CLINICAL, LABORATORY FEATURES AND AVAILABLE TREATMENT OPTIONS AMONG PATIENTS WITH APLASTIC ANAEMIA ATTENDING MUHIMBILI NATIONAL HOSPITAL, TANZANIA

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By

Mwashungi Ally

A Dissertation/ Thesis Submitted in (partial) Fulfillment of the Requirement for the Degree of Master of Science (Haematology and Blood Transfusion) of

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CERTIFICATION

The undersigned certify that he has read and hereby recommend for acceptance by Muhimbili University of Health and Allied Sciences a dissertation titled; **''Clinical, laboratory features and available treatment options among patients with aplastic anaemia attending Muhimbili National Hospital, Tanzania''** in (partial) fulfilment for the degree of Master of Medicine (Haematology and Blood Transfusion) of the Muhimbili University of Health and Allied Sciences.

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(Supervisor)

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Date

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Dr. Alex Magesa

(Co-supervisor)

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Date

DECLARATION AND COPYRIGHT

I, **Dr. Mwashungi Ally,** declare that this dissertation is my original work and that it has not been presented and will not be presented to any other university for a similar or any other degree award.

Signature……. **Date**……

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DEDICATION

To all patients with aplastic anaemia.

ABSTRACT

Background: Aplastic anaemia (AA) is a rare, potentially life-threatening disease presenting with hypocellular fatty marrow and reduction in the blood count of all blood cell lines; the red cells, white cells and platelets. A cell- mediated autoimmune mechanism has been implicated in the pathogeneis of AA. Gold standard treatment is with either allogeneic haemopietic stem cell transplant or intensive immunosuppressive therapy with ATG followed by cyclosporine. AA is associated with high morbidity and mortality if left untreated. There is a paucity of studies on AA in Tanzania and East Africa. A fraction of AA patients evolve to paroxysmal nocturnal haemoglobinuria (PNH): this is associated with the appearance of a population of blood cells deficient in GPI-anchored proteins (GPI-negative: sometimes referred to belonging to a PNH clone).

Aim: To describe the clinical presentation, laboratory features, treatment modalities, and presence of GPI negative red cells population among patients with AA attending Muhimbili National Hospital.

Participants: All patients with AA who attended MNH from September 2016 to March 2017.

Study design: Descriptive cross-sectional study

Methods: Consenting patients were sequentially recruited from inpatient and outpatient haematology unit. A structured questionnaire was used to obtain information on the socio-demographic characteristics, clinical history, physical signs and treatment modalities. Blood and urine samples were collected for investigations. Analysis was done using spss statistics version 20. Comparison between patients with AA alone and those with GPI negative red cell population with various characteristics was analyzed by using Fischer's exact test and statistical differences were considered to be significant if p value was less than 0.05

Results: A total of 40 patients with AA were recruited. Seventeen (43%) were males. The median (IQR) age at diagnosis was 24 (15-33) years. The male to female ratio was 1:1.4. Forty eighty percent of patients had severe AA, 20% had very severe AA and 32% had non severe

AA. Anaemia was the dominant clinical presentation (78%) followed by bleeding tendencies (70%) and fever (48%). The median (IQR) haemoglobin level was 6.6(5.4-8.8)g/dl, median (IQR) absolute reticulocyte count was 10 (6.25-16) $X10^9$/L, median (IQR) absolute neutrophil count was 0.49(0.01-1.04) $X10^9$/L, and the median (IQR) platelet count was 17.50(9.63-41.00) $X10^9$/L. None of the patients had haemoglobinuria or positive Ham's test. Twenty six samples were analyzed for presence of GPI deficient red cells, and in 42%, GPI deficient red cells were significantly higher than seen in normal controls (>0.2%). In three patients the PNH clone comprised more than 1% of red cells. Of the patients with significant GPI negative red cells population, 91% had been diagnosed with AA for more than one year before recruitment, and 82% were found to have less transfusion requirement within six months. Seventy eight percent of all patients were treated with a combination of supportive therapy and cyclosporine alone. Four patients (10%) were referred abroad and received intensive immune suppression therapy with ATG and cyclosporine. One patient (3%) received allogeneic haemopoietic stem cell transplant. Ten percent of patients had received only supportive treatment with blood transfusion and antibiotics.

Conclusion: AA mostly affects people below the age of 40 years. The majority of patients with AA attending MNH have either severe or very severe AA. Almost half of patients with AA have PNH clones, although they do not have as yet any clinical or laboratory evidence of haemolysis (PNH-sc). Treatment offered at MNH includes supportive treatment and immune suppression with cyclosporine alone.

Recommendation: There is a need of providing definitive therapy for AA in Tanzania.

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LIST OF ABBREVIATIONS

AA	Aplastic Anaemia
ANC	Absolute Neutrophil Count
ALC	Absolute lymphocyte count
ARC	Absolute Reticulocyte Count
ATG	Anti-thymocyte globulin
BMF	Bone Marrow Failure
CBC	Complete Blood Count
CPL	Central Pathology Laboratory
GPI	Glycophosphatidylinositol
HEMPAS	Hereditary erythroblastic multinuclearity with positive acidified serum lysis test
HLA-DR	Human Leukocyte Antigen- antigen D Related
IST	Immunosuppressive Therapy
MNH	Muhimbili National Hospital
MUHAS	Muhimbili University of Health and Allied Sciences
PNH	Paroxysmal Nocturnal Haemoglobinuria
TNF	Tumor Necrosis Factor
WBC	White Blood Cells
WHO	World Health Organization

DEFINITION OF KEY TERMS

Pancytopenia: We adhered to the WHO criteria, i.e. haemoglobin less than 10g/dl, neutrophil count less than 1.5 X 10^9/L, and platelet count less than 100 X 10^9/L.

Bone marrow failure: Deficient haemopoiesis resulting in pancytopenia

Severity of AA was classified using Camitta's criteria

SEVERITY	CRITERIA
Severe AA	Bone marrow cellularity of less than 25% plus at least two of the following; • ARC less than 20 X 10^9/L, • ANC less than 0.5 X 10^9/L, • Platelet count less than 20 X 10^9/L
Very severe AA	ANC less than 0.2 X 10^9/L
Non Severe AA	Not meeting criteria for severe AA

Table 1: Classification of AA

CHAPTERONE

1.0 INTRODUCTION

Aplastic anaemia is a rare, potentially fatal disease due to failure of haemopoiesis, presenting with pancytopenia and hypocellular bone marrow(1). AA can be inherited but the majority of cases are acquired and in the majority the cause is unknown. In inherited AA, depletion of stem cells is due to intrinsic defects. In acquired cases, the accumulated evidence points primarily to suppression of haemopoiesis being immune-mediated(2).

Paroxysmal nocturnal haemoglobinuria is an acquired clonal stem cell disorder in which inactivating mutation of the X-linked gene PIG-A result in deficient synthesis of the GPI anchor, a structure that attaches several surface proteins to the cell membranes in red blood cells, white blood cells and platelets. The lack of GPI-linked proteins renders red cells highly susceptible to lysis by complement (3-4).

An autoimmune mechanism has been postulated as the agent of bone marrow failure in both AA and PNH. There is evidence that GPI molecule is the target of autoimmunity in both diseases (5-8). Studies have shown that more than 50% of patients with AA have GPI-negative blood cells (PNH clone), and the presence of these GPI-negative cells is associated with higher response rate to immunosuppressive therapy and better prognosis (9-11).

In Tanzania, until now patients with AA have not been tested for the presence of GPI-negative blood cells. The results of this study will determine the proportion of patients with GPI-negative blood cells among patients with AA, and this will aid in predicting the prognosis and response to treatment. It will also review the treatment options offered at MNH, in the aim to improve the quality of care of patients with AA.

1.1 Literature review

1.1.1Pathophysiology

AA can either be inherited or acquired. Majority of cases are acquired. Most of acquired cases occur without an evident precipitating cause and are termed as idiopathic AA (12). Various environmental triggers have been found to be associated with the disease. Examples of the triggers are exposure to certain toxic chemicals such as benzene and organophosphates, viral infections such as EBV, drugs eg chloramphenicol and alkylating agents (12).The underlying pathophysiology has been found to be immune-mediated, in which CD8+ HLA-DR+, cytotoxic T lymphocytes produce inhibitory cytokines, such as interferon gamma and TNF, which suppress stem cell growth by affecting the mitotic cycle; cell killing through Fas-mediated apoptosis. In addition, the above mentioned cytokines induce nitric oxide synthase and nitric oxide production by marrow cells, which contributes to immune-mediated cytotoxicity and damage to haemopoietic cells. The immune pathophysiology has been inferred from improvement in blood counts following use of IST in over 75% of patients with AA(2)(13)

1.1.2 Epidemiology

The incidence of AA is estimated to be less than three per million per year(14). The annual incidence in Europe is estimated to be about 2 cases per million populations(12). A similar incidence was reported in Latin America(15). No accurate incidence data are available from the United States, but findings from several retrospective studies overlap those from Europe and suggests that the incidence is 0.6 – 6.1 cases per million populations(12).

In Eastern Asia, AA is 2-3 times more common. In Bangkok, the annual incidence was found to be 4 cases per million population(16). Based on the prospective studies done in the rural areas of Thailand, it may be closer to 6 cases per million populations(17).

In Africa, there is paucity of literature on epidemiological studies reporting population based incidence of AA (18). No studies about the prevalence and incidence of the disease have been done in Tanzania and Africa in general.

Although AA occurs in all age groups, the highest frequency of aplastic anemia occurs in persons aged 15 to 25 years; a second peak occurs at age 65 to 69 years. The male-to-female ratio for AA is approximately 1:1, although there are data that suggest a male preponderance in Thailand and Malaysia(17)(19).

1.1.3 Clinical presentation

The clinical presentation of patients with aAA includes symptoms related to the decrease in bone marrow production of haemopoietic cells. The onset is insidious, and the initial symptom is frequently related to anaemia or bleeding. Fever or infections may also be noted at presentation. Specific manifestations may include anaemia;Pallor, weakness, dyspnea, and fatigue, thrombocytopenia; petechiae, bruising, epistaxis, mucosal and gingival bleeding, vaginal bleeding and unexpected bleeding from other sites, and neutropenia which may manifest as overt infections involving various sites.

Physical examination generally is unrevealing, except for evidence of anemia (e.g., conjunctival and cutaneous pallor, resting tachycardia), cutaneous bleeding (e.g., ecchymoses and petechiae), or gingival bleeding and intraoral purpura. Lymphadenopathy and splenomegaly are not features of aplastic anemia.

Similarly to other autoimmune diseases, AA has a varied clinical course; some patients have mild symptoms with a stable clinical courses that necessitate little or no therapy, whereas others present with life-threatening pancytopenia representing a medical emergency (12) . When severe, and if left untreated, AA has a high mortality (20)

1.1.4 Laboratory features

AA is diagnosed with blood and bone marrow studies. Patients with AA have varying degrees of pancytopenia. Anemia is associated with a low reticulocyte index. The reticulocyte count usually is less than 1.0 percent and may be zero despite the high levels of erythropoietin (21). Macrocytes may be present. There are no abnormal cells in the peripheral blood.

Marrow aspirate typically contains numerous spicules with empty, fat-filled spaces comprising over 75% of the marrow, and relatively few hematopoietic cells. Lymphocytes, plasma cells, macrophages, and mast cells may be prominent, reflecting a lack of other cells rather than an increase in these elements (22).

AA is classified into three categories; Non severe AA, severe AA and very severe AA based on the marrow cellularity, absolute reticulocyte count, absolute neutrophil count, and platelet count (23).

Following the British Society of Haematology guidelines, patients with AA are screened for the presence of GPI-negative blood cells once a year. Dr. Ham demonstrated that the RBCs in PNH were lysed by complement when serum was acidified. If done properly, Ham test is a reliable way to diagnose PNH. The Ham test can detect GPI negative cells at about 5% of total cells. A false positive test result is seen in another very rare disease, congenital dyserythropoietic anaemia, type II (HEMPAS), but HEMPAS cells do not lyse in their own serum.

The gold standard investigation for diagnosis of PNH is flow cytometry, which can detect GPI negative cells at about 0.01% of total cells (24). Partial expression of GPI cells may be enough to prevent in vivo hemolysis.

1.1.5 Treatment options

Definitive treatment by either allogeneic SCT or IST has dramatically improved the prognosis of AA patients over the last 30 years, and more than 75% of patients can now be expected to have long term survival after either therapy(20).

Until recently, the two only main options for patients with PNH were either allogeneic bone marrow transplantation or supportive management, including blood transfusion, iron therapy and anticoagulation when required. Since the start of this millennium a major advance has been the introduction of eculizumab, a monoclonal antibody that targets the C5 protein of the complement system. Blockade of C5 prevents activation of the complement distal pathway,

and thus abolishes the complement-mediated intravascular haemolysis that severely plagues patients with PNH (15).

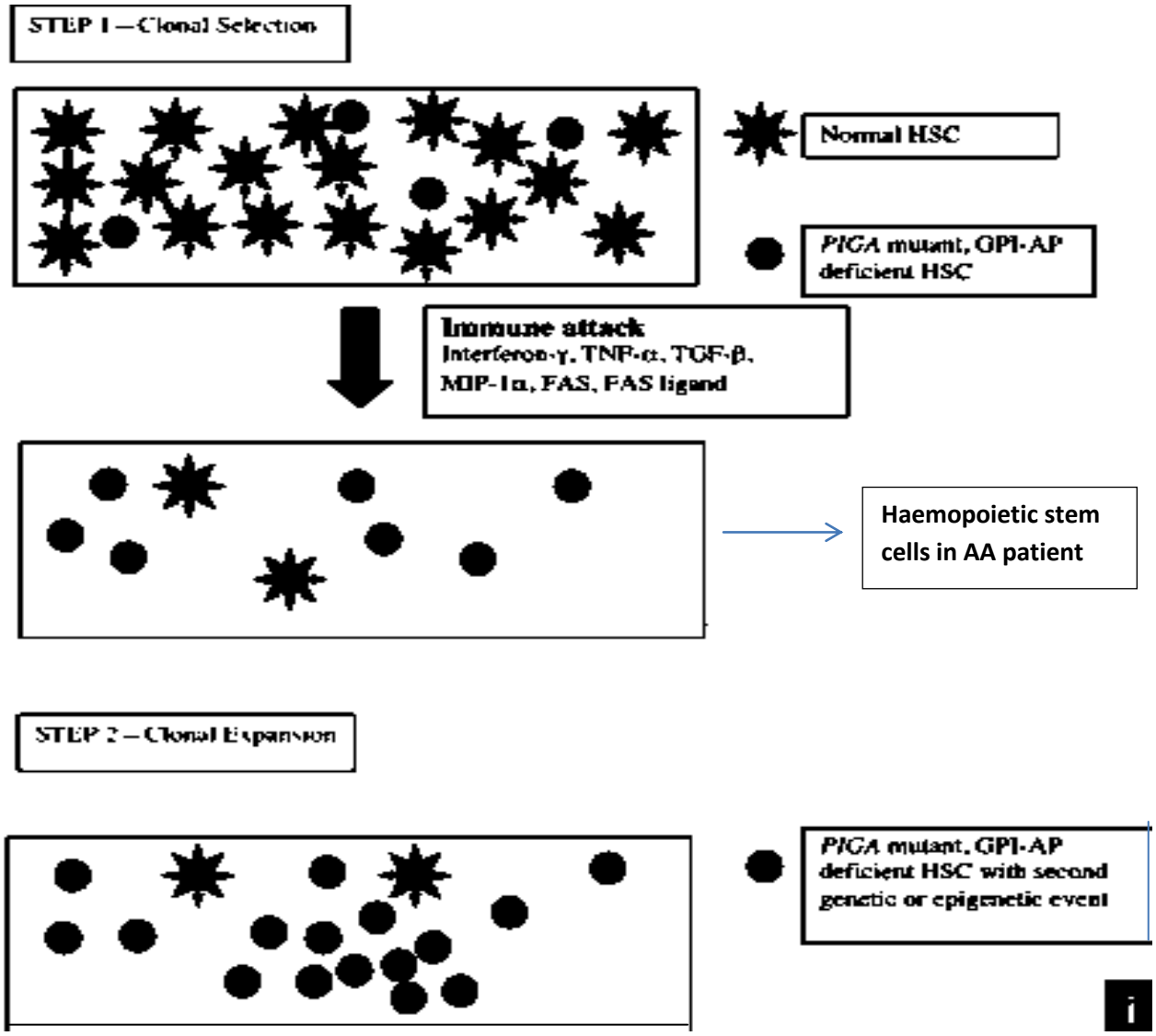


Figure 1: Model of two-step hypothesis of AA and PNH pathophysiology(25)

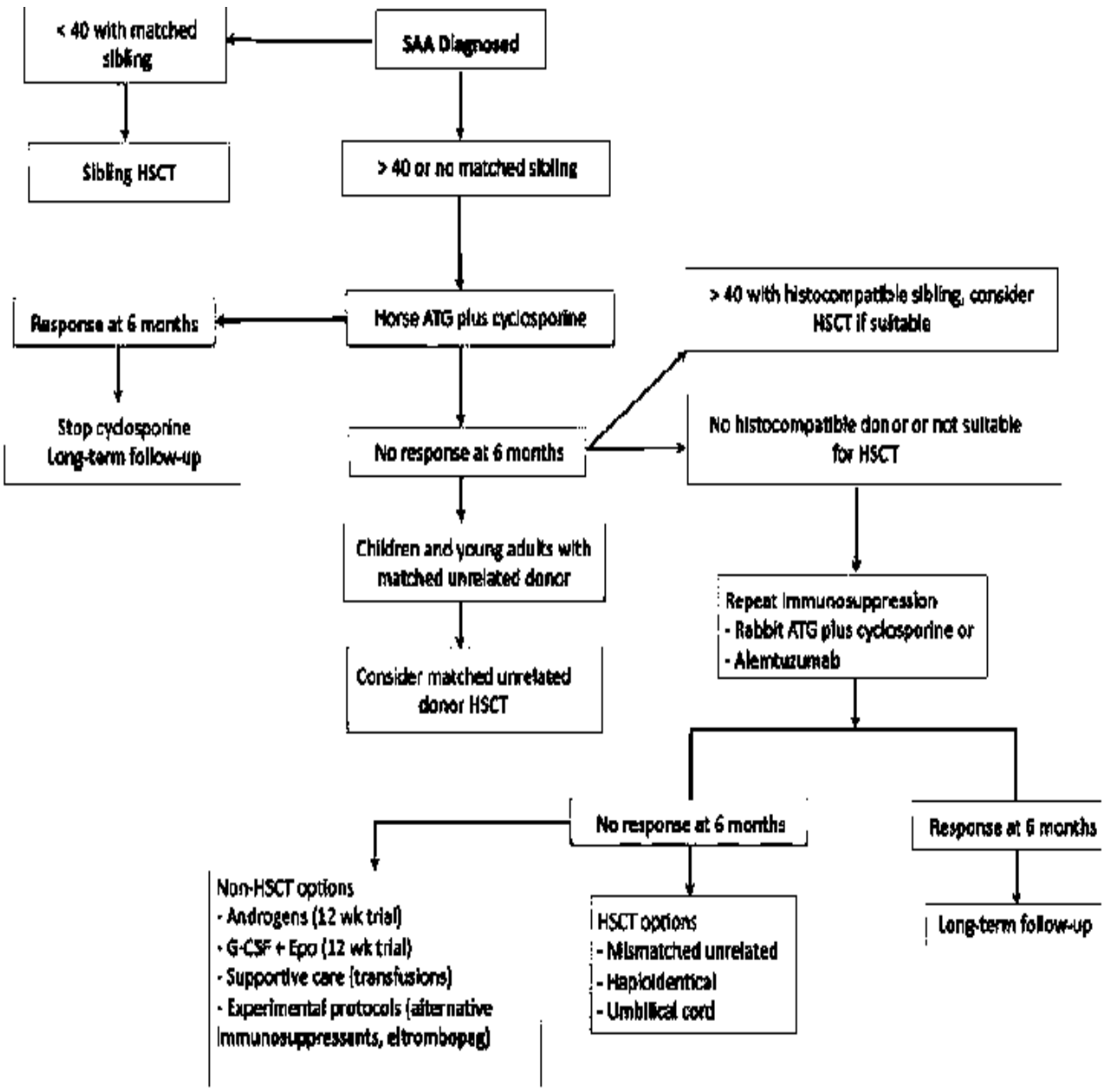


Figure 2: Algorithm for management of AA(26).

At MNH, neither allogeneic SCT nor intensive IST with ATG and cyclosporine is available. Patients with AA are either referred abroad for definitive treatment, or are managed with supportive treatment with blood transfusion and cyclosporine only. However not all of them can afford to purchase the medications

1.2 Conceptual framework

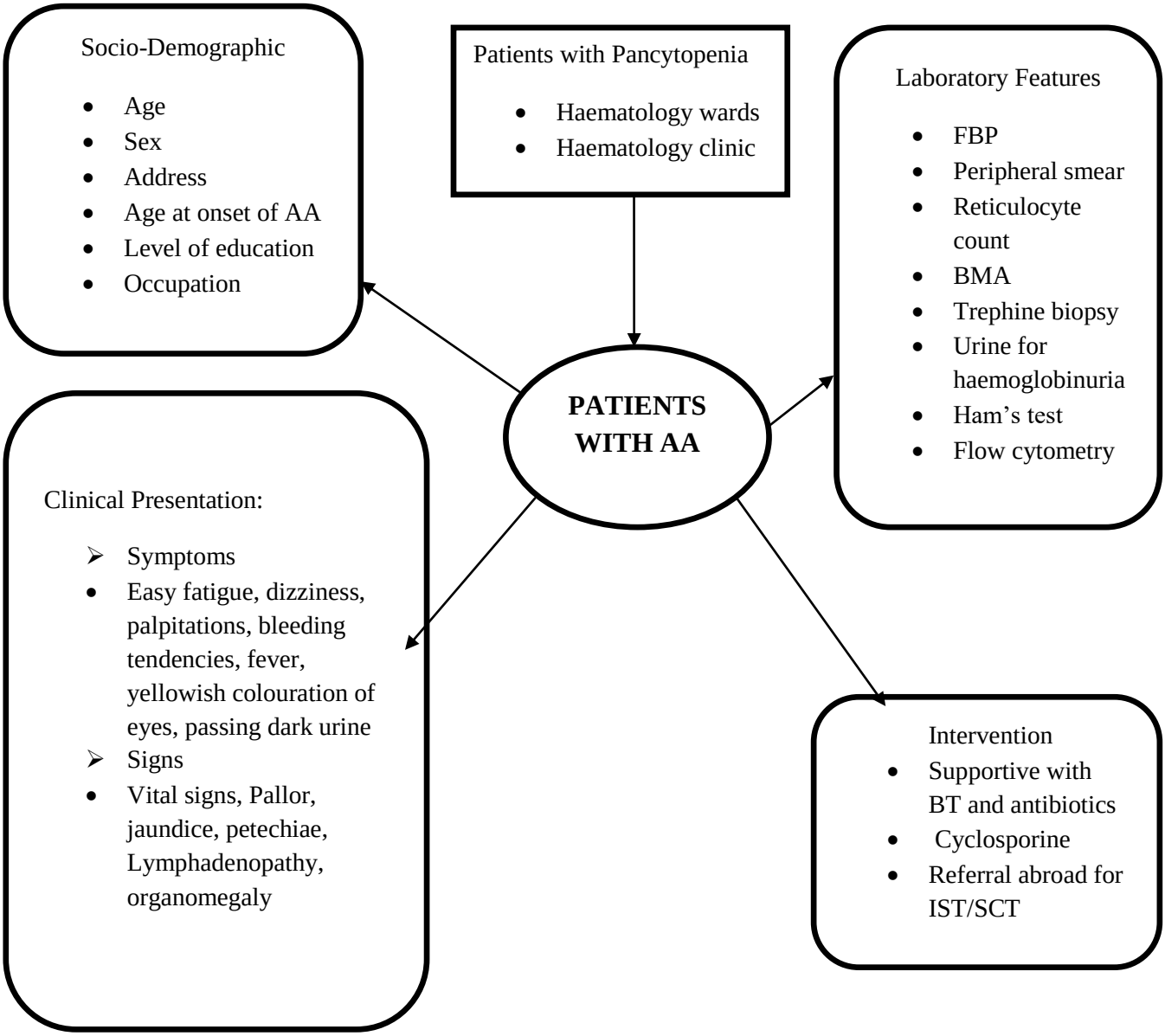


Figure 3: Conceptual framework

1.3 Problem statement

Studies done in other parts of the world have established a pathophysiological link between AA and PNH, thus patients with AA are screened for presence of GPI- deficient blood cells once every year. The link has not yet been established in Africa, and until now, in Tanzania, patients with AA have not been tested for the presence of GPI-deficient blood cells population.

1.4 Rationale

There is no registry for AA in Tanzania, but we can hope that this study will aid in establishment of a registry for AA in MNH and Tanzania at large. Until now, patients with AA have not been given priority in the formation of health policies and programs.

Providing the treatment is beyond the scope of this study, but through this study, we will be able to understand the magnitude of the disease, and the treatment options accessible to these patients. This may be used as an eye-opener for possible initiation of the definitive treatment modalities.

This being one of the first studies of AA in Tanzania will form a basis for further, more extensive studies to be done on AA.

1.5 Research Questions

This study aims to answer the following questions in the setup of MNH;

1. What are the clinical and laboratory features of patients with aplastic anemia?.
2. What proportion of patients with AA have GPI negative blood cells?
3. What are the available treatment modalities offered to patients with aplastic anemia at MNH?

1.6 Objectives

1.6.1 Broad Objective

To determine the clinical and laboratory features, available treatment options and presence of significant GPI negative red cells among patients with aplastic anaemia attending at Muhimbili National Hospital, Tanzania.

1.6.2 Specific Objectives

1. To describe the clinical and laboratory presentation of patients with AA.
2. To determine the proportion of patients with significant GPI negative blood cells among patients with AA.
3. To describe the available treatment options offered to patients with AA at MNH.

CHAPTER TWO

2.0 METHODOLOGY

2.1 Study design

This was a hospital based, descriptive, cross sectional study.

2.2 Study duration

The study was conducted for a period of seven months, from September 2016 to March 2017.

2.3 Study area and populations

The study was conducted at Muhimbili National Hospital in Dar es Salaam. MNH is the largest referral hospital in Tanzania, catering for patients from all over the country including patients referred from regional hospitals (Amana, Temeke and Mwananyamala) and private hospitals in Dar es Salaam. It is the only government hospital diagnosing and managing AA in Tanzania. This study involved all patients with AA, both inpatients and outpatients, attending Haematology department at MNH from September 2016 – March2017.

2.4 Inclusion criteria

Patients were recruited into the study included only when the diagnosis of AA was confirmed using clinical, laboratory data, and by bone marrow aspirates ± trephine biopsy results, old and new cases, all age groups, both sexes, who have consented to participate in the study.

2.5 Exclusion criteria

Patients who did not consent to participate in the study, those with no BMA reports confirming the diagnosis of AA and patients with features suggestive of inherited bone marrow failure syndromes were excluded from the study.

2.6 Sampling method

Convenient sampling technique was used. Since AA is a rare disease, all patients with AA attending MNH during the stated study duration were recruited into the study. At least five patients were recruited every month making a total of 40 patients.

2.7 Procedure and Data collection

Data collection commenced after attainment of ethical clearance. I was done using a questionnaire and it entailed the focused history taking on the socio demographic characteristics, clinical presentational symptoms of anaemia, fever, bleeding tendencies, yellowish colouration of the eyes, weight loss, number of blood transfusion and number of admissions. Date of diagnosis, age at diagnosis and onset of presentation were also recorded. I examined all patients thoroughly for relevant physical signs which included assessment for vitals (blood pressure, respiratory rate and pulse rate) pallor, fever, petechiae, active bleeding, jaundice and organomegaly.

Also, patients were asked about the treatment given ie cyclosporine tablets, prednisolone tablets, supportive treatment with blood transfusions only, and history of using ATG or SCT for those who were referred for management abroad.

2.8 Sample collection

For each of the study participant, 10mls of venous blood was drawn for laboratory tests (full blood count, peripheral smears, reticulocyte count, Ham's test and Flow cytometry analysis for GPI-deficient red blood cells). The blood was collected into sterile vacutainers containing EDTA anticoagulant. Samples for ham's test and flow cytometry analysis for GPI deficient cells were put in a vacutainers containing CPD-A anticoagulant. . Full blood counts and reticulocyte count were done within 6 hours of collection. Ham's test and flow cytometry analysis for GPI deficient red cells were done within 3 weeks of collection at CPL, haematology section. However, flow cytometry analysis for GPI deficient blood cells is not done as routine at MNH haematology laboratory; it took some time (three months) for the settings to be complete. Therefore a few samples haemolysed before analysis was done.

A total of 29 samples out of 40 samples collected were therefore analyzed for presence of GPI deficient red cells.

2.9 Laboratory procedures

CBC was done using the 3700 Celldyn machine. Peripheral blood films were made by staining the samples with Leishman stain. I performed reticulocyte counts manually by adding equal amount of blood samples with new methylene blue stain, the mixture was incubated for 20 minutes in water bath at 37 °C, then films were made, and reticulocytes were counted microscopically.

I assessed urine samples for presence of haemoglobinuria by macroscopic examination after centrifugation of urine samples.

I did Ham's test in all study participants. 0.05mls of patients' red cells were added in a test tube containing acidified serum (0.5mls of normal serum and 0.05 mls of 0.2M HCL acid),0.05mls of patients' red cells were added in test tube containing acidified heat inactivated serum, 0.05 mls of patients' red cells were added in another test tube containing patients serum. The test tubes were incubated for an hour followed by centrifugation. For each test, a negative control was done using samples from normal healthy people. Results were interpreted by the principal investigator and the senior laboratory technologist.

Flow cytometry analysis for GPI - deficient red cells was done using BD FACS Callibur machine. CD59 PE antibodies were used for red cells analysis. Samples were analyzed by the senior laboratory technologist and the results were interpreted by the principal investigator, and the chief laboratory scientist.

2.10 Data management and statistical analysis

Data were collected using pre-tested data collection questionnaires. Information from the questionnaires was entered into SPSS v.20.0 statistical software for further data cleaning and analysis.

Data analysis included calculation of frequencies and percentages for categorical data. Medians and inter-quartile ranges were used for numerical data which were not normally distributed. Proportion of AA patients with significant GPI deficient red cells was presented as a percentage. The numerator was the number of patients with GPI deficient red cells and the denominator was the total number of patients with AA whose samples were analysed for presence of GPI deficient red cells.

Fischer's exact test was used for comparison between patients with AA and GPI negative deficient red cells and those with AA alone.

2.11 Ethical consideration and confidentiality

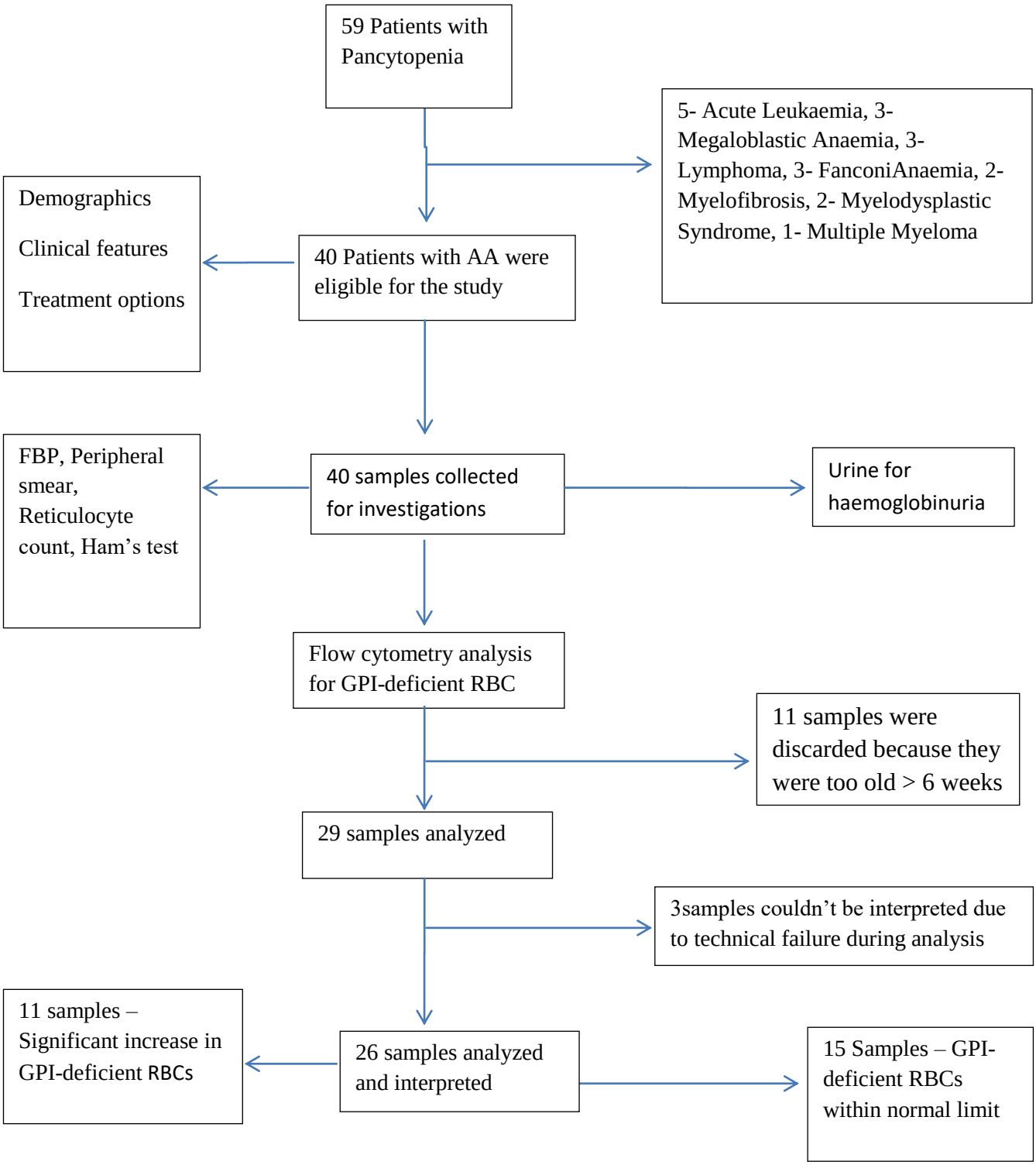
Ethical clearance was sought from the Research and Publications Committee of Muhimbili University of Health and Allied Sciences (MUHAS). The permission to conduct this study was sought from authorities of Muhimbili National Hospital. A formal written informed consent / assent, in Swahili, were sought from the participants.Patients' names were not used; information collected on questionnaires was entered into computer using identification numbers to maintain confidentiality.

CHAPTER THREE

3.0 RESULTS

I recruited patients from haematology units in ward 3, ward 5, paediatric ward and general haematology clinic.I screened a total of 59 patients with pancytopenia. Of these, 5 had acute leukaemia, 3 had megaloblastic anaemia, 3 had lymphoma, 2 had myelodysplastic syndrome, 2 had myelofibrosis, 1 had multiple myeloma. Therefore these 16 patients were excluded. 3 patients had congenital skeletal malformations and café au lait spots suggestive of Fanconianaemia, therefore they were also excluded.40 patients were eligible for the study and I recruited them after obtaining their consent or assent .In 18, the diagnosis of AA had already been established before recruitment. 22 patients were recruited after confirming the diagnosis during the study period. All 40 patients had bone marrow aspirates results and 26 of them had both BMA and trephine biopsy results. In 14 who did not have trephine biopsy results, the aspirates were adequate and highly suggestive of AA.

Figure 4: Flow chart.



3.1 Demographic characteristics

Seventeen out of 40 patients recruited (43%) were male. The male to female ratio was 1:1.4. The median age at diagnosis was 24(15-33) years. Majority of the patients were in the second and third decade of life, 12(30%) and 10(25%) respectively. Twenty eight (70%)patients had secondary or higher education. Majority of patients were students and those with employment, 14(35%) and 16(40%) respectively. Four (10%) patients were peasants. Sixteen (40%) patients were from Dar es Salaam and other regions comprised of less than 3(10%) patients each.

Table 2: Demographic characteristics of patients with AA

Variable		Number	Percentage (%)
Sex	M	17	43
	F	23	58
	Median age at diagnosis	24(15-33)	
Age (years)	(IQR)		
	<10	1	3
	10-19	12	30
	20-29	10	25
	30-39	9	23
	40 and above	8	20
Level of education	No formal education	1	3
	Primary education	11	28
	Secondary education	18	45
	Higher education	10	25
Occupation	Employed	16	40
	Peasants/ Farmers	4	10
	Students	14	35
	Others	6	15

3.2 Clinical Features

The most common symptoms were those related to anaemia; easy fatigue 29 (73%), awareness of heart beats 27(68%) and dizziness 26 (65%). Fevers occurred in19 (48%) and bleeding occurred in 18(45%). Other symptoms included yellowish discolouration of eyes, abdominal pain, bone pain, dark urine, cellulitis of the fore arm, leg and oral ulcers, 1 patient (3%) each. No patient had history of thrombosis or weight loss. The commonest physical signs were pallorwhich was found in 31(78%) and petechiaein 28 (70%). One patient (3%) had jaundice, 1 patient (3%) had swollen left arm and no patient presented with lymphadenopathy or organomegaly. Seven (18%) presented with menorrhagia and 5(13%) presented with conjunctival haemorrhage.

Table 3:Clinical features of patients with AA

Clinical features		Number (n)	Percentage (%)
Symptoms	Easy fatigue	29	73
	Awareness of heart beats	27	68
	Dizziness	26	65
	Fever	19	48
	Bleeding tendencies	18	45
	Others	6	15
Signs	Pallor	31	78
	Jaundice	1	3
	Conjunctival haemorrhage	5	13
	Gum bleeding	10	25
	Petechiae and ecchymoses	28	70
	Others	1	3

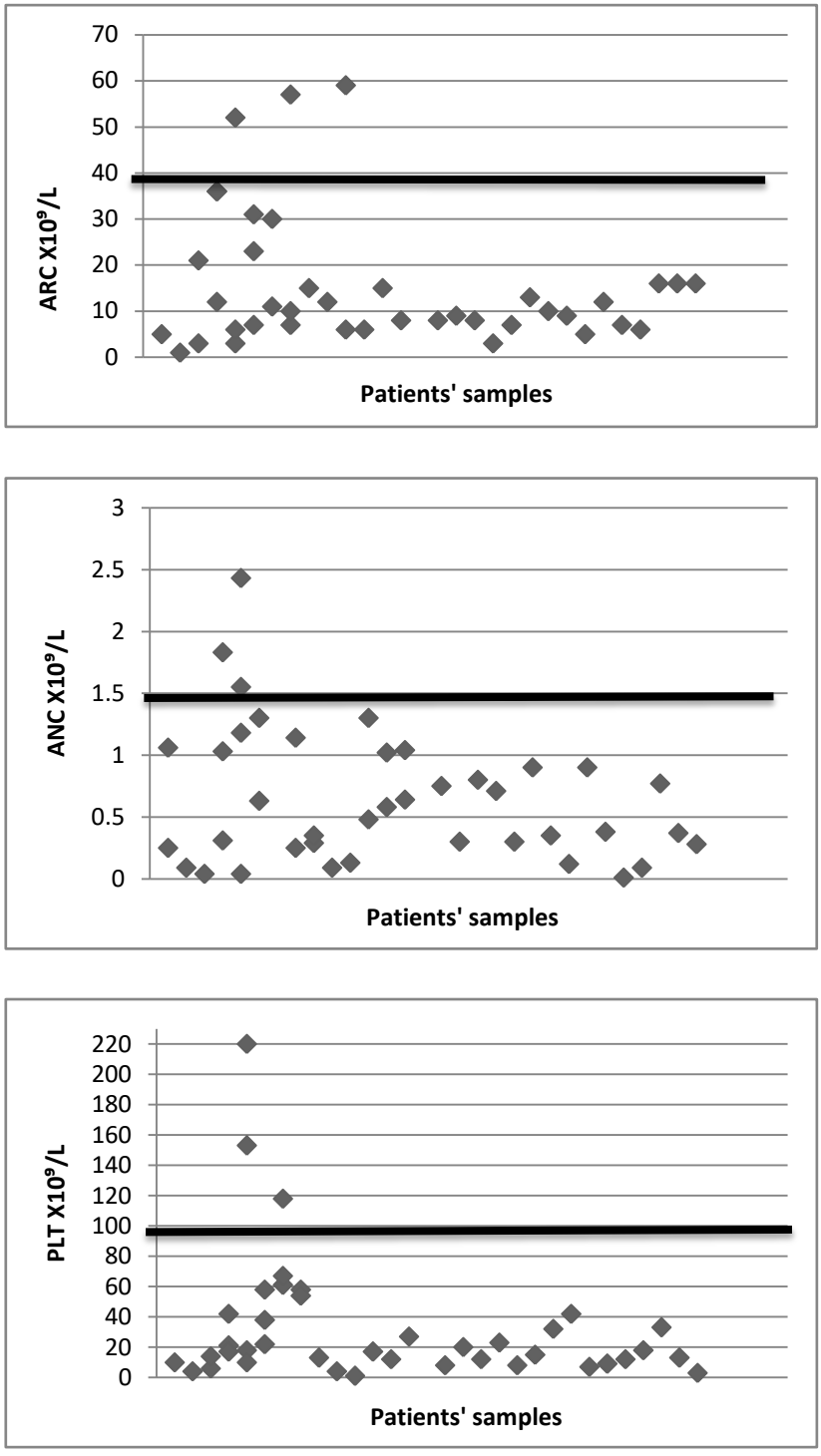
3.3 Laboratory Features

FBC and ARC were done to all patients, and the results were used to classify the severity of AA based on Camitta's criteria. The median (IQR) Hb level was 6.6 (5.4 -8.8) g/dl. 31(78%) had ARC of less than 20 X 10^9/l, with the median (IQR) ARC of 10 (6.25-16) X10^9/l. Three patients (8%) had ARC of above 40 X10^9/L. Five patients (22%) had normal WBC count whereas 35(88%) had leukopenia. Only one patient (3%) had a normal ANC of 2.43 X10^9/l. 20 (50%) of patients had ANC of less than 0.5 X10^9/l. The median (IQR) ANC was 0.49 (0.01-1.04) X10^9/l. 6(15%) had lymphopenia, the remaining 34(85%) had normal ALC. The median (IQR) ALC was 1.30 (0.82-1.69) X10^9/l. 24(60%) had PLT count of less than 20X10^9/l. Only two patients (5%) had normal platelet count of 158 X10^9/l and 220 X10^9/l. The median (IQR) PLT was 17.50 (9.63 – 41.00) as shown in table 4.

Table 5: Full blood counts of patients with AA

	Hb(g/dl)	MCV (fl)	ARC (X10^9/L)	WBC (X10^9/L)	ANC (X10^9/L)	ALC (X10^9/L)	PLT (X10^9/L)
Median	6.6	86.50	10.00	2.01	0.49	1.30	17.50
(IQR)	(5.4-8.8)	(83.0-90.7)	(6.25-16.00)	(1.41-3.18)	(0.26-1.04)	(0.82-1.69)	(9.63-41.00)
Minimum	2.4	74	1.00	0.40	0.01	0.10	1
Maximum	14	113	59.00	5.00	2.43	3.31	220

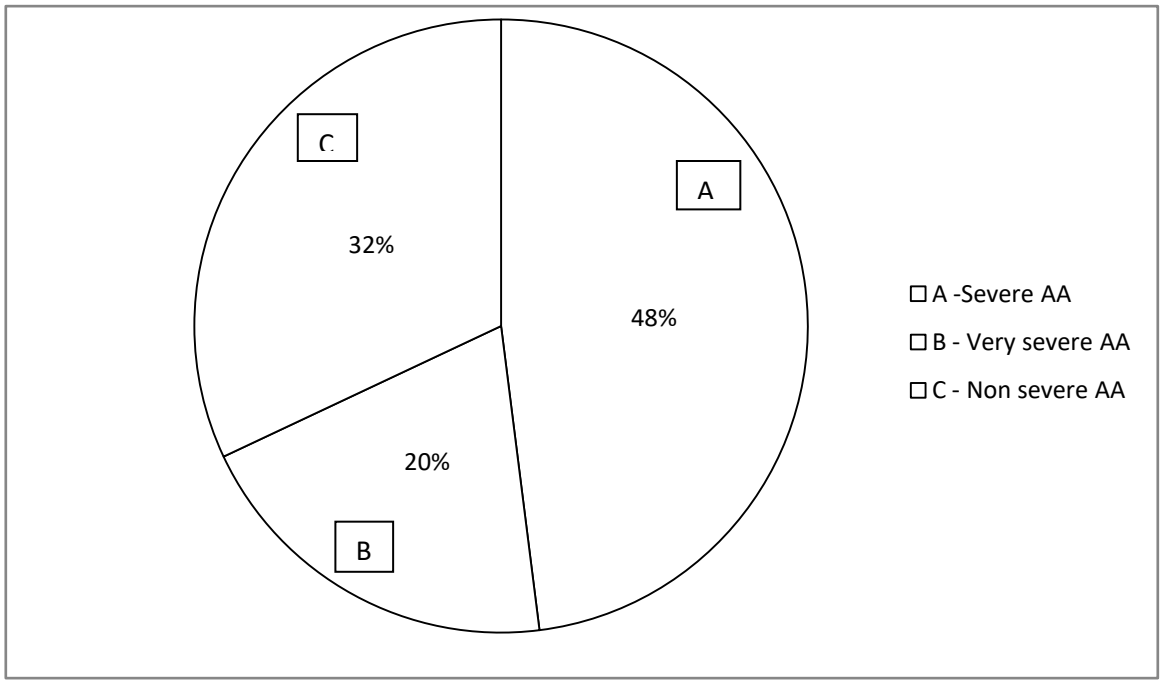
Figure 5: Absolute reticulocyte counts, absolute neutrophil counts and platelet counts of patients with AA



Peripheral blood film assessment revealed normal film in one patient (3%), 2(5%) had unilineage cytopenia (neutropenia), one(3%) had bicytopenia (anemia and neutropenia), and pancytopenia in 36(90%) patients. The patient with normal counts had undergone allogeneic haemopoietic SCT and those with unilineage cytopenia and bicytopenia had received intensive IST.

Twenty seven (68%) patients presented with severe or very severe AA based on Camitta's criteria. Thirteen (32%) had non severe AA.

Figure 6: Distribution of patients according to severity of AA.



3.4 Proportion of AA patients with GPI-deficient red cell population

Urine for haemoglobinuria assessment was done in all 40 patients and none of the patients had haemoglobinuria. Ham's test was also done in all patients and none of them had a positive Ham's test results.

Flow cytometry analysis for presence of GPI deficient red cells was done on 29 samples. Three samples could not be interpreted because of technical failure during analysis. Four samples taken from normal healthy individuals had GPI-deficient red cells populations ranging from 0.01% to 0.04%. Out of 26 patients whose samples were analyzed for presence of GPI deficient red cells,15 had GPI deficient red cells of less than 0.1% which is within the normal limit. Eleven(42%) had significant increase in GPI deficient red cells of more than 0.2%.The distribution of GPI-deficient red cell populations among AA patients and the four normal individuals showed that 15 AA patients had GPI-deficient red cells within the same range as the normal individuals (figure 8). The median (IQR) percentage of GPI deficient red cells in patient without PNH clone was 0.07 % (0.04% – 0.08%). The minimum was 0.02% and maximum 0.09%.The median (IQR) percentage of GPI deficient red cells in patient with significant PNH clone was 0.68 % (0.38% – 1.63%). The minimum was 0.26% and maximum 3.87%.Three patients (12%) had GPI-deficient red cell population (PNH clone) of more than 1%.

Ten out of 11 patients (91%) with significant GPI negative red cells (PNH clone) were diagnosed with AA more than one year prior to recruitment. Eleven out of 15(73%) patients with AA for less than a year had GPI deficient red cells within normal limit. This was found to be statistically significant. Also 9 out of 11patients (82%) with significant GPI negative RBCs had less transfusion requirement of less than 10 units within six months. Ten out of 15patients (67%) with history of more than 10 units of PRBCs transfusion had no significant GPI negative RBCs. This was also found to be statistically significant. Severity of AA and type of treatment received were not found to be related with development of significant GPI negative RBCs.

Figure 7a: Patient with AA with 0.02% GPI-deficient red cells

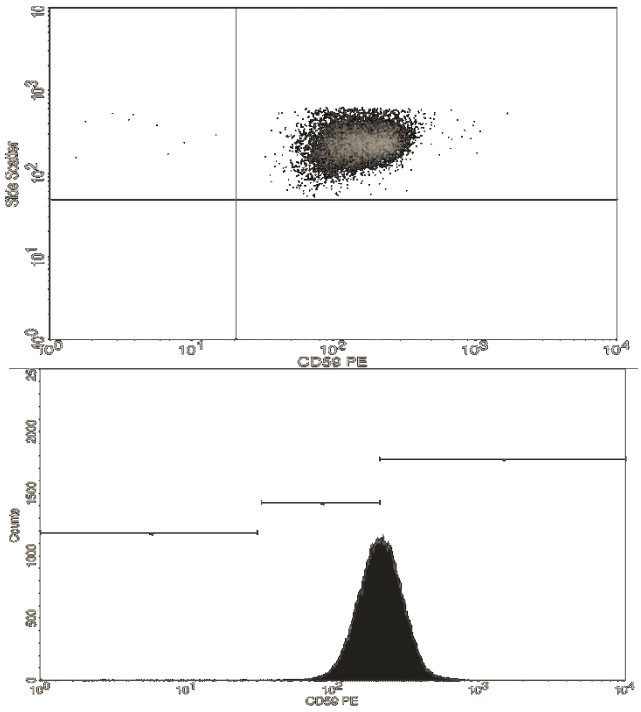


Figure 7b: Patient with AA with 3.86% GPI-deficient red cells

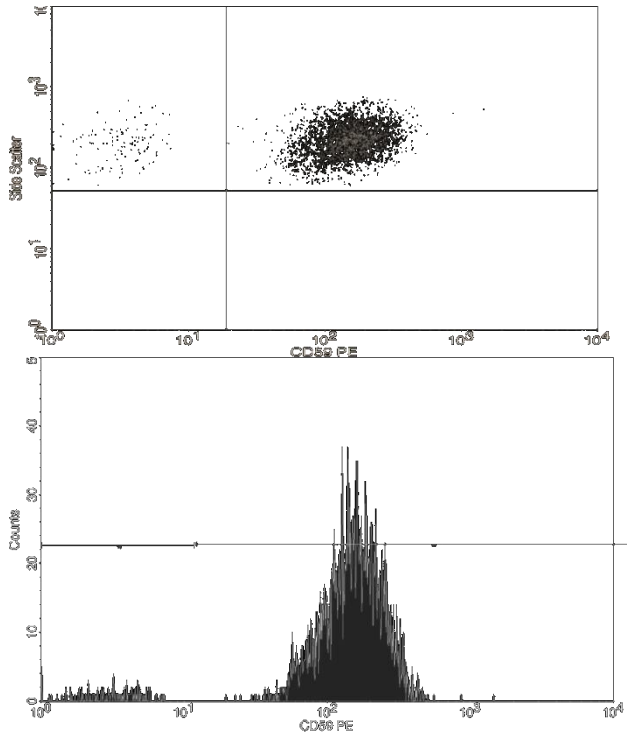


Figure 8: Distribution of Percentage of GPI-deficient red cell population in patients with AA and normal healthy individuals

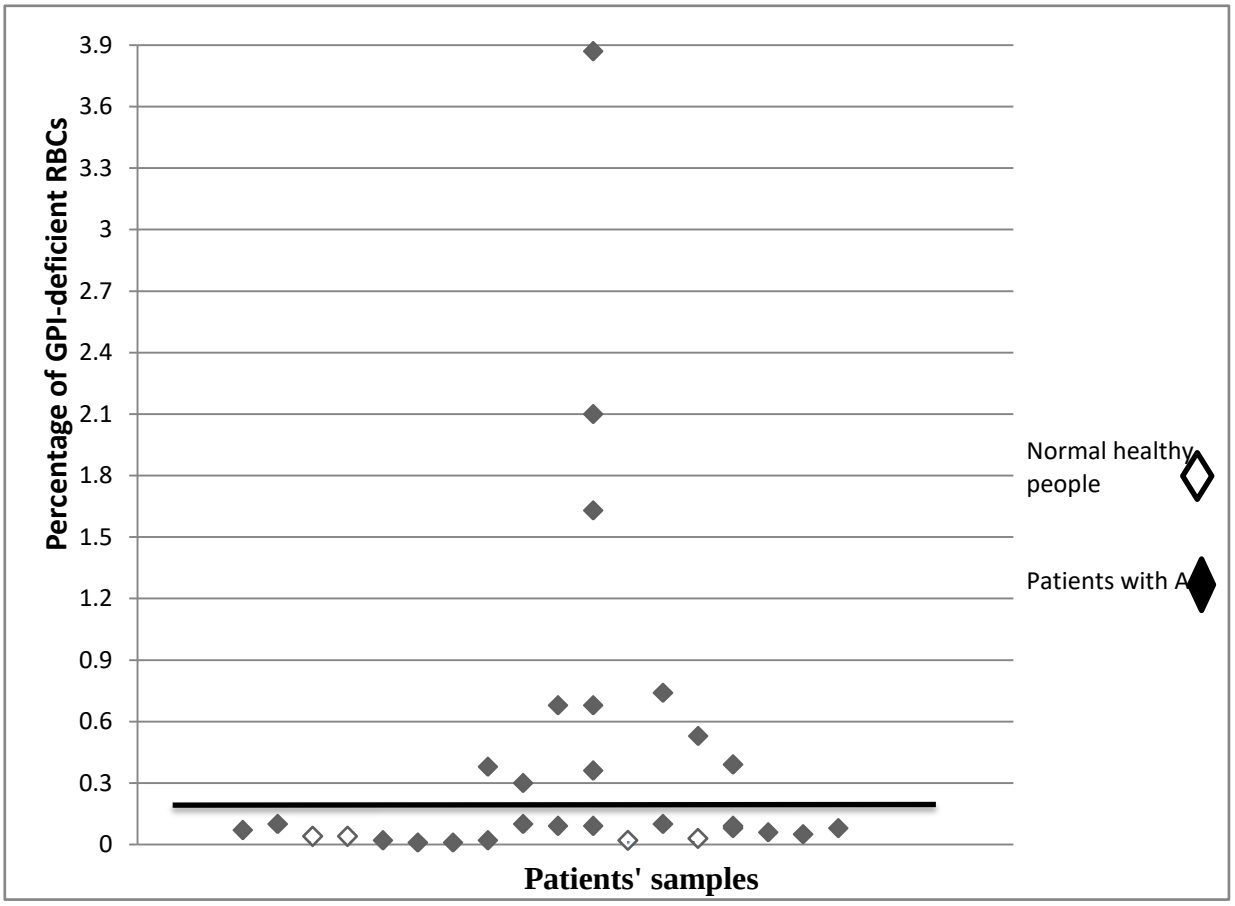


Table 5: Comparison of AA patients with and without significant GPI negative red cells with various characteristics

		AA patients with significant GPI deficient RBCs. n (%)	AA patients with GPI deficient RBCs within normal limit. n (%)	P value
Number of samples analyzed for GPI deficient red cells	Total 26 (100)	11 (42)	15 (58)	
Duration of illness	Less than 1 year	1 (9)	11 (73)	0.002
	More than 1 year	10 (91)	4 (27)	
Transfusion within 6 months	Less than 10 units	9 (82)	5 (33)	0.019
	More than 10 units	2 (18)	10 (67)	
Severity of AA	Non severe AA	6 (55)	3 (20)	0.079
	Severe A	5 (45)	12 (80)	
Treatment received	Supportive and cyclosporine	7 (64)	14 (93)	0.18
	Supportive and definitive treatment	4 (36)	1 (7)	

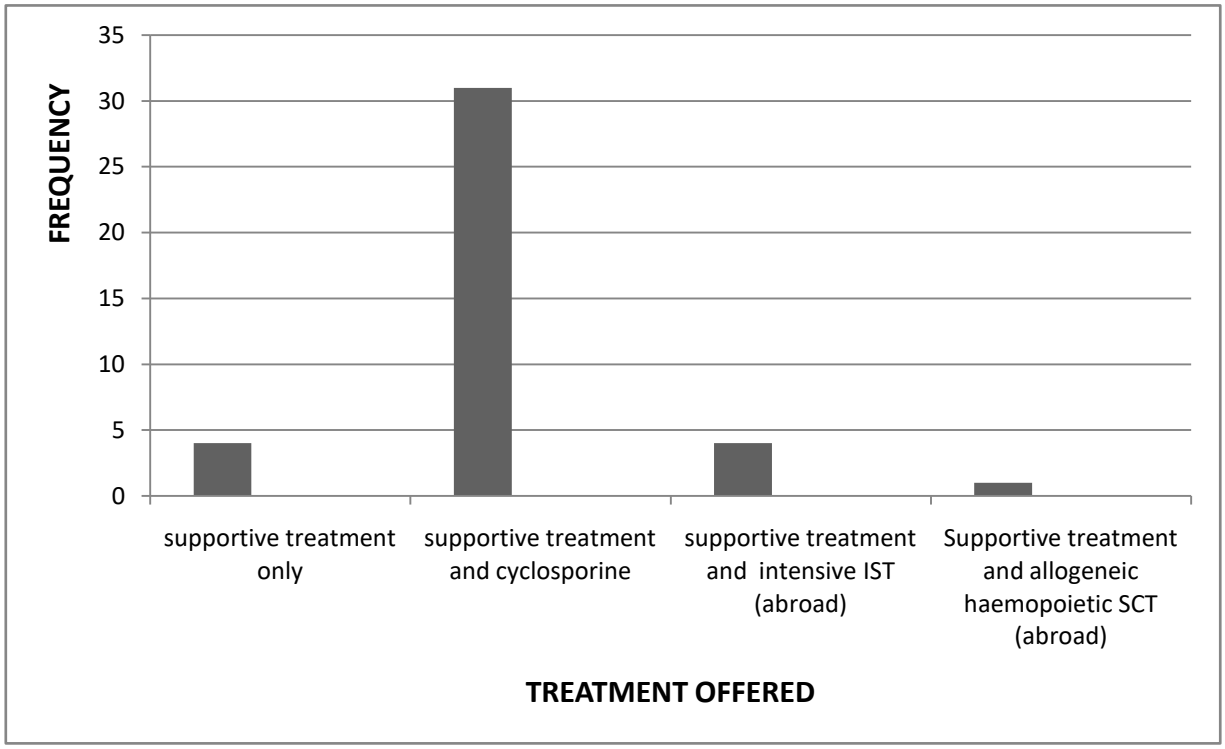
3.5 Available Treatment modalities

Thirty one patients (78%)were treated with a combination of supportive treatment and immune suppression therapy with cyclosporine only. Four patients (10%) had received supportive treatment with blood products and antibiotics only.

Four patients (10%) were treated with intensive IST and one patient (3%) had undergone allogeneic haemopoietic SCT making a total of five patients (13%) who were referred abroad for definitive treatment.

The median (IQR) number of admission per patient within six months was 2(0-3) and the median (IQR) duration of hospital stay was 6 (1-9) days. The commonest blood product transfused was PRBCs with the median (IQR) number of 6 (1-12) units per patient. Although 24 patients (60%) had platelet count of less than $20X10^9$/L, only one patient had received adult therapeutic dose of platelets at once. A total of 15 patients received platelet transfusion. The median (IQR) was 0 (0-4) platelet packs per patient

Figure 9: The treatment modalities offered to patients with AA



CHAPTER FOUR

4.0 DISCUSSION

This study has described the demographic, clinical and laboratory features, treatment modalities and the proportion of AA patients having a significant GPI negative red cell population among patients with AA attending at MNH.

In this study, the minimum age of the patient was 5 years and the maximum age was 69 years. The median (IQR) age at onset was 24 (15 -33) years in which 22 patients (55%) were of age below 30 years. This finding was similar to findings from other studies(16)(19).The second peak at 65 years of age which is described in literature was not clearly observed in this study.

Previous studies have shown that the male to female ratio is equal, and the study done in Thailand had shown a slight male predominance(16). In this study there was a female predominance with a male to female ratio of 1:1.4

Sixteen (40%) patients in the study were residents of Dar es Salaam. Twenty eight (70%) had either secondary or higher education. Only 10% of patients were farmers. The population of Dar es Salaam contributes 9.7% of the country population (Tanzania- population and housing census 2012), and over 70% of Tanzanian citizens are farmers(National survey and segmentation of smallholder households in Tanzania). It is possible that patients from other regions are attending hospitals at their regions and this suggests that the incidence and prevalence of AA in Tanzania could be even higher.

Anaemia was the commonest clinical finding occurring in 29 (73%) patients followed by fever in 19(48%) and bleeding tendencies in 18(45%). No patient had lymphadenopathy or organomegaly. This was also observed in other studies (27-28). One patient had jaundice and this was due to Malaria infection. Other symptoms which included abdominal pain, leg ulcers and cellulitis of the fore arm were related to neutropenia.

Out of 26 samples analyzed for presence of significant GPI negative red cells, 42% of patients had PNH clone. This is in keeping with the available literature which shows that up to 50% of AA patients have PNH clone. A study done in Egypt which involved 11 children with AA revealed that 36.3% of patients had PNH clone(10). Other studies done in France, USA, and Russia showed the prevalence of 35%, 40% and 58% respectively (29-31).

This study showed that there is a relationship between duration of illness (AA) and development of PNH clone. Only one out of eleven patients with PNH clone (9%) had AA for less than a year. This may be explained by the hypothesis that the PIGA mutant, GPI-deficient haemopoietic cells are spared after an autoimmune attack on normal haemopoietic stem cells. These mutant cells require some time for clonal expansion to occur (5-7).However the PNH clones have been found even in patients with AA at diagnosis. This is shown in various studies in which up to 50% of patients with AA have PNH clone at diagnosis(10)(32).

Patients with AA and PNH clone had less transfusion requirements compared to those with AA alone. This may be due to the fact that the GPI negative haemopietic stem cells are more resistant to an autoimmune environment. Therefore they are spared by the cytotoxic T cells, repopulate the marrow and alleviate symptoms of anaemia(6)(33)(11).

None of the patients with PNH clone had evidence of haemolysis as suggested by negative haemoglobinuria assessment, reticulocytopenia and absence of features of haemolysisin peripheral blood films. Serum LDH levels was not assessed in this study. This reflects that majority of patients with AA have subclinical PNH (PNH-sc), and this has also been found in other studies (28)(34).

Supportive care with blood products to maintain safe blood counts is vital in management of AA. All patients recruited in this study received supportive blood transfusions and antibiotics when indicated. As in most African countries, in Tanzania neither intensive IST nor haemopoietic SCT is available. This study showed that 78% of patients had received a combination of supportive therapy and cyclosporine alone, and 10% had received supportive therapy only. In a study done in Congo Brazzaville involving 30 patients, and another one

done in Nigeria involving 25 patients with AA, all patients received supportive blood transfusion, antibiotics and methyl prednisolone only(18)(35). Use of immuno suppression therapy with cyclosporine alone has been found to be a viable therapeautic option in the treatment of SAA in which up to 40%of patients may respond to cyclosporine(36). The definitive treatment with either intensive IST or allogeneic haemopoietic SCT has a 60-80% response rate(2)(20)(37). In this study, 5 patients were referred abroad for definitive therapy, and a good response was seen in four of them; one patient died within 3 months of receiving intensive IST. In Tanzania, cyclosporine is expensive, and only a few patients can afford to use it regularly. Egypt and South Africa are the only countries offering both haemopoietic SCT and IST in Africa.

Although this was a cross-sectional study, it was observed that 17 out of 40 recruited patients (43%) died during the study period. The leading causes of death being sepsis and bleeding. This agrees with the literature that if left untreated, AA is fatal(12)(27).

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Aplastic anaemia mostly affects people below the age of 40 years. The clinical presentation is due to bone marrow failure; anaemia, bleeding due to thrombocytopenia, and infections related to neutropenia. Almost half of patients with AA attending MNH have PNH clones, although they do not have yet any clinical or laboratory evidence of haemolysis (PNH-sc).The majority of patients have either severe or very severe AA. There is evidence of improved survival in the outcome of patients with AA globally following the use of intensive IST or allogeneic haemopoietic SCT. The situation is different in Tanzania where a small percentage of patients are referred abroad for IST or allogeneic haemopoietic SCT. At MNH patients with AA are treated with combination of supportive therapy and immune suppression with cyclosporine alone. Management of thrombocytopaenic bleeding remains a challenge in the absence of adequate platelet support services.

5.2 Recommendations

1. Since AA is a fatal disease; there is a need of providing definitive therapy in Tanzania.
2. Improving blood products transfusion services to maintain an adequate platelet and packed red cell supply.
3. Regular screening for presence of PNH clones among patients with AA which will help in monitoring, prevention, and management of intravascular haemolysis and thrombosis.

5.3 Study limitations

Lack of comparative group of patients with classical PNH.

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APPENDICES

Appendix 1: Questionnaire (English Version)

PROFORMA FOR AA STUDY Study No |___|___|___|

REGISTRATION

- Date (DD-MM-YY) ……|___|___|-|___|___|-|___|___|
- Telephone number 1)…… 2)…….

DEMOGRAPHIC HISTORY

- Hospital ID Number ……|___|___|___|___|___|___|___|___|___|___|
- Name ……...|_____|
- Date of birth ……|___|___|-|___|___|-|___|___|
- Sex ……...|___|
- Place of origin …… |_____|
- Current residence …… |_____|
- Level of education ……..|_____|
- Occupation ……. |_____|

HISTORY (Y for Yes, N for N)

- Date of diagnosis of AA ……|_____|
- Age at diagnosis or presentation ……|_____|
- Easy fatigue ……..|___|
- Dizziness ……....|___|
- Awareness of heart beats …….....|___|
- Fever …….....|___|
- Bleeding tendencies ……. |___|

- Dark urine …… |___|
- Yellowish colouration of eyes ……....|___|
- Abdominal pain ……..|___|
- History of thrombosis …….....|___|
- Weight loss ……|____|
- Others (specify) …….....|_____|
- History of blood transfusion ……..|___|
- Number of units of red blood cell transfused…..|_____|
- Number of platelet packs transfused ……..|_____|
- Number of admissions ……|_____|
- Mean duration of hospital stay per admission ……..|_____|

PHYSICAL EXAMINATION

- Pulse rate……...|_____|
- Respiratory rate ……|_____|
- SBP……|_____|
- DBP……...|_____|
- Height……|_____|
- Weight ……..|_____|
- Pallor ……....|___|
- Jaundice ……|___|
- Petechiae ……...|___|
- Edema ……...|___|
- Lymphadenopathy …….|___|
- Liver …….|___|
- Spleen ……...|___|
- Kidneys ……....|___|

TREATMENT OPTION GIVEN

- Supportive treatment with blood transfusions and antibiotics
- Cyclosporine
- Referred abroad for IST
- Referred abroad for allogeneic SCT
- Others (specify) ……|_____|

INVESTIGATIONS

- Hb……|_____|
- MCV ……..|_____|
- WBC ……..|_____|
- ANC……|_____|
- ALC ……|_____|
- PLT ……|_____|
- ARC ……...|_____|
- Peripheral smear ……|_____|
- Hams test ……...|_____|
- Haemoglobinuria ……|_____|

FLOW CYTOMETRY ANALYSIS

- Red cells – CD 59 PE…….|_____|

BMA slide number ……

Trephine biopsy HP number……..

Appendix II: Consent/ Assent form (English Version)

Consent / Assent to participate in the study of clinical features, laboratory features and treatment options given to patients of aplastic anaemia among patients with aplastic anaemia attending at MNH.

Dear Sir/Madam,

Greetings!

My Name is Dr. Mwashungi Ally, a resident doctor in the Department of Haematology and Blood Transfusion at MUHAS. I am conducting a study aiming to find out the clinical features, laboratory features, treatment options given to patients with aplastic anaemia, and the percentage of patients with aplastic anaemia having GPI-negative blood cells. I am requesting your participation.

How to participate:
Patients/ relative of patients who will be ready to participate will sign a consent/ ascent form to approve his/her willingness. Short interview will be done and blood sample for investigations will be taken.

Confidentiality:
Information obtained from you will be confidential and will be of help in this study and better care for patients with Aplastic anaemia in the future.

Costs:
You will not be required to pay anything for your participation.

Voluntary participation & rights to withdraw:
Your participation is voluntary and you have the right to withdraw from participating in our study at any time. Whatever your decision may be, it will not affect in any way your rights to care and treatment.

Risks

Blood sample will be drawn from your/ your child's arm. And you will be given five bottles for collection of urine overnight and early in the morning. We don't expect risk by drawing blood although you/your child will feel some pain when the needle pierces your/ your child's skin for drawing this blood (on the arm). The skin on your/your child's arm will be thoroughly cleaned prior to prevent infections.

Benefits:

Your participation in this study will help you know whether you/ your child have GPI negative blood cells or not, and thus determining prognosis of your/ your child's disease. You/your child will as well get the benefit of getting appropriate treatment of PNH if needed. We hope that the information from this research will be useful in contributing to improve the quality of care in patients with aplastic anaemia.

Contact persons:

If you have any inquiries about this study, please do not hesitate to contact:

Dr. Mwashungi Ally
Principal Investigator
Muhimbili University of Health and Allied Sciences (MUHAS)
Department of Haematology and Blood Transfusion
P.O. Box 65001 Dar es Salaam.
Tel. 0653 986 798

OR in case of any information about your rights as a participant in this study please contact:

Professor Said Aboud
The Chairman
Research and Publication Committee Research and Publication Committee
Muhimbili University of Health and Allied Sciences (MUHAS)
P.O. Box 65001 Dar es Salaam
Tel. 2151489

I will be grateful if you willingly agree to participate in this study.

I _____Have understood the above information and my questions have been answered by the investigator to my satisfaction. I willingly agree to take part in this research.

Signature of the participant/ caretaker ……..,

Date ……..

Signature of the investigator …….

Appendix III: Consent/ assent form (Swahili Version)

FOMU YA MAKUBALIANO YA KUSHIRIKI KATIKA UTAFITI

Habari! Mimi ni Dk Mwashungi Ally nani Daktari mwanafunzi wa shahada ya Uzamili katika Chuo Kikuu Cha Sayansi Za Tiba cha Muhimbili. Nafanya utafiti kuhusu ugonjwa wa Aplastic anaemia hapa Muhimbili hospitali.

Ninaomba ushirikiano wako.

Nia ya Utafiti;
Dhumuni ni kujua jinsi ugonjwa wa aplastic anaemia unavyojitokeza na kujua idadi ya wagonjwa wenye seli zisizo na GPI kati ya wagonjwa wenye aplastic anemia.

Jinsi ya Kushiriki:
Mgonjwa ambaye yuko tayari kushiriki ataweka sahihi yake ,ili kuonyesha utayari. Yatafuata maswali machache ya Utangulizi, kasha vipimo vya damu, ya mshipa vitachukuliwa.

Usiri:
Taarifa ya magonjwa yako/ ya mtoto wako hazitatangazwa kwa yoyote zaidi ya mtafiti. Matokeo ya utafiti kwa ujumla yatasaidia kuboresha huduma ya tiba kwa wagonjwa wa aplastic anaemia.

Gharama:
Hutatakiwa kulipa gharama yoyote kwa kushiriki kwako/kwa mtoto wako.

Utayari wa kushiriki au kujitoa:
Kushiriki kwako/ kwa mtoto wako ni hiyari na waweza kujitoa. Lakini haitakunyima/ haitamnyima haki ya kupata tiba zingine.

Athari:

Damu kwa ajili ya vipimo itatolewa kwenye mkono. Hatutegemei athari yoyote damu itakapovutwa, isipokuwa waweza/ mtoto anaweza kusikia maumivu kidogo. Ili kuepuka kusababisha maambukizi, mara zote ngozi yako/yake itasafishwa vema na dawa kabla ya kuchomwa sindano yoyote.

Faida:

Kushiriki kwako/kwake katika utafiti huu, kutakusaidia kujua uwepo wa seli zisizo kuwa na GPI hivyo kusaidia kuelewa maendeleo ya ugonjwa wako/wake, pia utapata tiba husika ya PNH kama itahitajika.

Ni tumaini letu kuwa utafiti huu utasidia kuboresha uelewa na huduma kwa wagonjwa wa aplastic anaemia nchini kwetu na penginepo. Nitakushukuru kwa kushiriki kwako katika utafiti huu. Ahsante.

Iwapo utakuwa na swali lolote kuhusu utafiti huu wasiliana na

Dr. Mwashungi Ally,
Chuo kikuu Cha Afya Na Sayansi za Tiba Muhimbili;
Idara ya Tiba; S.L.P 65001 Dar Es Salaam.
Simu 0653 986 798.

Endapo utakuwa na swali lolote kuhusu haki zako kama mshiriki katika utafiti huu wasiliana na:

Prof Said Aboud;
Mwenyekiti wa Kamati ya Tafiti na Matoleo Chuoni.
Chuo Kikuu Cha Afya na Sayansi Shirikishi Muhimbili;
S.L.P 65001 Dar EsSalaam .
Simu 2151489.

Mimi……nimeelezwa/ nimesoma yaliyomo katika fomu hii na nimeelewa maana yake. Nakubali kushiriki katika utafiti huu.

Sahihi……..(Mshiriki) Tarehe……..

Sahihi…….. (Mtafiti) Tarehe…….