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A Pilot Study Investigating the Aetiology of Aplastic Anaemia and the Response to Immunosuppressive Therapy at the Charlotte Maxeke Johannesburg Academic Hospital.

by

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A research report submitted to the Department of Clinical Medicine, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg,

in fulfilment of the requirements for the degree of

Master of Medicine (MMed)

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Declaration:

I, Tyral Dean Ramsamy declare that this research report is my own, unaided work. It is being submitted in a submissible format together with an extended literature review, for the Degree of Master of Medicine at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.



Tyral Dean Ramsamy

15 day of October 2020 at Oakdene

Dedication:

For Bhavisha,

My life, light, guide and soulmate

Publications/Presentations arising from this Project:

None, at the time of submission. An article has been submitted to the South African Medical Journal; feedback is pending.

Abstract

Introduction

There is a paucity of South African data documenting the various causes of Aplastic Anaemia (AA), as well as the response to combination immunosuppressive therapy. The vast differences in treatment protocols internationally may be as a result of the poor representation in literature owing to the rarity of the disorder. Here, an analysis of possible secondary causes of AA as well as individual responses to treatment was reviewed at the Charlotte Maxeke Johannesburg Academic Hospital.

Objective

To determine the absolute number of cases, possible causes and response to immunosuppressive therapy in aplastic anaemia at the Charlotte Maxeke Johannesburg Academic Hospital.

Method

A retrospective cross-sectional analysis of all laboratory confirmed cases of aplastic anaemia at the Charlotte Maxeke Johannesburg Academic Hospital for the period of January 2014 to June 2019.

Results

There were 35 confirmed cases of AA for the given study period; 21 of which were males and 14 females. Sixty-five percent of the study sample had no identifiable cause for AA and were defined as idiopathic. Twelve patients had identifiable secondary causes for AA. Of these, 5 patients were exposed to a toxin (hydrocarbons), 2 patients were exposed to drugs (azathioprine and zidovudine), two patients had active Hepatitis B infection, two patients had a documented auto-immune condition (Systemic Lupus Erythematosus and Rheumatoid Arthritis) and one patient was pregnant at initial presentation. All patients were treated with a combination of anti-thymocyte globulin (ATG), rabbit or horse derived, cyclosporine and corticosteroids. None of the patients had received a stem-cell transplant.

Based on the World Health Organization (WHO) Haematological Toxicity Scale, 46% of patients had a poor response to treatment, 26% had a partial response to treatment and 28% had a complete response to treatment.

Twenty-seven percent of patients died, many of whom had no clearly documented or suspected immediate cause of death. However, mortality cannot be commented on, due to the absence of complete follow-up data.

Conclusion

Aplastic Anaemia is a rare disease which carries a high mortality rate. All possible secondary causes should be sought by taking a detailed patient history and conducting relevant viral serology and immunological assays. All patients must be treated with a standard treatment protocol and any exposure to toxins or offending drugs should be terminated. A South African Bone Marrow Registry (SABMR) donor search should be conducted on all patients eligible for a stem-cell transplant to improve the number of patients receiving transplant therapy. The rationale for the absence of bone marrow transplants conducted must be investigated and acted upon. Every effort to improve patient follow-up and continuation of care should be made to establish concrete data on mortality and long-term complications.

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List of Abbreviations

AA	Aplastic Anaemia
AdsDNA	Anti-Double Stranded Deoxyribose Nucleic Acid
ANA	Anti-Nuclear Antibody
ATG	Anti-Thymocyte Globulin
Beta-hCG	Beta – Human chorionic gonadotropin
CMV	Cytomegalovirus
EBV	Ebstein-Barr Virus
FISH	Fluorescent in-situ Hybridization
HB	Haemoglobin
HCT	Haematocrit
HIV	Human Immuno-Deficiency Virus
IL	Interleukin
MCHC	Mean Corpuscular Haemoglobin Concentration
MCH	Mean Corpuscular Haemoglobin
MCV	Mean Corpuscular Volume
MPV	Mean Platelet Volume
NSAIDs	Non-Steroidal Anti-Inflammatory Drugs
PNH	Paroxysmal Nocturnal Haemoglobinuria
RCC	Red Cell Count
RDW	Red Cell Distribution Width
SABMR	South African Bone Marrow Registry
WCC	White Cell Count
WHO	World Health Organization

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Chapter one: Protocol with Extended Review of Literature

1. Background

1.1 Definition

Although somewhat misleading in the nomenclature, aplastic anaemia (AA) is defined as a pancytopaenia with hypocellular bone marrow, in the absence of an abnormal bone marrow infiltrate or bone marrow fibrosis [1]. Camitta *et al* [1] described criteria to aid in the diagnosis; two out of the following three criteria must be met for diagnosis:

- Haemoglobin less than 100g/L,
- Platelet count of less than 50 x 10^9/L
- Neutrophil count of less than 1.5 X 10^9/L

These criteria were further modified by Bacigalupo *et al* to grade the severity of AA as described below in table 1 [1]. Non-severe disease is defined as not meeting criteria for either severe or very-severe AA.

Table 1: Classification of Severity of Aplastic Anaemia

Parameters	**Severe Aplastic Anaemia**	**Very Severe Aplastic Anaemia**
Marrow Cellularity	<25% or 25-50% with <30% residual haemopoietic cells	<25% or 25-50% with <30% residual haemopoietic cells
Neutrophils	<0.5 x 10^9/L	<0.2 x 10^9/L
Platelets	<20 x 10^9/L	<20 x 10^9/L
Reticulocyte Count **Manual Count** **Reticulocyte Count** **Automated Analyzer**	<20 x 10^9/L <6 x 10^9/L	<20 x 10^9/L <6 x 10^9/L

AA is an immune-mediated disease characterized by haemopoietic failure as a result of targeted apoptosis of progenitor cells due to the clonal expansion of cytotoxic T-cells [2]. Evidence-based response to immunosuppressive therapy bears testimony to the hypothesis of an immune-mediated disorder [2]. An inadequate response to immunosuppressive therapy may support the

presence of severe stem cell depletion; however, some may argue a non-immune cause in such patients [1].

Current evidence suggests a genetic basis for disease, characterized by progressive shortening of telomere length. A 1998 study evaluated the mean telomere length from 79 patients diagnosed with aplastic anaemia. It concluded that a significant shortening of telomere length in both granulocyte and mononuclear cell fractions were present, with progressive telomere erosion in patients with ongoing disease [3].

A Canadian study conducted on 56 subjects, used a flow-fluorescent in-situ hybridization (FISH) method of telomere measurement. These subjects were also found to have significantly shorter telomeres compared to age-adjusted controls. This study found that telomere length was poorly correlated with the degree of marrow suppression [4].

Long-term complications include the development of myelodysplastic syndrome and the expansion of paroxysmal nocturnal haemoglobinuria (PNH) clones [1]. This is especially true for patients who are refractory to immunosuppressive therapy [1]. The presence of PNH clones has been described in up to 50% of patients with AA [5]. A study conducted at the Chris Hani Baragwanath Hospital in Soweto showed the presence of a PNH clone in 15 out of 22 patients with AA (68%) in whom a test was conducted. There were no cases in which evolution to myelodysplastic syndrome was documented [6].

1.2 Epidemiology

Aplastic anaemia is known to have a biphasic distribution, usually presenting in the second to third decade of life, with some cases presenting after the fifth decade of life [2].

Much focus has been drawn to the markedly increased prevalence of AA in South East Asia as opposed to western counterparts. The estimated incidence in western countries roughly equates to two cases per million population [7]; whereas a study in Thailand revealed an incidence of 3.9 per million for the Bangkok metropolitan population [8]. Other studies in China show an even higher incidence, and AA is thus estimated to be two to three-fold more common in Asia [1]. This higher relative incidence in South East Asia may be explained by the higher exposure

to toxic chemicals and pesticides and a higher prevalence of infections implicated in the cause of aplastic anaemia [8].

In a Thai cohort of 541 patients, it was found that exposure to benzene, thiazides, mebendazole, sulphonamides, carbamates and organophosphates predominated [8]. Hydrocarbon exposure may be of particular interest, considering the high accidental and non-accidental exposure in South Africa [9]. Hydrocarbons are organic compounds composed of hydrogen and carbon atoms and may be classified into four distinct types; aromatic, aliphatic, halogenated and terpene based. Aromatic hydrocarbons are those which possess a benzene ring. These include benzene, toluene and xylene. They are used as primary solvents in the manufacturing of paint, paint removers, nail polish and various glues. Aliphatic hydrocarbons are petroleum distillates commonly used in gasoline and kerosene. Halogenated hydrocarbons include fluoridated and chlorinated natural carbon compounds. These compounds are often used in the manufacture of various pesticides, including organophosphates. The terpene group of hydrocarbons include pine oil and commercial turpentine [10].

Although considered to be rare, the prevalence of AA depends largely on the interplay between individual immune response, environmental exposure to offending agents as well as a diversity of predisposing genetic factors [2].

There is limited data representing the South African population. However, a recent study in 2015 at the Chris Hani Baragwanath Academic Hospital attributed Human Immunodeficiency Virus infection (HIV), pregnancy and hepatitis B infection as the leading cause of AA in that population [6]. This study was conducted in a predominantly black, sub-urban population in Johannesburg and thus caution should be exercised before extrapolation and application of data as this may not be representative of all South African populations. The study included one hundred subjects over an eighteen-year period who were treated for aplastic anaemia at the Chris Hani Baragwanath Hospital. Infectious agents implicated as a secondary cause for this cohort included HIV infection, Hepatitis B virus infection and Cytomegalovirus infection [6].

The hepatitis B burden had significantly reduced, following the implementation of the Hepatitis B vaccination in the National Expanded Programme on Immunization and the protocol of

initiating earlier vaccination and immunoglobin to babies exposed to maternal antigens. A 2015 study showed that there has been a reduction in occult Hepatitis B infection from 70.4% pre-vaccination era to 66.0% in the post-vaccination era. However, there is limited data on its effect on reducing the burden of aplastic anaemia [11].

Prior to the use of blood and blood products, a high mortality was attributable to complications as a result of thrombocytopaenia such as intracranial haemorrhage. Current evidence suggests that infection has become the leading cause of death in patients with AA [12].

1.3 Pathogenesis

Current literature broadly classifies AA as either primary or secondary [13]. A large proportion of cases have been labelled as idiopathic. Below is a non-exhaustive list of congenital causes as well as chemicals, drugs and environmental factors implicated in the cause of aplastic anaemia.

Table 2: Causes of Aplastic Anaemia

PRIMARY CAUSES	SECONDARY CAUSES
Congenital • Fanconi's Anaemia • Dyskeratosis Congenita • Schwachman-Diamond Syndrome • Reticular dysgenesis • Amegakaryocytic Thrombocytopaenia • Familial Aplastic Anaemia	Chemical Exposure • Benzene • Pesticides
	Drugs • Carbimazole • Chloramphenicol • Chlorpromazine • Phenytoin
	Ionizing radiation
	Infection • Epstein Barr Virus (EBV) • Cytomegalovirus (CMV) • Parvovirus B19 • Human Immunodeficiency Virus • Hepatitis Virus
Idiopathic	Pregnancy

1.4 Clinical Features

AA is described as having an insidious course. Patients may present with an array of symptoms, based on the fundamental biochemical abnormalities. A patient may present with epistaxis, retinal haemorrhage, spontaneous bruising or even a gastrointestinal bleed, as a result of the associated thrombocytopaenia. Fatigue, dyspnoea, dizziness and palpitations, are all suggestive of severe anaemia. Severe neutropaenia may result in sepsis and thus a patient may present with signs and symptoms of systemic infection [13].

While most cases are said to be idiopathic, all possible causes should be considered and thus a comprehensive family history, drug history and occupational exposure history must be explored.

When considering congenital causes of AA, one should be aware of associated physical features that may suggest a clinical syndrome. In children, for example, a patient presenting with short stature, skin hypo/hyperpigmentation and microcephaly, should be investigated for Fanconi's Anaemia [1].

Hepatosplenomegaly and lymphadenopathy are not commonly described and thus one would have to consider other diagnoses such as a haematological malignancy.

1.5 Diagnostic Investigations

Idiopathic aplastic anaemia is a diagnosis of exclusion. All patients with pancytopaenia on peripheral blood count should be investigated fully for the causes of bone marrow failure. Thus, a full anaemia workup, as well as autoimmune studies, should be conducted. Viral serology for Ebstein Barr Virus, Cytomegalovirus, Human-Immunodeficiency virus, Hepatitis virus (A, B, C and D) and Parvovirus B-19 is also necessary. A bone marrow aspirate and trephine biopsy must be done to validate the presence of marrow hypocellularity and to rule out other causes of marrow dysfunction [1]. A pregnancy test should be conducted in all women of child-bearing age. A thorough history must be taken to establish the possibility of drug and toxin exposure.

1.6 Treatment Modalities

Following a confirmed diagnosis, a patient should be managed at a dedicated oncology or haematology centre under the direct management of a medical oncologist or clinical haematologist [7]. Should a drug-induced AA be suspected, the offending drug should be immediately discontinued. In the acute setting, the presenting complaint must be addressed. Should a patient present with bleeding, for example, epistaxis, haemostatic measures should be undertaken with supportive blood and blood products as is necessary. Should there be any indication of sepsis, a source should be investigated, and the patient should be managed with appropriate antibiotics.

The consensus around the use of blood and blood products is to limit its use to cases where a clear indication is present [7]. A patient with symptomatic thrombocytopaenia should be transfused with platelets. Likewise, a patient with symptoms of severe anaemia (shock or cardiac failure) must be transfused with leuco-depleted matched blood. The use of iron chelation therapy may be necessary to prevent transfusional iron overload should the patient require multiple transfusions.

In the treatment of AA, the two most commonly employed treatment strategies include stem-cell transplant and immunosuppressive therapy. The choice between the two therapies is dependent on the presence of a matched donor, the age at presentation, the severity of the disease and the resources available at the treating centre. Younger patients have shown better outcomes with stem-cell transplants as first-line therapy. Significantly higher mortality is noted in patients over the age of 40 years, who undergo stem cell transplant. In these patients, immunosuppressive therapy is still considered first-line [14]. Age is considered a strong predictor of clonal evolution to myelodysplastic syndromes. This may account for the directly proportional relationship between age and mortality in AA [15].

Allogeneic stem cell transplant has proven to be of benefit, especially in adolescents with AA [1]. If a patient is found to have a Human Leukocyte Antigen (HLA) matched sibling, this can be considered first-line (upfront) therapy in adolescents and young adults. A well-matched, unrelated donor may be considered should all other explored modalities fail [16].

Anti-thymocyte globulin (ATG) is horse or rabbit antibodies against human T-Cells. Its use has been popular in transplant medicine and, together with cyclosporine, is considered first-line immunosuppressive therapy in AA [1]. Horse ATG is given at a dose of 40 mg/kg/day for a total of four days. Rabbit ATG is given at a dose of 3.5 mg/kg/day for five days. ATG is usually well tolerated with a minority of patients experiencing serum sickness characterized by arthralgia, fever and skin rash. This is prevented by the concomitant use of corticosteroids [1].

Cyclosporine is a cyclic peptide extracted from a soil fungus. It preferentially suppresses cell-mediated immune reactions by effectively reducing IL-2 levels [17]. It is commonly used as an immunosuppressive agent in transplant patients as well as an alternative to methotrexate in the treatment of severe, active rheumatoid arthritis [17]. Together with ATG, cyclosporine may achieve up to 70% response rates in AA [2]. In the treatment of AA, a dose of 6 mg/kg/day is used to achieve an acceptable trough level of 200-400 ng/mL. The most common adverse effect experienced is nephrotoxicity and thus renal function should be monitored closely [17].

There are multiple on-going clinical trials in the domain of new treatment strategies for AA [18][19]. The addition of eltrombopag to the standard immunosuppressive regimen has shown better haematological response in patients with severe aplastic anemia. Eltrombopag, ATG and cyclosporine are now considered the new standard of care [20]. An American study investigated the use of alemtuzumab, a monoclonal antibody against CD52, in patients with severe aplastic anaemia who were refractory to ATG [19]. This would be useful in resource-limited settings, where the high cost of eltrombopag may limit its availability. It was found that 37% of patients with severe AA, who were previously unresponsive to ATG had a haematological recovery. Another study compared the use of mycophenolate mofetil as a combination immunosuppressant, to ATG and cyclosporine therapy alone [21]. No improvement in response or relapse rates were observed with the use of mycophenolate mofetil.

Other supportive measures in the treatment of AA include [22]:

- Progesterone in the suppression of menstruation.
- Strict infection control measures: patients should be managed in an isolation cubicle with neutropaenic precaution.
- Avoidance of non-steroidal anti-inflammatory drugs (NSAID’s).

2. Justification for the study

There is a paucity of South African data on aplastic anaemia. This includes data on the response to immunosuppressive agents and the distribution of infectious agents that cause aplastic anaemia. Knowledge of the most common infectious agents and their respective incidence in an HIV population may prove beneficial in early diagnosis and institution of treatment. Furthermore, costs may be saved by choosing appropriate investigations based on the prevalence of specific infectious causes for a particular population.

3. Aim

To determine the absolute number of new cases and the various causes of aplastic anaemia, as well as the response to immunosuppressive therapy in a Johannesburg population.

4. Objectives

4.1 To determine the absolute number of new cases of aplastic anaemia at the Charlotte Maxeke Johannesburg Academic Hospital for the period of 1st January 2014 until 30th June 2019.

4.2 To identify the most common causes of aplastic anaemia in the sample group.

4.3 To evaluate the clinical and haematological response to immunosuppressive therapy in the sample group.

5. Materials and Methods

5.1 Study Site

The study will be conducted at the department of Adult Medical Oncology, wards 594 and 495, Charlotte Maxeke Johannesburg Hospital.

5.2 Study Period

1 January 2014 to 30 June 2019

5.3 Study Design

A retrospective cross-sectional analysis of laboratory confirmed cases of aplastic anaemia, treated at the Adult Medical Oncology Unit at the Charlotte Maxeke Johannesburg Academic Hospital. Patient files will be retrieved from the Oncology Clinic at the said institution, where relative data will be collected and captured onto a data sheet. (See appendix A)

All patient files stored at the Adult Medical Oncology unit will be surveyed to identify those patients with a diagnosis of Aplastic Anaemia. These records will be compared against a pharmacy record of all patients treated with ATG and cyclosporine in the Oncology wards for the given study period. Electronically captured discharge summaries will also be used to identify information such as complications, the use of blood and blood products and the laboratory investigations conducted during the hospital stay.

5.4 Study Population

All Subjects with laboratory confirmed aplastic anaemia (based on bone marrow and trephine biopsy findings) seen at the Adult Medical Oncology Unit at CMJAH.

5.4.1 Inclusion Criteria

All subjects greater than eighteen years of age, irrespective of the cause of AA or HIV status.

5.4.2 Exclusion Criteria

Subjects younger than eighteen years of age. Subjects without fully documented bone marrow and trephine biopsy results. Subjects with lost or missing case records will also be excluded from the study but will be documented as such.

5.5 Measurement Tools

- Data collection sheet will be used to capture relevant information. (Appendix A).
- The severity scale defined by Bacigalupo *et al* [1] above, will be used to grade the severity of aplastic anaemia in each subject.
- Using the WHO haematological toxicity scale, a generated response-to-treatment-scale will be applied (see below). Each parameter on the toxicity scale will be awarded a point according to the grade. A grade 0 will be awarded 0 points, a grade 1 will be awarded 1 point and so on. A tally of the scores will be done; this number will be divided by the total number of variables (4) and the average score will be recorded from 0 to 4.
- A complete response to immunosuppressive therapy will be defined as a score of 0 or a score of 1. Partial response to treatment will be defined as a score of 2. A poor response to treatment will be defined as a score of 3 or a score of 4.
- The best full blood count parameters after treatment will be recorded, irrespective of the duration post-treatment.

Table 3: WHO Haematological Toxicity Scale

Parameter	**Grade 0**	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**
Haemoglobin (g/dL)	≥11.0	9.5-10.9	8.0-9.4	6.5-7.9	<6.5
Neutrophils ($X10^9$/L)	≥2.0	1.5-1.9	1.0-1.4	0.5-0.9	<0.5
Leucocytes ($x10^9$/L	≥4.0	3.0-3.9	2.0-2.9	1.0-1.9	<1.0
Platelets ($x10^9$/L	≥100	75-99	50-74	25-49	<25

5.6 Source of Data

Patient bed-letters, discharge summaries, documented results, prescription charts and clinic notes.

5.7 Study Definitions

I. Immunosuppressive therapy: The use of cyclosporin, corticosteroids and anti-thymocyte globulin (ATG).

II. Neutropaenic Precaution: Observe strict hygiene, close monitoring for signs of infection, avoidance of out-of-hospital food products.

III. Neutropaenic Sepsis: Sepsis precipitated by reduced neutrophil counts.

IV. Severe Disease: Meeting Bacigalupo criteria of haematological parameters for severe disease.

V. Very Severe Disease: Meeting Bacigalupo criteria of haematological parameters for very severe disease.

VI. Non-Severe Disease: Not meeting Bacigalupo criteria of haematological parameters for either severe disease or very severe disease.

VII. Partial Response to Treatment: Average score of 2 obtained using the WHO Haematological Toxicity Scale.

VIII. Complete Response to Treatment: Average score of 0 or 1 obtained using the WHO Haematological Toxicity Scale.

IX. Poor Response to Treatment: Average score of 3 or 4 obtained using the WHO Haematological Toxicity Scale.

5.8 Limitations and Potential Bias

5.8.1 Data collection may be limited by the adequacy of doctor's notes in patient files.

5.8.2 Data may be limited owing to poor adherence to follow-up appointments and/or medication.

5.8.3 The best response to treatment recorded may not be defined by a period post-treatment as no specific departmental protocol for the periodic measurement of the full blood count exists.

5.8.4 The population under investigation is an urban population at a quaternary level hospital in Johannesburg and may not reflect the South African population in its entirety.

5.8.5 A high prevalence of HIV and its resultant opportunistic infections may favour a higher incidence of infective aetiologies.

5.8.6 Patients are not always worked up comprehensively, e.g. some will not have had virological tests or results of these tests may not be traceable. Most patients will not have had a screening for congenital abnormalities. This may confound data interpretation. Missing variables will thus be excluded from the analysis of data.

5.8.7 Due to the low world-wide prevalence of AA and the paucity of data reflecting the South African Population, it may be difficult to generate a recommended sample size to achieve statistical significance. However, a study done in the Soweto population in 2015 [8], will be used to evaluate the power of this study.

5.9 Data processing and analysis

Data will be collected and analysed from data collection sheets (Appendix 1). The computer programme RedCap™ will be used to facilitate data capture. The data will then be exported to Microsoft Excel for analysis. The IBM SPSS® (New York, United States of America) Version 22 software will be used to statistically analyse variables.

The frequency of age categories will be calculated for male and female patients as well as for the entire sample group. Ages of patients will be categorised as per the data collection sheet. This will be graphically represented to determine if the sample population follows a typical bimodal distribution of AA.

The relative racial distribution will be analysed and grouped as Black, Caucasian, Asian, Indian and Coloured. Each group will be expressed as a percentage of the total sample population.

The aetiology will be categorised as idiopathic and secondary causes. It will be sub-categorised into specific secondary causes and infectious causes (drugs, chemicals, pregnancy, etc.) and expressed as a percentage of the sample population.

Patients will be classified as HIV infected and HIV non-infected at time of diagnosis. The number of HIV infected patients will be expressed as a percentage of the total sample population.

Patients receiving immunosuppressive therapy will be grouped based on the ATG derivative received (Rabbit-ATG group and Horse-ATG group).

Patients will be categorised as very severe, severe and non-severe as per the Bacigalupo classification; these will be expressed as a percentage. Response to treatment will be graded as per the WHO toxicology scale (grade 0 to grade 4) recorded as the best full blood count parameters after treatment. The response will be grouped as a complete response (score 0 and score 1), partial response (score 2), or poor response (score 3 and score 4) to treatment based on the WHO toxicology scale (see above). Response to treatment between the HIV infected and HIV non-infected group will be compared using the chi-squared test. Response to treatment between the Rabbit-ATG and Horse-ATG group will also be compared using the chi-squared test. It must be noted that some patients remain transfusion dependent after therapy and thus, the use of blood products may confound the best full blood count used.

The total number of patients who received stem cell transplant will be recorded and expressed as a percentage of the sample population.

6. Ethics

6.1 Patients will be allocated a sample number and will not be identified by name.

6.2 Data will be collected from patient files by the researcher and will be kept confidential.

6.3 A list of patients who have been lost to follow up will be tabulated, with their contact details, and be forwarded to the treating consultant to re-establish contact with them.

6.4 This protocol will be submitted to the Postgraduate Committee of the University of the Witwatersrand as well as the Human Research Ethics Committee of the University of the Witwatersrand, for permission to conduct the study.

6.5 Consent will be obtained from the head of the division of adult medical oncology as well as from the chief executive officer at the Charlotte Maxeke Johannesburg Academic Hospital.

7. Timing

	JAN '19	FEB '19	MAR '19	APR '19	MAY '19	JUN '19	JUL 19	AUG '19	SEP '19	OCT '19	NOV '19	DEC '19	JAN '20	FEB '20
Literature Review	■	■												
Preparing Protocol	■	■												
Protocol Assessment by Supervisor			■	■										
Post Graduate Submission					■									
Ethics Application						■	■	■						
Data Collection									■	■	■			
Data Analysis												■		
Write up												■	■	■

8. Funding

Costs for stationery and transport will be self-funded and are projected to be R1000,00. There are no other identifiable costs in the study.

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Chapter Two: Submissible Article

A Pilot Study Investigating the Aetiology of Aplastic Anaemia and the Response to Immunosuppressive Therapy at the Charlotte Maxeke Johannesburg Academic Hospital.

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Author's contributions: Tyral Dean Ramsamy was the principal investigator and primary author of the protocol and final manuscript. Cleo Solomon and Georgia Demetriou conceptualized the study and assisted with the study design, editing and approving of the final manuscript.

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Abstract

Introduction

There is a paucity of South African data documenting the various causes of Aplastic Anaemia (AA), as well as the response to combination immunosuppressive therapy. The vast differences in treatment protocols internationally may be as a result of the poor representation in literature owing to the rarity of the disorder. Here, an analysis of possible secondary causes of AA as well as individual responses to treatment was reviewed at the Charlotte Maxeke Johannesburg Academic Hospital.

Objective

To determine the absolute number of cases, possible causes and response to immunosuppressive therapy in aplastic anaemia at the Charlotte Maxeke Johannesburg Academic Hospital.

Method

A retrospective cross-sectional analysis of all laboratory confirmed cases of aplastic anaemia at the Charlotte Maxeke Johannesburg Academic Hospital for the period of January 2014 to June 2019.

Results

There were 35 confirmed cases of AA for the given study period; 21 of which were males and 14 females. Sixty-five percent of the study sample had no identifiable cause for AA and were defined as idiopathic. Twelve patients had identifiable secondary causes for AA. Of these, 5 patients were exposed to a toxin (hydrocarbons), 2 patients were exposed to drugs (azathioprine and zidovudine), two patients had active Hepatitis B infection, two patients had a documented auto-immune condition (Systemic Lupus Erythematosus and Rheumatoid Arthritis) and one patient was pregnant at initial presentation. All patients were treated with a combination of anti-thymocyte globulin (ATG), rabbit or horse derived, cyclosporine and corticosteroids. None of the patients had received a stem-cell transplant.

Based on the World Health Organization (WHO) Haematological Toxicity Scale, 46% of patients had a poor response to treatment, 26% had a partial response to treatment and 28% had a complete response to treatment.

Twenty-seven percent of patients died, many of whom had no clearly documented or suspected immediate cause of death. However, mortality cannot be commented on, due to the absence of complete follow-up data.

Conclusion

Aplastic Anaemia is a rare disease which carries a high mortality rate. All possible secondary causes should be sought by taking a detailed patient history and conducting relevant viral serology and immunological assays. All patients must be treated with a standard treatment protocol and any exposure to toxins or offending drugs should be terminated. A South African Bone Marrow Registry (SABMR) donor search should be conducted on all patients eligible for a stem-cell transplant to improve the number of patients receiving transplant therapy. The rationale for the absence of bone marrow transplants conducted must be investigated and acted upon. Every effort to improve patient follow-up and continuation of care should be made to establish concrete data on mortality and long-term complications.

Introduction

Aplastic anaemia (AA) is a haematological disorder characterized by pancytopaenia, with hypocellular bone marrow, in the absence of any marrow infiltrate or bone marrow fibrosis [1]. Criteria for the diagnosis was first described by *Camitta et al*, in which two out of the following three blood count parameters must be met [2].

- Haemoglobin less than 100g/L,
- Platelet count of less than 50 x 10^9/L
- Neutrophil count of less than 1.5 X 10^9/L

Bacigalupo et al further classified AA as very severe, severe and non-severe based on the degree of bone marrow cellularity, platelet count, neutrophil count and reticulocyte count [3]. This classification was used in defining the severity of disease in this study.

The epidemiology of AA is often described as biphasic in its age distribution, with most cases being documented in the 2nd decade of life, peaking again in the 5th decade [4]. This distribution was contrary to the findings of a South African study [5], in which the second peak in age distribution was not observed. Much focus has been placed on the higher prevalence of AA in South East Asia, with some evidence identifying a 2-3-fold increase compared to western counterparts [4] [8]. Many reasons for this have been postulated, including higher exposure to toxic chemicals and pesticides, as well as a higher prevalence of infections (such as viral hepatitis), all of which have been described as secondary causes of AA [8] [9].

The pathogenesis of AA involves targeted destruction of progenitor haemopoietic cells as a result of clonal expansion of cytotoxic T-cells [6]. This results in haemopoietic failure which manifests as pancytopaenia. Causes of AA are broadly classified as primary or secondary. Viral infection, toxin/drug exposure, pregnancy and autoimmune disease are among the common secondary causes of AA [7].

Table 1: Causes of Aplastic Anaemia

<table>
<tr><th>PRIMARY CAUSES</th><th>SECONDARY CAUSES</th></tr>
<tr><td rowspan="4">Congenital
Fanconi’s Anaemia
Dyskeratosis Congenita
Schwachman-Diamond Syndrome
Reticular dysgenesis
Amegakaryocytic Thrombocytopaenia
Familial Aplastic Anaemia</td><td>Chemical Exposure
Benzene
Pesticides</td></tr>
<tr><td>Drugs
Carbimazole
Chloramphenicol
Chlorpromazine
Phenytoin</td></tr>
<tr><td>Ionizing radiation</td></tr>
<tr><td>Infection
Epstein Barr Virus (EBV)
Cytomegalovirus (CMV)
Parvovirus B19
Human Immunodeficiency Virus (HIV)
Hepatitis Virus</td></tr>
<tr><td>Idiopathic</td><td>Pregnancy</td></tr>
</table>

Patients may present with an array of signs and symptoms which are secondary to the loss of function of individual cell-lines. These may include spontaneous haemorrhage, sepsis or even heart failure as a result of the profound anaemia. The disease presentation is variable and dependent on the severity of AA [1].

All patients should have complete workup prior to commencing therapy [1]. This includes a full blood count, auto-immune screening tests (such as anti-nuclear antibody and anti-double-stranded DNA), viral serology (including HIV, CMV, EBV, Parvovirus B19, Hepatitis B and C), and a bone marrow aspirate and trephine biopsy. A thorough history of toxin and drug exposure should be explored in search of other possible secondary causes for the disease. A pregnancy test (beta-hCG) on all females of childbearing age should be conducted [4].

Patients diagnosed with aplastic anaemia should be referred to and treated by a clinician experienced in its management [1]. The addition of eltrombopag to the standard immunosuppressive regimen has shown better haematological response in patients with severe aplastic anemia. Eltrombopag, ATG and cyclosporine are now considered the new standard of care. [8] Stem cell transplants have shown superior results in adolescents and young adults and should be explored as upfront therapy. [9,10] There are multiple on-going studies on the use of monoclonal antibodies and other immune-modulating therapy for the treatment of AA. Supportive therapy includes the use of blood and blood products, iron-chelation, suppression of menstruation and maintenance of neutropaenic precautions. [4]

Aim of the Study

Due to the paucity of data for AA in South Africa, a pilot study may be justified to provide data that may aid larger studies in identifying true demographic information and possible causal relationships. This study sought to identify the prevalence of AA at the selected institute, as well as to identify possible secondary causes of AA. Further, a response to current recommended protocols for immunosuppressive therapy in the treatment of AA was evaluated.

Materials and Methods

Study Population and Study Design

This is a retrospective cross-sectional study, conducted at the Charlotte Maxeke Johannesburg Academic Hospital for the period 01-01-2014 until 31-06-2019. All patient files at the Adult Medical Oncology Clinic were surveyed for patients with laboratory confirmed diagnoses of AA. This was compared against pharmacy records of all ATG administered to patients in the oncology wards 495 and 594. Clinic files with blood and histology results, electronic hospital records of bed-letters and prescriptions, discharge summaries, and pharmacy records were used in data collection. Exclusion criteria included patients under the age of eighteen and those patients with missing laboratory confirmation of diagnosis.

Measurement Tools

A data collection sheet (Appendix 1) was uploaded to RedCap^{TM} and used to electronically capture data. This data was later exported to Microsoft Excel for ease of statistical analysis.

Statistical Analysis

The IBM SPSS® (New York, United States of America) Version 22.0 software was used to analyse the data. Categorical variables were represented as frequencies and percentages. Nominal variables (blood results) were reported as minima, maxima, median, and interquartile ranges. Comparisons between categorical variables were analysed using the Chi-Square test with confidence intervals set at 95%, with p-values <0.05 to be considered statistically significant.

Study Definitions

I. Immunosuppressive therapy: The use of cyclosporin, corticosteroids and anti-thymocyte globulin (ATG).
II. Neutropaenic Precaution: Observe strict hygiene, close monitoring for signs of infection, avoidance of out-of-hospital food products.
III. Neutropaenic Sepsis: Sepsis precipitated by reduced neutrophil counts.
IV. Severe Disease: Meeting Bacigalupo criteria of haematological parameters for severe disease.
V. Very Severe Disease: Meeting Bacigalupo criteria of haematological parameters for very severe disease.
VI. Non-Severe Disease: Not meeting Bacigalupo criteria of haematological parameters for either severe disease or very severe disease.
VII. Partial Response to Treatment: Average score of 2 obtained using the WHO Haematological Toxicity Scale.
VIII. Complete Response to Treatment: Average score of 0 or 1 obtained using the WHO Haematological Toxicity Scale.
IX. Poor Response to Treatment: Average score of 3 or 4 obtained using the WHO Haematological Toxicity Scale.

Limitations

1. Due to the rarity of the disease, statistical significance for many parameters could not be generated from the small sample size.
2. A degree of missing data, which includes investigations not conducted or documented, resulted in difficulty identifying all patients with true secondary causes for aplastic anaemia.

3. The lack of bone marrow transplant cases does not allow for any comparison between transplant and non-transplant treatment modalities.
4. The small sample population of individuals infected with HIV, make any comparison between the severity of disease or response to treatment between HIV infected and non-infected individuals problematic.
5. True disease mortality is not represented here, due to the high number of cases that were lost to follow-up.
6. The Haematological response may be confounded by the use of blood products in patients who remained transfusion dependent.

Ethical Considerations

Each patient was assigned a patient number. Data was subsequently captured with patient anonymity being maintained. Permission to collect data was obtained from the Head of the Department of Adult Medical Oncology, as well as the Chief Executive Officer at the Charlotte Maxeke Johannesburg Academic Hospital. Ethical clearance was obtained from the Human Research Ethics Committee (Medical) at the University of the Witwatersrand, Johannesburg (Clearance Certificate Number M190738). The protocol was submitted to the South African National Health Research Database (Reference: GP_201909_061).

Results

Demographic Information

The study sample comprised of data from a total of 35 patients and of these, 21 (60%) were males and 14 (40%) were females. The majority (89%) of the patients were of African ethnicity and only 9% were Caucasian and 3% Indian. Most of the patients were in the 18-29 years age category (43%) followed by 30-39 years age category (31%). The remaining 26% of the patients were aged 40 years or older.

Table 2: Frequency of age, sex and ethnicity in the sample population

Variables	**Frequency**	**Percentage**
Sex		
Male	21	60%
Female	14	40%
Ethnicity		
African	31	89%
Caucasian	3	9%
Indian	1	3%
Age		
18-29	15	43%
30-39	11	31%
40-49	4	12%
≥50	5	14%

Figure 1: Distribution of sex (n=35)

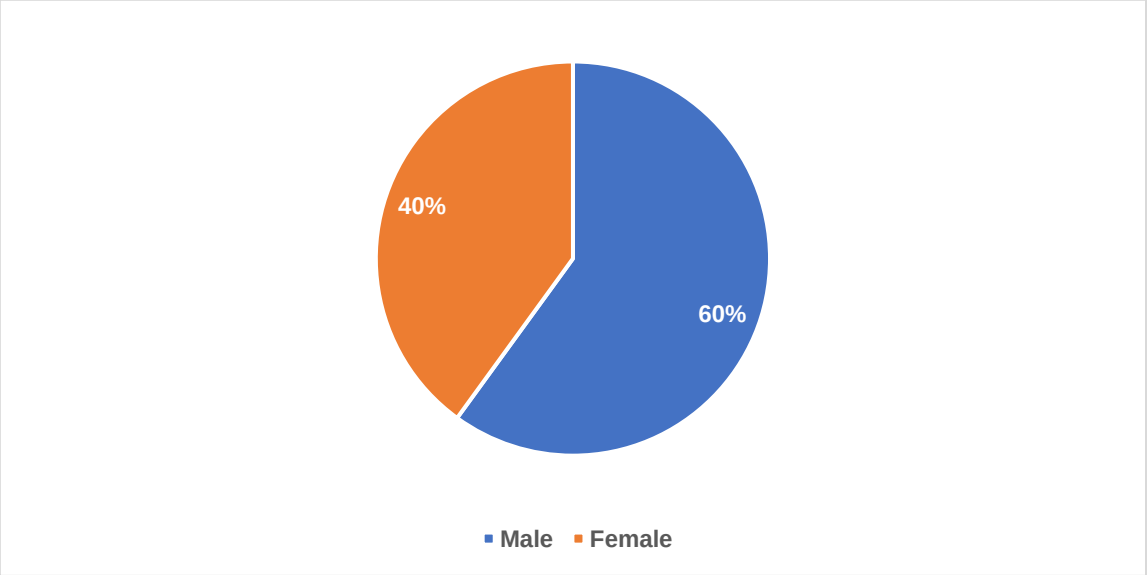


Figure 2: Distribution of ethnicity (n=35)

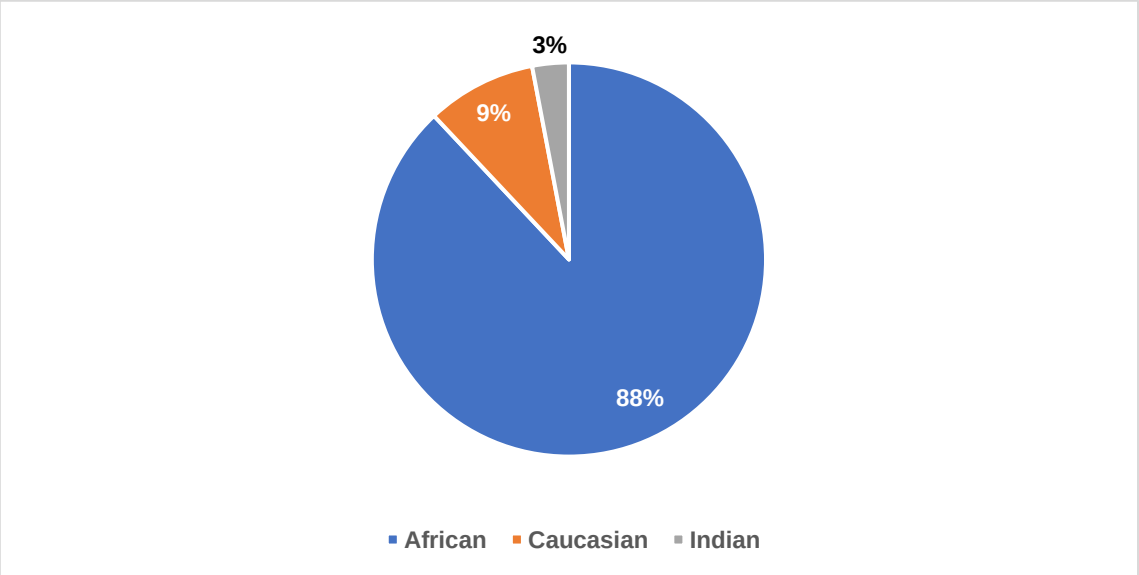
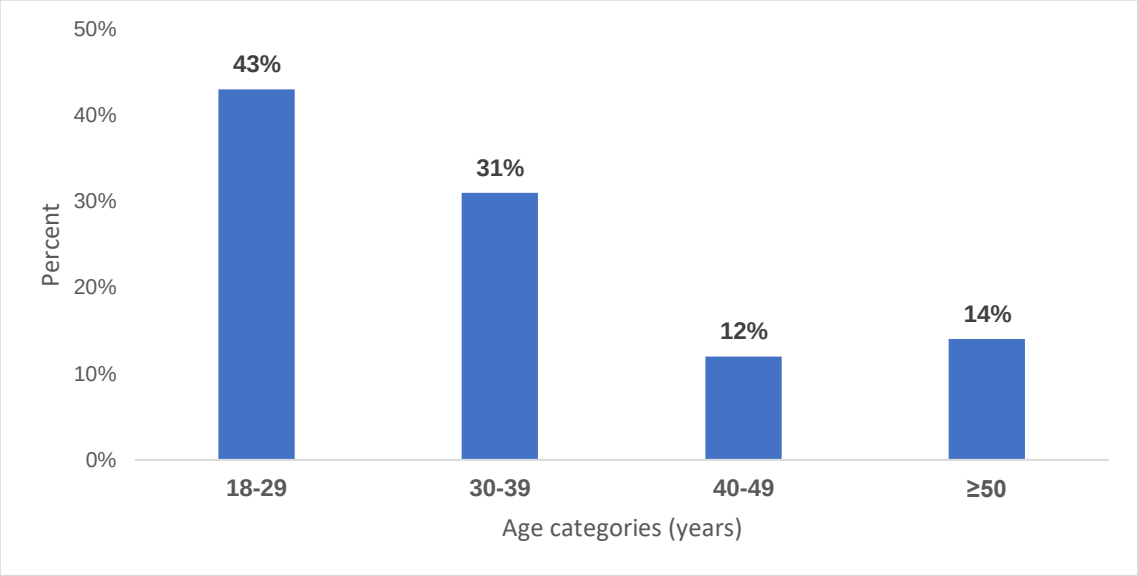
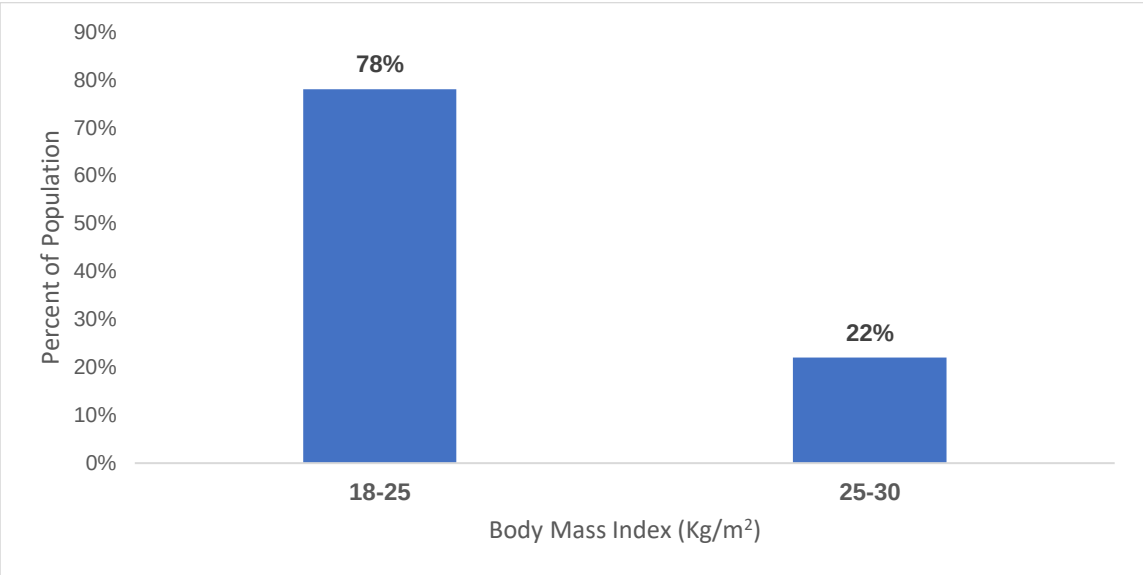


Figure 3: Distribution of age categories (n=35)



Eighteen patients from the sample had documented body mass indices (BMI) calculated. The majority of these patients had a normal BMI (18-25 kg/m^2), whilst 22% were classified as overweight (25-30 kg/m^2). None of the patients with recorded BMI were obese (BMI >30 kg/m^2) or underweight BMI <18 kg/m^2).

Figure 4: Distribution of body mass index (N=18)



Immunosuppressive Regimen

All patients were treated with a standard protocol regimen which included the use of ATG, cyclosporine and steroid (Intravenous and oral). Seventy-four percent of patients were given Rabbit-ATG, whilst only 26% were given Horse-ATG. The doses of all drugs were weight-based. The duration of ATG therapy was documented for 34 out of the 35 patients. The majority of these patients (83%) were treated with ATG for a duration of 7-10 days. Seventeen percent of patients were given ATG for 5 days. The use of rabbit versus horse ATG was dictated by the availability of either type at the treating centre and not necessarily by the choice of the physician.

Figure 5: Distribution of type of Anti-thymocyte globulin used in the treatment regimen (n=35)

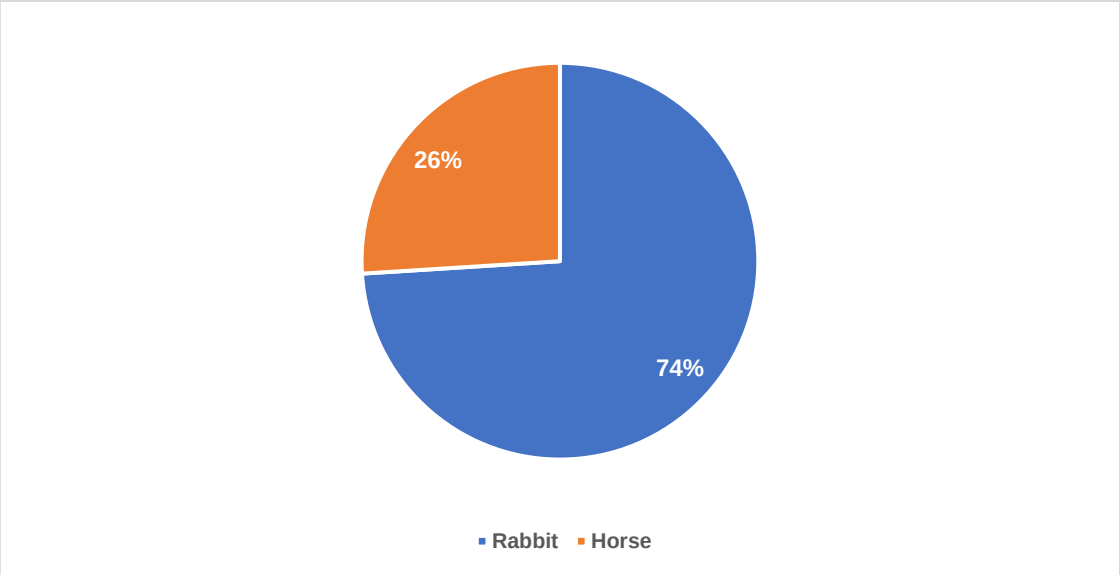
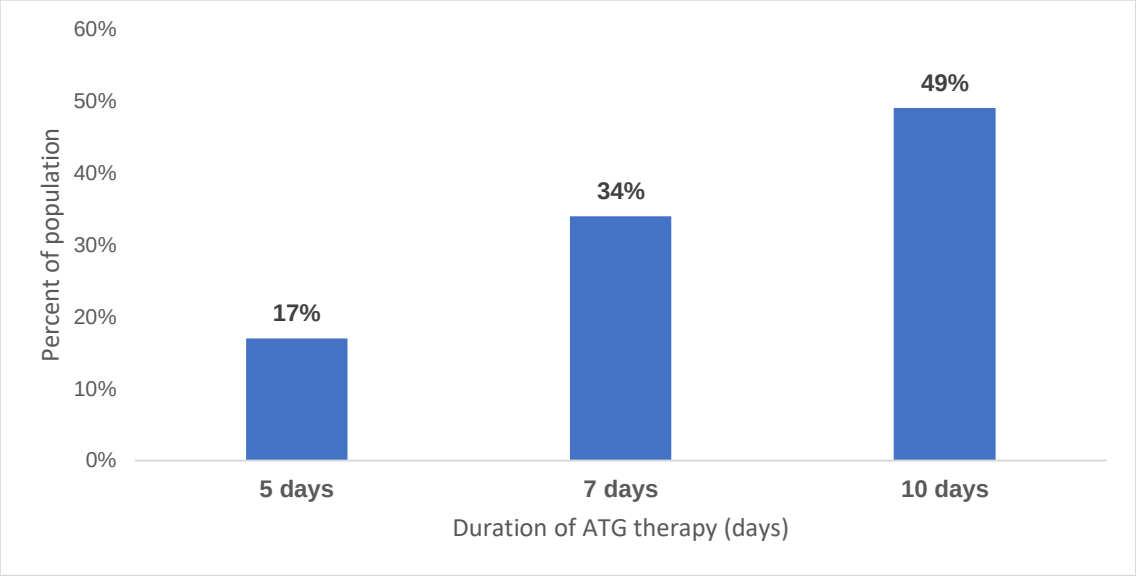


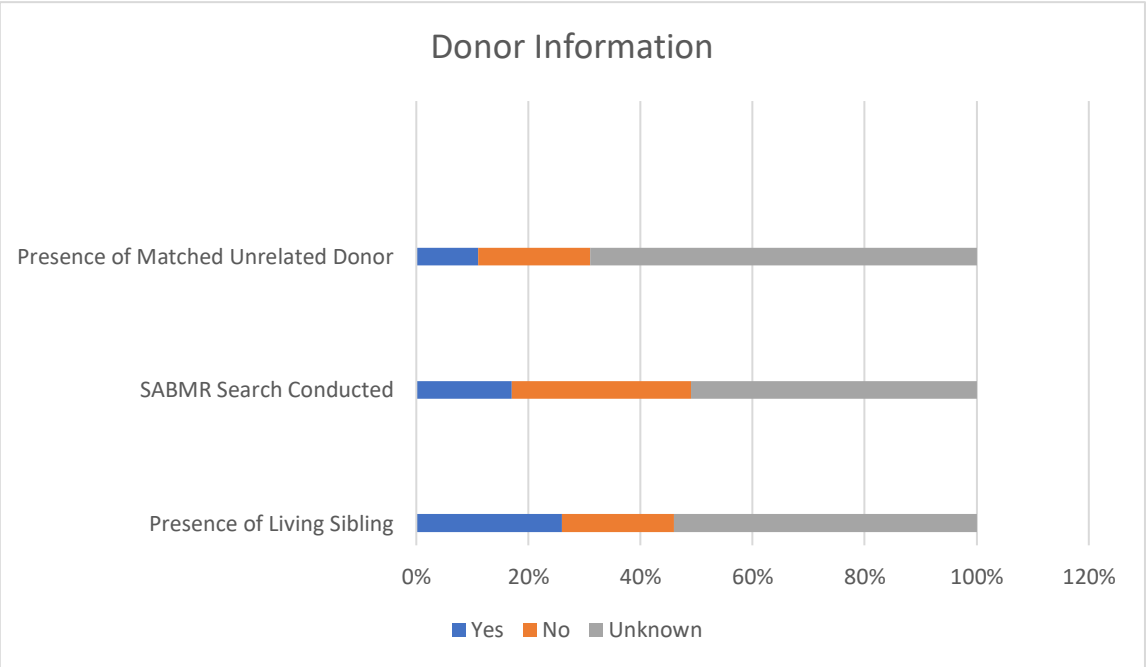
Figure 6: Duration of ATG therapy (n=34)



Donor Information

More than half the number of patients (54%) did not have the presence of a related donor documented. Twenty-six percent had a living related sibling, whilst 20% had no living sibling. Seventeen percent of patients had a South African Bone Marrow Registry (SABMR) search conducted. Eleven percent of patients had the presence of a local or international matched unrelated donor. None of the patients received an allogeneic stem cell transplant during the study period.

Figure 7: Donor Information



Laboratory Investigations

The full blood count was recorded at diagnosis for each subject. For standardization, the full blood count results used for the reporting of the bone marrow aspirate and trephine biopsy results were used.

Table 3: Blood count parameters at diagnosis

	Minimum	**Maximum**	**Median**	**Range**
White cell count	0.99	5.49	2.35	4.50
Neutrophils	0.04	1.73	0.63	1.69
Red cell count	0.92	3.52	1.73	2.60
Haemoglobin	3.40	11.00	5.80	7.60
Platelets	1.00	101.00	9.00	100.00

A high proportion of patients were noted to be exposed to the common viruses implicated in aplastic anaemia. There were no active EBV, CMV or Parvovirus infections. Two patients (6%) were Anti-nuclear antibody positive. Of these, one patient was diagnosed with SLE whilst the other patient was diagnosed with rheumatoid arthritis, prior to the diagnosis of AA. A significant proportion of the sample had not undergone routine laboratory investigations. Eighty-three percent of patients did not have anti-double-stranded DNA tests conducted.

Table 4: Viral serology and auto-immune assays (n=35)

	Positive (%)	**Negative (%)**	**Not Done (%)**
EBV IgG	71	6	23
EBV IgM	0	80	20
CMV IgG	60	3	37
CMV IgM	0	66	34
Parvovirus IgM	41	26	32
Parvovirus IgG	0	68	32
Parvovirus PCR	3	27	70
ANA	6	77	17
AdsDNA	0	17	83
HIV	11	86	3

EBV (Ebstein-Barr Virus), CMV (Cytomegalovirus), ANA (Anti-nuclear Antobody), AdsDNA (Anti-double stranded DNA), HIV (Human Immunodeficiency Virus), Ig (Immunoglobulin)

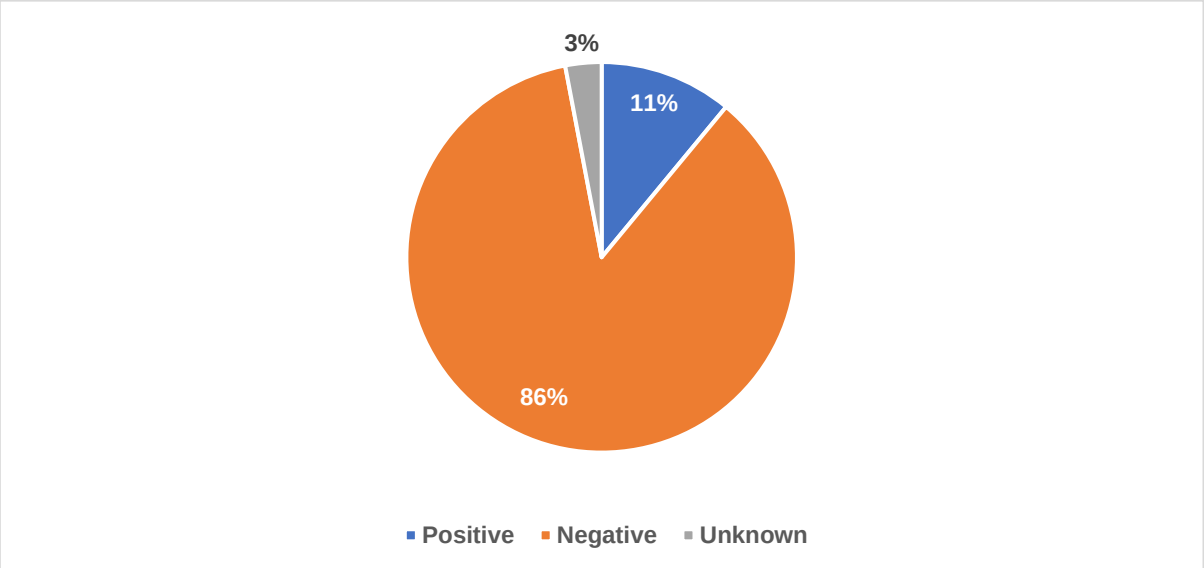
Two patients tested positive for hepatitis B and two patients for hepatitis C. Of these patients, only one patient was found to have active hepatitis (Hepatitis B) and was co-infected with malaria at the time of diagnosis. Treatment with Lamivudine and Artemether/Lumefantrine was initiated. This patient had a complete response to treatment for AA and has had continued follow-up at the oncology clinic.

Table 5: Hepatitis Infection (n=35)

	Yes	No	Not done
Hepatitis B	6%	80%	14%
Hepatitis C	6%	77%	17%

Eleven percent of the patients were HIV positive. All of these patients were on antiretroviral treatment at the time of diagnosis. One of these patients were on a Zidovudine based regimen, which was subsequently changed on the diagnosis of AA. One patient did not have an HIV test done as part of their initial workup.

Figure 8: HIV status at diagnosis of AA (n=34)

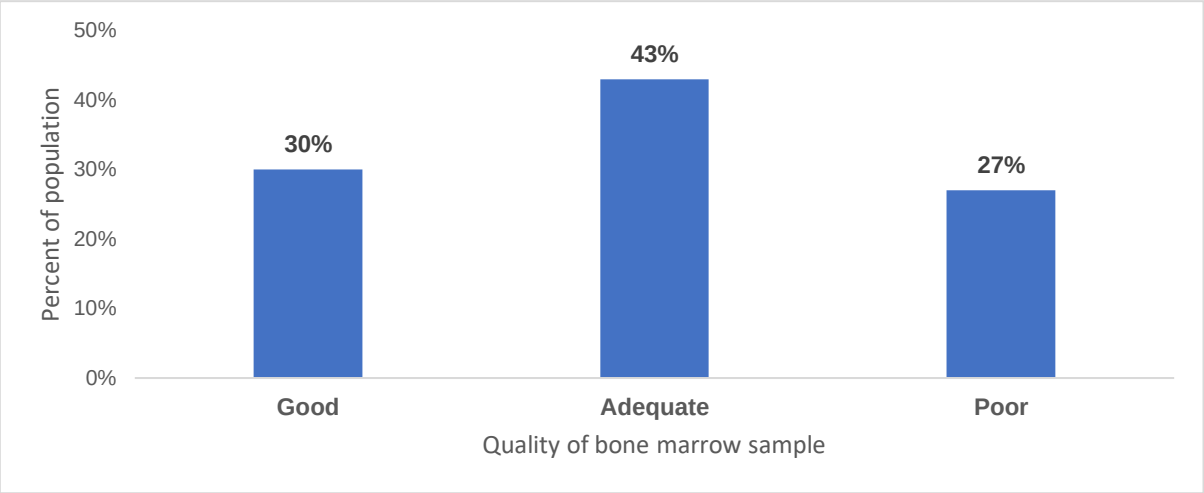


One patient was pregnant and presented during the third trimester of pregnancy (35 weeks gestation). She had delivered a live child. The outcome of the child is unknown, and the patient was eventually lost to follow-up.

Bone Marrow Aspirate and Trephine Biopsy Results

A majority of the samples (73%) were of a satisfactory standard, enabling appropriate interpretation. Twenty-seven percent of the samples reviewed were reported as 'poor quality' although interpretation was still possible. Two patients had the presence of blasts reported on their bone marrow aspirate results. Both patients had blasts less than 10%. These patients did not have other marrow findings suggestive of hypoplastic MDS.

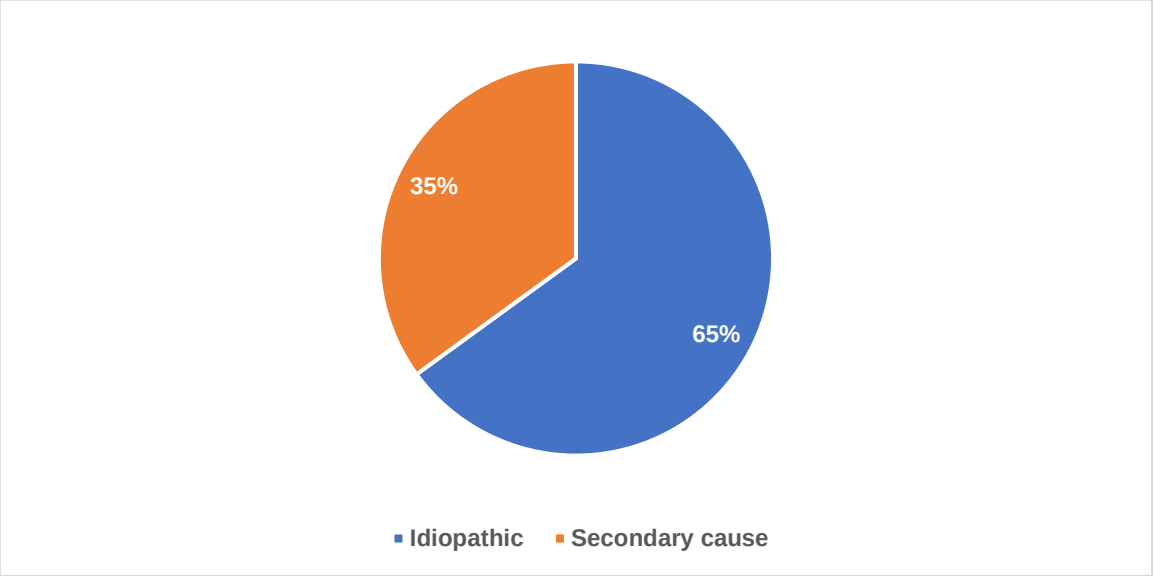
Figure 9: Quality of Bone Marrow Aspirate & Trephine Biopsy (n=35)



Diagnosis

In the majority of patients (65%), no attributable cause for aplastic anaemia could be found, and the diagnosis was said to be idiopathic. Thirty-five percent of patients were documented as having a possible secondary cause.

Figure 10: Cause of aplastic anaemia

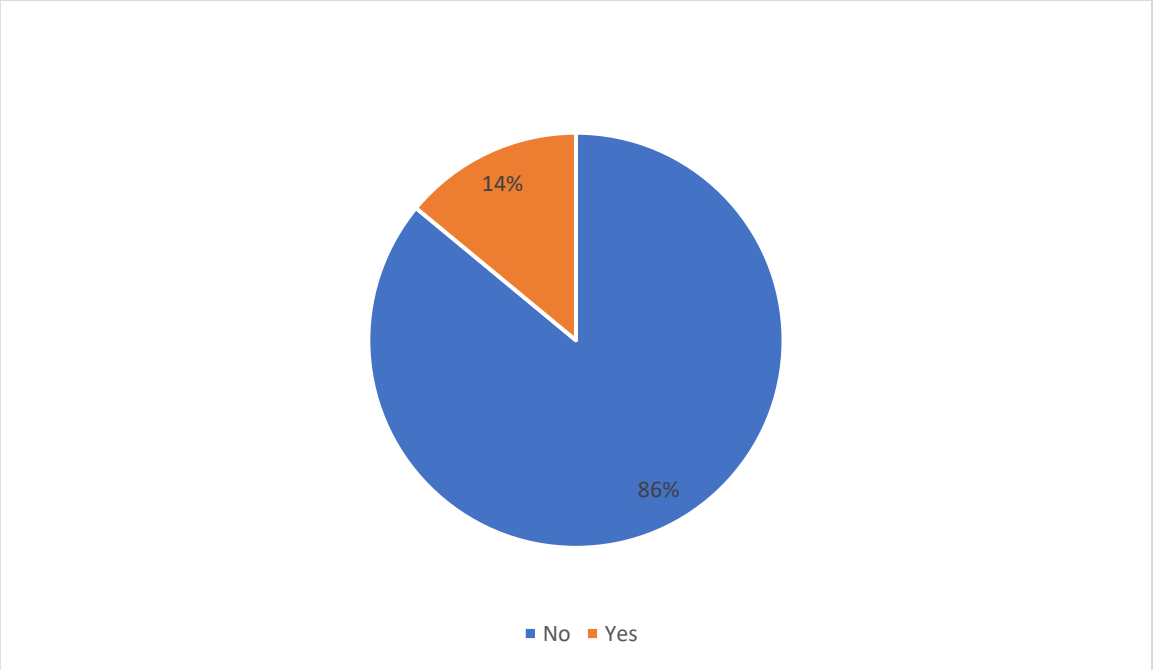


Five patients had a documented toxin exposure, namely hydrocarbons. Of these, 3 patients (all male) had an occupational exposure to hydrocarbons (one patient had worked in the petroleum industry whilst two others worked in the gold and steel mining industries). Two patients had been exposed to organophosphates, one of which was a documented previous intentional ingestion, whilst the other was recorded as an accidental exposure whilst using illicit drugs. Two patients in the sample had documented illicit drug use; one recorded as cannabis use, whilst the other disclosed their use of heroin. Two patients had documented auto-immune disease. One was diagnosed with SLE during the work-up for their pancytopaenia. The other patient had been receiving treatment for rheumatoid arthritis. One patient was pregnant at 35 weeks gestation at diagnosis. One patient was diagnosed with active hepatitis B during their initial workup and was subsequently treated with lamivudine. Two patients were receiving drugs that have been shown to suppress bone marrow activity [11][12]. One patient was on a Zidovudine based ARV regimen which was subsequently changed after the diagnosis of AA. Another patient was receiving Azathioprine as part of their treatment for rheumatoid arthritis, which was continued during and after receiving therapy for AA.

Table 6: Secondary causes of aplastic anaemia (n=12)

	Frequency	Percentage
Toxin	5	42%
Autoimmune	2	17%
Drug-Induced	2	17%
Hepatitis	2	17%
Pregnancy	1	8%

Table 14: Toxin Exposure



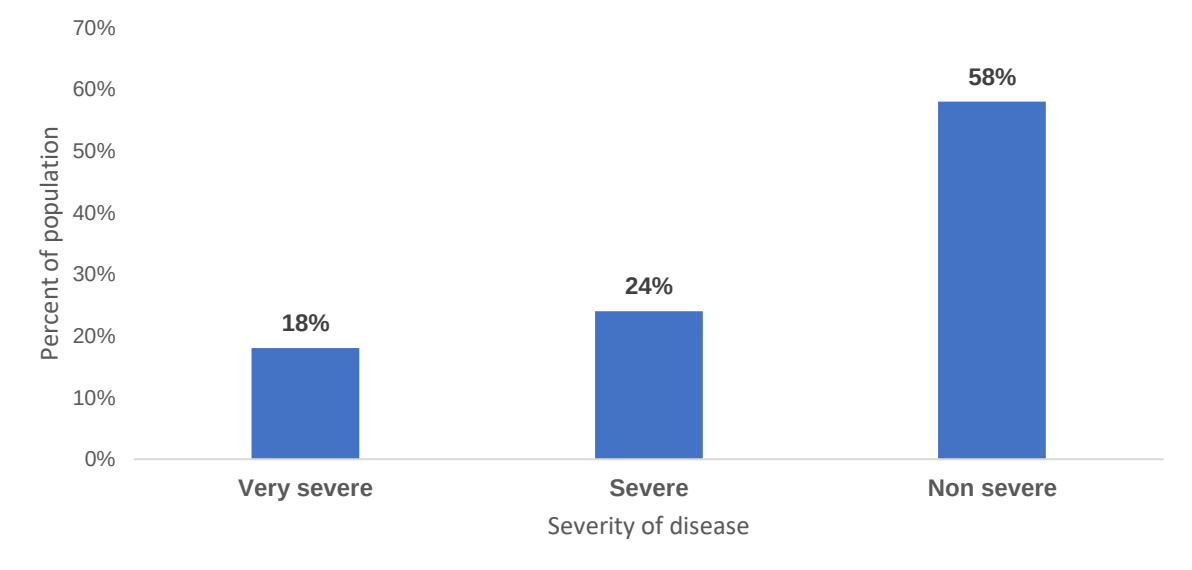
Diagnosis Severity

More than half of the sample population (58%) presented with non-severe disease, according to the Bacigalupo classification for severity of the disease [3].

Table 7: Classification of the Severity of Aplastic Anaemia

Parameters	**Severe Aplastic Anaemia**	**Very Severe Aplastic Anaemia**
Marrow Cellularity	<25% or 25-50% with <30% residual haemopoietic cells	<25% or 25-50% with <30% residual haemopoietic cells
Neutrophils	$<0.5 \times 10^9/L$	$<0.2 \times 10^9/L$
Platelets	$<20 \times 10^9/L$	$<20 \times 10^9/L$
Reticulocyte Count **Manual Count** **Reticulocyte Count** **Automated Analyzer**	$<20 \times 10^9/L$ $<6 \times 10^9/L$	$<20 \times 10^9/L$ $<6 \times 10^9/L$

Figure 11: Diagnosis severity based on Bacigalupo classification (n=35)



There was no statistically significant difference in diagnosis severity by sex (p=0.37), age (p=0.49) or HIV Status (p=0.47). However, a higher proportion of males (21%) presented with very severe disease compared to females (14%). The majority of females (71%) presented with non-severe disease.

The highest proportion of very severe disease was noted in the 30-39 age category; whilst individuals greater than 40 years of age usually presented with non-severe disease (86%).

One HIV positive patient presented with very severe disease, with the remaining patients diagnosed with non-severe disease at presentation. Three out of 4 HIV positive patients presented with non-severe disease and thus no firm conclusions can be made regarding the impact of HIV on disease severity.

Table 8: Diagnosis severity by patient profiles

Variables	**N**	**Very severe**	**Severe**	**Non-severe**
Sex				
Male	21	21%	32%	47%
Female	14	14%	14%	71%
Age				
18-29	15	20%	27%	53%
30-39	11	27%	27%	45%
≥40	9	0%	14%	86%
HIV status				
Positive	4	25%	0%	75%
Negative	30	18%	29%	54%

The severity of disease was compared between toxin-exposed and non-exposed patients in the population sample. In the toxin-exposed arm, 50% of patients had non-severe disease, whilst 25% had severe and another 25% had very severe disease. There were no statistically significant differences in disease severity between the two arms (p=0.81). The toxin-exposed patients who presented with non-severe disease were treated with immunosuppression, without a period of observation following withdrawal of the exposure.

Table 9: Disease Severity by Toxin Exposure (p=0.81)

Toxin Exposure	**N**	**Very Severe**	**Severe**	**Non-severe**
Yes	5	25%	25%	50%
No	30	17%	21%	63%

The severity of disease was compared against the diagnosis of AA. Fifty-two percent of patients in the idiopathic arm were recorded as non-severe disease whilst 67% of patients with secondary

causes had non-severe disease. No significant difference existed between disease severity and diagnosis of AA (p=0.69).

Table 10: Disease severity by diagnosis (p=0.69).

Diagnosis	**N**	**Very Severe**	**Severe**	**Non-severe**
Idiopathic	23	19%	29%	52%
Secondary Cause	12	17%	17%	67%

Response to Treatment

The response to treatment was categorised as poor, partial or complete based on the WHO Haematological Toxicity Scale [13]. Each parameter on the toxicology scale was awarded a point according to the grade. A grade 0 was awarded 0 points, a grade 1 was awarded 1 point and so on. The score for all the parameters was tallied and divided by the total number of parameters (4). An average overall score of 0 or 1 was categorised as a complete response to treatment. A score of 2 was defined as a partial response to treatment. A score of 3 or 4 was recorded as a poor response.

Table 11: WHO Haematological Toxicity Scale

Parameter	**Grade 0**	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**
Haemoglobin (g/dL)	≥11.0	9.5-10.9	8.0-9.4	6.5-7.9	<6.5
Neutrophils ($X10^9$/L)	≥2.0	1.5-1.9	1.0-1.4	0.5-0.9	<0.5
Leucocytes ($x10^9$/L	≥4.0	3.0-3.9	2.0-2.9	1.0-1.9	<1.0
Platelets ($x10^9$/L	≥100	75-99	50-74	25-49	<25

Twenty-eight percent of the sample population had a complete response to treatment. Twenty-six percent had a partial response; whilst 46% had a poor response to treatment. The male population had an equal number of subjects in each of the categories (poor, partial and complete). More female subjects were shown to have responded poorly to treatment (64%). The highest proportion of complete response was seen in individuals older than 40 years of age. A higher proportion of complete response to treatment was seen in subjects who had received Horse-ATG (33%), compared to those who had received Rabbit-ATG (28%). Three out of 4

HIV-positive patients had a complete response to treatment, whilst one of them had a poor response to treatment. Again, only 4 patients in the study sample were HIV positive, hence these results should be interpreted with caution. Given the sample size however, there were no statistically significant differences in response to treatment by sex (p=0.19), age (p=0.15), ATG type (p=0.74) or HIV status (p=0.09).

Figure 13: Response to treatment (n=35)

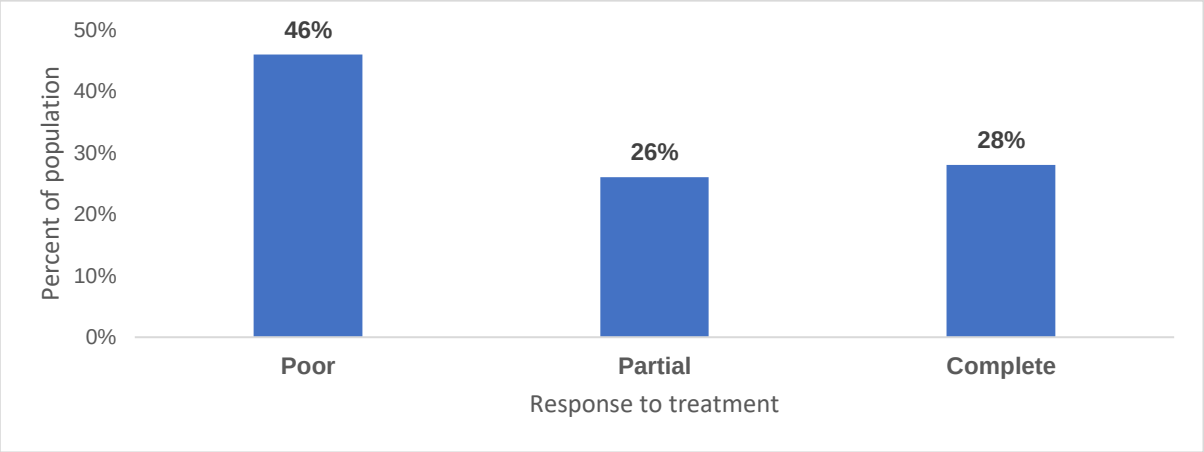


Table 12: Response to treatment by patient profiles

Variables	N	Poor	Partial	Complete
Sex				
Male	21	33%	33%	33%
Female	14	64%	14%	21%
Age				
18-29	15	18-29	40%	33%
30-39	11	30-39	55%	0%
≥40	9	≥40	44%	44%
ATG type				
Rabbit	25	48%	24%	28%
Horse	9	33%	33%	33%
HIV status				
Positive	4	25%	0%	75%
Negative	30	50%	27%	23%

Patients were treated with variable durations of ATG. The treating centre's protocol recommends 7-10 days of ATG. Current literature suggests 4 days of treatment with Horse-ATG and 5 days with Rabbit-ATG. The duration of treatment of ATG was compared to the response to treatment. Six patients were treated with 5 days of ATG. Half of these patients responded poorly to treatment whilst only 17% showed a complete response to treatment. Patients treated with ATG for a duration of 7 days displayed the highest proportion of complete response (33%). However, a chi-square analysis reveals no statistically significant differences in response to treatment by the duration of ATG therapy (p=0.96). Again, this may be attributed to the relatively small sample size.

Table 13: Response to treatment by Duration of ATG therapy (p=0.96)

ATG Duration	**N**	**Poor**	**Partial**	**Complete**
5 days	6	50%	33%	17%
7 days	12	42%	25%	33%
10 days	17	47%	24%	29%

A comparison between response to treatment by diagnosis revealed a higher proportion of idiopathic AA had a poor response to treatment (55%). Of the patients with a probable secondary cause for AA, 42% had a complete response to treatment.

Table 14: Response to Treatment by Diagnosis

Diagnosis	**N**	**Poor**	**Partial**	**Complete**
Idiopathic	23	55%	23%	23%
Secondary Cause	12	33%	25%	42%

Outcome

Thirty-five percent of patients are currently following up at the Oncology Clinic at Charlotte Maxeke Johannesburg Academic Hospital. Twenty-seven percent of patients had demised owing to various causes including neutropaenic sepsis and fatal haemorrhagic events. A high

proportion of patients had been lost to follow-up (38%). None of these patients could be contacted using the personal details provided in their hospital registration information.

There were no statistically significant differences in outcome by sex (p=0.10), age (p=0.97), ATG type (p=0.63) or HIV status (p=0.86). The high proportion of missing data owing to poor patient follow-up makes interpretation of this data unreliable.

Figure 14: Outcome of Patients (n=35)

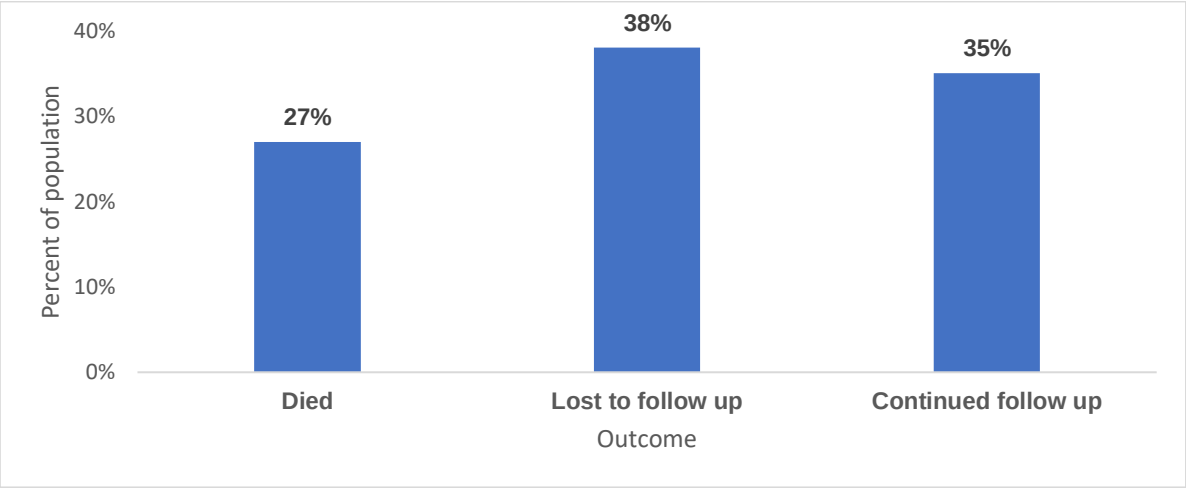


Table 15: Outcome by Patient Profiles

Variables	N	Died	Lost to follow up	Continued follow-up
Sex				
Male	21	20%	30%	50%
Female	14	36%	50%	14%
Age				
18-29	15	27%	33%	40%
30-39	11	27%	45%	27%
≥40	9	25%	38%	38%
ATG type				
Rabbit	25	28%	40%	32%
Horse	9	25%	25%	50%
HIV status				
Positive	4	25%	50%	25%
Negative	30	24%	38%	38%

Discussion

A total of 35 cases of aplastic anaemia were identified at the Charlotte Maxeke Johannesburg Academic Hospital for the study period of five and a half years. This equates to an annual average of 6.4 cases. This is consistent with a study at the Chris Hani Baragwanath Hospital in which the average number of cases seen were 5.8 per year for the study period, although inclusion criteria for age proved to be broader [5].

The male to female ratio for this study was 1.5:1. This is consistent with two previous studies in the Soweto population in South Africa [5] [14]. More than half of the study sample were young, African males. The biphasic distribution often described in the literature was not noted here as 74% of patients were aged between 18 and 39 years of age, with the remainder being aged older than 40 years [4].

All patients were treated with a standard protocol regimen recommended at the said institution. Rabbit-ATG is recommended as part of the standard treatment protocol and it reflects that 74% of patients were treated as such. The remaining 26% were treated with Horse-ATG. According to the treatment protocol at the institution, the recommended duration for ATG therapy is 7 to 10 days. The longer duration of ATG (compared to that recommended in current literature) may be associated with several complications such as serum sickness and infection. Seventeen percent of patients received ATG for five days. Reasons for the deviation in the institution's protocol are not clear. It may be suggested that these patients did not tolerate therapy or had developed sepsis during treatment, which prompted the treating physician to pause therapy. The treating physician's knowledge of the recommended standard duration of therapy must likewise be sought.

Many patients did not have clear donor information documented. A SABMR donor search was conducted in only 17% of patients. This may be justified by the high proportion of patients being foreign nationals and thus not qualifying for stem cell transplant, making use of the SABMR donor search unnecessary. The prevalence of foreign national patients, however, does not account for the remaining total of 83% of patients in which the SABMR donor search was not conducted or status of which was unknown. Other reasons for this must be further explored.

None of the patients had received an allogeneic stem cell transplant. A non-exhaustive list of possible explanations for the paucity of transplants conducted include:

1. the high burden of the cost related to the acquisition of international donors.
2. the high number of patients in whom a donor search was not performed.
3. The number of patients not willing to take the associated transplant risks.
4. The number of patients who demise before such plans can be discussed.

A high proportion of patients did not have complete viral and immunological serology conducted at diagnosis. Of note, 70% of patients did not have Parvovirus PCR testing and 83% did not have anti-double-stranded DNA testing. One patient was not tested for HIV. Reasons for this remain unclear, although it must be noted that patients referred from other institutions may have had these investigations conducted and the results may have erroneously been excluded from the referral documentation. Thus, a true representation of secondary causes may not be evident in this study due to the lack of complete secondary work-up [3] conducted.

The majority of patients had an acceptable bone marrow sample, whilst 27% had a poor-quality sample recorded in the findings. This may reflect a degree of difficulty experienced in obtaining an acceptable quality bone marrow sample in patients with aplastic anaemia.

The majority of the sample population had no identifiable cause for AA. Twelve patients were documented as having a probable cause for AA. Of these, 5 patients had been exposed to a variety of hydrocarbons. Exposure to hydrocarbons in the petroleum and agricultural industries may also exist [13], however it has not been well described in South African literature. A study in Thailand described organophosphates to have a relative risk estimate of 2.1 [16]. South Africa has a high burden of organophosphate exposure in both the domestic and agricultural domains. Domestically, concern exists for both accidental and intentional ingestion [17] [18]. Long-term complications of such exposures may require further investigation.

Two drugs were identified as possible implicates: azathioprine and zidovudine. Azathioprine has been previously described as a secondary cause for AA. Zidovudine, although more commonly associated with pure red-cell aplasia, may also cause a pancytopaenia [11]. Drug

and toxin induced aplastic anaemia should be observed for spontaneous remission following withdrawal of the offending agent. This is especially true for non-severe disease. If no spontaneous remission is observed, the patient should be escalated to standard immunosuppressive therapy. A period of observation was not noted in the drug and toxin exposed patients.

Pregnancy is an established secondary cause of AA [19]. One patient in the study presented in her third trimester of pregnancy.

Human-immunodeficiency Virus is a documented secondary cause of AA [20]. There were four patients with HIV, all of whom were already on antiretroviral therapy for at least one year prior to presentation. Viral load studies were not conducted on all patients, however, given the duration of ARV treatment prior to presentation, HIV was excluded as a secondary cause for AA in these patients. These patients presented with less severe disease and responded better to immunosuppressive therapy than the HIV negative counterparts. However, given the sample size, no statistically significant conclusions can be made. A 2015 study reviewed 8 HIV positive patients; four of which were treated with stem-cell transplants and the remaining 4 were treated with immunosuppressive therapy [20]. A poor outcome was noted in the non-transplant arm with a 75% mortality rate.

Forty-six percent of patients had a poor response to treatment. There were no significant differences in response to treatment, based on the duration of ATG received, type of ATG received, HIV status, or cause of AA.

Males were shown to have a more severe disease at presentation, however, they responded better to immunosuppressive therapy. The majority of females presented with less severe disease and had a poorer response to immunosuppressive therapy compared to male counterparts.

AA has been shown to have a high untreated mortality rate worldwide. Twenty-seven percent of patients in this study succumbed to their illness. Causes of death could not be established in most cases. However, neutropaenic sepsis and fatal haemorrhage were documented in many

cases. There were no statistically significant associations between disease outcome and severity of disease at diagnosis, duration of ATG received, type of ATG received, HIV status or cause of AA. A high number of patients were lost to follow-up. Patients may have returned to their home for continued follow-up without prior notification of the oncology clinic. Patients may have also succumbed to their illness before returning to the health care facility for follow-up. Unsuccessful attempts had been made to contact patients who had been lost to follow up.

Conclusion

Aplastic anaemia is a rare haematological condition that has a preponderance for young males in this study population. Males present with more severe disease but respond better to immunosuppressive therapy than female counterparts.

Twelve possible secondary causes for AA were identified in this study, some of which required treatment outside of the realm of immunosuppressive therapy alone (Hepatitis B, SLE). Thus, secondary causes must be sought in all cases and the involvement of a multi-disciplinary team should be considered. Clerking data sheets should be made available to clinicians to combat incomplete work-up.

There were no stem-cell transplants conducted during this study period. Reasons for the paucity of transplants conducted (mentioned above), should be thoroughly explored and recommendations should be made to improve in this regard.

The variable treatment duration here, highlights the need for a national recommended protocol to avoid gross discrepancies in treatment algorithms within and between institutions.

Care should be taken in maintaining patient follow-up to ensure adequate morbidity and mortality data.

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Chapter Three: Appendices

Appendix 1: Data Collection Sheet

APLASTIC ANAEMIA STUDY – CHARLOTTE MAXEKE JOHANNESBURG ACADEMIC HOSPITAL

Sample Number: _____

Weight: _____ Height: _____

Ethnicity: African ☐ Caucasian ☐ Asian ☐ Coloured ☐ Indian ☐

Gender: Male ☐ Female ☐

Age 18-29 ☐ 30-39 ☐ 40-49 ☐ >50 ☐

Family History _____

Treatment Received

ATG ☐ Duration _____ Rabbit: ☐ Equine: ☐

Cyclosporine ☐ Duration _____

Steroid ☐ Duration _____

Red Cells ☐ No of Initial Units _____

Platelets ☐ No of Initial Units _____

Plasma ☐ No of Initial Units _____

Other _____ Duration _____

Donor Information

Presence of related donor Yes ☐ No ☐ Unknown ☐

Presence of matched, unrelated donor Yes ☐ No ☐ Unknown ☐

SABMR donor search conducted Yes ☐ No ☐ Unknown ☐

Allogeneic Stem Cell Transplant Yes ☐ No ☐

Blood Results

	On Diagnosis	Best Response
WCC		
• **Neutrophils**		
• **Lymphocytes**		
• **Monocytes**		
• **Basophils**		
RCC		
Hb		
Hct		
MCV		
MCH		
MCHC		
RDW		
Platelet		
MPV		
Morphology Comment		
Hepatitis Studies		[redacted]
EBV		
CMV		
ANA		
AdsDNA		
Pregnancy Test		

WHO Haematological Toxicity Scale after treatment (tick)

Parameter	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Haemoglobin (g/dL)	≥11.0	9.5-10.9	8.0-9.4	6.5-7.9	<6.5
Neutrophils ($X10^9$/L)	≥2.0	1.5-1.9	1.0-1.4	0.5-0.9	<0.5
Leucocytes ($x10^9$/L	≥4.0	3.0-3.9	2.0-2.9	1.0-1.9	<1.0
Platelets ($x10^9$/L	≥100	75-99	50-74	25-49	<25

Severity (According to Bacigalupo *et al*)

Very Severe ☐

Severe ☐

Non-Severe ☐

Response to Treatment

Poor ☐

Partial ☐

Complete ☐

HIV Status

Positive ☐ Negative ☐

On Treatment ☐ Pre-Treatment ☐ Defaulted Treatment ☐

Duration of Treatment _____ Regimen_____

CD4 count_____

Bone Marrow & Trephine Results

Sample	Good ☐	Adequate ☐	Poor Quality ☐
Hypocellularity	Y ☐	N ☐	
Fibrosis	Y ☐	N ☐	
Fatty Changes	Y ☐	N ☐	
Haemodilute	Y ☐	N ☐	
Blasts	Y ☐	N ☐	_____%
Parvo B19	Y ☐	N ☐	Not done ☐

Other _____

General Comments _____

_____

Clinical History

Illicit drug use	Yes ☐	No ☐	Name_____	Duration _____
Medication Exposure	Yes ☐	No ☐	Name_____	Duration _____
			Name_____	Duration _____
Toxin Exposure	Yes ☐	No ☐	Name_____	Duration _____

Appendix 2: HREC Clearance Certificate

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R14/49 Dr Tyral Dean Ramsamy

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M190738

NAME: (Principal Investigator)	Dr Tyral Dean Ramsamy
DEPARTMENT:	Internal medicine Adult Medical Oncology (Wards 495 and 594) Charlotte Maxeke Johannesburg Academic Hospital
PROJECT TITLE:	A Pilot Study Investigating the Aetiology of Aplastic Anaemia and the Response to Immunosuppressive Therapy at the Charlotte Maxeke Johannesburg Academic Hospital
DATE CONSIDERED:	26/07/2019
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof Georgia Demetriou and Dr Cleo Solomon
APPROVED BY:	Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	30/09/2019

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 Research Office Secretary on the Third Floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. 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**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in **July** and will therefore be due in the month of **July** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES