OUTCOMES OF PAEDIATRIC PATIENTS WITH ACQUIRED APLASTIC ANAEMIA TREATED WITH IMMUNOSUPPRESSIVE THERAPY AT TWO JOHANNESBURG HOSPITALS

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Declaration

I, Alison van der Nest, declare that this research report is my own, unaided work. It has been submitted 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 this or any other university.
Declarations of interest: None.

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Alison van der Nest

Dedication

To the children and their families living with this condition, and to my family, for their unwavering support and encouragement.

Abstract

Background

Aplastic anaemia (AA) is a life-threatening disorder of bone marrow failure, due to an autoimmune destruction of stem cells in the bone marrow. Treatment options include haematopoietic stem cell transplantation (HSCT) and immunosuppressive therapy (IST). Most frequently, IST is used for treatment of AA in South Africa as HSCT is often unavailable. This study describes the population of paediatric patients with acquired AA in South Africa and determines their response to IST.

Methods and materials

A retrospective record review was done of patients diagnosed with AA between January 2002 and December 2011 at two Johannesburg centres. Forty-one records were included. The patient demographics are illustrated. The main outcomes reviewed include overall survival, survival with treatment, response to therapy, rate of relapse and progression to clonal disorders and malignancy, and causes of death.

Results

61% of patients were male. The median age at diagnosis was 10.1 years. The majority (67%) had severe AA. Overall survival rates were 68% for all patients and 75% for patients treated with IST. 83% of patients received IST (38% one course, 62% two courses). At the six month evaluations, 6% had a complete response, 59% had a partial response and 15% had no response. The relapse rate was 15% and progression to clonal disorder was 5%. Thirteen patients (32%) demised.

Conclusion

This study demonstrates patient demographics, survival rates and response to IST in keeping with international findings. The use of IST can be justified in this setting when HSCT cannot be provided.

Acknowledgements

Firstly, I would like to thank my supervisor, Professor Gita Naidu, for her invaluable input and guidance throughout the process of completing this research report. I would like to thank the staff of the CHBAH and CMJAH Haematology Oncology units for their assistance in the retrieval of patient records. I would also like to thank my sister, Megan, for her help with proofreading this report. Lastly, I would like to thank the patients and their families for allowing their records to be used as part of important research into this condition.

Content

List of Abbreviations

AA	Aplastic anaemia
AML	Acute myeloid leukaemia
ANC	Absolute neutrophil count
ATG	Antithymocyte globulin
BMI	Body mass index
BSH	British Society for Haematology
CMV	Cytomegalovirus
CRE	Carbapenem-resistant Enterobacteriaceae
CsA	Cyclosporine A
EBV	Ebstein Barr virus
ESBL	Extended-spectrum beta-lactamase
HIV	Human immunodeficiency virus
HSCT	Haematopoietic stem cell transplantation
IST	Immunosuppressive therapy
LMIC	Low- and middle-income countries
MDS	Myelodysplastic syndrome
PNH	Paroxysmal nocturnal haemoglobinuria
SAA	Severe aplastic anaemia
vSAA	Very severe aplastic anaemia

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1. Introduction

Acquired aplastic anaemia (AA) is a rare, life-threatening disorder of bone marrow failure. It is characterised by peripheral blood cytopenias and a hypocellular bone marrow, in which the normal bone marrow milieu is replaced by fatty tissue[1]. The annual incidence is estimated at 2 per 1 000 000 per year in North America and Europe, with a slightly higher incidence of 7 per 1 000 000 per year in Asian countries[1,2]. The frequency peaks in the age group 10 – 25 years, with a second peak after age 60[3].

Acquired aplastic anaemia must be distinguished from the inherited bone marrow failure syndromes, such as Fanconi Anaemia, as the underlying pathophysiology and management of these conditions differs[4]. The pathophysiology of acquired AA is thought to be due to an aberrant immune response resulting in destruction of haematopoietic stem cells in the bone marrow by autoreactive T-cells[5]. The cause of this may be an inciting event such as a viral infection or may be drug-induced, but in many cases the cause is unknown[5]. Acquired AA has been associated with viruses such as Cytomegalovirus (CMV), Epstein Barr virus (EBV), Human immunodeficiency virus (HIV), Parvovirus B19 and the Hepatitis viruses. It is also associated with pregnancy, autoimmune conditions such as systemic lupus erythematosus, and various drugs and toxins [1].

Over the decades, survival outcomes have improved in response to targeted therapies [6]. The two forms of targeted therapies are haematopoietic stem cell transplantation (HSCT) and immunosuppressive therapy (IST). HSCT from a human leukocyte antigen-identical sibling donor is recommended as first line therapy for newly diagnosed patients who have severe or very severe aplastic anaemia and are less than 40 years of age[7], as it is potentially curative[8]. However, HSCT may not be possible for various reasons. These include lack of a matched donor, lack of adequate facilities to perform the procedure, prohibitive costs associated with the procedure, patient age, and patient choice[9]. In these cases, immunosuppressive therapy is recommended [7].

Immunosuppressive therapy consists of a combination of horse or rabbit-derived antithymocyte globulins (ATG) and cyclosporine A (CsA)[10]. Some studies have shown that patients treated with horse ATG have better response rates than those treated with rabbit ATG[11].

It is hypothesised that patients with AA have abnormally activated T-cells that destroy haematopoietic stem cells in the bone marrow. By inhibiting these T-cells and dampening the immune response, further destruction is prevented and the bone marrow can recover[2]. Antithymocyte globulins are polyclonal antibodies that have several proposed immunomodulatory effects, including depletion of T-cell populations, induction of B-cell apoptosis, and modulation of various molecules and cytokines involved in immune cell interactions[12,13]. ATG can cause severe allergic phenomena, including anaphylaxis and serum sickness. It must be administered in an inpatient setting[13].

CsA acts by inhibiting calcineurin, a protein phosphatase important in activating T-cells. This leads to decreased T-cell activation and overall decreased immune response[14]. The toxic side effects associated with CsA include nephrotoxicity, neurotoxicity, hypertension, hyperlipidaemia, and electrolyte abnormalities[14]. Other immunosuppressive therapies such as Tacrolimus have been studied as a replacement drug for CsA. In 2017, Chiu *et al*[15] published a study investigating the efficacy and safety of Tacrolimus + ATG as upfront therapy for AA in adult patients. The study showed good overall response rates to this therapy comparable to those treated with CsA, and had a favourable safety profile.

There are late complications associated with AA and treatment with IST, including relapse and development of a clonal disorder. Relapse is defined as once again meeting the criteria for severe aplastic anaemia (SAA) or very severe aplastic anaemia (vSAA) after initial response to treatment, and can occur in 10-30% of patients[16]. These patients then require a repeat course of IST or exploration of bone marrow transplantation options[17]. Approximately 10-15% of patients may develop a clonal disorder such as paroxysmal nocturnal haemoglobinuria, or may develop myelodysplastic syndrome/acute myeloid leukaemia (MDS/AML)[16]. Patients have worsening blood cell counts that are unresponsive to therapy and samples of their bone marrow show prominent dysplasia of cells with cytogenetic abnormalities[10]. These patients have a poorer prognosis and decreased long term survival[10].

In 2015, a study by Waja *et al*[18] evaluated 100 patients over the age of 14 with aplastic anaemia at Chris Hani Baragwanath Academic Hospital (CHBAH). 72% were adult patients and 28% were children (l4 to 19 years of age). Most patients (82%) had idiopathic acquired

AA, and 64% were treated with IST. A response to IST of 61% was recorded, with an overall 5-year survival rate of 69%. In this study, the response to treatment and survival rates were not analysed according to the age of the patient.

This study describes the basic demographics and clinical characteristics of paediatric patients with aplastic anaemia at two major treatment centres in Johannesburg, South Africa. The aims of this study were to explore the outcomes of patients with AA that were treated with first line IST, including the response to treatment, relapse rates, progression to malignancy or clonal disorders, survival rates, and causes of death.

2. Methods and Materials

2.1 Materials

Patient records from the Paediatric Haematology Oncology Units at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) and Chris Hani Baragwanath Academic Hospital (CHBAH) were used for this study. These units are in Johannesburg, South Africa. The hospitals receive patients from Gauteng province and the North West province of South Africa, as well as patients from the Kingdom of Eswatini. In 2018, there were 19.7 million children under the age of 18 (34% of the population) in South Africa, 21% of which resided in Gauteng province[19]. There is an equal split between male and female children in the South African population[19].

Ethics approval for this study was granted by the Medical Human Research Ethics Committee of the University of the Witwatersrand (M180322). Permission was obtained from the Heads of the paediatric Haematology-Oncology Units and Chief Executive Officers of CMJAH and CHBAH to access data and conduct this research at their institutions.

2.2 Methods

Patients

A retrospective review of the medical records of all children and adolescents <19 years of age diagnosed with AA between January 2002 and December 2011 was done. Patients diagnosed

with inherited bone marrow failure syndromes were excluded. Additionally, any patient treated with a HSCT as upfront therapy was excluded. The information retrieved from the patient records included age at diagnosis, weight and height at diagnosis, time from diagnosis to initiation of IST, details of treatment received, full blood counts at various time points throughout the course of patient care, and bone marrow aspirate and trephine results. Serology results for CMV, EBV, HIV, Parvovirus B19 and Hepatitis A, B and C were obtained.

Diagnosis

Diagnosis was confirmed based on history, physical examination, and laboratory investigations. These included a full blood count and differential count, reticulocyte count, and a bone marrow aspirate for microscopy and trephine for histology. Serology was done at the time of diagnosis for the viral infections stipulated above. Fanconi Anaemia was excluded using chromosomal breakage tests and gene sequencing as required.

Severity classification

The severity of AA of each patient was classified according to the modified Camitta criteria[20]. These criteria are shown in the table below.

Table 1: Camitta criteria for the classification of severity of aplastic anaemia

	Bone marrow cellularity	ANC	Platelet count	Reticulocyte count
Non severe AA	Hypocellular	<1 x 10^9/L	<50 x 10^9/L	<60 x 10^9/L
Severe AA	<25%	<0.5 x 10^9/L	<20 x 10^9/L	<20 x 10^9/L
Very severe AA	<25%	<0.2 x 10^9/L	<20 x 10^9/L	<20 x 10^9/L

AA Aplastic anaemia, ANC Absolute neutrophil count

Treatment

All patients in the study received a standardised regimen of horse ATG and CsA for the first and second courses of treatment, as per the British Society for Haematology (BSH) guidelines for the treatment and management of aplastic anaemia[8]. Both hospitals used the same doses and duration of therapy. At CMJAH, a modified BSH treatment protocol was used, and patients received a second course of IST 28 days after the first course was given. At CHBAH, patients received a second course of IST if they had an inadequate response to the first course of IST (still fulfilled the criteria for SAA or vSAA), or if the patient relapsed after response to the first course. The following regimen was used, as per the BSH treatment guidelines[8]: Day 1 – 4: Horse ATG 40mg/kg daily IVI, together with methylprednisolone 1mg/kg IVI twice daily. Day 5 – 11: oral prednisone 1mg/kg twice daily, tapered thereafter. CsA was commenced on Day 1 at 2.5mg/kg twice daily and continued for at least six months, maintaining blood concentration levels between 150 – 200ng/mL. CsA was tapered thereafter at a rate of 0.25mg – 0.5mg/kg/month.

Supportive therapies, such as transfusion of blood products and the administration of antimicrobial medications for suspected or confirmed infections, were tailored for each patient as per hospital protocols.

Definitions

Response to IST was evaluated six months after initiation of therapy, and again at various time points. Time to best response after the six-month evaluation was also recorded. In this study, response was defined by the parameters shown in Table 2[7].

Table 2: Criteria defining response to IST

	Haemoglobin	ANC	Platelet count
Complete response	>12g/dL	>1.5 x 10^9/L	>150 x 10^9/L
Partial response	>8g/dL	>0.5 x 10^9/L	>20 x 10^9/L

	maintained without transfusion for three months		
No response	Still meeting the criteria for SAA six months after treatment initiation		
Relapse	Meeting criteria for SAA after partial or complete response		

ANC Absolute neutrophil count, SAA Severe aplastic anaemia

Nutritional status was defined by weight-for-height Z-scores for children 0 – 5 years[21,22], shown in Table 3. Body mass index (kg/m^2) categories were used for patients 6 – 19 years, shown in Table 4. The World Health Organisation (WHO) body mass index (BMI) charts for sex and age were used[23].

Table 3: WHO weight-for-height Z-score classification (0 – 5 years)

WHO weight-for-height classification	Z-score
Normal weight	>-2 to <+2
Wasting	<-2
Severe wasting	<-3
Overweight	>+2
Obese	>+3

Table 4: Body mass index categories (6 – 19 years)

Body mass index category	**Percentile**
Underweight	$<5^{th}$
Normal weight	5^{th} to $<85^{th}$
Overweight	85^{th} to $<95^{th}$
Obese	95^{th} or greater

2.3 Statistical Analysis

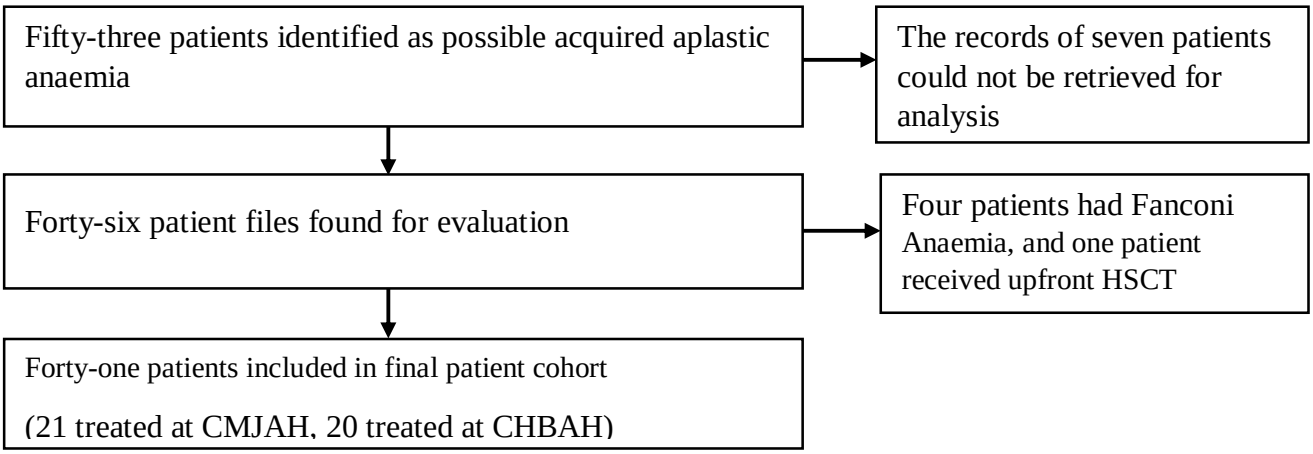
Statistical analysis was done using the IBM SPSS 24.0 software platform and Microsoft Excel 2013. Percentages were used for nominal data such as sex, severity of disease, and nutritional status classification. Medians were calculated for age at presentation, time from diagnosis to initiation of treatment, and duration of CsA therapy. The Kaplan-Meier method was used to estimate survival[24]. Overall survival was estimated from the time of diagnosis to death or last visit, and the actuarial ten-year survival rate was reported with 95% confidence intervals. Survival of those who received IST was estimated from time of initiation of the first course of IST to death or last visit, and the actuarial ten-year survival rate was reported with 95% confidence intervals. Log rank testing was done to determine statistical significance in survival rates when comparing those who received one course of therapy to those who received a second course. P-values of < 0.05 were considered statistically significant in all analyses.

3. Results

3.1 Study Population

Fifty-three patient records were identified for possible inclusion in the study. The records of seven patients could not be retrieved for analysis. Four patients were noted to have Fanconi Anaemia and were excluded from the study. One patient received upfront HSCT and was excluded. Forty-one patients were included in the final analysis (Figure 1).

Figure 1: Study flow diagram



3.2 Demographics and clinical characteristics of patient cohort

The demographics, disease severity, and anthropometry of the 41 patients that were included in the analysis are shown in Table 5. Twenty-five patients (61%) were male and sixteen (39%) were female. Twenty-eight patients (67%) were classified as having severe AA and thirteen (33%) as very severe AA. None had non-severe AA. The median age at time of diagnosis was 10.1 years (range, 0.9 – 15.9 years, IQR 7.5 – 12.2 years).

Table 5. Demographics and clinical characteristics of patient cohort

Characteristic	Entire cohort (total = 41)	CMJAH patients (total = 21)	CHBAH patients (total = 20)
Sex			
Male	25/41 (61%)	13/21 (62%)	12/20 (60%)
Female	16/41 (39%)	8/21 (38%)	8/20 (40%)
Severity of disease			
Severe aplastic anaemia	28/41 (67%)	11/21 (52%)	17/20 (85%)
Very severe aplastic anaemia	13/41 (33%)	10/21 (48%)	3/20 (15%)
Nutritional classification			
Normal weight	34/41 (83%)	18/21 (86%)	16/20 (80%)
Severe wasting	1/41 (2%)	0/21 (0%)	1/20 (5%)
Underweight	2/41 (5%)	0/21 (0%)	2/20 (10%)
Overweight	4/41 (10%)	3/21 (14%)	1/20 (5%)
Obese	0/41 (0%)	0/21 (0%)	0/20 (0%)

CMJAH Charlotte Maxeke Johannesburg Academic Hospital, CHBAH Chris Hani Baragwanath Academic Hospital

3.3 Causes of aplastic anaemia and associated comorbidities

Viral results are shown in Table 6. All patients were tested for HIV, CMV, EBV, and Parvovirus B19 infections, as well as Hepatitis A, B and C infections. All patients had documented negative HIV ELISA tests, taken at the time of diagnosis. Two patients were IgM positive for EBV and two patients were IgM positive for Parvovirus B19 at the time of

diagnosis. These patients were also IgG positive for these viruses. In these four patients, the viral infections may have caused the AA. None had a clear drug-related cause of AA. Four patients had documented comorbidities at the time of diagnosis. These were disseminated tuberculosis, diabetes mellitus, absence seizures, and hypertension.

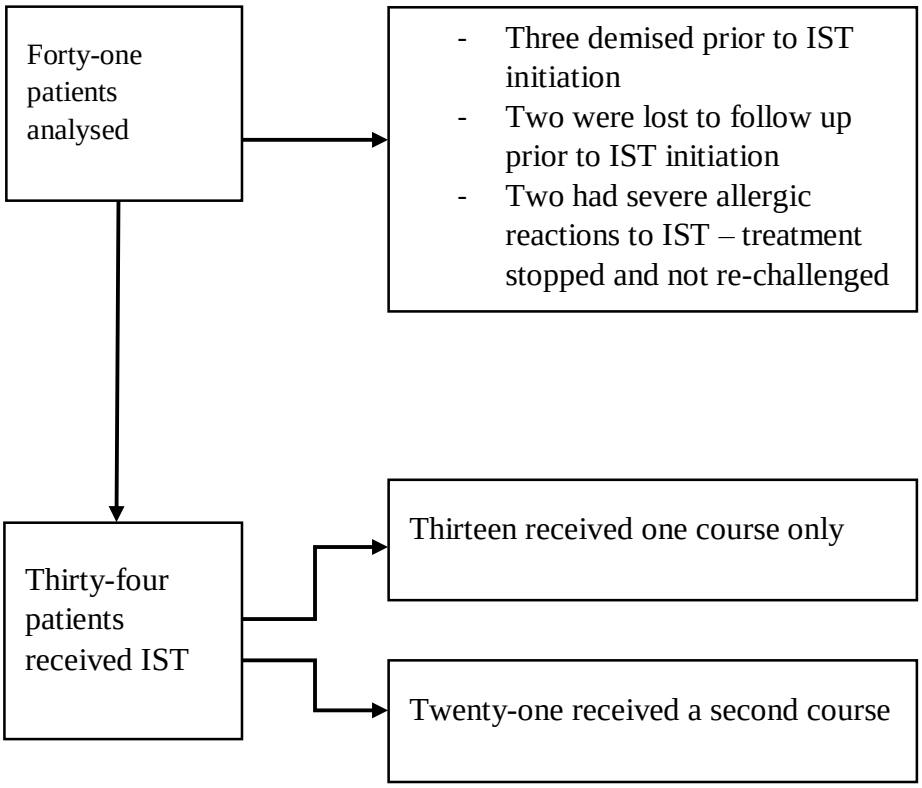
Table 6. Viral serology results at time of diagnosis

Virus	Negative n = 41 (%)	Positive (IgM and IgG) n = 41 (%)
Cytomegalovirus	41 (100)	0
Ebstein Barr virus	39 (95)	2 (5)
Parvovirus B19	39 (95)	2 (5)
Hepatitis A	41 (100)	0
Hepatitis B	41 (100)	0
Hepatitis C	41 (100)	0
Human immunodeficiency virus	41 (100)	0

3.4 Response to therapy

Figure 2 shows analysis of therapy received. Three patients died before the first course of IST could be given. The causes of death were *Escherichia coli* infection confirmed on blood culture, suspected nosocomial infection, and pneumonia. Two patients did not return for follow up after diagnosis and so did not receive IST. Two patients had severe allergic reactions to ATG and the ATG was aborted. They were not re-challenged with ATG. Both patients went on to have a complete recovery at 11 months and 24 months respectively. Neither patient had a documented relapse or evolution to clonal disorder or malignancy.

Figure 2. Analysis of therapy received



Thirty-four patients received IST. The median time from diagnosis to initiation of therapy was 36 days (range, 8 – 261 days). CsA was started on the first day of ATG administration and was then weaned to stop. The median time to stop CsA was 12 months (range, 0.5 – 103 months).

Thirteen patients received one course of IST and twenty-one received a second course. The second course was administered as per the treatment guidelines of each hospital. Of the twenty-one patients treated at CMJAH, sixteen patients were given a second course 28 days after the first course as standard protocol, one patient was lost to follow up after the first course of IST, and four patients died prior to receiving any IST. At CHBAH, the second course was given after relapse (two patients), or after failure to achieve adequate response to the first course (three patients). The time between the first and second courses of IST for the patients at CHBAH who relapsed was seven and forty-seven months respectively. The time between the first and second courses of IST for the patients at CHBAH who failed to achieve

an adequate response to the first course was three, twelve and fifteen months respectively. At the end of the study period, 25/34 patients (74%) had achieved a response to therapy. Table shows the characteristics of patients who received one course of IST and patients who received two courses.

Table 7. Comparison of characteristics of patients who received one course IST vs two courses IST

Characteristic	One course of IST (n = 13)	Two courses of IST (n = 21)
Sex		
Male	6/13 (46%)	12/21 (57%)
Female	7/13 (54%)	9/21 (43%)
Severity of disease		
Severe aplastic anaemia	10/13 (77%)	16/21 (76%)
Very severe aplastic anaemia	3/13 (23%)	5/21 (24%)
Treatment centre		
Charlotte Maxeke Johannesburg Academic Hospital	2/13 (15%)	16/21 (76%)
Chris Hani Baragwanath Academic Hospital	11/13 (85%)	5/21 (24%)

IST Immunosuppressive therapy

Of the thirteen patients who received one course of IST, 1/13 (8%) died from bleeding complications within a week of receiving treatment, and 1/13 (8%) was lost to follow up 50 days after treatment initiation. At the six-month evaluation after initiation of therapy, 1/13 (8%) had a complete response, 9/13 (69%) had a partial response, and 1/13 (8%) had no response to therapy. Two patients who had a partial response to IST at six months died 26 and 49 months after receiving IST, from suspected sepsis and bleeding complications respectively.

Of the 9/13 patients who received one course of IST and had a partial response at six months, six went on to have a complete response without further IST. The mean time to complete

response was 21 months (range, 13 to 29 months). One remained a partial responder at last follow up, and two died at 26 and 50 months after initiation respectively. One patient who had no response at 6 months achieved a partial response at 17 months without further treatment.

The twenty-one patients who received a second course of IST were evaluated for response to therapy six months after administration of the second course. Two patients were lost to follow up prior to the six-month evaluation, and three patients died soon after administration of the second course. One patient died from bleeding complications and two patients died from blood culture-confirmed infections (*Klebsiella pneumoniae* and *Enterobacter cloacae* respectively). At the six-month evaluation, 1/21 (5%) had a complete response, 11/21 (52%) had a partial response, and 4/21 (19%) had no response to the second course of IST.

Of the eleven patients who had a partial response to the second course of IST, five remained as partial responders, and six went on to have a complete response. Mean time to complete response was 26 months (range, 16 – 37 months). One of these patients relapsed 35 months after the second course was given and died from pneumonia.

Of the four patients who had no response to the second course of IST after six months, two went on to have a partial response at 29 and 44 months respectively, and two died. One patient died from bleeding complications. The cause of death of the other patient is unknown.

A comparison of response rates of those who received treatment with SAA (26 patients) to those with vSAA (8 patients), is shown in Table 8 below. Of the SAA patients, 10/26 (38%) received one course of IST, and 16/26 (62%) received a second course of IST. 1/26 had a complete response, 17/26 had a partial response and 4/26 had no response to IST. 2/26 demised less than six months after receiving a second course of treatment. 2/26 were lost to follow up and their response is unknown.

Of the vSAA patients who received treatment, 3/8 (37%) received one course of IST, and 5/8 (63%) received a second course. 1/8 had a complete response, and 4/8 had a partial response. 1/8 demised seven days after initiation of IST, and 2/8 was lost to follow up.

Table 8. Comparison of response to treatment based on disease severity

	SAA (n = 26)	vSAA (n = 8)
Complete response	1/26 (4%)	1/8 (13%)
Partial response	17/26 (65%)	4/8 (50%)
No response	4/26 (15%)	0
Died	2/26 (8%)	1/8 (13%)
Lost to follow-up	2/26 (8%)	2/8 (25%)

3.5 Survival

As shown in Figure 3, the ten-year survival rate for all 41 patients in the sample was 68%. The mean survival time was 84 months (95% CI 67 – 100). The ten-year survival rate of the patients who received IST was 75%. The mean survival time was 93 months (95% CI 77 – 101). Figure 4 shows a comparison of survival rates of those who received one course of IST and those who received a second course. The ten-year survival rate for patients who received one course of IST was 79% and for those who received a second course was 74%. There was no statistically significant difference between the two groups ($p = 0.697$).

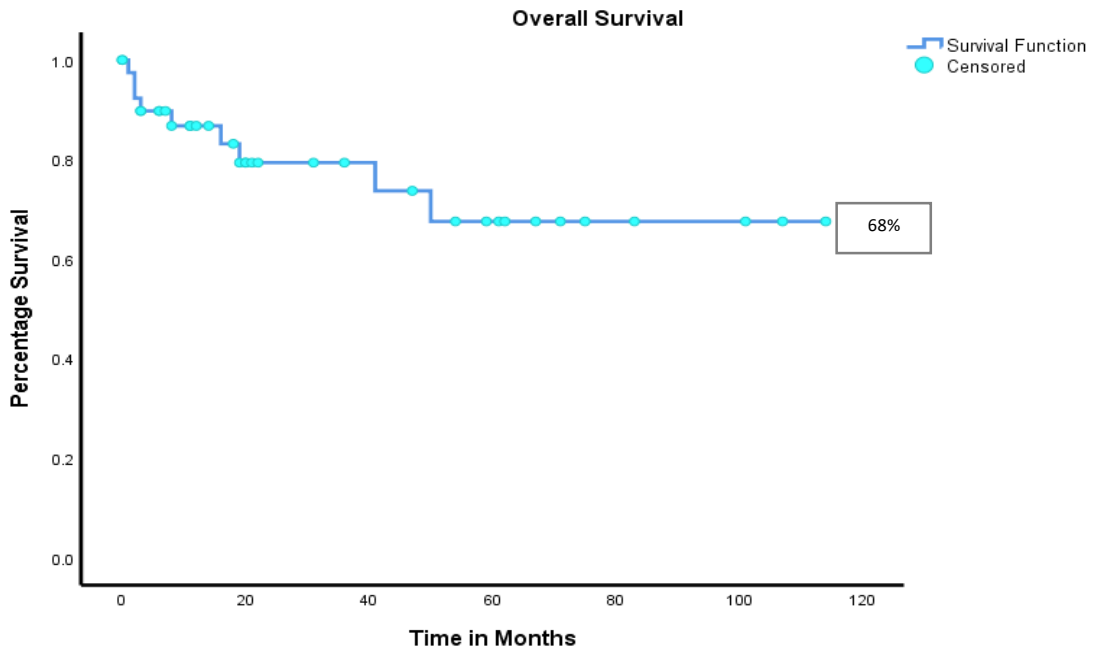


Figure 3. Kaplan-Meier survival curve for all patients

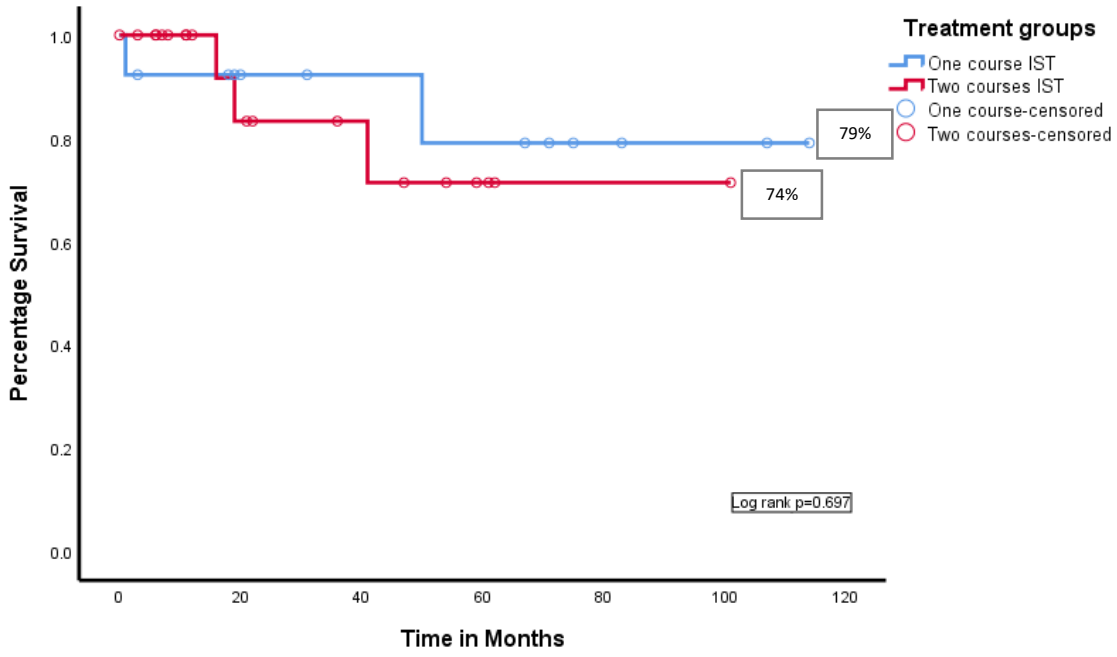


Figure 4. Kaplan-Meier survival curves comparing two groups who received IST (one course IST vs two courses IST)

3.6 Relapse

Five out of thirty-four patients (15%) relapsed after an initial response to IST. One patient died from suspected sepsis before a second course could be given. A second course of IST was administered to four patients after relapse. One survived and had a partial response to the second course, and three died soon after the second course was given. Two died from pneumonia and the other from *Klebsiella pneumoniae* infection confirmed on blood culture.

3.7 Progression to clonal disorder and malignancy

One patient developed paroxysmal nocturnal haemoglobinuria (PNH) 21 months after diagnosis. This patient had received a second course of IST 14 months after failure to respond to the first course and had a partial response to the second course. This patient was alive at the end of the study period. One patient developed myelodysplastic syndrome 47 months after diagnosis and initiation of IST. This patient had received one course of IST, with a partial response noted at the six-month evaluation. This patient died from bleeding complications two months after development of myelodysplastic syndrome.

3.8 Deaths

Table 9 shows the causes of death in this cohort. Thirteen out of forty-one patients (31.7%) died. Ten of these patients received IST prior to death. The median time from diagnosis to death was 12 months (range, <1 - 54 months), and median time from IST initiation to death was 20 months (range, <1 - 52 months). Three patients had positive blood cultures at the time of death, and their deaths were attributed to these infections. One patient cultured a carbapenem-resistant *Klebsiella pneumoniae*, and two patients cultured extended-spectrum beta-lactamase producing organisms, namely *Escherichia coli* and *Enterobacter cloacae*. Two patients had suspected nosocomial infection at the time of death, however their blood cultures remained sterile. Five patients died from bleeding complications related to thrombocytopenia. Two patients died from pneumonia. The cause of death for one patient is unknown.

Table 9. Causes of Death

Cause of death	Number of patients
Confirmed infection	3/41 (23%)
- *CRE Klebsiella pneumoniae*	- 1/3 (33%)
- *ESBL Escherichia coli*	- 1/3 (33%)
- *ESBL Enterobacter cloacae*	- 1/3 (33%)
Suspected infection	2/41 (15%)
Bleeding complications	5/41 (39%)
Pneumonia	2/41 (15%)
Unknown	1/41 (8%)

CRE Carbapenem-resistant Enterobacteriaceae, ESBL Extended-spectrum beta-lactamase

4. Discussion

Aplastic anaemia is a rare disease with a high morbidity and mortality rate requiring specialised treatment in dedicated centres. While HSCT offers a potential cure, many patients do not have a matched donor. In South Africa, there is limited capacity to offer HSCT due to lack of facilities and the high cost associated with transplantation. IST is therefore the mainstay of treatment in South Africa. A study from India describes similar difficulties in offering HSCT due to high cost and lack of transplantation facilities[(25)].

The cohort of this study is similar to other paediatric studies regarding patient demographics and age at diagnosis. Data from Europe, India and North America show a slight male predominance of patients at 58%, 51% and 52% respectively. A median age of 7.8 to 9 years at time of diagnosis is noted in these studies[(25,26,27)]. Our study shows a slightly higher male predominance of 61%, and a median age of 10.5 years at diagnosis.

Regarding survival, a recent Cochrane review using data from Europe and North America reported overall survival rates between 45% and 87% for patients treated with IST[(28)]. Dufour *et al*[(29)], Ramzan *et al*[(25)] and Rogers *et al*[(30)] report three-year, four-year and five-year survival rates of 87%, 74% and 93% respectively. In 2007, Locasciulli *et al*[(31)] reported a ten-

year survival rate of 81% for children <16 years treated with IST. A five-year survival rate of 69% was reported in the study by *Waja et al*[18] at CHBAH. The study by *Waja et al* included twenty-eight patients between the ages of 14 and 19 years, but did not stratify survival by age. In our cohort, nineteen of the twenty patients diagnosed and treated at CHBAH were less than 14 years old at diagnosis, and so were not included in the study by *Waja et al.* The cohort for the present study demonstrated a ten-year overall survival rate to be 75% for those who received IST, comparable to these local and international studies.

In comparison, studies looking at HSCT as first line therapy have demonstrated higher survival rates of 85 – 97% and offers the potential for a cure[32]. However, many low-and middle-income countries, including South Africa, do not have the facilities required to offer HSCT to those who are eligible, or the ability to bear the costs associated with transplantation. The demonstration of good survival outcomes with IST suggests that IST is a reasonable option for these countries to offer as first line therapy if HSCT cannot be offered.

With regards to survival, no statistically significant difference was found in survival rates when comparing patients who received one course of IST to those who received a second course. However, when considering response to therapy, some patients who failed to respond adequately to the first course of IST did have a response to the second course. As IST and the associated supportive therapies are expensive, this has important cost implications for patients and the treatment centres alike, consideration should be given to each case prior to administration of the second course of IST. Survival outcomes related to nutritional status were not analysed as too few patients fell outside the defined normal weight range.

The definition of response to therapy differs across studies, which affects the comparability of response rates[33]. The reported response to therapy ranges from 60% to 75% in various studies[5,31,34]. The response rate documented in a previous study of patients with AA at CHBAH showed an overall response rate of 61%[18]. In our cohort, the response to therapy was 74% at the end of the study period, in keeping with these reported outcomes.

Viruses associated with the development of AA include CMV, EBV, Parvovirus B19 and the hepatitis viruses A, B and C[35]. In the study by *Waja et al*[18] at CHBAH, the viral studies showed that 3% of patients were positive for Hepatitis B, and 2% were positive for EBV[18]. In the cohort for this study, all patients were tested at the time of diagnosis for the above

viruses. Two patients (5%) were found to be IgM positive for EBV and two patients (5%) were IgM positive for Parvovirus B19, which may have precipitated the development of AA in these patients.

With regards to HIV, *Waja et al*[18] documented that 16% of their study cohort were living with HIV. The study demonstrated that patients living with HIV had a statistically significant lower survival probability than those who had tested negative for the virus (37.5% vs 78.5%, p-value 0.01)[18]. In South Africa in 2019, it was estimated that 7.5 million people were living with HIV, 340 000 of those being children less than 14 years old[23]. Considering the relatively high prevalence of HIV in the South African population, it was surprising that in our cohort all patients had negative HIV tests at the time of diagnosis. The impact of HIV on the survival and response outcomes of paediatric patients with acquired AA could not be determined in this study.

The long term complications associated with aplastic anaemia include relapse after response and clonal evolution or development of malignancy[10]. The risk of relapse after initial response to treatment is estimated at 10-30%[32], and the risk of developing MDS/AML is approximately 10% within 10 years of treatment with IST[36,37,38]. In this study, 15% of patients relapsed, and 5% demonstrated clonal evolution to PNH and MDS, in keeping with international data.

The main causes of death in our study were infection, pneumonia and complications from excessive bleeding such as intracranial haemorrhage, similar to the causes of death reported in studies by Shah *et al* and Rogers *et al*[27,29]. Fungal infections and gram-negative sepsis are known to be frequent causes of morbidity and mortality in patients with AA[7]. A study of adult AA patients in Thailand noted that gram-negative bacilli were isolated in 67% of patients who died from an infectious episode[39]. In our study, three patients who demised had culture-confirmed infections, all of which were gram-negative bacilli. Other reported causes of death in published literature include multi-organ failure and death after development of a malignancy[30].

5. Conclusion

There is a scarcity of published data regarding paediatric AA in Africa. Although it is a rare disease, it can be well managed or possibly cured if treated correctly. HSCT offers a potential cure, but donors may not be available, and financial and technical constraints may not allow for bone marrow transplantation. Immunosuppressive therapy then becomes the only definitive treatment option available.

Our study was limited to two centres in South Africa, and had a relatively small sample size. Some records were incomplete, and several patients were lost to follow up, thus the long-term outcomes of these patients are unknown. None of the patients in the cohort were living with HIV, and so the impact of HIV infection on survival outcomes could not be ascertained.

This study demonstrated a response to therapy similar to international studies, and so the use of IST in our setting is justifiable when HSCT cannot be offered. Other therapeutic agents such as tacrolimus and eltrombopag have been studied internationally and could be an area of further research in the South African population.

A complete analysis of the cost of IST, including the cost of blood products and costs associated with inpatient therapy, was not undertaken in this study. However, future research could be done on the cost implications of IST compared to HSCT. This may assist low- and middle-income countries in deciding if provision of HSCT is financially viable when compared to IST.

Many other important aspects of management of this disease, including the use of blood products, use of adjuvant therapies, and use of antimicrobial therapies were not addressed in this study. As gram-negative sepsis was noted to be a significant cause of death in our cohort, future research may include analysis of prophylactic antibiotic use and treatment of infectious episodes in these patients. There are definite opportunities for further research in this area, especially in the context of resource limited countries.

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