unit at Chris Hani Baragwanath Academic Hospital during the period 1 January 1995 – 31 December 2012 (17 years) were reviewed. During this period, a total of 100 patients with AA were found. In view of the fact that this was a retrospective study, data were missing for some patients and the statistics were calculated based on the available data. For some statistics, the number of patients for whom data was available is indicated (e.g. symptoms in 99 rather than 100 patients; see next page). There were 64 males and 36 females with a male to female ratio of 1.77:1. The median age was 24.5 years (range 14-78 years). The median age for males was 23.5 years (range of 14-78 years) and for females 26.5 years (range of 14-63 years). The peak frequency was in the second (28%) and third decade (42%). The age at presentation in the different decades is shown in Table 1.1. Eighty seven percent of patients presented before the age of 40 years.

Table 3.1 Age at presentation

Age (years)	Number	Percentage
10-19	28	28
20-29	42	42
30-39	17	17
40-49	4	4
50-59	3	3
60-69	5	5
70-79	1	1

The ethnicity profile of the patients was 92% black, 3% mixed ethnicity, 2% white and 2% asian.

The most common symptoms were fatigue, occurring in 79.2% of patients and bleeding manifestations, occurring in 83.3% of patients. The symptoms at presentation are shown in Table 3.2. The most common symptoms were those related to anaemia and bleeding.

Table 3.2 Symptoms at presentation

Symptoms	Number	%
Fatigue	76	79.2
Dyspnoea	13	13.5
Palpitations	2	2.1
Dizziness	17	17.7
Swelling of legs/body	8	8.3
Fever	12	12.5
Night sweats	8	8.3
Bleeding	80	83.3
Other (e.g. headache, weight loss)	29	30.2

The signs at presentation are shown in Table 3.3. The dominant clinical sign was pallor (anaemia) occurring in 92/99 patients (92.9%). Bleeding manifestations was the second most common clinical finding. Data was missing in 1 patient.

Clinical features suggestive of IBMFS were noted in 7 patients, of which pigmentary changes was the commonest occurring in 5/7 patients (71%).

Table 3.3 Signs at presentation in Aplastic Anaemia patients

Signs	Number	Total (number)	%
Pallor	92	99	92.9
Jaundice	2	99	2
Fever	7	99	7
Petechiae	37	99	37.3
Purpura	11	99	11.1
Ecchymosis	14	99	14.1
Epistaxis	13	99	13.1
Gum bleeding	27	99	27.2
Lymphadenopathy	14	99	14.1
Cardiac failure	3	99	3
LRTI	5	99	5
Hepatomegaly	3	99	3
Splenomegaly	0	99	0
Fundal changes	21	99	21.2
Short stature	2	99	2
Craniofacial dysmorphism	1	99	1
Pigmentary changes	5	99	5
Nail dystrophy	1	99	1
Leukoplakia	0	99	0
Bone deformity	0	99	0

Occupational, family and drug histories are shown in Table 3.4. There were 29/98 (29.6%) of patients who had a positive drug history. The drugs associated with AA in this cohort included antiepileptics, antibiotics, anti-inflammatories, illicit drugs and traditional/herbal substances. There were 5/98 (5.1%) who had a positive occupational history. These patients had exposure to petroleum products. None of the patients had a positive family history including those with IBMFS.

Table 3.4 Drug, occupational and family history

History	Number	Total	%
Drug history	29	98	29.6
Occupational history	5	98	5.1
Family history	0	98	0

The Full Blood Count and differential white cell count together with the reticulocyte count and reticulocyte production index is vital in the assessment and staging of Aplastic Anaemia. Table 3.5 shows details of these haematological parameters that are normally assessed in the work up and staging of an individual with suspected Aplastic Anaemia. The variable, number of patients who had results available for the defined variable, the minimum and maximum value for the variable (range), the mean and the median are shown in Table 3.5. Some of the values were noted to be in the normal range. A possible explanation for this is that these patients were transfused with blood products prior to coming to Chris Hani Baragwanath Academic Hospital. However, on review, they fulfilled the criteria for the diagnosis of hypoplastic/aplastic anaemia.

The grades of the cytopenias are shown in Table 3.6. This is based on the World Health Organisation (WHO) haematological toxicity scale. According to this scale; leucocytes,

haemoglobin, neutrophils and platelets are graded into five categories: Grade 0 to Grade 4 based on their degree of severity (see Methods section). The majority of patients presented with Grade 3 and Grade 4 cytopenias.

Table 3.5 Haematological characteristics of the patients at presentation

Variable	Number	Minimum	Maximum	Mean	Median
White cell count ($x10^9$/l)	99	0.72	7.59	2.80	2.60
Haemoglobin (g/dl)	99	1.70	14.10	5.78	5.50
Mean cell volume (fl)	95	77.20	144.0	99.01	96.30
Platelets ($x10^9$/l)	99	1.0	147	23.48	26.0
Neutrophils ($x10^9$/l)	87	0.02	5.30	0.88	0.65
Lymphocytes ($x10^9$/l)	86	0.02	4.14	1.61	1.57
Corrected reticulocyte count (%)	72	0	3.50	0.65	0.4
RPI	73	0	1.80	0.34	0.20

Table 3.6 WHO grades of the blood counts

Variable	Number (patients)	Grade 0 (%)	Grade 1 (%)	Grade 2 (%)	Grade 3 (%)	Grade 4 (%)
Anaemia	99	2	4	12	19	62
Leucopenia	99	18.2	20.2	31.3	25.3	5.1
Neutropenia	87	11.5	5.7	10.3	28.7	43.7
Thrombocytopenia	99	4	1	6.1	15.2	73.7

The work up of a patient with suspected AA includes:

I. Confirmation of the diagnosis of AA after excluding other causes of pancytopenia.
II. Exclusion of secondary causes of bone marrow hypoplasia.
III. Assessment of the severity of AA.
IV. Exclusion of possible IBMFS.
V. Documenting the presence or absence of a PNH clone.

Table 3.7 shows the laboratory characteristics that were assessed at presentation. In the first column is the variable of interest together with a defined limit. The number of patients with the abnormality is shown in the second column, with the total number of patients tested for the defined variable in brackets. The percentage positive of the variable is noted in the third column and the mean value and range are shown in the subsequent two columns.

The ferritin level was high in 89% of patients and low in only 1.2%.

Table 3.7 Laboratory characteristics of patients at presentation

Variable	Number	%	Mean	Median	Range
Bilirubin Total bilirubin >21umol/l	5 (82)	6	12.38	8.0	2-218
Direct bilirubin >6umol/l	9 (82)	11	5.88	3.0	1-196
AST >40U/l	19 (81)	23.5	64.07	22.0	8-1315
ALT >40U/l	17 (81)	20.9	74.93	19.0	5-1770
Vitamin B12 <145ng/l	3 (86)	3.5	565.80	366.0	102-2000
Firritin <30ng/l >150ng/l >1000ng/l	 1 (82) 73 (82) 20 (82)	 1.2 89 24.4	900.82	453.50	15-10000
RCF <55ng/l	5 (75)	6.7	1563.25	961.10	760-9718.90
LDH >430U/l	20 (47)	42.5	636.23	414	21.0-3918.0
Alpha fetoprotein >6.6ku/l	3 (18)	16.7	3.61	2.20	0.9-15.70

Table 3.8 Viral studies and ANF results of the aplastic anaemia patients

Investigation	Positive (no.)	Negative (no.)	No result (no.)
Hepatitis B	2 (2.9%)	69	29
Hepatitis C	0 (0%)	69	31
EBV	1 (1.8%)	57	42
CMV	0 (0%)	55	45
Parvo virus B19	0 (0%)	53	47
HIV	12 (15.8%)	76	12
ANF	1 (1.4%)	69	30

Two patients were positive for Hepatitis B (2.9%), one for EBV (1.8%) and twelve patients for HIV (15.8%). The range of the CD4 (/µl) count was 4-840/µl with a mean of 309/µl and median of 275/µl. In Table 3.8, the percentages are calculated based on the number of available results and not the total number of patients.

In patients for whom a PNH result was available in the patient records, a PNH clone was detected in 15/22 (68%) patients. The Hams test or more recently flow cytometry was used to confirm the diagnosis.

Genetic tests for inherited bone marrow failure syndromes were documented in the files in 4 patients. An abnormality was detected in 2 of the patients. One patient was homozygous for Fanconi Anaemia and the other had a deletion in one of the FANCG genes. In South Africa, Fanconi Anaemia occurs in the white Afrikaans population as a result of mutations in the FANCA gene and in the black population mutations have been found largely in the FANCG gene (Morgan et al, 2005; Tipping et al, 2001).

Results of the HLA studies done were available for 28 patients. A matched sibling donor was found in only 5/28 (17.8%) patients.

The severity grading of aplastic anaemia is important for treatment decisions and is based on the blood count and bone marrow parameters (Camitta et al, 1975). The severity grading in the 100 patients reviewed in this study is shown in Table 3.9. In the first column is the severity, the second column contains the number of patients and the third is the percentage of all the patients who were classifiable.

Table 3.9 Severity grading of the aplastic anaemia patients (AA)

Severity	Number	%
Very severe AA	14	14
Severe AA	44	44
Non-severe AA	25	25
Unclassifiable	17	17

The majority of patients (58%) presented with severe and very severe AA. Seventeen patients were not classifiable due to insufficient data. If one excludes the patients who were unclassifiable due to missing data, then 69.9% would have severe and very severe AA.

Patients with aplastic anaemia often require blood product support due to significant anaemia and thrombocytopenia. The blood product usage is shown in Table 3.10.

A mean of 21.04 units per patient of platelets and 18.52 units per patient of leucodepleted packed red blood cells were transfused during the entire course of illness.

Table 3.10 Blood product usage in patients with aplastic anaemia

Blood product	Number (patients)	Minimum (units)	Maximum (units)	Mean (units)	Median (units)
Leucodepleted packed red blood cells	95	0	222	18.52	8
Platelets	95	0	263	21.04	9

Growth factors were used in 3 patients and iron chelation in 11 patients.

The standard regimen is a combination of ATG, ciclosporin with or without corticosteroids. The treatments received by the patients are documented in Table 3.11.

Table 3.11 Specific treatment received by aplastic anaemia patients

Treatment	Number	%
Stem cell transplant	2	2
ATG + ciclosporin + prednisone	56	56.5
ATG +prednisone	7	7
Ciclosporin +prednisone	21	21
Prednisone	13	13

The 2 patients who had a SCT received prior immunosuppressive therapy. Eleven patients were given a second course of immunosuppressive therapy. Anabolic steroids were used in 28 patients. In 3 patients the records were incomplete.

The response to therapy is categorised into complete, partial and less than a partial response (see Methods for definitions). Table 3.12 shows these results.

Table 3.12 Response to therapy

Response	Number	%
Complete response	35	35.4
Partial response	25	25.3
Less than a partial response	39	39.4

The overall response rate was 60.6%. Twenty one (21%) patients relapsed. Three patients (3%) transformed to AML and 5 patients had associated haemolytic PNH. As far as the status of the patients at the last clinic or hospital visit is concerned; 25 had a complete response, 26 had a partial response and 45 had less than a partial response. Of the 25 patients with a complete response, 10 were on treatment and 15 had been weaned off treatment. In the patients with a partial response, 19 were on treatment and 7 had been weaned off treatment.

A total number of 45 patients were lost to follow-up. Twenty six patients demised. Of the patients who demised the mean survival was 30.4 months with a lower median of 14.5 months. The main causes of death were infection/sepsis, bleeding and transformation to acute myeloid leukemia.

The response according to the severity of aplastic anaemia is shown in Table 3.13. The severity in 17 patients and the response in 1 patient could not be determined due to insufficient data.

Table 3.13 Response according to the severity of Aplastic anaemia

Severity	Number	Complete response	Partial response	Less than partial response
Very severe AA	14	3 (21.4%)	1 (7.1%)	10 (71.4%)
Severe AA	44	15 (34%)	15 (34%)	14 (32%)
Non severe AA	24	12 (50%)	6 (25%)	6 (25%)

In patients with severe aplastic anaemia, there was an even distribution in the number of patients who attained a complete, partial and less than partial response; whereas most patients with very severe aplastic anaemia obtained a less than partial response. In patients with non severe aplastic anaemia, 12/24 (50%) achieved a complete response.

In table 3.14, the response according to the treatment modality is shown. The number of patients as well as the percentages achieving various degrees of response is shown. The degree of severity at diagnosis is also documented. The best response was seen in patients who underwent allogeneic stem cell transplantation with both achieving a complete response. The patients who received ATG, ciclosporin and prednisone had a response of 69.4%. Surprisingly, patients who had ciclosporin and prednisone had a better response (58.8%) than patients who received ATG and prednisone (16.6%), however this may be explained by the larger number of patients with very severe AA in the ATG group. Patients who received prednisone only had a response of 55.5%. The majority of these patients had non-severe AA.

Table 3.14 Response according to treatment modality

Treatment modality	Severity of AA			Response to treatment			Number of patients
	Very severe	Severe	Non-severe	Complete	Partial	Less than partial	
Stem cell transplant	0	2 (100%)	0	2 (100%)	0	0	2
ATG, ciclosporin, prednisone	10 (21.7%)	27 (58.6%)	9 (19.5%)	20 (43.4%)	12 (26%)	14 (30.4%)	46
ATG, prednisone	2 (33.3%)	2 (33.3%)	2 (33.3%)	0	1 (16.6%)	5 (83.3%)	6
Ciclosporin, prednisone	1 (5.9%)	10 (58.8%)	6 (35.3%)	5 (29.4%)	5 (29.4%)	7 (41,2%)	17
Prednisone	0	2 (22.2%)	7 (77.8%)	3 (33.3%)	2 (22.2%)	4 (44.4%)	9

To see if there was any relationship between the HIV status and response to treatment, the response in relation to HIV status was assessed. Twelve patients were positive, 76 patients were negative and the status in 12 patients was not known. The response in one HIV positive patient could not be assessed due to a lack of data.

Table 3.15 Response to treatment according to HIV status

HIV status	Number	Complete response	Partial response	Less than partial response
Positive	11	3 (27.3%)	2 (18.2%)	6 (54.5%)
Negative	76	27 (35.5%)	20 (26.3%)	29 (38.2%)
Unknown	12	5 (42%)	3 (25%)	4 (33.3%)

The response to treatment in the HIV positive patients was less favourable than in the HIV negative population with more than 54.5% not attaining a partial or complete response as compared to 38.2% in the HIV negative patients (p: 0.33). However, this was not statistically significant due to the small number of HIV positive patients. The relative risk was 0.73 with a confidence interval of 0.33-1.43.

Five (45.5%) of the HIV positive patients demised. Their average survival was 13.7 months with a range of 1.6-50 months. Fourteen (18.4%) HIV negative patients demised. Their average survival was 35.9 months with a range of 1.2-167 months.

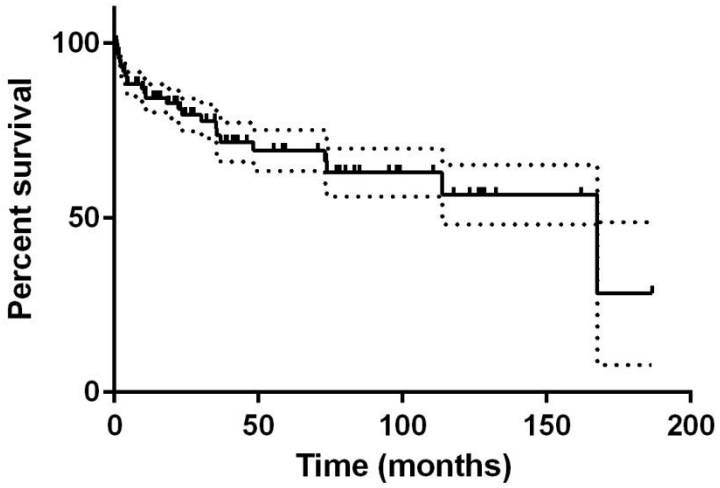

Figure 1: Kaplan-Meier survival curve for patients with aplastic anaemia (1995-2012)

The Kaplan-Meier survival curve is shown above (solid line) with the 95% confidence intervals (dotted lines). The horizontal lines are the censored variables.The 5-year survival probability was 69.2%.

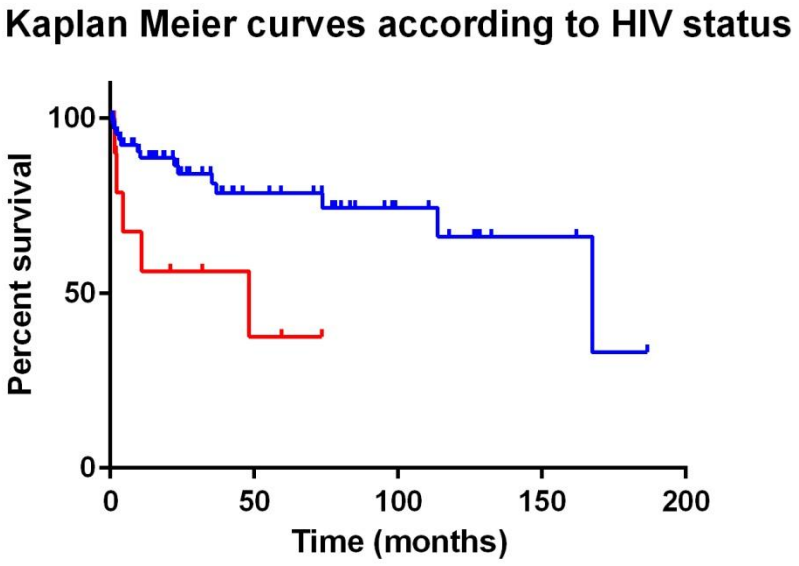

Figure 2: Kaplan-Meier survival curve according to HIV status

The above Kaplan-Meier survival curve compares the HIV negative (blue line) patients with the HIV positive patients (red line). The survival probability in the HIV patients is worse and is statistically significant (p value: 0.01). The 5-year survival probability was 37.5% for the HIV positive patients and 78.5% in the HIV negative patients.

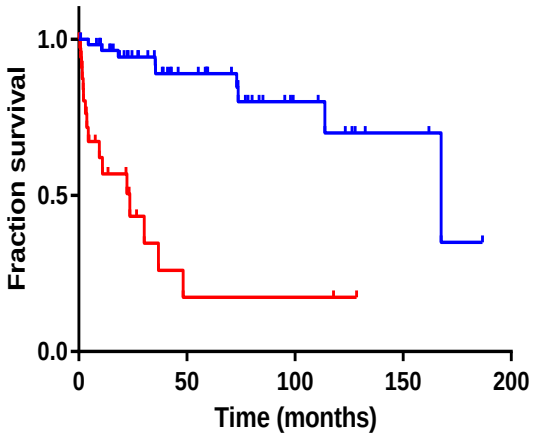

Figure 3: Kaplan-Meier survival curve in patients with AA according to Response

The patients who responded (blue) had a better survival than the non-responders (red). The 5-year survival probability was 88.9% compared to 17.3% respectively (p= 0.0001).

Table 3.16 Causes of death in patients with Aplastic anaemia

Cause	Number	%
Infection	16/26	61.5
Haemorrhage	1/26	3.8
Secondary malignancy	3/26	11.5
Other	3/26	11.5
Unknown	3/26	11.5

The most common cause of mortality was infection accounting for 61.5% of all deaths.

CHAPTER 4

DISCUSSION

Aplastic anaemia is a rare disease with an incidence of 2-3 per million per year (Montane et al, 2008; Young et al, 2008, Montane et al, 2008). It is 2-3 times more common in Asia (Issaragrisil et al, 2006). Most of the data on this disease are from North America, Europe and Asia. The aim of this study was to present the epidemiological, clinical and haematological features, the treatment modalities and the outcomes of patients with Aplastic anaemia at Chris Hani Baragwanath Academic Hospital (Johannesburg, South Africa).

One hundred patients were reviewed over a period of seventeen years. The average number of patients seen per year was 5.8. The majority of patients were of black ethnicity (92.9%). Aplastic anaemia is typically a disease of young individuals with the peak incidence occurring between 10-25 years. A second peak at 65 years is described in the literature (Heimpel, 2000). The median age at diagnosis in this study was 24.5 years and there was no clear second peak. The majority of cases (42%) presented between 20-30 years. The male to female ratio is equal in some studies and other studies report either a male or female predominance depending on the geographical area. There was a male predominance in this cohort of patients with a ratio of 1.7:1 which is similar to a previous study done on aplastic anaemia at this centre which reported a male-to-female ratio of 2.15:1 (Patel, 1994).

Anaemia was the dominant clinical sign occurring in 92.9% of patients followed by signs of mucocutaneous bleeding. These included petechiae, purpura, ecchymoses, epistaxis and gum bleeding. Of note, fundal haemorrhages occurred in 20% of patients. A fever was documented in only 7% of patients at presentation. Lymphadenopathy and hepatosplenomegaly are not features of aplastic anaemia unless there is a concomitant infection or a secondary cause of AA (Gordan-Smith et al, 1991).

Hepatomegaly was evident in 3 (3%) of the patients. One of the patients was HIV positive and had concomitant tuberculosis which could explain the reason for the hepatomegaly, the second patient was positive for hepatitis B and no obvious cause could be determined in the third.

Lymphadenopathy was documented in 14 (14%) patients. Five of these patients were HIV positive, 2 had tuberculosis, one had tonsillitis and another presented with pneumonia. There was no clear explanation in the other 5 patients.

There were two patients who had jaundice at presentation. In one patient it was due to haemolytic PNH and the other patient had deranged liver function tests with the ALT and AST raised more than the GGT and ALP. Hepatitis studies and viral studies for EBV, CMV, HIV, Parvovirus B19 were negative in this patient. There was, however, a significant alcohol history, and the liver dysfunction resolved spontaneously.

Clinical features suggestive of an IBMFS were present in 7 patients of which skin pigmentary changes and short stature were the commonest manifestations. Results of genetic studies which were available for 2 patients confirmed FA.

A number of drugs and toxins have been associated with aplastic anaemia. These are based mainly on case reports and case control studies (Issaragrisil et al, 1997; Kauffmann et al, 1996; Baumelou et al, 1993). Drugs which are associated with aplastic anaemia and were taken by some patients in this study included: sulphonamides (bactrim), anti-malarials (quinine) was used in a patient who developed malaria, antihypertensive agents (thiazides), anti-convulsants (phenytoin, carbamazapine), and anti-inflammatory agents. There were five patients who had an occupational history where exposure to substances associated with aplastic anaemia may have occurred. These included: exposure to benzene, petroleum

products and agricultural pesticides. There was no history of prior exposure to radiation, cytotoxic or chemotherapeutic agents in this cohort.

None of the patients had an autoimmune disorder. One patient was positive for ANF (titre of 1:40) but had no clinical features of a rheumatological disorder.

Aplastic anaemia can manifest for the first time in pregnancy and there is an increased risk of relapse after immunosuppressive therapy in a patient with aplastic anaemia during pregnancy. Supportive care is the mainstay of treatment (Kwon et al, 2006; Tichelli et al, 2002). Ciclosporin has been used safely in pregnancy. Four patients were diagnosed with aplastic anaemia during pregnancy. Two patients were treated with ciclosporin and prednisone and two with prednisone only. One patient obtained a complete response and subsequently had an uneventful second pregnancy. Two patients obtained a partial response and one patient relapsed after a partial response.

Clones of paroxysmal nocturnal haemoglobinuria (PNH) cells are characterised by a deficiency of glycosylphosphatidylinositol-anchored proteins on the cell surface due to an acquired mutation of the PIG-A gene on the X chromosome. This results in increased susceptibility to complement-mediated lysis due to a deficiency of CD 55 and CD 59 on the cell membranes. The response to immunosuppressive therapy is not different in patients with or without a PNH clone (Scheinberg, et al, 2010) and these patients are treated no different to patients without PNH clones which can occur in 40-50% of patients with aplastic anaemia. In most patients, the clone remains subclinical. Clinical features such as haemolysis and thrombosis is seen in only a few patients and occurs more commonly in those patients with large clones (>50% of cells). Fifteen out of 22 patients for whom PNH results were documented had PNH clones. Five patients had features of haemolysis. None of them had thrombosis. The clone size ranged from 1.1-95%. In 3 patients with haemolytic PNH the

clone size was >50% and in 2 patients it was <50%. The prevalence of PNH clones could not be determined due to insufficient data.

Hepatitis-associated aplastic anaemia is described in the literature as a syndrome of bone marrow failure occurring 2-3 months after an episode of acute hepatitis. The cause is often uncertain and patients are negative for Hepatitis A, B and C viruses. Brown et al, 1997 reported a series where there was good response to immunosuppressive therapy (Brown et al, 1997). In this review there were two patients who had hepatitis B. Both had severe aplastic anaemia. One of the patients was also diagnosed with HIV, had an infiltrative picture on liver function tests and a chest X-Ray in keeping with tuberculosis. He was initiated on anti-TB therapy. The patient was also started on ciclosporine and prednisone but was lost to follow-up. The second patient had unremarkable liver function tests. Immunosuppressive therapy was given with a poor response. There was one positive result for EBV. There were no positive results for Hepatitis A, hepatitis C or Parvovirus B19.

Haematologic manifestations of HIV (i.e. cytopenias) are common and include anaemia, neutropenia, lymphocytopenia and thrombocytopenia. They have a multifactorial aetiological basis. Aplastic anaemia due to HIV is rare and there is a paucity of data regarding this association in the literature. However, there has been a case report of aplastic anaemia due to HIV in a child who responded to antiretroviral therapy (Shah et al, 2005). HIV1, subtype C, the most common subtype in southern Africa, has also been shown to infect haematopoietic progenitor cells in a study conducted in Botswana, resulting in higher rates of anaemia (Andrew et al, 2007). It remains uncertain at this stage whether HIV is a cause of aplastic anaemia or is coincidental as both diseases affect teenagers and young adults.

There were 12 patients who were HIV positive. The grades of cytopenias were similar to the HIV negative subset manifesting predominantly with grade 3 and grade 4 anaemia,

neutropenia and thrombocytopenia. There did not appear to be any obvious relationship between the development of aplastic anaemia and the CD 4 count. The mean CD4 count was $309x10^6$/l with a range of 4-$840x10^6$/l and a median of $274.5x10^6$/l. The response to therapy appeared to be less favourable in HIV seropositive patients compared to seronegative patients with a complete response achieved in 27.3% versus 35.5% and a partial response of 18.2% versus 26.3% respectively. There were 54.5% of HIV positive patients and 38.2% of HIV negative patients who had less than a partial response. The survival also appeared worse in the HIV positive subset. Sepsis was the cause of death in all 5 patients with HIV, who demised. Four of the five patients died in the first year of diagnosis. Three patients were diagnosed with tuberculosis (TB); 2 with pulmonary TB and 1 with TB lymphadenitis. Only three patients were on combined antiretroviral therapy prior to the diagnosis of aplastic anaemia. ATG, cyclosporine and prednisone were given in 4 patients; 2/4 patients achieved a complete response and 2/4 had less than a partial response. Cyclosporine and prednisone was given in 6 patients; 1 had a complete response, 2 had a partial response and 3 had less than a partial response. In one patient prednisone was given with no response.

Supportive care with blood products in order to maintain a safe blood count is part of the management of aplastic anaemia. Prophylactic platelet transfusions are given when the platelet count is $<10x10^9$/l or $<20x10^9$/l in the presence of fever/sepsis/DIC, and are invariably given in response to bleeding manifestations (Kelsey et al, 2003). Fatal haemorrhages are more common when the platelet count is $<10x10^9$/l. Packed red blood cells are given to maintain the haemoglobin >8g/dl, depending on co-morbidities especially cardiorespiratory. Leucodepleted packed red blood cells and single donor platelets which are leucodepleted and irradiated are routinely given in order to prevent alloimmunisation which may result in increased graft rejection and platelet refractoriness in multi-transfused patients. Irradiated blood products prevent Transfusion Associated Graft-Versus Host Disease as AA

patients are candidates for stem cell and bone marrow transplants. The average number of platelets transfused per patient during the course of disease was 21.04 with a median of 9 and packed red blood cells 18.52 with a median of 8. Iron chelation in the form of deferrioxamine was given to 11 patients. The number of packed red blood cells transfused in these 11 patients ranged from 16-222 per patient during their treatment course.

It is necessary to determine the severity of aplastic anaemia as it is important with regard to treatment decisions. Severe and very severe aplastic anaemia is treated with haemopoietic stem cell transplantation; either an HLA identical sibling donor or a matched unrelated donor or anti-thymocyte globulin (ATG)-based immunosuppressive therapy (IST). The use of ATG and ciclosporine has resulted in a significant improvement in haematopoietic recovery and survival. In severe aplastic anaemia, the response rate to ATG alone is less than the combination of ATG and cyclosporine (Bacigalupo et al, 2000a) and the response to ATG and ciclosporine is greater than ciclosporine alone in non-severe aplastic anaemia (Marsh et al, 1999). In the majority of cases, IST is used initially as most patients are not suitable candidates for stem cell transplanion due to lack of a suitable donor, age and co-morbidities. In this review, 53% of patients presented with severe aplastic anaemia and 16.9% with very severe aplastic anaemia. Thirty percent had non- severe aplastic anaemia. In a study done in 2007 where 2479 consecutive patients with AA were analysed using data from 257 centers reporting to the European group for Blood and Marrow Transplantation Severe Aplastic Anaemia Working Party between 1991-2002, 1421 patients had data on severity. There were 40.8% who had VSAA, 24.4% with SAA and 34.6% with moderately severe AA (Locasciulli et al, 2007). It appears that severe and very severe aplastic anaemia is common in both settings. In this study, there were 87 (87%) patients who were $\leq$ 40 years making them eligible for SCT based on age; and based on severity criteria, 69.9% of patients who were

classifiable were potential SCT candidates. However, HLA sibling matches were found in 5/28 patients for whom results were available and is a major limiting factor.

Only 2 patients underwent HLA compatible sibling SCT. The first was a 20 year old male with SAA who initially received ATG. He had complete HLA compatibility with his sister and attained a complete response following alloSCT. He developed mild to moderate chronic graft vs host diseases which was controlled on therapy and he has a follow up of 11 years without relapse. The second was a 17 year old female patient with SAA who also received IST initially. She subsequently underwent a SCT as HLA studies revealed a 10/10 match with her sister. A complete response was attained. Complications included: acute GVHD, CMV infection and therapy-related hypertension. Unfortunately she demised 10 months later from a drug overdose (unrelated to aplastic anaemia). Her blood counts were normal at the time of her death. Both patients received cyclophosphamide and fludarabine as part of their conditioning regimen.

Immunosuppressive therapy was used in 97 patients. ATG, ciclosporine and prednisone was given in the majority (56). However, 7 patients were given ATG and prednisone, 21 ciclosporine and prednisone and 13 prednisone only. Results using immunosuppression with ATG and ciclosporine are good and this regimen is considered the standard protocol. The 10 year actuarial survival in patients receiving first line immunosuppressive therapy is 80% in children and 70% in adults (Locasciulli et al, 2007). A second course of ATG was given to 11 patients: 7 of which relapsed and 4 who did not respond to the first course of ATG. Of the non-responders, only 1 patient had a partial response. Of those who relapsed, 4/7 (57%) of the patients responded. The response rate to a second course of IST is between 30-60% with the response being better in previous responders compared to non-responders. All 4 of the patients who responded previously attained a complete response.

A complete response occurred in 35/99 (35.4%), a partial response in 25/97 (25.3%) and a less than partial response in 39/97 (39.4%).Thus, the overall response rate was 60.6% which is comparable to that reported in the literature from studies in Europe and the USA. Immunosuppressive therapy using ATG and ciclosporine is associated with response rates of 60-80% (Bacigalupo et al, 2000b). Responders (complete and partial response) had a better survival when compared to non-responders with a cumulative survival probability at 5 years of 88.9% and 17.3% respectively (p=0.0001). There were 16/26 (61.5%) deaths that occurred in the non-responders. The inferior survival in non-responders was also found by Scheinberg et al, 2006a where re-treatment with IST in patients who had refractory or relapsed disease was being evaluated. (Scheinberg et al, 2006a). In the HIV positive patients, the response was less favourable than the HIV negative patients with 54.5% not achieving a response compared to 38.2% (p: 0.33). One possible explanation for the poor response in this study could be the high rate of early deaths with 33% mortality in the first year in the HIV positive patients.

There were 21 patients out of 60 patients who responded initially and subsequently relapsed (35%). Among the complete responders relapse occurred in 14 of 35 (40%) and in 7 of 25 (28%) of partial responders. The mean time to relapse was 33.13 months with a range of 2.7-82.1 months. Relapse is reported to occur in approximately 10- 30% (Schrezenmeier et al, 1993; Bacigalupo et al, 2000b).

Patients are at risk for clonal disease and follow-up of these patients should be life-long. In this study, 45 patients were lost to long-term follow-up, which is significant. The median duration of follow-up was 23.4 months with a mean of 40.6 months. Three patients transformed to acute myeloid leukemia (AML) and all 3 demised subsequently. The times to

transformation after the diagnosis of aplastic anaemia were 3months, 2years 7 months and 2 years 10 months. Two of the patients attained partial responses and relapsed prior to their transformation to AML and 1 patient had less than a partial response. The transformation rate has been reported to be 8% for MDS/AML at 11 years (Frickhofen et al, 2003). This is higher than was noted in our setting in which only 3/100 (3%) patients were reported to have transformed to AML. The same study reported transformation to solid tumours to be 11%. There was no transformation to solid tumours in this study.

The overall 5-year survival in this study was 69.2%. In the literature, the long-term survival probability is 70-85% using immunosuppressive therapy. There is not much data on survival in the setting of HIV and aplastic anaemia. The survival in HIV positive patients was significantly worse with a survival probability at 5 years of 37.5% compared to 78.5% in the HIV negative population (p:0.01). The majority of deaths occurred in the first year (4/5 patients demised in the first year). All the deaths were due to sepsis. The majority of deaths in this cohort (HIV positive and negative) were due to infection (61.5%). Fatal haemorrhage occurred in 1 patient and malignancy accounted for 3 deaths; all transforming to acute myeloid leukemia.

CHAPTER 5

CONCLUSION AND LIMITATIONS OF THE STUDY

This study was conducted with the objective of obtaining data regarding the demographics, clinical presentation, treatment, complications and outcome of patients with aplastic anaemia at the Clinical Haematology Unit at Chris Hani Baragwanath Academic Hospital.

The clinical presentation was similar to that of other studies reported in the literature with features of anaemia and bleeding manifestations predominating. The severity of AA was also similar with most of the patients classified as severe and very severe aplastic anaemia. Acquired idiopathic aplastic anaemia was the commonest. The most frequent secondary causes of aplastic/hypoplastic anaemia were HIV, pregnancy and hepatitis B respectively accounting for 18% of the patients. Congenital AA was diagnosed in 2 (2%) patients.

Immunosuppressive therapy was the mainstay of treatment. Haematopoietic stem cell transplantation with a HLA matched sibling donor was the treatment modality in the minority of patients. The reasons for this are the low number of patients with siblings from the same parents and low number of patients with HLA compatibility. Matched unrelated donor transplantation was not done. Searching for a matched unrelated donor is not feasible for financial reasons at our public sector hospital.

The response rate, relapse rate and overall survival are similar to that reported in the published literature from studies done in Europe and the USA. Transformation occurred in a few patients to acute myeloid leukemia and to PNH. The rate of transformation was less in this study relative to that reported in the literature.

The most common cause of mortality was infection. HIV positivity and non-response to therapy were associated with inferior survival.

With regards to HIV positive patients, the clinical presentation was similar to HIV negative patients. The CD4 counts are variable, and most patients were not on antiretroviral therapy at the time of diagnosis. All were treated with immunosuppressive therapy and none underwent transplantation. There was a high rate of early mortality due to infection and the overall survival was inferior to the HIV negative patients.

LIMITATIONS

It was a retrospective study conducted at a single institution. Data collection was dependent on the patient files and some of the data was missing. The institution serves the Soweto region which is constituted predominantly of patients of black ethnicity and it is possible that the findings may be different in other regions in South Africa.

Compliance remains a major problem at this hospital with a significant number of patients being lost to long term follow- up.

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APPENDICES A AND B

DATA COLLECTION SHEET

APPENDIX A: # DATA SHEET

STUDY NUMBER:

AGE:
GENDER:
ETHNICITY:

CLINICAL PRESENTATION

HISTORY

SYMPTOMS :	**NO**	**Yes**	**Details(if Yes)**
FATIGUE:			
DYSPNOEA:			
PALPITATIONS:			
DIZZYNESS:			
SWELLING:			
FEVER:			
NIGHT SWEATS:			
BLEEDING:			
OTHER:			

DRUG HISTORY :	**NO**	**Yes**	**Details(if Yes)**
ANTIBIOTICS:			
ANTI-INFLAMMATORY:			
ANTI-CONVULSANTS:			
ANTI-THYROIDS:			
ANTI-MALARIALS:			
OTHER:			

	NO	**Yes**	**Details(if Yes)**	
FAMILY HISTORY:				

OCCUPATIONAL/ENVIRONMENTAL EXPOSURE:	NO	**Yes**	**Details(if Yes)**	
BENZINE AND OTHER SOLVENTS:				
AGRICULTURAL PESTICIDES:				
CUTTING OILS AND LUBRICATING AGENTS:				
RECREATIONAL DRUGS:				
OTHER:				

CLINICAL EXAMINATION	NO	Yes	Details(if Yes)	
PALLOR:				
FEVER:				
INFECTION:				
PETECHIAE				
PURPURA:				
ECCHYMOSIS:				
FUNDAL CHANGES:				
EPISTAXIS:				
GUM BLEEDING:				
NEUROLOGICAL-FALLOUT				
OTHER:				

APPENDIX B: FLOW CHART OF INVESTIGATIONS

	Initial Presentation	**Best Response**	Last Visit/Loss to Follow up /death
Duration from initial presentation			
WCC			
RCC			
HCT			
HB			
MCV			
PLATELETS			
NEUTROPHILS			
LYMPHOCYTES			
EOSINOPHILS			
BASOPHILS			
MONOCYTES			
BLASTS			
SMEAR COMMENTS			
CORRECTED RETICULOCYTE COUNT			
RPI			
NA			
K			
UREA			
CREATININE			
SERUM FE			
%SATS.			
TRANSFERRIN			
FERRITIN			
RED CELL FOLATE			
VIT. B12			
HEP. A ANTIBODY			
HEP B s Ag			
HEP. B cAb			
HEP. B s Ab			
HEP. C ANTIBODY			
ANA			
CMV			
EBV			
PARVOVIRUS B 19			
HIV			
CD4 COUNT (IF +)			
VIRAL LOAD (IF +)			

TOTAL BILIRUBIN			
DIRECT BILIRUBIN			
TOTAL PROTEIN			
ALBUMIN			
ALP			
GGT			
AST			
ALT			
LDH			
ds - DNA			
u - HAEMOSIDERIN			
HAPTOGLOBIN			

BONE MARROW ASPIRATE AND TREPHINE

CELLULARITY ……..
ERYTHROPOIESIS .....
GRANULOPOIESIS .....
MEGAKARYOPOIESIS .....
LYMPHOCYTES .....
PLASMA CELLS .....
MONOCYTES .....
MACROPHAGES .....
OTHER / ABNORMAL CELLS .....
FIBROSIS .....
CONCLUSION .....

CHEST X -RAY :.....

ABDOMINAL ULTRA SOUND :.....

OTHER IMAGING :.....

.....

.....

.....

TRANSFORMATION:

	NO	YES	DURATION FROM DIAGNOSIS	OUTCOME
TO MDS:				
TO AML:				
TO HAEMOLYTIC PNH:				
TO SOLID TUMOURS:				

TREATMENT

SUPPORTIVE THERAPIES:

TRANSFUSIONS:	QUANTITY		FREQUENCY		INDICATIONS	
LEUCODEPLETED PACKED CELLS						
SINGLE DONOR PLATELETS						
GRANULOCYTE TRANSFUSIONS						

GROWTH FACTORS:	NO	YES	DETAILS(IF YES)	
G-CSF:				
IRON CHELATOR:				

SPECIFIC TREATMENT INCLUDING DATE OF INITIATION AND TERMINATION:

	NO	YES	INITIATION	TERMINATION
HLA - IDENTICAL SIBLING DONOR:				
ATG AND CYCLOSPORIN:				
MATCHED UNRELATED DONOR:				
CYCLOSPORIN:				
OXYMETHALONE:				
SUPPORTIVE THERAPY:				
OTHER:				

TIME TO PARTIAL RESPONSE FROM INITIAL TEATMENT DATE: .....

TIME TO COMPLETE RESPONSE FROM INITIAL TREATMENT DATE: .....

RELAPSE:	**NO**	**YES**	**DETAILS**

CURRENT STATUS:

	NO	**YES**	**DETAILS**
COMPLETE RESPONSE:			
PARTIAL RESPONSE OFF TREATMENT:			
PARTIAL RESPONSE ON TREATMENT:			

DEATH:

DATE OF DEATH : .....

CAUSE OF DEATH : .....

SURVIVAL (DATE OF HISTOLOGICAL DIAGNOSIS UNTIL DATE OF DEATH) : .....

LOSS TO FOLLOW UP:

LAST DATE PATIENT SEEN : .....

STATUS OF PATIENT AT THAT DATE : .....

OTHER:

....

....

.....

.....

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.....

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