ACUTE PROMYELOCYTIC LEUKAEMIA IN ADULTS AT CHRIS HANI BARAGWANATH ACADEMIC HOSPITAL

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A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree of Master of Medicine (Internal Medicine)

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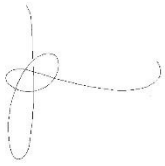
ETHICS COMMITTEE APPROVAL

This study was approved by the Human Research Ethics Committee (HREC) (Medical), of the University of the Witwatersrand. Clearance certificate number: M191010. Approval was later granted by the HREC to extend the study inclusion period by 12 months (see appendix D).

DECLARATION

I declare that this research report is my own work. It is being 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.



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4th August 2022

Johannesburg

ABSTRACT

Background

Acute promyelocytic leukaemia (APL) is a subtype of acute myeloid leukaemia (AML). APL is a medical emergency due to its high early mortality rate, most commonly due to bleeding complications resulting from an associated coagulopathy including a DIC (disseminated intravascular coagulation). However, APL occurs predominantly in young patients and is eminently treatable with supportive care, all-trans retinoic acid (ATRA) and appropriate chemotherapy.

Aims and Objectives

- To investigate the demographic and clinical profile of patients diagnosed with APL during the period 01/01/1994 to 31/12/2019.
- To determine the factors responsible for morbidity and mortality.
- To investigate the relationship with HIV (human immunodeficiency virus).
- To describe the management and outcome of the patients, including response to treatment and survival.

Patients and Methods

This was a retrospective study in which the records of patients diagnosed with APL from 01/01/1994 to 31/12/2019 at the Clinical Haematology Unit, Department of Medicine, Chris Hani Baragwanath Academic Hospital were assessed. Data relating to patient demographics, clinical presentation, laboratory parameters, management and outcomes was reviewed and analysed.

Results

There were 79 evaluable patients included in this study. Although 16.87% of the patients with APL were HIV seropositive, there was no clear association with HIV, implying that the relationship between the two diseases is likely coincidental. The APL coagulopathy was evident at presentation, with 82.05% of patients presenting with bleeding, and 4.29% with thrombosis. Laboratory evaluation revealed that 93.42% of patients had anaemia and 97.37% had a

thrombocytopenia. 63.93% of patients had evidence of a DIC. There was rapid improvement of all of these parameters following initiation of therapy. The Sanz risk score was used to assess risk, and 26.32% of patients had a high-risk APL. There was also a high percentage of patients with an immunophenotype associated with a high white cell count (WCC) and a poorer prognosis.

Management

Management involved supportive therapy with blood and blood products, and specific therapy, most commonly with ATRA and daunorubicin during induction and consolidation. Maintenance therapy included ATRA, methotrexate and mercaptopurine. There were 21 patients who received re-induction therapy, five of whom were for failed induction and the remaining patients for relapsed disease. Early complications, defined as complications occurring within the first 30 days from initiation of therapy, were found in 89.33% of patients, and 83.54% of these were bleeding complications. Thirty-seven patients achieved remission following induction therapy. At the last date seen, 39.24% of patients were in remission, 49.37% of patients had died and 3.8% were experiencing relapsed disease. Overall, the median survival was 7.8 months, which improved to 43.7 months once early mortality was excluded. There was no statistically significant difference in the survival time between HIV seropositive and seronegative patients. There was also no statistically significant difference in survival time across the low, intermediate and high-risk groups as calculated by the Sanz score.

Conclusion

Acute promyelocytic leukaemia (APL) most commonly presents with bleeding manifestations, anaemia, thrombocytopenia and DIC. These complications contribute to a high early mortality rate. Therefore, recognition and awareness of this entity, early diagnosis and timeous referral is of paramount importance in order to decrease the high early mortality of APL, a malignancy that is eminently treatable with appropriate supportive care and specific therapy.

DEDICATION

With special thanks to my family and friends for their unwavering love and support.

ACKNOWLEDGEMENTS

Thank you to Prof Patel and Dr Rahman for their patience, teaching and guidance throughout this process.

I would like to acknowledge the Clinical Haematology team and the Department of Medicine at Chris Hani Baragwanath Academic Hospital, including all the medical, nursing and allied staff, and in particular the patients whose data was used in this study.

I would also like to thank Mazvita Muchengeti who assisted with my statistical analysis.

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AIDA	All-trans retinoic acid and idarubicin
AIDS	Acquired immunodeficiency syndrome
AML	Acute myeloid leukaemia
ANC	Absolute neutrophil count
APL	Acute promyelocytic leukaemia
APTT	Activated partial thromboplastin time
ATO	Arsenic trioxide
ATRA	All-trans retinoic acid
cART	Combined/combination anti-retroviral therapy
CD	Cluster of differentiation
CHBAH	Chris Hani Baragwanath Academic Hospital
CMJAH	Charlotte Maxeke Johannesburg Academic Hospital
CNS	Central nervous system
CSF	Cerebrospinal fluid
CVA	Cerebrovascular accident
DIC	Disseminated intravascular coagulation
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
FAB	French-American-British
FFP	Fresh frozen plasma
FISH	Fluorescence in situ hybridization
FLT3-ITD	FMS-like (Feline McDonough Sarcoma gene) tyrosine kinase 3 - internal tandem duplication)
GFR	Glomerular filtration rate
GI	Gastrointestinal
GIMEMA	Gruppo Italiano Malattie Ematologiche dell'Adulto
Hb	Haemoglobin
HIV	Human immunodeficiency virus
HLA-DR	Human leucocyte antigen – DR isotype

HREC	Human Research Ethics Committee
HSCT	Haematopoietic stem cell transplantation
ICH	Intracranial haemorrhage
INR	International normalised ratio
IQR	Interquartile range
ISTH-BAT	International Society of Thrombosis and Haemostasis - Bleeding Assessment Tool
IT	Intrathecal
KDIGO	Kidney Disease: Improving Global Outcomes
LDH	Lactate dehydrogenase
LLETZ	Large loop excision of the transformation zone
LPA	Leucemia promielocitica aguda
MCC	Medicines Control Council
MCV	Mean corpuscular volume
NCCN	National Comprehensive Cancer Network
NPM1	Nucleophosmin protein-1
PETHEMA	Programa de Estudio y Tratamiento de las Hemopatias Malignas
Plt	Platelets
PLZF	Promyelocytic leukaemia zinc finger
PML	Promyelocytic leukaemia
PT	Prothrombin time
PTT	Partial thromboplastin time
PRKAR1A	Protein kinase A regulatory subunit type 1/alpha
PV	Per vaginal
RARA	Retinoic acid receptor alpha
RT-PCR	Reverse transcriptase – polymerase chain reaction
SAHPRA	South African Health Products Regulatory Authority
STAT5b	Signal transducer and activator of transcription 5b
TED	Thromboembolic disease
t(1;17)	Translocation chromosome 1 to 17
t(5;17)	Translocation chromosome 5 to 17

t(15;17)	Translocation chromosome 15 to 17
TB	Tuberculosis
TF	Tissue factor
tPA	Tissue plasminogen activator
UA	Uric acid
USA	United States of America
WCC	White cell count
WHO	World Health Organization

1. CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

1.1. Haematopoiesis

Blood is formed in the bone marrow by a process called haematopoiesis. Stem cells in the bone marrow differentiate into either myeloid or lymphoid progenitor cells. Myeloid progenitor cells are then able to differentiate into red blood cells, granulocytes, monocytes and platelets.[1]

1.2. Pathogenesis of APL

Acute myeloid leukaemia (AML) is a haematological malignancy that results in a clonal expansion of cells of the myeloid series, referred to as myeloblasts.[2] The World Health Organization (WHO) classification of myeloid neoplasms includes all granulocytes, monocytes, macrophages, erythroid cells, megakaryocytes and mast cells in this definition.[3] The clonal expansion of cells results in a maturation block, as these cells are unable to further differentiate and mature. Myeloblasts and, in the case of acute promyelocytic leukaemia (APL), promyelocytes accumulate in the bone marrow, blood and other tissues. The clinical picture that results is a consequence of this accumulation, as well as the subsequent reduced production of mature cells of the myeloid series. Acute promyelocytic leukaemia is a subtype of AML, with a characteristic genetic abnormality. The most common chromosomal translocation in APL is t(15;17)(q22;q21) which results in a fusion of the genes that code for the promyelocytic leukaemia (PML) protein on chromosome 15 and retinoic acid receptor alpha (RARA) on chromosome 17, creating the oncogene PML-RARA.[1, 4] PML facilitates the control of cell growth, aging and apoptosis, and the RARA gene is involved in encoding specific transcription factors.[1] The fusion product, PML-RARA, thus prevents gene transcription and results in a maturation block of myeloid cells, which become arrested in the promyelocyte phase of differentiation.[1]

As a result of the development of new therapeutic agents such as ATRA (all-trans retinoic acid) and arsenic trioxide (ATO), as well as improved supportive care, APL is now a potentially curable disease. Cure rates in the literature are currently 75-80%.[5] This is particularly true if the

patient is able to receive timeous treatment and is supported appropriately through the early life-threatening phase of the disease.

1.3. Presentation

Acute promyelocytic leukaemia (APL) is a medical emergency, with a high early mortality rate primarily due to an associated coagulopathy (DIC – disseminated intravascular coagulation and hyperfibrinolysis), with a resultant increased bleeding and thrombotic tendency.[6] Patients with APL typically present with a thrombocytopenia as a result of bone marrow infiltration as well as evidence of the APL coagulopathy (including a DIC) with clinical features ranging from minimal to severe haemorrhage and / or both venous and arterial thromboses.[6]

At presentation, in comparison to patients with other subtypes of AML, APL patients have relatively lower platelet counts and fibrinogen levels and higher prothrombin and thrombin times as well as prolonged partial thromboplastin times and fibrin degradation products, including D-dimers.[4, 7]

1.4. Risk factors for the development of APL

Acute myeloid leukaemia (including APL) may be either a primary or a secondary malignancy, depending on whether it arises de novo or following another event or exposure. In most cases, AML is a primary event, however, there are indications that there may be contributing factors including radiation, occupational exposure and drugs, such as alkylating agents, topo-isomerase II inhibitors, chloramphenicol and phenylbutazone.[1]

There has been a suggestion that there may be a seasonal influence on the incidence of APL, as alluded to in a few studies.[8] Furthermore, ethnic background may also play a role, as some studies have found an increased prevalence of AML with recurrent cytogenetic abnormalities (including APL) in Black patients.[8] African studies, and South African studies in particular, have found a much younger median age at presentation of AML than that seen globally (i.e. 68 years in the United States of America). The median age was 38.5 years in a study performed at the

Universitas Academic Hospital in Bloemfontein, 41 years in a study done at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) and 45 years in a study conducted at Chris Hani Baragwanath Academic Hospital (CHBAH).[8-11]

1.5. Classification of APL

There are many classifications used for subtyping AML. The current standard is the World Health Organization classification. In the revised 2016 WHO classification of tumours of haematopoietic and lymphoid tissues, APL is defined as an AML with recurrent genetic changes, resulting in the PML-RARA fusion oncogene (to highlight the significance of this gene, regardless of whether it arises from the typical translocation or not), called APL with PML-RARA.[12] The FAB classification subtypes AML into groups (M0 – M7), based on the type of cell from which the leukaemia developed, and the degree of maturity of these cells. According to the FAB classification, APL is defined as either a typical M3, or an M3 variant where there is minimal granulation and an association with a high white cell count (WCC).[13]

1.6. Epidemiology

The incidence of APL in the United States of America (USA) is estimated to be between 600 and 800 new cases annually.[14] In South Africa, APL appears to be more prevalent as a proportion of AML cases than what is found in the literature worldwide, with a rate of 40% of all cases of AML with recurrent genetic abnormalities in one study.[8] At CHBAH, 23% of AML patients had APL.[9]

APL has an unusual age-related incidence which is uncommon under the age of ten, rises in the teenage years, plateaus in early adulthood and decreases after the age of sixty.[14] In essence APL is primarily a disease of young to middle aged individuals. This is markedly different from the rest of the AML subtypes which gradually increase leading up to the age of fifty five, and then increase exponentially with advancing age.[14]

1.7. Diagnosis

The WHO criteria for the classification of AML can be applied to any peripheral blood smear or bone marrow sample (taken at initial diagnosis) prior to the patient receiving any treatment for the suspected malignancy.[3] Cell lineage and the extent of maturation are determined by looking at morphologic, cytochemical and immunophenotypic characteristics.[3] An acute leukaemia can be diagnosed when there is a blast percentage of more than 20%, however, in the case of APL, it is enough to make a diagnosis based on the genetic features alone (in the relevant clinical circumstances), even if there are less than 20% blasts in the peripheral blood or bone marrow.[3] Although a bone marrow biopsy is stated as not always required for diagnosis, it assists in making a more precise assessment of the state of the haematopoietic cell lines and allows clinicians to have a better understanding of bone marrow cellularity, topography, the state of the stromal cells and the microenvironment at baseline.[3] It also provides additional diagnostic and prognostic information from an immunohistochemical aspect, and is important in the detection of minimal residual disease monitoring.[3] Cytogenetic testing is important in assessing the patient's baseline karyotype, and later in determination of response to treatment and for assessing genetic evolution. It is especially important in APL to use quantitative RT-PCR (reverse transcriptase – polymerase chain reaction) and FISH (fluorescence in situ hybridisation) to diagnose variants and to find other prognosticating factors such as the NPM1 (nucleophosmin protein-1) and FLT3-ITD (FMS-like (Feline McDonough Sarcoma gene) tyrosine kinase 3 - internal tandem duplication) gene mutations.[3] The presence or absence of NPM1 and FLT3-ITD mutations are not required for the diagnosis of APL, but rather the presence of these mutations is of prognostic value and may impact on the choice of treatment.

While the majority of patients with APL have a demonstrable translocation between chromosomes 15 and 17, i.e. t(15;17), other variant translocations have been identified. A group in Italy studied 60 patients with APL, and found that a variant PML-RARA translocation had occurred most commonly through insertions, or by simple or three-way variant translocations.[15] The variant translocations include t(1;17) and t(5;17) which are much less common than the t(15;17), but are well described.[15]

The morphology of APL is typically characterised by the predominance of abnormal promyelocytes with heavy granulation, a nucleus of variable size that is usually bilobed with tightly packed granules in the cytoplasm (hypergranular) and bundles of Auer rods ('faggots').[13] The usual APL immunophenotype is dependent on whether the patient has a hypergranular or hypogranular variant, but is generally characterized by the following immunophenotype: CD34 (cluster of differentiation) negative to weak positive, HLA-DR (human leucocyte antigen – DR isotype) negative, CD13 and CD33 positive, CD11b negative, CD15 negative or weak positive, CD117 variable and occasionally CD2 and CD56 positive. Certain immunophenotypes of APL (specifically CD2, CD34 and CD56 positive) have been associated with a poorer outcome, and CD2 and CD34 positive APL subtypes have specifically been associated with a more dramatic leucocytosis.[16] A higher white cell count at diagnosis (especially if it is more than 10×10^9/l) has been found to be the most important risk factor for early mortality, complete remission failure and overall survival failure.[16, 17]

1.8. Prognosis

The Sanz score, which is based on the presentation white cell and platelet counts, enables risk profiling to be done. Based on this scoring system, low risk patients have a WCC $\leq 10 \times 10^9$/l and platelet count $> 40 \times 10^9$/l, intermediate risk patients have a WCC $\leq 10 \times 10^9$/l and platelet count $\leq 40 \times 10^9$/l and high risk patients have a WCC $> 10 \times 10^9$/l.[18]

Other factors that predict a worse prognosis are the hypogranular M3 variant or subtype of APL and a high level of PML-RARA expression.[19] Variant translocations can also adversely affect prognosis, as seen in patients with a PLZF-RARA (promyelocytic leukaemia zinc finger) rearrangement which occurs in 0.8% of patients and who have a poor response to ATRA as well as to ATO.[15] However, these patients can still achieve a complete remission with chemotherapy and thus the prognosis may not be as poor as previously thought.[15] Patients with the usual t(15;17) have a favourable prognosis associated with a good response to ATRA. Most patients with APL that do not have this typical translocation may still harbour the PML-RARA gene (and thus have a good response to ATRA). This fusion gene comes about through either insertions in 4% of patients, or complex rearrangements in 2% of patients.[15]

A recent meta-analysis has found that the presence of a FLT3-ITD mutation is likely to indicate a worse prognosis in that the complete remission, 5 year overall survival and disease free survival rates are lower.[19] In a study conducted at Cairo University, FLT3-ITD mutations were detected in 35.5% of patients with APL, which was significantly higher than the 18.5% of patients with AML.[20] The overall survival of those without this mutation was significantly better, thus a worse outcome is predicted in those that are FLT3-ITD positive.[20] In a study conducted in Bloemfontein on AML patients, it was found that FLT3-ITD mutations were present in 37.5% of APL patients, and the majority of those positive for this mutation were also noted to have long ITD lengths as well as allelic ratios of more than 50%, both of which are also associated with a worse prognosis.[8] In the study at CMJAH, 12% of patients were found to have the FLT3-ITD mutation, which was lower than anticipated.[11] The median age of patients with this mutation was also much lower than in those without the mutation, at 33 years compared to 41 years.[11]

The other gene in which a mutation occurs recurrently is NPM1 which is associated with a better outcome, although if found concurrently with the FLT3-ITD mutation, the favourable effect is mitigated.[8] The Bloemfontein study did not find a single NPM1 mutation in their population, which they suggest could be due to the low median age in their population, as it has been noted that this mutation becomes more prevalent with increasing age.[8] This finding was also consistent with a study conducted in Sudan, although in two Egyptian studies there was a 28.3% and a 19.5% prevalence.[21-23] In the South African study conducted at CMJAH, a 7.5% prevalence of NPM1 mutations was found in AML.[8]

1.9. Early mortality

The PETHEMA (Programa de Estudio y Tratamiento de las Hemopatias Malignas) study indicated that the only independent risk factors for early death were a high white cell count and renal dysfunction. A poorer response to induction was shown in those with white cell counts >10 x 10^9/l, and patients older than 70 years of age.[24]

The National Comprehensive Cancer Network (NCCN) recommends that treatment for APL should be stratified according to the patient's prognosis, and therefore it is important to determine a patient's prognostic profile before initiating therapy.[19] In a patient with a suspected

diagnosis of APL (e.g. characteristic leukaemic morphology, severe coagulopathy or suggestive immunophenotype), it is recommended that ATRA should not be delayed and should be started urgently while awaiting molecular and genetic confirmation.[14]

The high early mortality rate is a significant concern in APL. As much as 29% of patients die in the first 30 days following diagnosis.[24] Immediate mortality in one study was defined as including the first three days after treatment was initiated, while early mortality was defined as death during the induction phase of chemotherapy or within 30 days of initial treatment.[25] Immediate mortality was largely a result of haemorrhage, while early mortality was more likely to be a result of infection or the differentiation syndrome.[25] In this study regarding early mortality, it was concluded that those patients with a higher tumour burden had faster disease progression accounting for a higher early mortality rate.[25] The most important risk factors for early mortality were found to be a high white cell count, high ISTH-BAT (International Society of Thrombosis and Haemostasis - Bleeding Assessment Tool) score, prolonged PT (prothrombin time) and rate of disease progression.[25] In addition, the authors concluded that disease severity is more important than the timing of initiation of treatment as a predictor for early mortality.[25] In a single centre study from Tunisia, the early mortality rate was noted to be higher than that found in industrialised countries at 14%, which is likely attributable to the higher proportion of patients with a high Sanz score, the low socioeconomic status in the country and delays in presentation.[5]

Another study looking into haemorrhage as a cause of early mortality showed that an increased risk of bleeding was found in those patients with low platelet and fibrinogen levels, and that there was no association with PT, activated partial thromboplastin time (APTT) or D-dimer levels.[17] These severe haemorrhages tended to be intracranial (65%) and pulmonary (33%), were found to occur regardless of whether induction therapy had been initiated and occurred especially early.[6] In the PETHEMA study, the median time to death from pulmonary haemorrhage was nine days, and six days for intracranial haemorrhage.[6] Sixty nine percent (69%) of the patients died within 24 hours from the time these bleeds started.[6] Unfortunately, most of the literature on early haemorrhagic mortality is retrospective, but risk factors are noted to include a high blast percentage at presentation, poorer ECOG (Eastern Cooperative Oncology Group) performance status, older age, low platelet count, biochemical evidence of coagulopathy

and high lactate dehydrogenase and creatinine levels.[6] Timeous supportive therapy, including appropriate transfusion of blood products, also reduced the risk of adverse bleeding outcomes which resulted from abnormal coagulation profiles.[4]

Thrombosis, both arterial and venous, is more common than previously appreciated, and while ATRA assists in facilitating time to resolution of the coagulopathy, it can worsen the procoagulant state in association with the differentiation syndrome.[6] There is a wide range (5 – 20%) of reported incidences of thrombotic complications in both prospective and retrospective studies.[6] These include catheter-related thrombosis, deep vein thrombosis, stroke, pulmonary embolus and myocardial infarction.[6] Again, there is limited data currently available, but risk factors are reported to include a high presentation WCC, short PML-RARA isoform, FLT3-ITD mutation, and expression of CD2 and CD15.[6]

Ideally, ATO should be initiated at diagnosis, especially in high risk patients, in order to decrease early mortality. [26] Unfortunately, ATO was not readily available in the South African public health sector until the end of 2021. At the time of this study, it was not licenced for induction treatment of APL and was only permitted for use on a named-patient basis once approval was granted by the Medicines Control Council (MCC) / South African Health Products Regulatory Authority (SAHPRA). In addition, due to the prohibitive costs, ATO tends to be used as a salvage therapeutic option in patients who relapse with APL in the South African public health sector.

1.10. Differentiation syndrome

One of the complications of treatment with ATRA is the differentiation syndrome, which occurs due to the large burden of differentiating cells producing inflammatory cytokines and a clinical picture which can include fever, fluid retention (and thus oedema and effusions), hypotension and acute renal failure.[1] This syndrome is as a result of differentiated malignant cells adhering to the endothelium in the pulmonary vasculature, and typically occurs in the first three weeks of therapy.[1] It can also occur in response to therapy with ATO, although this is less common.[7] There is a higher risk of the development of this syndrome in patients who are high risk (i.e.

according to the Sanz score classification) as the WCC can increase rapidly following initiation of treatment with ATRA.[1] The Tunisian study observed a high rate of differentiation syndrome which they surmised may be due to their high percentage of patients with high risk disease (35%) or possibly due to ethnicity.[5] This was associated with a high WCC and creatinine at presentation and they also suggested an interesting correlation with obesity and hypertensive episodes prior to the development of differentiation syndrome. The latter two factors they suggested to be due to the presence of leptin receptors on APL cells (not found on normal promyelocytes) that would result in sympathetic stimulation as well as an increase in production of reactive oxygen species in endothelial cells, and secretion of cytokines such as tumour necrosis factor and interleukin 6.[5]

Differentiation syndrome can be defined as moderate if two or three signs or symptoms are present, and as severe if four or more symptoms are present.[7] The signs and symptoms to be considered include the following; unexplained fever, dyspnoea, pleural effusions, pericardial effusions, pulmonary infiltrates, renal failure, weight gain of > 5kg that is otherwise unexplained and hypotension.[7] At least two or more of the above signs and symptoms are required for the diagnosis of the differentiation syndrome (in the absence of an alternative diagnosis that would better explain them).[7]

1.10.1. Prophylaxis for the differentiation syndrome

Prednisone at a dose of 0.5 mg/kg/day orally was used as prophylaxis in all patients in the LPA99 trial (leucemia promielocitica aguda), and in the LPA2005 trial dexamethasone ($2.5 mg/m^2$ 12 hourly) was used instead, and only in patients with a WCC > 5×10^9/l at presentation or within the first two weeks of treatment with ATRA.[7] There was a small increase in the rate of moderate differentiation syndrome in the LPA2005 trial as compared to LPA99, but the conclusion that this may be due to fewer patients receiving prophylaxis is only speculative.[7]

1.10.2. Management of the differentiation syndrome

The mainstay of therapy includes glucocorticosteroids (e.g. dexamethasone 10mg 12 hourly intravenously), chemotherapy (e.g. an anthracycline alone or combined with cytarabine) and

other supportive management such as diuresis, mechanical ventilation and dialysis.[1, 7] Cessation of ATRA (or ATO if this is the precipitating agent) may be required in severe cases, such as those requiring dialysis or ventilation.[1]

1.11. Coagulopathy

The pathophysiology of the coagulopathy seen in APL is multifaceted and research into the underlying mechanisms is ongoing. Multiple presumptive mechanisms are proposed, as detailed below.

1.11.1. Coagulation

APL blasts have high levels of tissue factor (TF), and PML-RARA activates the TF promotor.[7] TF expression activates the coagulation cascade, thus resulting in the clinically apparent consumptive coagulopathy and DIC.[7] It forms a complex with cell membrane phospholipids and factor VII which then activates factor X. Cancer procoagulant (another procoagulant factor found in high quantities in malignancies, and at especially high levels in APL cells) also acts on factor X directly, and in this way the coagulation cascade is activated.[6] High levels of TF-bearing microparticles have been found in patients with APL, and this is noted to correspond with their WCC and D-dimer levels.[6] These factors all contribute towards a pro-thrombotic state in patients with APL.

1.11.2. Hyperfibrinolysis

Hyperfibrinolysis is mediated by the leukaemic cells themselves through exposure of tissue factor and Annexin II.[7, 27] Annexin II, which is also present in high concentrations in APL cells, binds plasminogen and tissue plasminogen activator (tPA), thereby increasing the conversion of plasmin sixty-fold. There is a resultant increased consumption of fibrinogen; thus contributing to the coagulopathy as well as an increased bleeding tendency.[7]

1.11.3. Proteolysis

A third mechanism in the pathophysiology of the APL coagulopathy is that of direct proteolysis by non-specific proteases that degrade coagulation factors (fibrinogen in particular, as well as inhibitors of fibrinolysis).[6]

1.11.4. Other mechanisms

An acquired von Willebrand syndrome with impaired platelet function can produce platelet aggregation and increase the risk of bleeding.[6] APL cells themselves can also activate the endothelium resulting in clotting activation and it has been found that heparin could modulate this effect in a recent study.[6] A cell death process called ETosis can also activate coagulation, hyperfibrinolysis and cause endothelial damage.[6]

These processes are further complicated by chemotherapy induced cytopenias and infections, as well as bone marrow infiltration (common to all leukaemias) that can also contribute to thrombocytopenia.[6]

ATRA is able to correct the coagulopathy associated with APL through enabling differentiation of the leukaemic blasts, resulting in a decrease in the expression of tissue factor and Annexin II (which are expressed at lower levels in mature, differentiated myeloid cells).[27] ATRA has a pro-coagulant effect and is also thought to increase the risk for thrombosis as a result of the differentiation syndrome and an increase in cytokine production.[28]

Other factors to take into account, especially in a South African setting, would be the influence of comorbidities such as HIV (human immunodeficiency virus) and tuberculosis (TB) on the coagulopathy in APL, as these conditions have a known aetiological association with thrombotic disease.[29]

Multiple mechanisms are responsible for the coagulopathy in APL that results in bleeding (more commonly) and thrombosis (less commonly). The bleeding and thrombosis seen in APL needs to be managed carefully, with a multifaceted approach taking into account patient profile and

prognostic risk factors. It should include transfusions of blood products where indicated, meticulous monitoring of blood counts and coagulation parameters and specific treatment with differentiating agents and chemotherapy.[6]

1.12. Management

1.12.1. Supportive measures

Holistic care including patient and family counselling and education should be provided throughout the management and follow up of these patients, making use of social work, palliative care, psychology and rehabilitation services where indicated.

Transfusions of platelets, fresh frozen plasma and cryoprecipitate may be necessary in the management of the coagulopathy.[7] It is recommended to transfuse cryoprecipitate to keep the fibrinogen level above 100 – 150 mg/dl and platelets to keep the platelet count above 30 – 50 x 10^9/l. High and intermediate-risk patients have lower levels of fibrinogen at presentation, and have been shown to need more platelet transfusions than low-risk patients.[4] Subsequently, the upper limit of these ranges should be targeted in those at high risk (according to the Sanz criteria) or those who are actively bleeding.[6] Platelet counts, prothrombin, thrombin and activated partial thromboplastin times, fibrinogen and fibrin degradation product levels should be monitored at least daily until the coagulopathy has resolved, both clinically and biochemically.[6] The LPA2005 trial did not use tranexamic acid, which was found to be associated with an increased risk of thrombosis and did not decrease the mortality rate from haemorrhage.[6, 7] Thus, the use of tranexamic acid and other antifibrinolytic agents is not advised.[6] This includes the use of prothrombin complex concentrates and recombinant factor VIIa as, although some case reports suggest benefit, the evidence is of low quality.[6] Similarly, use of heparin and other anticoagulants is not recommended outside the scope of clinical trials, as evidence for their benefit is lacking.[6] In the case of a severe thrombosis, unfractionated heparin (or dose adjusted low molecular weight heparin – depending on the platelet counts) can be considered, but this must take into account the high risk of haemorrhagic transformation.[6]

1.12.2. Specific measures

Treatment in APL is given in different phases, namely induction, consolidation and maintenance.

1.12.2.1. Induction

Combination chemotherapy including ATRA, an anthracycline and ATO (which is a newer addition) is the current standard of therapy for APL during induction.[4] The combination of ATRA (at 45mg/m^2/day) with an intravenous chemotherapy regimen has been validated by a series of randomised clinical trials and appears to be more effective than either agent alone.[4, 30] The type of anthracycline to be used for induction remains controversial, although idarubicin (at 12mg/m^2/day) may have a better outcome in terms of survival in younger patients when compared to daunorubicin in combination with cytarabine.[14, 30] Chemotherapy may be withheld in some patients on a case-to-case basis if there are contraindications such as organ failure and in those older than 80 years of age.[14]

ATRA can be thought of as a "differentiating agent" that overcomes the differentiation block brought about by the presence of the PML-RARA protein, which prevents gene transcription.[1] It should be started as soon as possible, while definitive genetic confirmation of the diagnosis of APL is awaited.[14] It is unlikely to cause harm in patients if the diagnosis is not confirmed, and is also capable of rapidly reversing the coagulopathy. This is especially important in patients with a high Sanz score who have a high risk of early mortality and the development of the differentiation syndrome.[14] Side effects of ATRA include the differentiation syndrome, hypertension, skin changes (dry skin, exfoliation), cheilitis, dry mucous membranes, hypertriglyceridaemia, teratogenicity, pseudotumour cerebri, splenic infarction and Sweet's syndrome.[5]

ATO is considered the single most effective agent when the available treatments are considered individually.[31] Indeed, improved survival has been demonstrated in regimens using ATO, which has also been noted to improve the outcome regardless of poor prognostic indicators such as FLT3-ITD mutations and a high WCC at presentation.[4, 19, 25] Electrolyte disturbances (such as

hypomagnesaemia) can occur as a result of treatment with this agent, which can lead to prolongation of the QT interval and ventricular arrythmias including torsade de pointes.[14] This necessitates careful electrolyte, ECG (electrocardiogram) and symptom monitoring (especially for palpitations and syncope). Cessation of other agents known to cause QT prolongation is often necessary and withholding ATO if the QT interval is longer than 500 msec is recommended.[14] Hyperglycaemia is another common side effect of ATO that requires monitoring, and the differentiation syndrome may also complicate treatment with ATO.[14]

In low and intermediate risk APL, the combination of ATRA and ATO has been proven to be superior to ATRA and intravenous chemotherapy both in terms of decreased toxicity and increased anti-leukaemic effects.[31] Studies by the PETHEMA group adjusted the treatment regimens received by patients depending on their risk profile.[7] A randomised control trial of all risk groups of APL indicated that the ATRA/ATO combination is efficacious; with a higher rate of cure, a lower rate of relapse and no difference in survival when compared to ATRA and intravenous chemotherapy.[26] Induction therapy with ATRA and ATO improves the coagulopathy and fibrinolysis seen in APL as evidenced by a decreased need for blood product transfusion, as well as an improvement in haemostatic abnormalities.[4] This may be achieved through specific binding of ATRA and ATO to PML-RARA which results in the degradation of the fusion protein and thus induces leukaemic cell apoptosis.[4] Through this mechanism, ATO also decreases the transcription of genes and thus reduces the coagulopathic effects of the condition.[27] Interestingly though, there is no acceleration in recovery time or decrease in transfusion requirements seen when ATO is added to ATRA.[4]

1.12.2.2. Consolidation

Consolidation therapy based on risk was evaluated in the LPA2005 trial. Those patients who were found to be in complete remission following induction were given three cycles of consolidation therapy at monthly intervals with ATRA and an anthracycline.[7] This trial also showed that low and intermediate risk patients with APL benefited from a lower dose of mitoxantrone (as compared to the LPA99 study) in that it reduced toxicity (as well as the [7]duration of neutropenia and thrombocytopenia) and shortened duration of hospital admission,

while being as effective therapeutically.[7] High-risk patients younger than 60 years of age in this study were given treatment with cytarabine, ATRA and idarubicin.[7] This regimen resulted in a lower three year relapse rate and improved disease-free survival than that seen in those who were treated without cytarabine in the LPA99 study.[7] ATRA at a dose of 45 mg/m^2 daily for fifteen days was associated with a reduced risk of relapse in consolidation therapy.[14] ATO was used in a large randomised control trial (two courses of 25 days) followed by two courses of ATRA and daunorubicin.[14] This regimen demonstrated a better overall survival than that seen in those who did not receive ATO.[14] In general, for patients receiving ATRA and chemotherapy, remission status should not routinely be assessed after induction, but only after consolidation (unless ATO is used).

Consolidation therapy can be risk adapted. Older patients are thought to be more susceptible to the toxicities of chemotherapy and thus APL protocols tend to reduce the doses of chemotherapy used in both induction and maintenance treatment in these patients.[14] The mortality rate of patients who achieve complete remission is much higher in older patients, for example in the PETHEMA trial (where the therapy used had a comparatively lower toxicity rate), mortality was < 1% in patients under 60 years of age, but 19% in patients older than 70 years of age.[14]

In patients who achieve molecular remission following consolidation therapy, there is no evidence for routine haematopoietic stem cell transplantation (HSCT).[14] However, HSCT can be considered in patients with persistent minimal residual disease as they have a poorer prognosis.[14] In these patients, ATO or other additional therapy should be considered prior to transplantation in an attempt to achieve complete remission.[14] In patients where HSCT is not an option, ATO or gemtuzumab ozogamicin can be considered and if the patient achieves a molecular complete remission in the bone marrow, autologous HSCT is an option if a PCR negative harvest is obtained.[14] Gemtuzumab ozogamicin is a CD33 monoclonal antibody (CD33 is a receptor present in high concentrations on APL cells).[32] A sustained remission can also be achieved in patients who receive ATO or gemtuzumab ozogamicin.[14]

1.12.2.3. Maintenance

There is some controversy around the need for maintenance therapy in patients who have achieved a molecular remission following consolidation.[14] Two randomised trials (which involved the use of ATRA) showed benefit, but two other randomised trials did not.[14] Low-risk and intermediate-risk patients who achieved a complete molecular remission after ATO and ATRA consolidation did not benefit from maintenance therapy.[14] Maintenance therapy used by the PETHEMA group included oral mercaptopurine (50mg/m^2/day), intramuscular methotrexate (15mg/m^2/week) and ATRA (45mg/m^2/day) for 15 days every three months, and continued for two years.[30] The Gruppo Italiano Malattie Ematologiche dell'Adulto (GIMEMA) group evaluated patients who were PCR negative following consolidation therapy in the AIDA-0493 (ATRA and idarubicin) trial, and found that the arms in which patients received ATRA as part of maintenance therapy had better outcomes.[33] In the AIDA-2000 trial, patients who were PCR negative following consolidation received maintenance with the same regimen as in the PETHEMA trial above, as it was noted that those who received maintenance with ATRA had fewer relapses.[33] Those who had tested PCR positive were considered to have resistance and were thus treated with salvage therapy.[33]

1.12.3. Management of older patients (elderly), comorbidities, pregnancy, FLT3-ITD positivity and genetic variants of APL

There are some situations where the treatment of APL will need to be adjusted according to the patient's profile, for example in older patients, those with significant comorbidities and pregnant women.

1.12.3.1. Elderly

Patients over 70 years of age are less likely to develop APL, and tend to have a more favourable prognosis, with low-risk features of the disease and a lower rate of relapse.[14] It is noted, however, that they have a higher likelihood of adverse chemotherapy-related outcomes, such as a higher mortality rate and neutropenic sepsis.[14] Thus, patients who are unlikely to tolerate

chemotherapy could be considered for treatment with either ATO alone or ATRA and ATO, without chemotherapy.[14]

1.12.3.2. Comorbidities

Those with significant comorbidities should also have therapy adjusted to avoid the use of chemotherapy, with ATRA, ATO and gemtuzumab ozogamicin, especially if chemotherapy is directly contraindicated, as in those with cardiac disease or other organ failure.[14]

1.12.3.3. Pregnancy

There is little evidence available on the management of APL in pregnancy, but it is important to consider that any delays in initiating treatment could reduce the likelihood of obtaining a complete remission, and also that chemotherapy, ATRA and ATO are potentially teratogenic.[14] Careful planning of therapy, taking into account the patient's wishes and the gestational age of the foetus is necessary.[14] As ATRA is teratogenic, it should not be used in the first trimester of pregnancy, and either anthracycline-based chemotherapy alone should be given.[14] Alternatively, if termination is desired this could be considered once the patient is haemodynamically stable, followed by standard treatment protocols.[14] Daunorubicin is preferred over idarubicin as it is less lipophilic with less transfer across the placenta and lower toxicity, and has been more extensively studied in pregnancy.[14]

Where chemotherapy is used, there will be a higher bleeding risk as promyelocytes release procoagulants and plasminogen activators. However, once remission has been achieved, ATRA can be used in the second or third trimesters which will help to ameliorate the bleeding risk.[14] ATO should not be used at any stage in pregnancy, and has only been studied to a limited extent in humans but is noted to result in low birth weight, spontaneous abortion and stillbirths.[14] In the second and third trimesters, there are two proposed approaches. The two approaches are based on the toxicities of chemotherapy and ATRA. Chemotherapy can result in abortion, prematurity, low birth weight, neutropenia in the neonate and sepsis, and although ATRA is reasonably safe following the first trimester, it may cause cardiac toxicity in the foetus including arrythmias and

thus requires close monitoring.[14] Therefore, patients can be treated either with ATRA followed by chemotherapy after complete remission is achieved, or with both ATRA and chemotherapy together. The risk of an increased chance of developing the differentiation syndrome when ATRA is used alone in the former option should be weighed against the toxicity of the second option, while considering that the latter option has an increased chance of cure, and should be the first choice in those with a high Sanz score.[14]

Patients with APL may additionally have a positive FLT3-ITD mutation, which is associated with a high WCC at presentation.[14] These patients, however, do not need to be treated any differently and currently the need to perform this analysis routinely on all patients at diagnosis is unclear.[14]

Some genetic variants of APL may be resistant to ATRA. However, they should be treated with similar regimens as those used in APL. The variants include the relatively resistant PLZF-RARA, resistant STAT5b-RARA (signal transducer and activator of transcription 5b), and PRKAR1A-RARA (protein kinase A regulatory subunit type 1/alpha) which has unknown sensitivity to ATRA.[14]

1.12.4. Monitoring

Monitoring for minimal residual disease in the bone marrow following the completion of therapy should be undertaken every three months to allow for early detection of relapse. Once patients have passed the three year mark after completion of consolidation therapy, monitoring can then be stopped, or the interval thereof increased further, as the risk of relapse decreases over time.[14] There is a good correlation between peripheral blood and bone marrow PCR results early in the course of APL, but following consolidation there is a 1.5 log higher sensitivity in samples from the bone marrow.[14]

1.12.5. Relapse

ATO is considered to be the best agent to use in relapsed patients, as it is highly effective with relatively low toxicity.[14] A complete remission was achieved in 80 – 90% of patients in recent studies, and 50 – 70% of these patients had survived one to three years.[14] Prior to the use of ATO, there were two studies that showed benefit and recommended the use of therapy (which typically consisted of ATRA and chemotherapy, usually high dose cytarabine and an anthracycline, possibly followed by HSCT) in those with molecular relapse, before a clear haematologic relapse occurs.[14] This same benefit has yet to be proven with ATO, but the risks of haemorrhage and differentiation syndrome advocate for starting treatment as early as possible.[14] The best consolidation therapy following a second remission with ATO is not clear, but it is suggested that repeat cycles with ATO or chemotherapy with ATO and ATRA or HSCT should be considered based on patient profiles and prognostic features such as age, the length of the first remission, molecular status and the presence of a suitable donor for HSCT.[14] For those in whom HSCT is not advised, gemtuzumab ozogamicin is an option to add to the above therapies.[14] Either allogenic or autologous HSCT can be considered. Autologous HSCT has a lower transplant-related mortality and could be appropriate in those without minimal residual disease and who have had a complete molecular remission of more than one year.[14] Allogeneic transplant has a higher risk of transplant-related mortality but a better graft versus leukaemia effect.[14] This could be offered to those who have not had a second molecular remission or have had a short complete remission period.[14] A bone marrow biopsy should be repeated in any patient who has had a relapse (evidenced by PCR positivity after completion of consolidation) within two weeks to assess for minimal residual disease.[14] It has been shown that any patient who has disease detectable at the molecular level on two instances following completion of consolidation will relapse if there is no further treatment given.[14]

The most common site for extramedullary relapse in APL is the central nervous system (CNS) (in at least 10% of patients with relapsed APL).[14] Patients most likely to relapse are those with a high Sanz score, and some sources recommend that this subset of patients receive CNS prophylaxis, although there is no clear benefit yet reported.[14] This is advised to be given only once a complete remission has been achieved in order to avoid performing lumbar punctures in

the high risk setting of the early phase of this disease, and is not advised in those with a low or intermediate Sanz score due to their low risk of relapse.[14] Induction should involve weekly intrathecal therapy using methotrexate, hydrocortisone and cytarabine until blasts have disappeared from the cerebrospinal fluid (CSF), and then six to ten more cycles as consolidation, in conjunction with systemic therapy.[14] High dose cytarabine is recommended as it has a high CSF penetrance. HSCT should be performed as consolidation and cranio-spinal radiation should also be given for established CSF relapse.[14]

1.13. HIV and APL

1.13.1. Background

The management of HIV has changed dramatically in recent years and the prognosis and life expectancy have increased significantly in the era of combined anti-retroviral therapy (cART), which has resulted in an increased HIV prevalence.[7] However, due to factors such as immune activation and dysregulation with residual inflammation, chronic antigenic stimulation, medication use and direct viral pathogenicity and oncogenic considerations, these patients are at increased risk of developing cancer.[7, 34, 35] Acquired immunodeficiency syndrome (AIDS) defining malignancies include Kaposi's sarcoma, non-Hodgkin lymphoma, and invasive cervical carcinoma. There is an association with many other malignancies as well, with one study demonstrating a rate of 23.5% of non-AIDS-related deaths resulting from non-AIDS-defining cancers.[35] Haematological malignancies are also noted to be increasing, with lymphoid malignancies (e.g. Hodgkin lymphoma) more significantly increased than myeloid malignancies.[7] HIV seropositive patients have double the risk of developing AML when compared to the general population, as noted in a French cohort study, but patients with APL were rare.[7, 34, 36] However, there is no increase in incidence in AML in HIV seropositive patients with CD4 counts less than 200 cells/ul, neither has there been a change in incidence since the introduction of cART.[37] There is also a suggestion that the incidence of AML may be increased in association with immunodeficient states due to the high incidence in severe combined immunodeficiency and Wiskott-Aldrich syndrome, which are both diseases with chronic T-cell abnormalities.[7]

Myelosuppression and resultant opportunistic infections are especially likely in patients with both HIV and AML due to insufficient haematopoiesis and in particular where there has been zidovudine use.[7] Myelosuppression in patients with HIV tends to be associated with low blast counts in the bone marrow and minimal acute transformation.[36] The association between HIV and AML may be a result of the HIV itself that facilitates transformation to AML, or it may be due to inability of the immune system to control transformed cells.[36] There is very little published information on HIV and APL, and there are no currently available guidelines for the management of this dual pathology. This poses a problem, as both of these conditions are significantly myelosuppressive and their concomitant treatment and the management of the ensuing side effects is challenging. There is thus an increased rate of opportunistic infections and deaths. [7, 37] It is likely that the development of leukaemia in these patients has a multifactorial aetiology.[36] Medications such as nucleoside reverse transcriptase inhibitors and cytostatic agents may increase the leukaemic complications in patients living with HIV.[7] It has been suggested that the use of integrase inhibitors should be encouraged as this will limit interactions with other medications and result in fewer side effects.[7]

1.13.2. Management

In order to determine the best treatment regimens for these patients, information from CD4 counts, viral loads, WHO staging and performance status are important, as it has been suggested that those with a good functional class and CD4 count above 200 cells/ul should be treated with standard regimens, which is associated with a longer survival.[34, 36] The outcomes of a French study which looked at all patients with this dual pathology of HIV and AML between 1990 and 1996 suggested that HIV infection (particularly in the short term) does not affect the outcome.[36] There does appear to be an association with CD4 count however, indicating that survival is shorter in patients with both HIV and AML, but that intensive treatment does have long term benefit and enables us to use the CD4 count as a prognostic factor.[37] A literature review demonstrated a median survival of only seven weeks as compared to seven months in patients with CD4 counts of ≤ 200 cells/ul and > 200 cells/ul respectively, and a similar conclusion was reached in the French study.[37] There appears to be no difference in the karyotype or cytogenetic abnormalities between HIV seronegative and seropositive patients, yet there was a trend (not

statistically significant) across the AML karyotypes, with favourable, intermediate and unfavourable karyotypes having progressively declining CD4 counts.[37] In severely immunocompromised patients, prior to the widespread use of combination antiretroviral therapy (cART), palliative care or low dose cytarabine was suggested.[36] Although there was a high relapse rate (which could, in part, be attributed to the challenges in giving consolidation and maintenance chemotherapy in these patients), there were no opportunistic infections or chemotherapy-related deaths noted, despite a long period of neutropenia with a median of 27 days.[36] It is, of course, difficult to differentiate between opportunistic infections and those as a result of neutropenia.

1.14. APL in Africa

There is a significant paucity of information on haematological malignancies in Africa, including information on APL in particular, with urban bias and poor reliability of data compounding the problem.[38]

A Tunisian study found that they had acceptable outcomes with an overall survival rate of 78% despite their limited resources, including lack of access to ATO, although their complete remission rate was lower than those found in international studies.[5]

1.15. APL in South Africa

There is a paucity of information on APL in South Africa. A recent study of AML at Chris Hani Baragwanath Academic Hospital (CHBAH) has shown that a significant percentage of the patients with APL have concurrent infection with HIV at 17.46%.[9] It is unclear whether this association is coincidental, reflecting a higher proportion of HIV seropositive individuals in the young-middle age group affected by APL, or whether there is a causative relationship.[9]

A study recently published on a series of patients in Cape Town from 1998 – 2019 demonstrated that the outcomes of APL patients at this centre were similar to those in developed countries with early death rates of 7% at seven days and 13% at 30 days, and an overall survival of 76.5% at

three years.[39] This is impressive in light of the fact that patients in South Africa have significant barriers to accessing healthcare, including long waiting times at local clinics and hospitals, large distances to travel to tertiary haematology facilities often relying on public transport and a low socioeconomic status in a large proportion of the country's population.[39] A total of 69 patients were found during this period at Groote Schuur Hospital, 39% of whom were high risk, 60% had a coagulopathy at diagnosis, and all patients had typical cytogenetics.[39] The median time from diagnosis to referral to this tertiary institution was 1.5 days, and 18.8% had a delay of more than five days.[39] Patients in this study were treated using a modified version of the PETHEMA LPA99 protocol (idarubicin was substituted with daunorubicin or doxorubicin).[39] Six patients were HIV seropositive, and two of these had high risk APL.[39] None of these patients had stage 4 HIV at presentation and only one died within the first 30 days; the other five all went into complete remission.[39] Following this, one was lost to follow up and one demised from disseminated tuberculosis after completing consolidation. The remaining three patients experienced long term complete remission, and no drug interactions between cART and chemotherapy and APL-specific therapy were noted.[39] There was also no need for a change in treatment regimen in this cohort of patients with this dual pathology, although it is noted that close observation for opportunistic infections is warranted, particularly during maintenance therapy.[39]

A study looking at the incidence of haematological malignancies in the Eastern Cape during the period 2004 – 2013 found 3603 cases, 17% of which were acute leukaemias, and 58 cases of APL.[38] The median age was noted to be 33.5 years, with incidence rates of 0.11 for males and 0.07 for females.[38] The median age for all haematological malignancies was estimated to be 52 years, which is significantly younger than that of the northern hemisphere at around 70 years.[38] This may be attributed to a lower median population age as a result of HIV, TB and migration of working-age adults out of the Eastern Cape and could also be postulated to result in an increase in incidence as life expectancy increases with cART and improved access to health care.[38]

In light of the increasing HIV prevalence, the challenges this poses to treatment in AML and APL, and the paucity of information available on APL in South Africa, further studies on APL are now timely.

2. CHAPTER 2: PATIENTS AND METHODS

2.1. Aim

The aim of this study was to investigate the demographic and clinical profile of patients diagnosed with APL at the Clinical Haematology Unit, Department of Medicine, Chris Hani Baragwanath Academic Hospital, during the period 01/01/1994 to 31/12/2019. Particular note was made regarding early death rates, presentation immunophenotypes and the effects that HIV may have had on presentation and outcomes.

2.2. Objectives

Specifically, the study had the following objectives:

2.2.1. To describe the demographic profile (including factors such as age, gender, ethnic group).

2.2.2. To describe the clinical and laboratory parameters at presentation.

2.2.3. To determine the factors responsible for morbidity and mortality, with particular attention to factors responsible for early mortality and complications (death and complications within the first 30 days of initial treatment). Late complications are defined as those occurring after the first 30 days of initial treatment.

2.2.4. To investigate the relationship with HIV, CD4 counts and the use of combination anti-retroviral therapy (cART), as it pertains to presentation of APL and patient outcomes.

2.2.5. To describe the management and outcome of the patients, including response to treatment and survival.

2.2.6. To investigate demographic features, laboratory parameters and different treatment strategies with outcomes in order to identify possible prognostic factors.

2.2.7. To compare and contrast the profile of patients seen in South Africa with published data in the literature.

2.3. Methodology

2.3.1. Study design

This study was a retrospective audit; with descriptive and comparative elements. Data was collected from patient files in the Clinical Haematology Unit, Department of Medicine, Chris Hani Baragwanath Academic Hospital, using REDCap (an application for creating surveys and managing data). Anonymity was ensured by the use of study numbers to identify patients (see Appendix I).

2.3.2. Study population

This study included all patients who presented to the Clinical Haematology Unit, Department of Medicine, Chris Hani Baragwanath Academic Hospital, with a confirmed diagnosis of APL, during the period 01/01/1994 to 31/12/2019 (i.e. 26 years). A sample size of 79 patients was obtained.

2.3.2.1. Inclusion criteria

- Adults ≥ 18 years of age.
- Patients with a confirmed diagnosis of APL, based on morphologic, immunophenotypic and genetic/molecular criteria.
- Patients diagnosed and/or managed with APL between 01/01/1994 and 31/12/2019, at the Clinical Haematology Unit.

2.3.2.2. Exclusion criteria

- Patients with inadequate records and clinical and laboratory information.
- Patients with inconclusive findings, and unclear or ambiguous diagnoses, as well as other subtypes of AML.

2.3.3. Confounding variables and limitations

Due to the retrospective nature of the study, some data was incomplete and / or missing. There was also a high rate of lost to follow up / non-compliance, which obscured some detail regarding longer term outcomes.

2.3.4. Data collection

Data was obtained retrospectively from patient files and captured into a data sheet (see Appendix A). The data was statistically analysed using STATA. Data included demographics, comorbidities, HIV prevalence and associated features, presentation, laboratory parameters, complications, management and outcomes.

2.3.5. Sampling

A total of 79 patients were identified and were evaluable for review during the study period.

2.3.6. Ethics

Ethics approval was obtained from the Human Research Ethics Committee (HREC) at the University of the Witwatersrand, Johannesburg. Consent for the use of the patients' records was obtained from the Head of the Clinical Haematology Unit, Academic Head of Internal Medicine and Chief Executive Officer of Chris Hani Baragwanath Academic Hospital. Patient identities were kept anonymous and raw data was only accessible to the supervisors, statistician and principal investigator. The study only commenced once permission and approval from all the above authorities had been obtained.

2.3.7. Statistical analysis

Frequencies and percentages were used to describe categorical variables. Continuous variables were described using median and interquartile range. A histogram was used to show the number

of patients with APL over time. Box plots were used to describe the changes in WCC, haemoglobin and platelets at presentation, 10 days post-treatment, after first cycle of treatment, at the first clinic visit and at treatment completion. Kaplan Meier (KM) curves were used to describe survival.

Survival time was measured in years from the date of diagnosis to the date last seen. The log rank test for equality of survivor functions was used to compare KM curves.

Statistical significance was set at 5% ($p<0.05$) and all analyses were done in Stata version 15. A statistician was consulted for assistance.

2.3.8. Study significance

There is a paucity of information available on AML, and APL in particular, in South Africa. A study describing our patients' characteristics is necessary based on the differences found in the haematological malignancies in Africa and South Africa compared to the rest of the world. The impact of HIV on these patients is especially interesting and important to consider in the light of the prevalence of this disease in our population, and the challenge that this dual pathology creates in the management of patients with APL.

2.3.9. Definitions

Relapse

Haematological relapse is defined as the presence of ≥ 5% blasts in the bone marrow, blasts in the peripheral blood or extramedullary disease.[40]

Molecular relapse is the recurrence of minimal residual disease as evidenced by a positive RT-PCR.[40]

Remission

Complete remission is the presence of ≤ 5% blasts in the bone marrow, no blasts in the peripheral blood, no extramedullary disease, an absolute neutrophil count of ≥ 1.0 x 10^9/l and a platelet count of ≥ 100 x 10^9/l.[40]

Response to treatment

The optimal outcome for patients with APL is to attain complete remission without minimal residual disease.[41]

Sanz risk score

The Sanz score, which is based on the presentation white cell count (WCC) and platelet counts, enables risk stratification. Based on this scoring system, low risk patients have a WCC ≤ 10 x 10^9/l and platelet count > 40 x 10^9/l, intermediate risk patients have a WCC ≤ 10 x 10^9/l and platelet count ≤ 40 x 10^9/l and high risk patients have a WCC > 10 x 10^9/l.[18]

Eastern Cooperative Oncology Group score

The Eastern Cooperative Oncology Group (EGOC) performance status scale is graded from 0 to 5.[42] Grade 0 indicates a patient that is fully active and capable of performing all activities without any difficulty.[42] Grade 1 indicates a patient only incapable of performing strenuous activity, grade 2 a patient incapable of performing vocational activities and is ambulant for more than 50% of their waking hours and grade 3 a patient only capable of self-care.[42] Grade 4 describes a patient that is completely disabled and restricted to their bed or a chair, and grade 5 a patient that has demised.[42]

Differentiation syndrome

The differentiation syndrome is a complication of the treatment of APL, occurring due to the large burden of differentiating cells producing inflammatory cytokines and affecting the endothelium. The clinical picture includes fever, fluid retention, hypotension and acute renal failure. It typically occurs in the first three weeks of therapy and can be seen during therapy with ATRA and ATO, the latter being less common.

Disseminated Intravascular Coagulopathy

Disseminated Intravascular Coagulopathy **(**DIC) is essentially an imbalance in the thrombotic and thrombolytic pathways, which usually work in concert to maintain homeostasis. As a result of excessive protease activity, fibrin formation in the vasculature can result in multi-organ dysfunction and failure. Activation of the coagulation cascade consumes clotting factors and platelets and can result in bleeding manifestations.[1] Suggestive laboratory tests include prolongation of the activated partial thromboplastin time (PTT), prothrombin time (PT) and elevated markers of fibrin degradation products; as well as a low or rapidly decreasing platelet count (< 100 x 10^9/l). The peripheral blood smear may show fragmented red blood cells.[1]

Renal failure

The KDIGO (Kidney Disease: Improving Global Outcomes) guidelines define kidney failure as a glomerular filtration rate (GFR) of < 15 ml/min/1.73m^2 or requiring kidney replacement therapy.[43]

3. CHAPTER 3: RESULTS

3.1. Number of patients per year

There was a total of 537 patients with AML (acute myeloid leukaemia) over the study period (01/01/1994 to 31/12/2019). 83 of these patients (15.46%) had APL (acute promyelocytic leukaemia), and 79 of these 83 patients were evaluable. The records for the remaining four patients were not able to be located, and thus there was insufficient data available for evaluation.

The average number of patients with APL presenting to the Clinical Haematology Unit, Department of Medicine, CHBAH per year, during the study period, is three. The average number of patients presenting with APL in the first half of the study period (13 years; i.e., from 1994 to 2006) is two per year, which doubles in the second half of the study period (2007 to 2019) to four patients per year. The highest number of patients that were seen in a year was six, which occurred in both 2012 and 2014.

The total number of patients with APL and the number of patients presenting per year is depicted in the graph below.

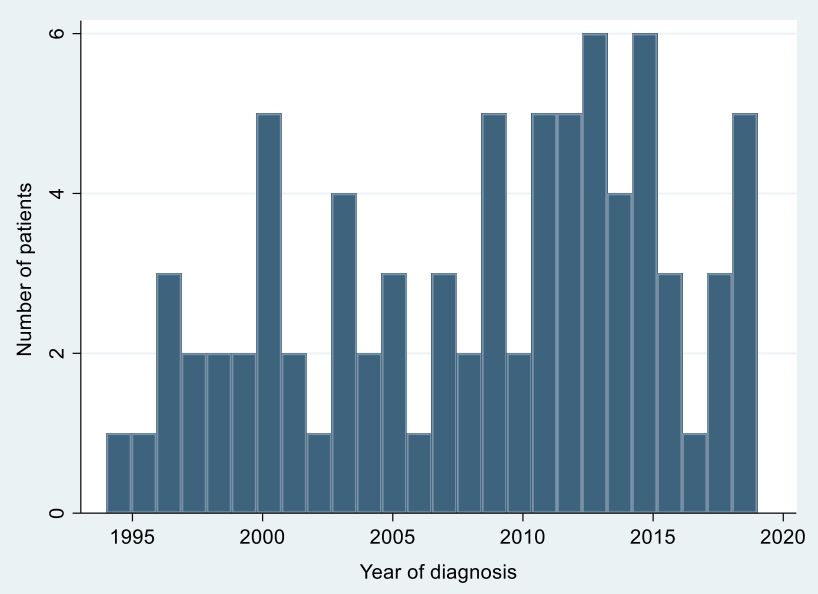

Figure 3.1: Number of APL patients at CHBAH (1994 – 2019)

APL: acute promyelocytic leukaemia

CHBAH: Chris Hani Baragwanath Academic Hospital

3.2. Demographics of APL

The demographic details of the patients with APL in this study are shown in Table 3.1 below. There was a total of 79 evaluable patients with APL, of which 39 (49.37%) were male, and 40 (50.63%) were female. The male: female ratio was 1:1.03. The median age at presentation was 32 years. The ethnicity of our population was as follows: Black patients - 96.2%, White patients - 2.53% and unknown (data missing) in one patient.

Table 3.1: Demographic characteristics of patients with APL

	N	**%**	**Median (IQR)**
Gender			
Male	39	49.37	
Female	40	50.63	
Age in years (range)			32 (24 – 46)
Ethnic Group			
Black	76	96.20	
White	2	2.53	
Unknown	1	1.27	

APL: acute promyelocytic leukaemia

IQR: interquartile range

Performance status was good at baseline with 50.63% of patients having an ECOG (Eastern Co-operative Oncology Group) status of one. However, the data was missing for 21.52% of the patients. The most common comorbidity was HIV at 17.72% of all patients, with hypertension and diabetes also being common at 12.66% and 7.59%, respectively. 5.06% of our patients also had tuberculosis. This is shown in Table 3.2 below.

Table 3.2: Performance status and comorbidities of patients with APL

	N	%
Performance status (ECOG)		
0	1	1.27
1	40	50.63
2	7	8.86
3	7	8.86
4	7	8.86
Unknown	17	21.52
Comorbidities		
HIV	14	17.72
Hypertension	10	12.66
Diabetes	6	7.59
Tuberculosis	4	5.06
Asthma	2	2.53
Epilepsy	2	2.53
Bipolar mood disorder	1	1.27
Kaposi sarcoma	1	1.27
Rheumatic heart disease	1	1.27
Psychosis	1	1.27
Gout	1	1.27

APL: acute promyelocytic leukaemia

ECOG: Eastern Cooperative Oncology Group

HIV: human immunodeficiency virus

3.3. HIV positive patients

Of the total number of AML patients (537) during the study period, 11.92% were HIV seropositive. In those with APL where HIV status was known (77 patients), 14 were HIV seropositive (16.87% of the total group of 83 patients).

In the first half of the study (1994 to 2006) there were four HIV seropositive patients with APL (28.57% of the total number of HIV seropositive patients with APL) and in the second half (2007 to 2019) there were ten (71.43% of the total number of HIV seropositive patients with APL). See table 3.3 below.

Table 3.3: HIV seroprevalence by year of diagnosis in patients with AML and APL

	Year	HIV seropositive	HIV seronegative	HIV unknown	Percentage of HIV seropositive patients
AML patients	1994 - 2019	64	368	105	11.92
APL patients	1994 - 2019	14	63	6	16.87

HIV: human immunodeficiency virus
AML: acute myelocytic leukaemia
APL: acute promyelocytic leukaemia

Of the APL patients in this study, 7.69% of male patients were HIV positive, and 27.5% of females were HIV positive. There were 4 male patients and 2 female patients for whom data on HIV status was missing. See table 3.4 below.

Table 3.4: HIV seropositivity by gender in patients with APL

	HIV Status, N (%)			Total
	Seronegative	**Seropositive**	**Unknown**	
Gender				
Male	32 (82.05)	3 (7.69)	4 (10.26)	39 (49.37)
Female	27 (67.50)	11 (27.50)	2 (5.00)	40 (50.63)

HIV: human immunodeficiency virus

APL: acute promyelocytic leukaemia

There was a total of 14 patients with HIV and APL, of whom half had a CD4 count of > 200 cells/ul, with five patients having a CD4 count of ≤ 200 cells/ul. Data was missing for two patients. One patient had a CD4 count of < 50 cells/ul, two with a count between 50 and 100 cells/ul, and two with a count between 101 and 200 cells/ul.

Four patients had an unsuppressed viral load (VL), all of whom had a CD4 count of > 200 cells/ul. This information was missing for six patients, and of the remaining four, all of whom had a suppressed viral load, one had a CD4 count between 50 and 100 cells/ul, two had a count between 101 and 200 cells/ul, and one had a count of > 200 cells/ul.

Only seven patients were documented to have been using cART, and all of these patients had a CD4 count ≥ 50 cells/ul.

Information on the CD4 counts and viral loads is depicted in Table 3.5 below.

Table 3.5: CD4 counts, VL and cART in HIV positive APL patients

CD4 Count cells/ul	N	Percentage	On cART	Unknown whether on cART	VL suppressed	VL unsuppressed	VL unknown
< 50	1	7.14		1			1
50 - 100	2	14.29	2		1		1
101 - 200	2	14.29	1	1	2		
> 200	7	50	4	3	1	4	2
Unknown	2	14.29		2			2

CD4: cluster of differentiation four

VL: viral load

cART: combined/combination anti-retroviral therapy

HIV: human immunodeficiency virus

APL: acute promyelocytic leukaemia

In five of the 14 patients with APL who were HIV seropositive, the diagnosis of HIV was made at the same time as the diagnosis of APL. There were six patients who were known to be HIV seropositive at the time of the APL diagnosis (two of whom were already on cART). Two of these six patients had been known with HIV for two years, two patients had been known with HIV for four years and two patients had had HIV for more than ten years. The time of HIV diagnosis for three patients was unknown. There were two patients who were diagnosed with HIV ten years after the diagnosis of APL was made. This information is shown in Table 3.6 below.

Table 3.6: Timing of HIV diagnosis in relation to APL diagnosis

When HIV diagnosed	N
New diagnosis	5
Known diagnosis	6
· On cART	2
· Unknown if on cART	4
Duration of HIV unknown	3
Total	14
HIV diagnosis after APL diagnosis	2
Grand total	16

HIV: human immunodeficiency virus

APL: acute promyelocytic leukaemia

cART: combined/combination anti-retroviral therapy

3.4. Presenting symptoms

Patients diagnosed with APL most commonly presented with bleeding (82.05%), followed by fatigue (74.65%), malaise (61.43%) and weakness (54.93%). Only 4.29% of patients had thrombosis as a presenting symptom. See table 3.7 below.

Table 3.7: Presenting symptoms of patients with APL

Symptoms	**N**	**%**
Bleeding	64	82.05
Fatigue	53	74.65
Malaise	43	61.43
Weakness	39	54.93
Weight loss	29	41.43
Fever	25	35.71
Night sweats	24	34.78
Anorexia	18	26.09
Bone pain	5	7.14
Thrombosis	3	4.29
Pruritis	3	4.29
Amenorrhoea	2	2.86
Impotence/erectile dysfunction	1	1.43

APL: acute promyelocytic leukaemia

The most common site of bleeding on admission in the patients in our study was gum bleeding (50.63%), followed by spontaneous cutaneous bleeding (ecchymosis, petechiae, purpura) (43.04%), epistaxis (30.38%) and bleeding at venepuncture sites (26.58%). 60.76% of patients presented with two or more sites of bleeding. See table 3.8 below.

Table 3.8: Site of bleeding in patients with APL

Site	**N**	**%**
Multiple sites (two or more sites)	48	60.76
Gums	40	50.63
Cutaneous (ecchymosis, petechiae, purpura)	34	43.04
Epistaxis	24	30.38
Venepuncture sites	21	26.58
PV bleeding	12	15.19
Buccal mucosa (petechiae, purpura, haemorrhagic bullae)	9	11.39
Haemoptysis	6	7.59
GI tract bleeding (melaena, haematemesis, haematochezia)	5	6.33
Haematuria	5	6.33
Ocular	3	3.80
Post-surgery (LLETZ, circumcision, biopsy)	3	3.80
Penile	2	2.53
Intracranial	1	1.27

APL: acute promyelocytic leukaemia

PV: per vaginal

GI: gastrointestinal

LLETZ: large loop excision of the transformation zone

3.5. Clinical signs

A significant majority of patients presented with pallor: 64 of the 79 patients in the study (81.01%). This is in keeping with bone marrow infiltration which is a universal feature of the disease, together with a high incidence of bleeding at presentation. Other clinical features included lymphadenopathy (29.11%) and hepatomegaly (21.52%). These latter two features may be related to the disease or may represent clinical manifestations of comorbidities such as HIV and tuberculosis. See table 3.9 below.

Table 3.9: Clinical signs in patients with APL

Sign	**N**	**%**
Pallor	64	81.01
Lymphadenopathy	23	29.11
Hepatomegaly	17	21.52
Splenomegaly	4	5.06
Jaundice	3	3.80

APL: acute promyelocytic leukaemia

3.6. Laboratory parameters

A summary of the laboratory findings can be found in Table 3.10 below. At presentation, the median WCC was 3.51 x 10^9/l, haemoglobin was 6.55 g/dl, platelet count was 24 x 10^9/l, INR was 1.3, PTT was 32.7 sec and fibrinogen 2.1 g/l, respectively. The median indices for renal function at presentation were within normal limits. The median uric acid was 0.32 mmol/l and the median LDH was raised at 601.5 U/l. These values improved when compared to those at both the first clinic visit and at completion of chemotherapy. At the first clinic visit and then at completion of chemotherapy, the median WCC improved to 5.39 and 5.03 x 10^9/l, haemoglobin to 11.9 and 12.85 g/dl and platelets to 356.5 and 270.5 x 10^9/l, respectively. The INR also normalised after the first cycle of chemotherapy to 1.08.

Table 3.10: Laboratory findings in patients with APL

	Median (Range)					
Parameter	**At presentation**	**10 days post treatment**	**After first cycle of treatment**	**First clinic visit**	**At completion of chemotherapy**	**At last date seen / last visit**
White cell count x 10^9/l	3.51 (0.51 – 144)	2.04 (0.46 – 26.9)	5.06 (1.77 – 24.08)	5.39 (2.3 – 11.72)	5.03 (3.42 – 7.8)	5.12 (0.19 – 203)
Haemoglobin g/dl	6.55 (3.1 – 13.6)	8.1 (6.1 – 10.7)	9.65 (7.01 – 14)	11.9 (6.5 – 15.8)	12.85 (6.5 – 15.7)	10.8 (3.1 – 16.5)
Haematocrit l/l	0.19 (0.10 – 0.37)	0.25 (0.19 – 0.34)	0.31 (0.23 – 0.42)	0.36 (0.27 – 0.43)	0.36 (0.27 – 0.45)	0.26 (0.10 – 0.41)
MCV fl	90 (73.3 – 109)	86.6 (78 – 97.5)	91.2 (82 – 104.1)	90.4 (82.1 – 103.1)	90.35 (83.8 – 98.8)	88.0 (74.1 – 111)
Platelets x 10^9/l	24 (2 – 178)	39.5 (10 – 225)	430 (42 – 821)	356.5 (87 – 987)	270.5 (87 – 467)	154.5 (2 – 512)
Na mmol/l	137 (124 – 154)	137 (122 – 179)	139 (132 – 148)	139 (138 – 142)	141 (141 – 141)	138 (128 – 172)
K mmol/l	3.7 (2.5 – 4.9)	4.1 (2.5 – 5.1)	4.1 (2.8 – 5.3)	4 (3.1 – 4.3)	4.5 (4.5 – 4.5)	3.5 (2.5 – 5.5)
Cl mmol/l	100 (89 – 111)	102 (84 – 135)	102 (89 – 111)	100.5 (99 – 102)	100 (100 – 100)	104 (89 – 133)
HCO3 mmol/l	19 (1.4 – 31)	18 (1.1 – 31)	21 (2 – 30)	25 (4.6 – 25)	22 (22 – 22)	20 (2.8 – 28)
Urea mmol/l	6.1 (1.5 – 40.3)	6.2 (1.9 – 22.2)	4.7 (1.7 – 96)	4.65 (3.4 – 50)	4 (4 – 4)	6.9 (2.4 – 38)
Creatinine umol/l	75 (45 – 450)	66 (40 – 149)	61 (38 – 105)	67 (56 – 84)	69 (69 – 69)	80 (36 – 399)
UA mmol/l	0.32 (0.08 – 0.8)	0.32 (0.32 – 0.32)	0.21 (0.19 – 0.33)	-	-	-
LDH U/l	601.5 (203 – 4506)	1010 (1010 – 1010)	620.5 (235 – 1006)	406 (406 – 406)	-	-
INR	1.3 (0.87 – 2.85)	1.21 (0.88 – 1.64)	1.08 (0.85 – 1.76)	-	-	1.39 (0.91 – 6.2)
Fibrinogen g/l	2.1 (0.5 – 21)	3.65 (1.1 – 6.8)	3.4 (1.9 – 8.5)	-	-	3.5 (2.03 – 40)
PTT sec	32.7 (20.7 – 60)	32 (20 – 59.5)	34.5 (27.8 – 64)	-	-	39.1 (11.5 – 60.8)

APL: acute promyelocytic leukaemia

MCV: mean corpuscular volume

UA: uric acid

LDH: lactate dehydrogegnase

INR: international normalised ratio

PTT: partial thromboplastin time

Table 3.11 depicts the laboratory values necessary for a diagnosis of DIC (disseminated intravascular coagulation) to be made. DIC is both a clinical and biochemical diagnosis. In this study, a patient was considered to have a DIC if they had low platelets, evidence of coagulation factor consumption (either a prolonged INR or PTT or low fibrinogen) and a high D-dimer indicating fibrinolysis. Due to the retrospective nature of the study, it was difficult to apply a scoring system for the calculation and diagnosis of DIC.

At presentation, 74 patients (97.37%) had a thrombocytopenia. There were 28 patients (43.76%) that presented with a low fibrinogen (the reference range used was 2 – 4 g/l) and six (9.38%) with a high fibrinogen. Fifteen patients (20.55%) had an INR $\geq$ 1.5. Fourteen patients (20%) had a high PTT, and 62 (100%) had a D-dimer of > 0.25 mg/l.

Fifty-one patients (82.26%) had a very high D-dimer of > 4 mg/l at presentation. Unfortunately, data for D-dimer values after ten days of treatment and on completion of the first cycle of therapy was less frequently available, but showed a definite improvement. After ten days, the D-dimer was still high in all patients (data available for 26 patients), but only seven patients (26.92%) had a D-dimer value of > 4 mg/l at this time. After the first cycle of therapy, there is only data on the D-dimer for 11 patients, but again while this value was high for almost all of these patients (90.90%), it was > 4 mg/l in only 18.18% of patients.

Similarly, the thrombocytopenia also improved from presentation, compared to the first clinic visit and at completion of chemotherapy, only one patient had a platelet count < 100 x 10^9/l.

Table 3.11: Laboratory parameters for diagnosis of DIC in patients with APL

Parameter	At presentation	10 days post treatment	After first cycle	First clinic visit	Completion of chemotherapy	Last date seen / last visit
Platelets x 10^9/l						
≤100	74 (97.37%)	52 (92.86%)	3 (6.38%)	1 (2.27%)	1 (6.25%)	31 (43.06%)
>100	2 (2.63%)	4 (7.14%)	44 (93.62%)	43 (97.73%)	15 (93.75%)	41 (56.94%)
Total	76	56	47	44	16	72
Unknown	3	23	32	35	63	7
Fibrinogen g/l						
<2	28 (43.76%)	3 (10.71%)	1 (9.09%)			0
2.0 - 4.0	30 (46.88%)	14 (50%)	6 (54.55%)			7 (63.64%)
>4	6 (9.38%)	11 (39.29%)	4 (36.36%)			4 (36.36%)
Total	64	28	11			11
Unknown	15	51	68	79	79	68
INR						
<1.5	58 (79.45%)	34 (94.44%)	15 (88.24%)			12 (66.67%)
≥1.5	15 (20.55%)	2 (5.56%)	2 (11.76%)			6 (33.33%)
Total	73	36	17			18
Unknown	6	43	62	79	79	61
PTT sec						
<40	56 (80%)	28 (80%)	11 (84.62%)			10 (66.67%)
≥40	14 (20%)	7 (20%)	2 (15.38%)			5 (33.33%)
Total	70	35	13			15
Unknown	9	44	66			64
D-dimer mg/l						
≤ 0.25	0	0	1			0
0.25 – 0.5	1	3	5			1
0.5 – 1	1	4	2			0
>1 <2	3	7				0
2.0 - 4.0	4	5	1			1
>4	51	7	2			6
Total >0.25	62 (100%)	26 (100%)	10 (90.90%)			8 (100%)
Total	62	26	11			8
Unknown	19	53	68			71

DIC: disseminated intravascular coagulation

APL: acute promyelocytic leukaemia

INR: international normalised ratio

PTT: partial thromboplastin time

Table 3.12 indicates the number of patients with DIC at different stages in the course of their illness. At presentation, 39 patients (63.93%) had evidence of DIC. At ten days post treatment, this had improved to 25.93% and then to 0% after the first cycle of therapy.

Table 3.12: Number of patients with APL with DIC at different stages of treatment

	Presentation	**10/7 Post treatment**	**After 1st cycle**	**1st clinic visit**	**Completion of treatment**	**Last seen**
DIC	39 (63.93%)	7 (25.93%)	0	0	0	0
No DIC	22 (36.07%)	20 (74.07%)	46 (100%)	44 (100%)	15 (100%)	43 (100%)
Total	61	27	46	44	15	43
Missing	18	52	33	35	64	36

APL: acute promyelocytic leukaemia

DIC: disseminated intravascular coagulation

Table 3.13 shows the grades of leucopenia, anaemia and thrombocytopenia of our population of APL patients at presentation. The majority of patients had a leucopenia (51.32%) at presentation (of varying grades), with 22.37% presenting with a normal WCC and 26.32% presenting with a WCC of > 10 x 10^9/l. Similarly, the majority of patients (61.29%) had a neutropenia at presentation, 20.97% of patients had a normal absolute neutrophil count (ANC) at presentation, and 17.74% had a neutrophilia. The vast majority of patients with APL in this study presented with anaemia (93.42%); 50% of all patients had a very severe, grade four anaemia with a haemoglobin of < 6.5 g/dl. There was a borderline anaemia in 5.26% of patients and only 1.32% had no anaemia at presentation. Again, the vast majority of patients in this study presented with a thrombocytopenia at 97.37% of patients, with only 2.63% of patients having a normal platelet count at presentation. More than half of these patients had a severe, grade four thrombocytopenia with a platelet count of < 25 x 10^9/l.

Table 3.13: Grades of leucopenia, anaemia and thrombocytopenia at presentation in patients with APL

		N	%
WCC x 10^9/l	< 1.0 (Grade 4)	9	11.84
	1.0 - 1.9 (Grade 3)	15	19.74
	2.0 - 2.9 (Grade 2)	10	13.16
	3.0 - 3.9 (Grade 1)	5	6.58
	4.0 - 10	17	22.37
	> 10	20	26.32
	Total	76	100.01
Haemoglobin g/dl	≥ 11	5	6.58
	9.5 - 10.9 (Grade 1)	8	10.53
	8.0 - 9.4 (Grade 2)	13	17.11
	6.5 - 7.9 (Grade 3)	12	15.79
	< 6.5 (Grade 4)	38	50
	Total	76	100.01
	Borderline anaemia (11-13 in males, 11-12 in females)	4	5.26
	No anaemia (>13 in males, >12 in females)	1	1.32
Platelets x 10^9/l	> 400	0	0
	150 - 400	2	2.63
	100 - 149	0	0
	75 - 99 (Grade 1)	3	3.95
	50 - 74 (Grade 2)	6	7.89
	25 - 49 (Grade 3)	26	34.21
	< 25 (Grade 4)	39	51.32
	Total	76	100
Absolute neutrophil count x 10^9/l	> 7.5	11	17.74
	2 - 7.5	13	20.97
	1.5 – 1.9 (Grade 1)	2	3.23
	1.0 – 1.4 (Grade 2)	4	6.45
	0.5 – 0.9 (Grade 3)	4	6.45
	< 0.5 (Grade 4)	28	45.16
	Total	62	100

APL: acute promyelocytic leukaemia

WCC: white cell count

The following three graphs are box and whisker plots demonstrating the changes in WCC, haemoglobin and platelet count from presentation, following the first ten days of therapy, the first completed cycle of therapy, the first clinic visit, completion of therapy and the last seen WCC.

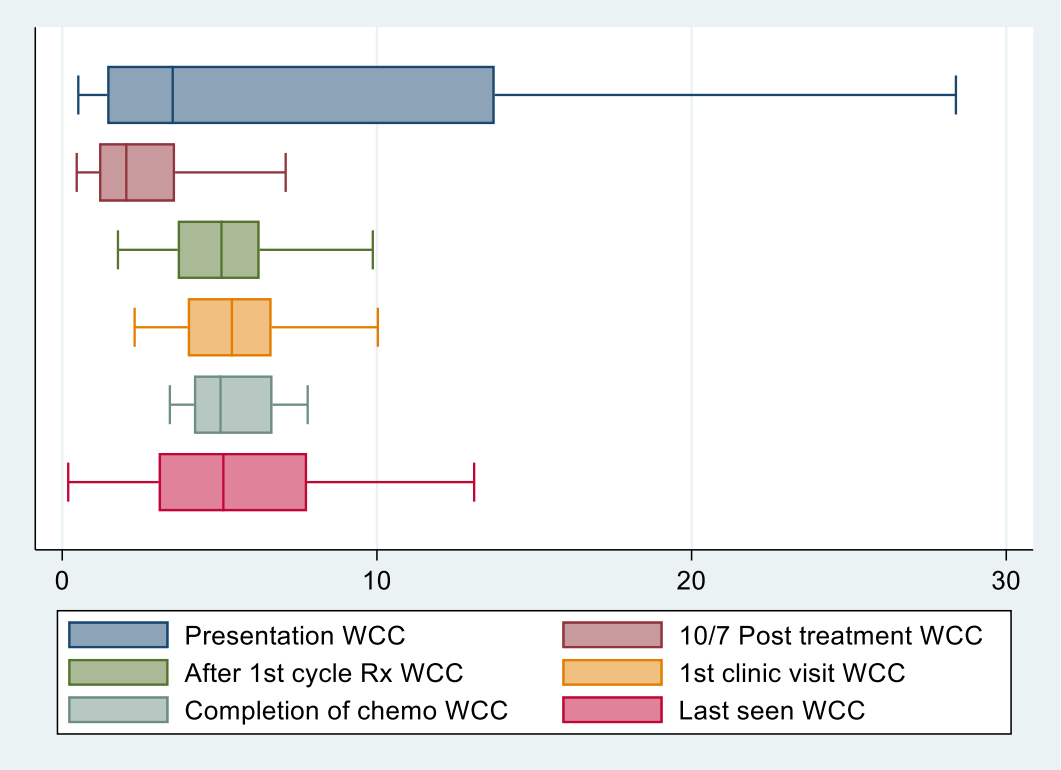

Figure 3.2: Changes in white cell count (WCC) in patients with APL

APL: acute promyelocytic leukaemia

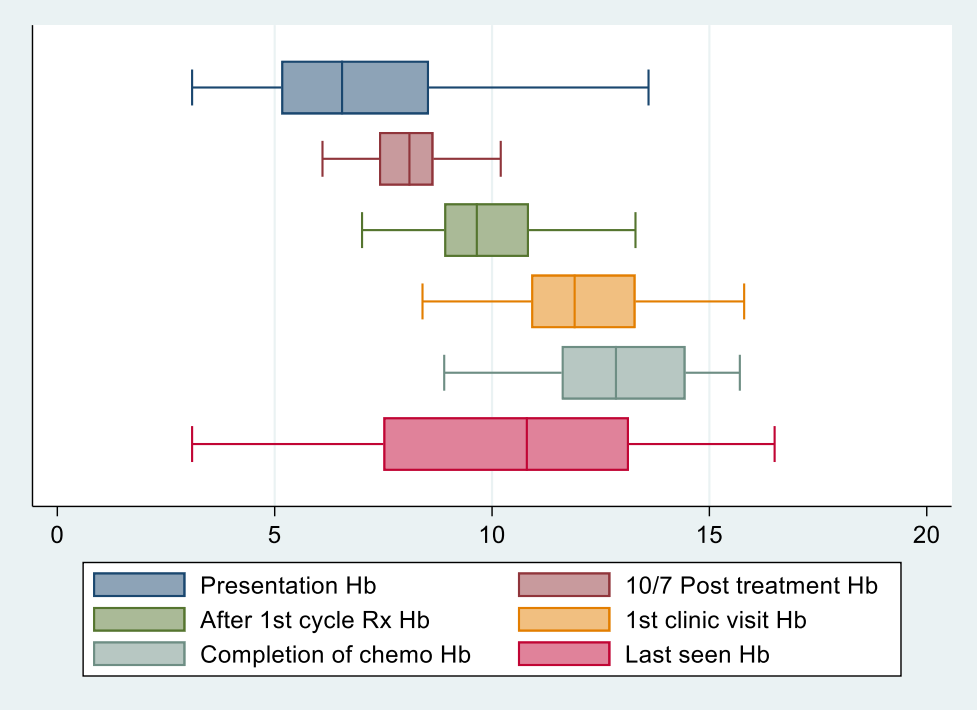

Figure 3.3: Changes in haemoglobin (Hb) in patients with APL

APL: acute promyelocytic leukaemia

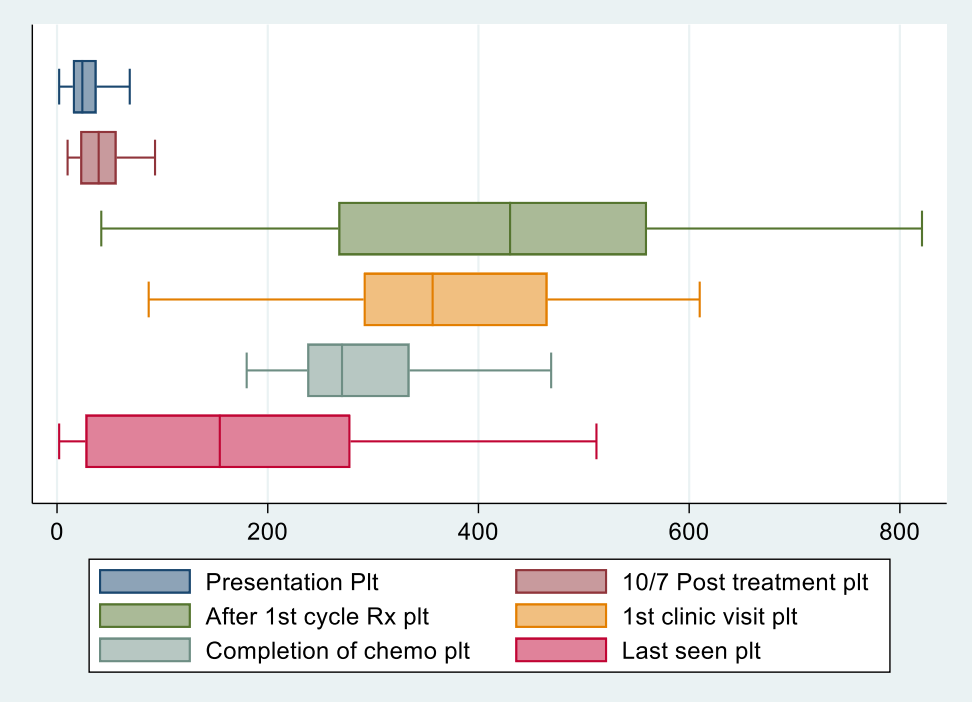

Figure 3.4: Changes in platelets (plt) in patients with APL

APL: acute promyelocytic leukaemia

The patients in this study were classified according to the Sanz score using the WCC and platelet values at presentation.

Most patients (59.21%) were intermediate risk, with a WCC $\leq 10 \times 10^9$/l and platelet count $\leq 40 \times 10^9$/l. 26.32% were high risk (WCC $> 10 \times 10^9$/l) and 14.47% were low risk (WCC $\leq 10 \times 10^9$/l and platelet count $> 40 \times 10^9$/l). See table 3.14 below.

Table 3.14: Sanz score risk stratification of patients with APL at presentation

Sanz group	**N**	**%**
Low risk	11	14.47
Intermediate risk	45	59.21
High risk	20	26.32
Total	**76**	**100**

APL: acute promyelocytic leukaemia

3.7. Bone marrow assessment

3.7.1. Morphology

Information on bone marrow morphology was available for 73 patients. The remaining six had missing data; bone marrow assessment may not have been done in two of these patients as they demised pre-treatment. Six patients in our study (8.21%) had a hypogranular variant, while the remaining 67 (91.78%) had the typical, hypergranular morphology.

3.7.2. Flow cytometry

Table 3.15 describes the percentage of patients displaying different immunophenotypes in our APL population. The most commonly found markers were CD33 and CD13, both in 62.03% of patients, followed by CD15 in 26.58% of patients.

Table 3.15: Immunophenotype in patients with APL

Parameter	N	%
CD33	49	62.03
CD13	49	62.03
CD15	21	26.58
CD117	16	20.25
CD2	15	18.99
HLA-DR	14	17.72
CD34	12	15.19
CD11b	7	8.86
CD56	2	2.53

APL: acute promyelocytic leukaemia

CD: cluster of differentiation

HLA-DR: human leucocyte antigen – DR isotype

The immunophenotype in table 3.15 was similar in patients with a high Sanz score (i.e. a high-risk APL), compared to Table 3.16 below.

Table 3.16: Immunophenotype in high-risk APL (WCC > 10 x 10^9/l)

Parameter	N = 20	%
CD33	13	65.00
CD13	13	65.00
CD15	6	30.00
CD117	4	20.00
CD2	6	30.00
HLA-DR	6	30.00
CD34	5	25.00
CD11b	2	10.00
CD56	1	5.00

APL: acute promyelocytic leukaemia

WCC: white cell count

CD: cluster of differentiation

HLA-DR: human leucocyte antigen – DR isotype

3.7.3. Cytogenetics

Of the 51 patients in whom cytogenetic studies were done, 49 demonstrated a t(15;17) (96.08%). Only two patients had differing cytogenetics, specifically one patient with t(15;17)(q24;q21) with additional material on the long arm of chromosome 7, and one patient with add(4q), del(9p) in addition to the t(15;17). Of the eight patients that were tested for FLT3-ITD, all of whom had a t(15;17), only one was positive (12.5%) (see Table 3.17 below).

Table 3.17: Cytogenetics in patients with APL

Parameter	**Positive**	**Negative**	**Unknown**
t(15;17)	49 (96.08%)	2 (3.92%)	28
FLT3-ITD	1 (12.5%)	7 (87.5%)	71
Other variants detected			
t(15;17)(q24;q21) with additional material on the long arm of chromosome 7	1 (1.96%)		
t(15;17) add(4q), del(9p)	1 (1.96%)		

APL: acute promyelocytic leukaemia

t(15;17): Translocation chromosome 15 to 17

FLT3-ITD: FMS-like (Feline McDonough Sarcoma gene) tyrosine kinase 3 - internal tandem duplication

3.7.4. Molecular assessment

Thirty eight patients had a positive PML-RARA fusion gene on PCR, and of these, 29 also had a positive t(15;17). In two patients the t(15;17) was negative, and in seven patients the t(15;17) was unknown.

3.8. Management

3.8.1. Delays in referral and initiation of treatment

There were some patients in this study who were diagnosed with APL and only referred to our Clinical Haematology Unit later. The majority of patients (63 patients - 80.77%) were diagnosed and seen by our unit on the same day, while 12.82% of patients were diagnosed with APL and

referred to our Clinical Haematology Unit the following day. There was one patient each with a delay of two, three, four, six and 15 days, respectively. One patient was excluded from this analysis as the diagnosis was made in another province and the patient was transferred later.

Unfortunately, due to the retrospective nature of this study, it is difficult to ascertain the duration of symptoms prior to referral and presentation to our Clinical Haematology Unit.

There were some delays in the initiation of treatment of APL. 51.38% of patients received therapy (supportive care and ATRA and / or chemotherapy) on the same day that they were seen by the Clinical Haematology Unit, and 16.67% of patients received treatment the following day. The remaining patients all had varying degrees of delay as outlined in Table 3.19 below, with the longest delays being 11 days and 19 days, each occurring in one patient, respectively. Seven patients were excluded; five did not receive any treatment at all (died before treatment could be initiated), it was unclear when one patient had been initiated on therapy due to incomplete data and one was treated at another facility prior to being transferred to our unit.

Table 3.19: Delays in treatment in patients with APL

Number of days between referral and treatment	**N = 72 (%)**
0	37 (51.38)
1	12 (16.67)
2	7 (9.72)
3	3 (4.16)
4	3 (4.16)
5	2 (2.78)
6	4 (5.56)
7	2 (2.78)
11	1 (1.39)
19	1 (1.39)

APL: acute promyelocytic leukaemia

3.8.2. Supportive management

The supportive care received by our patients included transfusion of blood products, other drugs (including analgesia, allopurinol and antibiotics) and psychosocial care.

Blood transfusion was required in 86.08% of patients, with a median of three units of packed red cells per patient. 75.95% of patients received platelet transfusions, again with a median of three megaunits of platelets per patient. 45.57% of patients received fresh frozen plasma (FFP), with a median of four units per patient. See table 3.20 below.

Table 3.20: Blood and blood product transfusion in patients with APL

Transfusion	**N**	**%**	**Median (IQR)**
Packed red blood cell transfusions	68	86.08	
Number of blood transfusion units			3 (2 – 5)
Platelet transfusion	60	75.95	
Number of platelet transfusion units			3 (2 – 4)
FFP transfusion	36	45.57	
Number of FFP transfusion units			4 (2 – 6)

APL: acute promyelocytic leukaemia

IQR: inter-quartile range

FFP: fresh frozen plasma

3.8.3. Chemotherapy

Specific management of the APL patients in this study (chemotherapy) is delineated in Table 3.21 below.

ATRA (all-trans retinoic acid) was documented to have been given to 72 patients (91.14%) with data missing for three patients, and ATO (arsenic trioxide) was received by only one patient at relapse. Sixty-nine patients received induction, 50 received a first consolidation cycle, 41 received a second consolidation cycle, 29 received a third consolidation cycle, and eight received a fourth consolidation cycle. Three patients received intrathecal (IT) chemotherapy and 23 were documented to have received maintenance therapy, although it is likely that this number was much higher given the number of patients who achieved remission. Given the retrospective nature of the study, it is difficult to account for this, but it is likely due to this information being undocumented or missing from the records.

Induction chemotherapy most commonly consisted of ATRA and daunorubicin (62.32% of patients) followed by ATRA, daunorubicin and cytarabine (20.29%). In terms of consolidation chemotherapy, the most common regimen received was ATRA with daunorubicin.

Maintenance therapy was comprised of ATRA twice daily orally for two weeks in every month, methotrexate weekly orally for four weeks and mercaptopurine daily orally for four weeks. These monthly maintenance cycles were continued for a period of two years.

Table 3.21: Management of patients with APL: chemotherapy

Management	**N**	**%**
ATRA	72	91.14
Induction	69	
ATRA + Daunorubicin	43	62.32
ATRA + Daunorubicin + Cytarabine	14	20.29
Other	12	17.39
Consolidation 1	50	
ATRA + Daunorubicin	25	50
ATRA + Daunorubicin + Cytarabine	11	22
Other	14	28
Consolidation 2	41	
ATRA + Daunorubicin	25	60.98
ATRA + Daunorubicin + Cytarabine	6	14.63
Other	10	24.39
Consolidation 3	29	
ATRA + Daunorubicin	13	44.83
ATRA + Daunorubicin + Cytarabine	3	10.34
Other	13	44.83
Consolidation 4	8	
ATRA + Daunorubicin	1	12.5
ATRA + Daunorubicin + Cytarabine	0	0
Other	7	87.5
Maintenance	**23**	
IT Chemotherapy	**3**	
HSCT	**1**	

APL: acute promyelocytic leukaemia

ATRA: all-trans retinoic acid

IT: intrathecal

HSCT: haematopoietic stem cell transplant

Twenty-one patients required re-induction chemotherapy and some went on to consolidation following this, as delineated in Table 3.22 below. Of these 21 patients, five received re-induction therapy for failed induction, and two of these five patients had a subsequent relapse in addition to the failed induction. Of those that only required one re-induction (12 patients), six were still in remission at the last date seen. One patient was lost to follow up following re-induction and was reported to have died a year later. The remaining five patients died; two only one month after completing their re-induction therapy, one died a month after completing a single cycle of

consolidation therapy following re-induction, and two died two months after completing four cycles of consolidation therapy after re-induction.

Seven patients required a second re-induction but none received any further consolidation chemotherapy following this as they all subsequently demised. One patient had a massive cerebral infarct, one had an intracranial haemorrhage, one had meningitis, one had fungal sepsis, one was lost to follow up and the cause of death for the remaining two patients was not clear.

Table 3.22: Management of patients with APL: re-induction and further management

Re-induction and further management	**N**
Re-induction	21
Re-induction: failed induction	5
Consolidation 1 post re-induction	11
Consolidation 2 post re-induction	9
Consolidation 3 post re-induction	3
Consolidation 4 post re-induction	2
Second re-induction	7
Consolidation post second re-induction	0

APL: acute promyelocytic leukaemia

In terms of the chemotherapy agents used for re-induction (both in those that required a first as well as a second re-induction), the most common regimen (used in 13 patients) was daunorubicin and cytarabine with ATRA. Five patients had therapy with mitoxantrone, cytarabine and ATRA, and five patients with daunorubicin and ATRA. There were differing regimens used in the remainder of the chemotherapy courses, as delineated in Table 3.23 below.

Table 3.23: Management of patients with APL: re-induction regimens

Re-induction regimens	N
ATRA + Daunorubicin + Cytarabine	13
ATRA + Mitoxantrone + Cytarabine	5
ATRA + Daunorubicin	5
ATRA + Mitoxantrone + Etoposide + Cytarabine	4
ATRA + Cytarabine	3
ATRA + Cytarabine + Etoposide	2
Cytarabine + Fludarabine	2
Mitoxantrone	1
Mitoxantrone + Idarubicin	1
Mitoxantrone + Daunorubicin + Cytarabine	1

APL: acute promyelocytic leukaemia

ATRA: all-trans retinoic acid

3.9. Complications

The most common early complication (occurring in the first 30 days following induction therapy) in the patients in our study was haemorrhage, which occurred in 83.54%. This was followed by infection, which occurred in 36.71% and renal failure at 26.58%. 10.13% of patients developed the ATRA differentiation syndrome during treatment and 7.59% had thromboembolic complications.

Late complications (more than 30 days following induction) were most commonly noted to be infection (22.78%) and renal failure (10.13%). TED (thromboembolic disease) only developed in two patients, and the ATRA differentiation syndrome occurred in only one patient.

Infection was a common cause of both early and late complications. In early disease, this was most often due to pneumonia, perineal infections, thrombophlebitis and abscesses. In late disease pneumonia, abscesses and meningitis were common. Tuberculosis, Enterococcus *faecium*, Escherichia *coli* and Klebsiella *pneumoniae* were the most commonly proven causative organisms in both scenarios.

Most complications occurred after induction therapy (46.84%), with 26.58% of complications happening after consolidation and 18.99% of complications being immediate; within three days of the patients' presentations to our Clinical Haematology Unit. See table 3.24 below.

Table 3.24: Complications in patients with APL

Early complications	**N**	**%**
Haemorrhage	66	83.54
Infection	29	36.71
Renal failure	21	26.58
Differentiation syndrome	8	10.13
TED	6	7.59
Other	6	7.59
Late complications		
Infection	18	22.78
Renal failure	8	10.13
Other	5	6.33
TED	2	2.53
Differentiation syndrome	1	1.27

APL: acute promyelocytic leukaemia

TED: thromboembolic disease

3.10. Outcomes

The outcomes of the patients in our study were based on the status of their disease at the last visit to the Clinical Haematology Unit. At this time, 50.63% had demised, 39.24% were in remission, 1.27% were experiencing a relapse, another 3.8% had an unclear remission status on their bone marrow and this information was unknown for 5.06% of patients. See table 3.25 below.

Table 3.25: Outcomes in patients with APL

Outcomes	**N**	**%**
Death	40	50.63
Remission	31	39.24
Unknown	4	5.06
Relapse	1	1.27
Disease status unclear	3	3.80
Total	79	100

APL: acute promyelocytic leukaemia

3.10.1. Remission

Thirty-seven patients (46.84%) achieved remission post induction therapy, while 35 did not. Data on remission post induction therapy was unfortunately not available for the remaining seven patients. Twenty-one patients were also in remission post consolidation, and as mentioned above, 31 were in remission at the time they were last seen in our unit. A higher response to therapy is expected shortly after induction therapy, and there are a few proposed reasons for a poor response later, such as poor adherence to therapy and follow up, drug-drug interactions, side effects, complications, treatment resistance and comorbidities. See table 3.26 below.

Table 3.26: Remission in patients with APL

Remission (N = 79)	N	%	Missing data
Post induction	37	46.84	7
Post consolidation	21	26.58	9
Last seen	31	39.24	9

APL: acute promyelocytic leukaemia

3.10.2. Relapse

A total of 19 patients relapsed in this study, and two patients had residual disease post induction. Of those that relapsed, two patients relapsed twice, and three patients relapsed following non-adherence to therapy. The majority of these relapses occurred while patients were receiving maintenance therapy (63.16%). One patient received ATO for relapse. See table 3.27 below.

Table 3.27: Relapse at different treatment phases in patients with APL

Relapse (N = 19)	N	%
Maintenance	12	63.16
During / post consolidation	4	21.05
Post induction	3	15.79

APL: acute promyelocytic leukaemia

3.10.3. Lost to follow up

Of the 26 patients that were lost to follow up, five were still alive and well with no complaints when they were contacted, and one had subsequently demised (possibly from relapsed disease; the family member reported haematuria and haemoptysis prior to the patient's death). The remaining 20 patients were unfortunately not able to be contacted. See table 3.28 below.

Table 3.28: Status of disease at last visit in patients with APL who were lost to follow up

Status of disease (N = 26)	**N**	**%**
Remission	17	65.38
Received induction	3	11.54
Received re-induction	2	7.69
Remission on maintenance	1	3.85
Significant cytoreduction, uncertain remission	1	3.85
Partial response	1	3.85
Second relapse	1	3.85

APL: acute promyelocytic leukaemia

3.10.4. Mortality

Six patients demised before any treatment could be initiated. Of these, three deaths were attributable to bleeding (one of which was the result of a combination of sepsis and bleeding), one to a stroke and the other two causes of death were unknown.

Twelve patients were classified as immediate mortalities, as they died within the first three days of treatment being initiated. The majority of these deaths were due to bleeding, with infection and the differentiation syndrome being other common causes at this time.

Another six patients died within the first 30 days of treatment being initiated and were classified as early mortalities. Of these six, four deaths were attributed to infection, and one each to bleeding and the differentiation syndrome.

There was a total of 15 late deaths (16 including the one patient who had been lost to follow up). Of these 15, eight deaths were the result of infection and two were from bleeding. Of these late deaths, nine patients achieved remission after induction therapy. Twelve of these 15 patients relapsed, and two patients had a second relapse. The other three patients never achieved remission. See tables 3.29, 3.30 and 3.31 as well as figure 3.5 below.

Table 3.29: Cause of mortality in patients with APL

Cause of death (N = 40)	N	%
Sepsis	12	30.00
Haemorrhage (including ICH)	10	25.00
Unknown	8	20.00
Multiple causes	4	10.00
Differentiation syndrome	3	7.5
CVA	2	5.00
Multiorgan failure	1	2.5

APL: acute promyelocytic leukaemia

ICH: intracranial haemorrhage

CVA: cerebrovascular accident

Table 3.30: Timing of mortality in patients with APL

	N	%
Pre-treatment	6	15.00
Immediate (1 - 3 days post induction)	12	30.00
Early (4 - 30 days post induction)	6	15.00
Late (> 30 days post induction)	16	40.00

APL: acute promyelocytic leukaemia

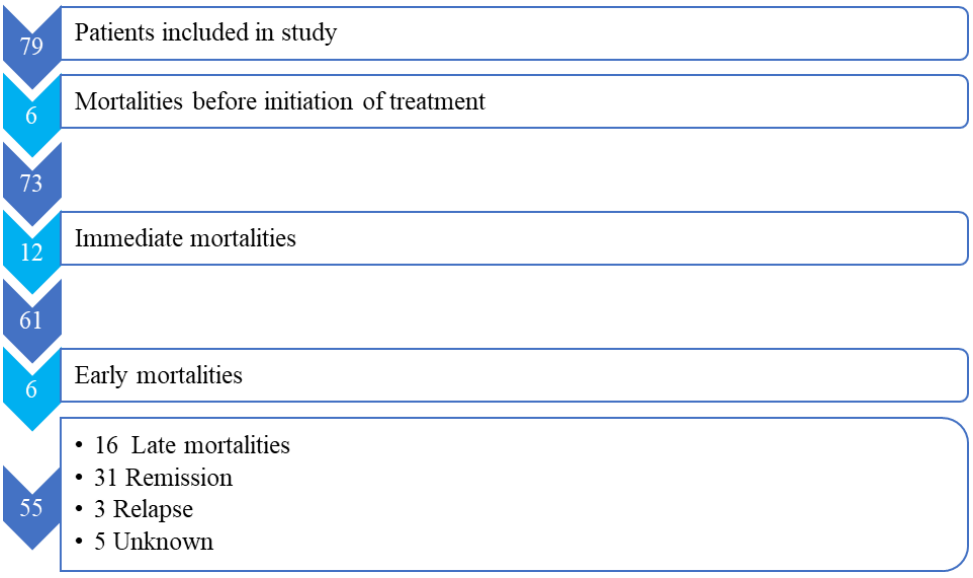

Figure 3.5: Number of patients with APL by outcome

Table 3.31: Cause of mortality in patients with APL at different treatment phases

Cause of death (N = 40)		**N**
Pre-treatment (N = 6)	Bleeding	2
	Unknown	2
	CVA	1
	Multiple causes	1
Immediate (N = 12)	Bleeding	5
	Multiple causes	3
	Differentiation syndrome	2
	Sepsis	2
Early (N = 6)	Infection	3
	Differentiation syndrome	1
	Unknown	1
	Multiple causes	1
Late (N = 16)	Sepsis	7
	Unknown	5
	Bleeding	2
	CVA APL: acute promyelocytic leukaemia	1
	Multiorgan failure	1

CVA: cerebrovascular accident

3.11. Survival

The median survival time in the APL patients in our study was seven months and 24 days. Of the total of 79 patients in the study, 74 were included in the survival analysis as there was insufficient data for three patients, and two patients demised on the same day that they presented to our unit. A total of 186 person-years contributed to this graph, figure 3.6 below.

Note that pre-treatment and immediate deaths accounted for 18 of 79 (22.78%) of the patients. When early deaths (six of 79 patients, 7.59%) are added, this amounts to 30.38%, and as such, impacts on the median survival of our patient population.

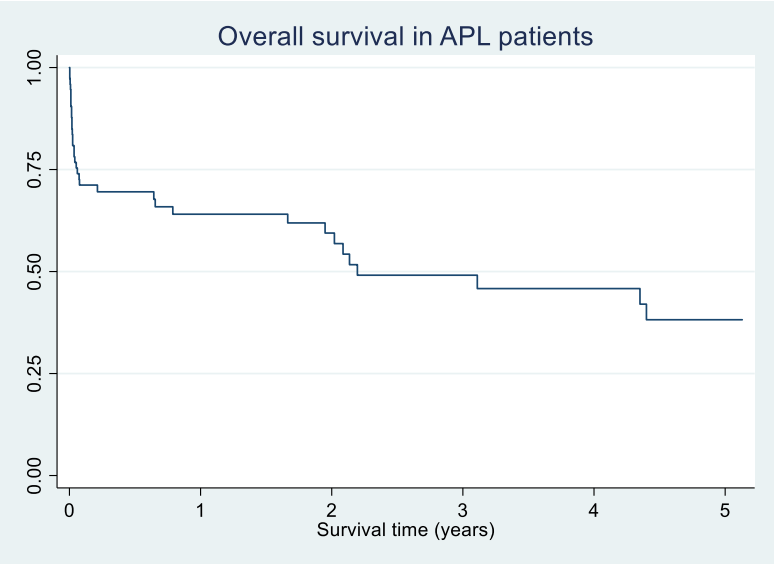

Figure 3.6: Overall survival from time of diagnosis in APL patients

When pre-treatment, immediate and early deaths were excluded, there were 50 patients with survival time that could be evaluated. The median survival in these patients is three years, seven months and 20 days. This is depicted in Figure 3.7 below.

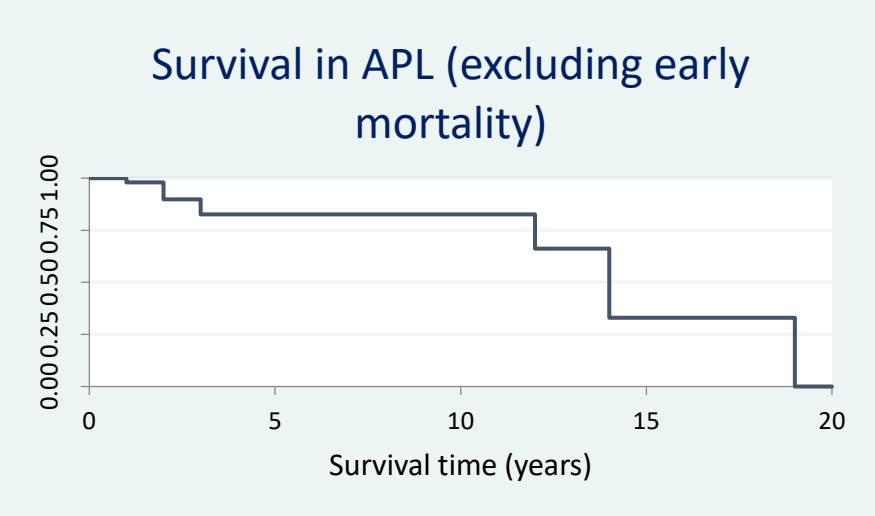

Figure 3.7: Survival in APL (excluding early mortality)

Survival data was compared between the populations of patients in our study who had a diagnosis of HIV to those that were HIV seronegative. Although the survival time is shorter in the HIV seropositive population, this was not statistically significant (p value = 0.153). See figure 3.8 below.

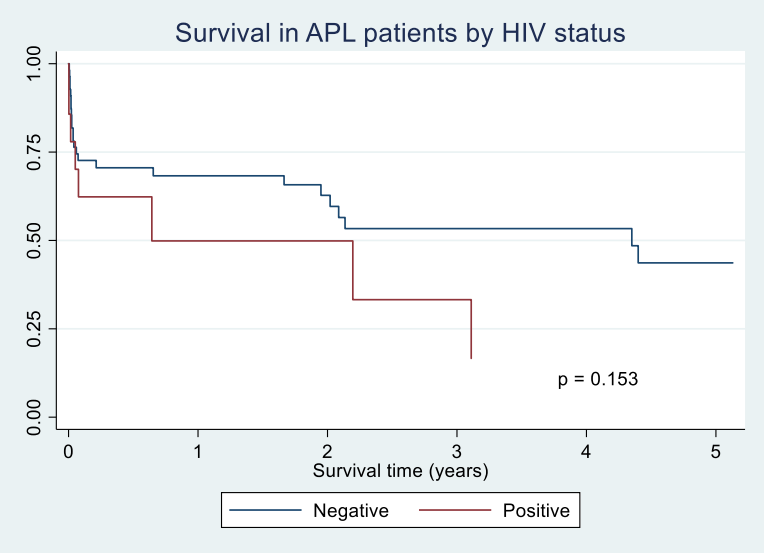

Figure 3.8: Survival in APL patients according to HIV status

The following graph (figure 3.9) depicts the survival trends in high, intermediate and low-risk patients with APL as classified by the Sanz score. There was no statistically significant impact on the survival time across these three risk groups.

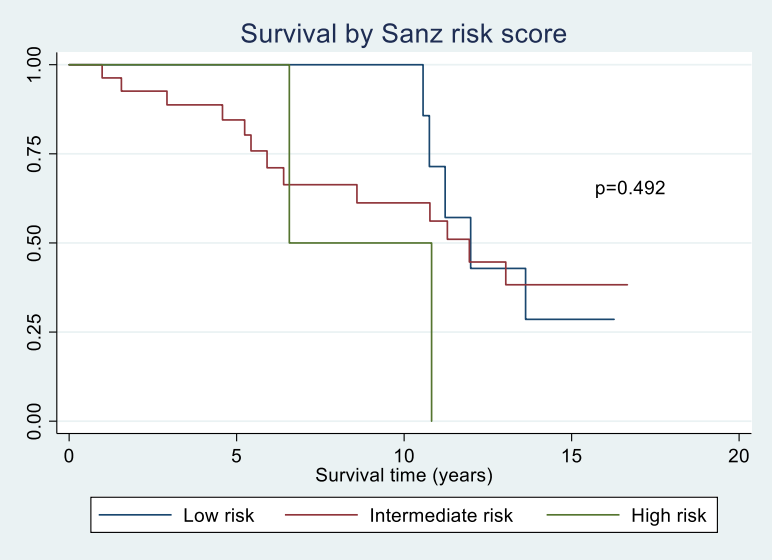

Figure 3.9: Survival in low, intermediate and high-risk APL patients as classified by the Sanz score

4. CHAPTER 4: DISCUSSION

4.1. Demographics of APL

Acute myeloid leukaemia (AML) is the most common acute leukaemia in adults. APL (acute promyelocytic leukaemia) is a subtype of AML. Compared to APL, AML has a much older age of incidence, while APL is predominantly a disease of young to middle-aged individuals.[14] Both AML and APL have a younger age of incidence in African studies which is reflected in the findings of this study. The median age at presentation of patients with APL in this study was 32 years with an interquartile range of 24 – 46 years. The median age of AML is 67 years, but in two studies in Johannesburg it was found to be 41 years and 45 years, respectively.[9, 11, 14]

The distribution of males and females was equal, as 49.37% of our patients were male, and 50.63% were female. The male: female ratio was 1:1.03. Studies of APL patients in Johannesburg found that 48% and 52.4% of patients were male, and 52% and 47.6%, were female, respectively, and a Cape Town study found that 44.93% of patients were male, and 55.07% were female.[9, 11, 14, 39] _ENREF_11 In the PETHEMA and GIMEMA studies performed in APL patients, 56% and 46.3% of patients were male, and 44% and 53.7% were female, respectively.[18] In the United States of America, the male: female ratio in AML patients is 1:0.69.[10]

The prevalence of APL in our Clinical Haematology Unit has increased over the study period, with the average number of patients presenting per year doubling from two to four patients from the first to the second half of the study. This could be attributed to the growth in the population that Chris Hani Baragwanath Academic Hospital (CHBAH) serves, better and improved case ascertainment (improved and more accurate diagnostic modalities) and the ongoing HIV pandemic.

The majority of patients in this study were of Black African ethnicity (96.2%), which reflects the demographics of the population that CHBAH serves.

HIV was the most common co-existing comorbidity in this study, being present in 17.72% of all patients. It is not clear if there is a causative link or an association between HIV and APL, or if this is simply a reflection of the high prevalence of HIV in this particular age group, in which APL commonly occurs. There was a clear and noticeable increase in the number of APL patients with HIV from the first to the second half of the study. From 1994 to 2006 there were only four HIV positive patients, and from 2007 to 2019 there were ten HIV seropositive patients. The number of patients diagnosed with APL had also increased over this timeframe, so this may reflect the increased numbers of HIV seropositive patients in the South African context as indicated above. The incidence of HIV was skewed with females being more commonly co-infected than males, at 27.5% compared to 7.69%, which likely reflects the demographic of younger female patients most commonly affected by HIV in South Africa. In the previous AML study at CHBAH mentioned above, of the HIV seropositive patients, 59.3% were females and 40.7% were males.[9]

In the current study, there were 14 patients that had concomitant HIV and APL. There was data missing on the CD4 counts and viral loads, but half of these patients had a CD4 count of > 200 and four had suppressed viral loads.

Hypertension (in 12.66% of patients) and diabetes (7.59%) were the next most common comorbidities. Again, this may reflect the high burden of lifestyle disease in the South African population. Four patients in the study had tuberculosis, two had asthma, two had epilepsy and there was one patient each with bipolar mood disorder, Kaposi sarcoma, rheumatic heart disease, psychosis and gout, respectively.

4.2. Presentation

4.2.1. Symptoms

The most common symptom at presentation in these patients was bleeding, found in 82.05% of APL patients. This is in keeping with the APL coagulopathy, including DIC, which is commonly found at presentation.[6] Most commonly, bleeding was noted at multiple (two or more) sites, in 60.76% of the patients, following by gum bleeding in 50.63%, cutaneous bleeding in 43.04%,

epistaxis in 30.38% and bleeding at venepuncture sites in 26.58% of patients on presentation. Twelve patients had vaginal bleeding, nine had bleeding from the buccal mucosae, six patients had haemoptysis, and there were also patients with bleeding at multiple other sites. Despite the increased clotting tendency in APL, only 4.29% of the patients had thrombosis at presentation. These findings at presentation are similar in comparison to the study performed in Cape Town, where 53 of the 69 patients presented with bleeding, as well as other international studies, emphasising the importance of bleeding in APL patients at presentation.[25, 28, 39]

Other symptoms noted at presentation included fatigue (74.65%), malaise (61.43%), weakness (54.93%), weight loss (41.43%), fever (35.71%), night sweats (34.78%) and anorexia (26.09%).

4.2.2. Clinical signs

As would be expected from the nature of the disease with bone marrow infiltration in all the patients and the high incidence of bleeding at presentation, the most common clinical sign at presentation was pallor, being clinically apparent in 81.01% of the patients with APL, and manifesting with anaemia based on the laboratory parameters in 93.42% (see below). Other clinical findings included lymphadenopathy (29.11%), hepatomegaly (21.52%), splenomegaly (5.06%) and jaundice (3.08%), all of which were more likely to be coincidental or associated with other concomitant comorbidities such as HIV and tuberculosis, as APL itself is less likely to cause organomegaly, lymphadenopathy or jaundice.

4.2.3. Laboratory parameters

At presentation, the median WCC was 3.51 x 10^9/l (grade one leucopenia), haemoglobin was 6.55 g/dl (grade three anaemia) and platelet count was 24 x 10^9/l (grade four thrombocytopenia).

Seventeen patients (22.37%) had a normal WCC, and 20 (26.32%) had a WCC of > 10 x 10^9/l; in keeping with a high-risk APL as classified by the Sanz score. 59.21% of the patients had an intermediate-risk APL at presentation with a WCC ≤ 10 x 10^9/l and platelet count ≤ 40 x 10^9/l, and 14.47% of patients were classified as low-risk, with a WCC ≤ 10 x 10^9/l and platelet count >

40 x 10^9/l.[7] In the Cape Town study, 39.13% of patients had a high-risk APL.[39] In a study in China, patients were split into an early death group, where the incidence of high-risk APL was 61.2%, and a group of patients who achieved complete remission, where the incidence of high-risk APL was 29.4%.[25] In the LPA99 and LPA2005 trials, 25% and 29% of patients, respectively, had a high-risk APL, which is most similar to the percentage seen in our study (26.32%).[7]

At presentation, the majority of the patients with APL in the study had a leucopenia, seen in 51.32% of patients. In keeping with this was a high incidence of neutropenia seen in 61.29% of the patients at presentation. The absolute neutrophil count was normal in 20.97% of patients and high in 17.74%.

As indicated above, there was a high percentage of patients with anaemia at presentation - 93.43%. 50% of all the patients with anaemia had a grade four anaemia with a haemoglobin of < 6.5 g/dl.

Clinical thrombocytopenia (platelet count < 100 x 10^9/l) was almost invariable at presentation in our population of patients with APL. 97.37% of patients had a clinical thrombocytopenia and there was a grade four thrombocytopenia in 51.32% of patients (platelet count < 25 x 10^9/l). This is consistent with observations in other studies and is expected based on the bone marrow infiltration, concomitant DIC and sepsis associated with an AML, in particular APL.[27]

As anticipated with the APL coagulopathy, there was a high percentage of patients who presented with DIC (63.93%). DIC is both a clinical and biochemical diagnosis and the criteria used here included the presence of thrombocytopenia, one of either a prolonged INR, PTT or low fibrinogen indicating coagulation factor consumption as well as a raised D-dimer level as evidence of fibrinolysis. This DIC picture was quick to resolve once treatment was initiated. At ten days post treatment 25.93% of the patients had evidence of DIC and following the first cycle of therapy there were no longer any patients with DIC. This is consistent with the findings in a study performed in China where induction therapy with ATRA / ATO and chemotherapy relieved the coagulopathy associated with APL.[4]

The full blood count also improved significantly following treatment, with the median WCC, platelets and INR normalising by the first clinic visit and haemoglobin improving to a median value of 11.9 x 10^9/l.

4.2.4. Bone marrow assessment

This study was performed over a long time period, and the diagnosis of APL was made based on methods available at the time. Initially, the diagnosis was made clinically, morphologically and with the use of immunohistochemistry. Later, flow cytometry and cytogenetics were available and these methods were also used. Finally, the diagnosis could be made molecularly. Towards the latter part of the study, the diagnosis was more comprehensive, using both molecular and cytogenetic techniques. When bone marrow morphology was assessed, 67 patients (91.78%) had the typical, hypergranular morphology. Six patients had a hypogranular variant, which is associated with a worse prognosis.[19] Four of these patients achieved remission post induction. In terms of outcomes, one of these six patients died before treatment could be initiated, one patient died at eight months, two patients were lost to follow up and two patients were in remission at the last date that they were seen.

The most commonly found immunophenotype in our population of APL patients was a positive CD33 (62.03% of patients), CD13 (62.03%), CD15 (26.58%) and CD117 (20.25%). Typically, APL is characterised by a negative or weakly positive CD34 (this was positive in 15.19% of our patients), HLD-DR negativity (positive in 17.72% of our patients), CD13 and CD33 positive (both positive in 62.03% of patients in our study), CD11b negative (this was only positive in 8.86%), CD117 variable (positive in 20.25%) as well as expression of CD2 and CD56 (which were positive in 18.99% and 2.53% of our patients, respectively).[16] It is also noted that a poorer prognosis can be expected with positivity of CD2, CD34 and CD56 which was not an uncommon immunophenotype in our patients.[16] CD2 and CD34 positivity have also been noted to be associated with a more significantly raised WCC.[16]

The immunophenotype in our high-risk population of APL patients as defined by the Sanz score was similar to that seen in the general population of APL patients. However, these patients had a

higher percentage of CD2 positivity at 30%, CD34 positivity at 25% and CD56 positivity at 5%, which is in keeping with the worse prognosis predicted by both the immunophenotype and the high-risk nature of the disease conferred by the high WCC.
There was a t(15;17) in 49 of the 51 patients that were tested. This is associated with a good prognosis due to a good response to ATRA.[15] Two patients had additional cytogenetic abnormalities, specifically a t(15;17)(q24;q21) with additional material on the long arm of chromosome 7, and t(15;17) with add(4q), del(9p). Eight patients were tested for FLT3-ITD and one was positive; this patient also had a positive t(15;17).

Additionally, there were 38 patients who tested positive for PML-RARA, 29 of whom also had a positive t(15;17). In patients with a positive PML-RARA, even without a t(15;17), the prognosis is favourable as they still have a good response to ATRA.[15] A high level of PML-RARA expression, however, is a predictive factor for a worse prognosis.[19]

4.3. Management

4.3.1. Delays in referral and treatment

The majority of patients diagnosed with APL were either referred to our facility immediately (80.77%) or the following day (12.82%), with the remaining 6.4% of patients experiencing a delay of between two and 15 days before being referred to, or being seen at our Clinical Haematology Unit. It is difficult to explain the reasons for the delays, due to the retrospective nature of the study, but it may be attributed to delays in getting results from the laboratory, transport issues or access to healthcare. It is also difficult to ascertain the duration of symptoms that the patients experienced before they were referred to our centre.

There were also delays once patients had been referred to the Clinical Haematology Unit before treatment was initiated. 51.38% of patients were initiated on therapy the same day, and 16.67% the following day, with the remaining patients experiencing delays between two and seven days, with one patient each experiencing a delay of 11 and 19 days, respectively.

4.3.2. Supportive management

There was a large number of patients in this study that needed supportive management of the coagulopathy associated with APL in the form of blood and blood products. 86.08% of patients required blood transfusions, with a median of three units of packed red cells per patient. 75.95% of patients required platelets, with a median of three units of platelets per patient. 45.57% of patients also received FFPs, with a median of four units transfused per patient.

Additionally, patients received allopurinol, analgesics, antibiotics and thromboprophylaxis (four patients) where indicated. Recent recommendations on the management of the APL coagulopathy by Sanz et al in 2020 suggest that heparin and other anticoagulants should not be used except in the setting of a clinical trial, as there is no clear evidence of benefit for such a therapeutic intervention.[6]

4.3.3. Chemotherapy

The standard of therapy for APL during induction currently includes the use of ATRA, an anthracycline and ATO in different combinations.[4] In our study, it was found that 91.14% of patients received ATRA (data was missing for three patients). Induction therapy also included the use of daunorubicin in 82.61% of patients, and 20.29% received cytarabine as well. ATO was not readily available in the South African public sector for induction therapy in APL during the study period. ATO was procured for one patient, at relapse.

At the time this study was conducted, ATO could only be obtained under specific circumstances. The Medicines Control Council (MCC) (now SAHPRA, the South African Health Products Regulatory Authority), would only authorise the use of ATO if a patient required two subsequent re-induction therapies for failed induction, or had a relapse of disease. Although it is not clear which anthracycline may be best for induction, there is some evidence to suggest that idarubicin may result in improved survival in comparison to daunorubicin.[14, 30]

Consolidation in APL is recommended to be given in three cycles of monthly therapy once remission has been achieved following induction as per the LPA2005 trial.[7] This trial used ATRA with an anthracycline and high-risk patients were also given cytarabine.[7] In our study, 50, 41, 29 and eight patients, respectively received one, two, three and four cycles of consolidation therapy for APL. The most commonly received regimen consisted of ATRA and daunorubicin, consistent with this evidence (50%, 60.98%, 44.83% and 12.5% in the first, second, third and fourth consolidation cycles, respectively). Eleven patients also received cytarabine (22%, 14.63%, 10.34% and 0%, respectively), and in this study it was noted that two of these patients had a high-risk APL according to the Sanz criteria, and the remaining nine were all evaluated as intermediate risk based on their WCC and platelet counts at presentation. Some patients received alternative consolidation regimens (28%, 24.39%, 44.83% and 87.5%, respectively – see Table 3.21).

A large randomised control trial also showed benefit in survival with the use of ATO combined with ATRA and daunorubicin for consolidation therapy, however, ATO was only available in South Africa on a named-patient basis through approval by the MCC / SAHPRA during the study period.[14]

There were 23 patients in our study who were documented to have received maintenance therapy, which consisted of ATRA, methotrexate and mercaptopurine in all of these patients, consistent with the regimen in the PETHEMA trial.[30] It is likely that many more patients at our Clinical Haematology Unit received maintenance given the much higher number of patients who achieved remission. The reasons for patients not receiving maintenance therapy could be death, relapse of disease or loss to follow up. Due to the retrospective nature of the study, some of this information was missing, likely either as it was not documented or misplaced. The GIMEMA group in the AIDA-0493 and AIDA-2000 trials, found that patients receiving ATRA as part of maintenance therapy had better outcomes and fewer relapses.[33]

Three patients received intrathecal chemotherapy, but it was not clear from the data what the indications for this were. Intrathecal chemotherapy is typically used for relapsed APL as there is

a higher risk of CNS disease in relapsed APL. One of these three patients was noted to be receiving re-induction therapy at the time for relapsed APL.

One of these patients (the only one in the study) also received an autologous haematopoeitic stem cell transplant (HSCT). Intrathecal therapy (in the same patient) was received with re-induction therapy for a relapse following induction and three cycles of consolidation therapy. Two cycles of consolidation were given, followed by the HSCT, but unfortunately the patient relapsed for the second time six months following this (one month following re-induction treatment) and subsequently demised.

There were 21 patients in this study that required re-induction therapy; five of whom had failed induction. Of these five patients, two failed induction and subsequently relapsed requiring another re-induction. There were 12 patients who had a single re-induction and of these, six were still in remission at the last date seen. Seven patients required two re-inductions (these patients all relapsed a second time) but were unable to receive any further therapy prior to their demise. Most commonly the regimen for re-induction used was ATRA, daunorubicin and cytarabine.

4.3.4. Complications

Bleeding complications were most commonly encountered in the first 30 days, in 83.54% of the patients with APL in our study. In the Cape Town study, four of the nine cases of early death were the result of intracranial haemorrhage.

Throughout the duration of the disease, the next most commonly occurring complications were infection and renal failure. In the Cape Town study, infection was also a common complication. Three patients died in the first 20 days of probable sepsis, and of the six patients that died during maintenance, one died of sepsis and one died of disseminated tuberculosis.[39] Three died after maintenance, one of whom died from pneumonia.[39] Renal failure in APL could have multiple aetiologies, with the most likely being prerenal failure from infection or hypovolaemia secondary to blood loss. It is thus important that renal protective strategies and monitoring as well as infection prevention measures are implemented in these patients.

A very high percentage (89.33%) of all patients had at least one early complication, and in addition to infection and renal failure, these patients developed the differentiation syndrome, haemorrhage and thromboembolic disease. In our study, 44.68% of patients had at least one late complication (i.e., from 30 days following initiation of therapy) (see Table 3.24).

4.4. Outcomes

At the last encounter with each patient in our unit, 39.24% of patients were in remission, 50.63% had demised, 1.27% had relapsed, the status of the disease in another 3.8% was unclear, and the outcome for the remaining 5.06% of patients was unknown.

A total of 37 patients achieved remission following induction, and 21 patients were in remission post consolidation. Thirty-one patients were in remission at the last date they were seen in the Clinical Haematology Unit.

There were 19 patients in this study who relapsed, three of whom relapsed during non-adherence to therapy. The majority of these relapses occurred during maintenance, at 63.16%.

There were 26 patients who were lost to follow up, and information was obtained for six of them; five were alive and well and one patient had demised.

4.4.1. Mortality

Six patients demised before treatment could be given. Of these, three deaths were attributable to bleeding. Immediate mortality (death in the first three days of treatment being initiated) occurred in twelve patients and was again most commonly caused by bleeding, but early mortality (death occurring after more than three days after treatment initiation), occurring in six patients, was more likely to be the result of infection, as was seen in late mortality, occurring in 15 patients (more than thirty days after treatment initiation). This is consistent with what is found in the literature, with immediate mortality likely to be caused by bleeding and early mortality likely to

be due to infection or the differentiation syndrome.[25] Overall, there were ten cases of mortality as a result of haemorrhage, and nine of these were attributable to intracranial haemorrhage. Again, this is consistent with other studies which have shown that bleeding is likely to be intracranial in 65% of cases.[6] In comparison to the Cape Town study, five patients (7.24%) died with seven days (four of which had intracranial haemorrhages), and nine patients (13.04%) died within 30 days (another four of whom had intracranial haemorrhages).[39] None of their patients died during consolidation, six died during maintenance and another three died following maintenance.[39]

4.4.2. Survival

The median survival time in our patients with APL was 7.8 months (0.65 years). When early mortality was excluded, the median survival time improved to 43.7 months. In the Cape Town study, overall survival at three years was 76.5%.[39] In the Tunisian study, there was a five year overall survival of 78%.[5] In the LPA2005 study, overall survival of all patients at three years was 89%, and 88% at four years.[7] In our study, overall survival at three years was closer to 50%, which is inferior to that described in local and international literature. There are a number of reasons that could explain this, including the high early mortality (removal of which demonstrated a much higher survival time) and barriers to receiving timeous treatment and to ongoing access to healthcare.

Survival time appeared shorter in the HIV positive population in this study, but this difference was not statistically significant.

Other factors that have an influence on prognosis include the Sanz risk score, FLT3-ITD and NPM1 mutations, variant translocations, a hypogranular APL variant and a high level of PML-RARA expression.[8, 15, 19, 20]

There were 20 patients in this study who were classified as high-risk APL based on a WCC > 10 x 10^9/l according to the Sanz score, 45 patients with an intermediate-risk APL (WCC ≤ 10 x 10^9/l and platelet count ≤ 40 x 10^9/l) and 11 patients with a low-risk APL. The differences in survival time in the different risk groups was not statistically significant.

There were eight patients who were tested for FLT3-ITD, and one who had a positive result. One patient was tested for NPM1 and had a negative result; therefore, no conclusions can be drawn about the effect of these mutations on prognosis, based on the very small number of patients tested for these mutations.

There were only two patients who had additional cytogenetic abnormalities in this study. Both of these patients had bleeding complications within the first 30 days, and one patient died before treatment could be initiated. The other patient achieved remission post induction, relapsed during maintenance and demised following an intracranial haemorrhage at 32 months following initial therapy.

There were six patients who had a hypogranular variant, four of whom had early complications in the form of haemorrhage, and one who had renal failure. Four of these patients achieved remission post induction. One patient died before treatment could be initiated, two were lost to follow up after two and three months, respectively, and one died at eight months following therapy. The remaining two survived 13 and 124 months, respectively, and were in remission at the last date that they were seen.

4.5. Limitations of the study

Due to the retrospective nature of the study, there were a number of patients for whom data was missing, either due to inadequate record keeping or missing information. There was also a large percentage of patients who were lost to follow up, i.e., 26 patients which equates to 32.91% of the entire study population.

5. CHAPTER 5: CONCLUSION

Acute Promyelocytic Leukaemia (APL) is a subset of AML. 79 patients with APL were seen and managed at the Clinical Haematology Unit, CHBAH, over the 26-year period during which this study was conducted.

In our patient population, the male: female ratio was 1:1.03. The mean age was 32 years, which is consistent with the younger age of presentation of APL. HIV was the most common comorbidity found in this study. There was no overt association or increased prevalence of patients with this dual pathology and the relationship between the two diseases is likely coincidental, as both occur in the young to middle age group.

The disease manifests with a coagulopathy including a DIC, which was reflected in the clinical presentation of these patients. 82.05% of patients presented with bleeding and 60.76% had bleeding at two or more sites. Conversely, only 4.29% of patients presented with features of thrombosis. The median haemoglobin was 6.55 g/dl, with anaemia being present in 93.42% of patients. The median platelet count was 24 x 10^9/l at presentation, and 97.37% of patients had a clinical thrombocytopenia. 63.93% of patients had evidence of a DIC at presentation. These laboratory parameters improved rapidly following initiation of supportive and specific treatment.

High-risk APL (based on the Sanz risk score) was evident in 26.32% of the patients. There was also a high percentage of patients with an immunophenotype which has been associated with a higher WCC and thus a worse prognosis, with positivity of CD2 in 30%, CD34 in 25% and CD56 in 5% of these high-risk patients.

There were some delays in referral, and some delays in the initiation of treatment which may have also contributed to poorer outcomes. However, what is unclear is the duration of patient symptoms prior to referral to our treatment centre, as this could have impacted directly on the prognosis and outcome of our patients. This evidently contributed to the significant early mortality (30.38%) of the patients.

Management consisted of supportive therapy with blood and blood products and specific modalities of treatment including ATRA and chemotherapy. Induction therapy included ATRA in 91.14% of patients and daunorubicin in 82.61% of patients. Consolidation was given with ATRA and daunorubicin, consistent with the recommendations from the LPA2005 trial. Maintenance therapy was given with ATRA, methotrexate and mercaptopurine in all 23 patients who received it, consistent with the regimen in the PETHEMA trial. However, it is likely that more than 23 patients received maintenance that was not documented given the much higher number of patients who achieved remission.

There were 21 patients who were given re-induction therapy, either for failed induction (five patients) or for a relapse of disease; this most commonly consisted of ATRA, daunorubicin and cytarabine.

Early complications (within the first 30 days following initiation of treatment) occurred in 89.33% of patients, most commonly with bleeding in 83.54% of patients), followed by infection and renal failure. Pre-treatment mortality in six patients and immediate mortality in a further 15 patients were most often the result of bleeding. Early and late causes of mortality, however, were more likely to be due to infection. 44.68% of patients also had at least one late complication.

There were 37 patients who achieved remission following induction therapy. At the last seen date there were 31 patients (39.24%) in disease remission, 50.63% of patients had demised and 3.8% had relapsed at this time.

The median survival time in this study was 7.8 months, and 43.7 months when early mortality was excluded. There was no statistically significant difference in the survival time when HIV positive and negative patients were compared, nor when the different risk groups as classified by the Sanz score were compared.

The recommendations following this study are that further information is needed on the relationship between HIV and APL and prospective studies looking more closely at this association is warranted.

Furthermore, education of health care professionals on the early diagnosis and timeous referral of patients with APL is of paramount importance, in order to prevent any unnecessary delays and facilitate early treatment, with a view to preventing pre-treatment, immediate and early mortality.

Broader education of the community with regard to anaemic symptomatology and signs and bleeding manifestations is also warranted and necessary to improve the outcome of this potentially treatable malignancy.

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Appendix A: Data collection sheet

Confidential

My First Instrument

Record ID	_____
Study number	_____
Gender	_____
Age at diagnosis	_____
Date of diagnosis	_____
Date of haematology referral	_____
Ethnic group	☐ Black ☐ White ☐ Mixed ☐ Asian
Height	_____
Weight	_____
BSA	_____
Performance status (ECOG)	_____
Comorbidities	_____
Chronic medication	_____
HIV	○ Yes ○ No
CD4	_____
VL	_____
Date of HIV diagnosis	_____

ART regimen

Date of ART initiation

Allergies

Family history

Past medical history

Symptoms

	yes	no
Weight loss	○	○
Anorexia	○	○
Fever	○	○
Night sweats	○	○
Thrombosis	○	○
Fatigue	○	○
Pruritis	○	○
Malaise	○	○
Weakness	○	○
Bone pain	○	○
Menorrhagia	○	○
Amenorrhoea	○	○
Impotence / erectile dysfunction	○	○
Bleeding	○	○

Site, duration, amount

Surgery, trauma

Other symptoms

Vitals

Pallor
○ Yes
○ No

Jaundice
○ Yes
○ No

Lymphadenopathy	
Mucus membranes, mouth	
CVS	
Resp	
Abdo	
CNS	
Fundoscopy	
Skin	
MSK	
Other	
CXR	
Other xrays	
US	
CT	
MRI	
presentation WCC	
10/7 Post treatment WCC	
After 1st cycle Rx WCC	

1st clinic visit WCC	
Completion of chemo WCC	
Last seen WCC	
presentation Hb	
10/7 Post treatment Hb	
After 1st cycle Rx Hb	
1st clinic visit Hb	
Completion of chemo Hb	
Last seen Hb	
presentation hct	
10/7 Post treatment hct	
After 1st cycle Rx hct	
1st clinic visit hct	
Completion of chemo hct	
Last seen hct	
presentation MCV	
10/7 Post treatment MCV	
After 1st cycle Rx MCV	

1st clinic visit MCV	_____
Completion of chemo MCV	_____
Last seen MCV	_____
presentation plt	_____
10/7 Post treatment plt	_____
After 1st cycle Rx plt	_____
1st clinic visit plt	_____
Completion of chemo plt	_____
Last seen plt	_____
presentation diff NLMEB	_____
10/7 Post treatment diff NLMEB	_____
After 1st cycle Rx diff NLMEB	_____
1st clinic visit diff NLMEB	_____
Completion of chemo diff NLMEB	_____
Last seen diff NLMEB	_____
presentation UE	_____
10/7 Post treatment UE	_____
After 1st cycle Rx UE	_____

1st clinic visit UE	
Completion of chemo UE	
Last seen UE	
presentation LFT	
10/7 Post treatment LFT	
After 1st cycle Rx LFT	
1st clinic visit LFT	
Completion of chemo LFT	
Last seen LFT	
presentation haematinics	
10/7 Post treatment haematinics	
After 1st cycle Rx haematinics	
1st clinic visit haematinics	
Completion of chemo haematinics	
Last seen haematinics	
presentation UA	
10/7 Post treatment UA	
After 1st cycle Rx UA	

1st clinic visit UA	_____
Completion of chemo UA	_____
Last seen UA	_____
presentation LDH	_____
10/7 Post treatment LDH	_____
After 1st cycle Rx LDH	_____
1st clinic visit LDH	_____
Completion of chemo LDH	_____
Last seen LDH	_____
presentation d dimer	_____
10/7 Post treatment d dimer	_____
After 1st cycle Rx d dimer	_____
1st clinic visit d dimer	_____
Completion of chemo d dimer	_____
Last seen d dimer	_____
presentation INR	_____
10/7 Post treatment INR	_____
After 1st cycle Rx INR	_____

1st clinic visit INR	_____
Completion of chemo INR	_____
Last seen INR	_____
presentation ptt	_____
10/7 Post treatment ptt	_____
After 1st cycle Rx ptt	_____
1st clinic visit ptt	_____
Completion of chemo ptt	_____
Last seen ptt	_____
presentation fibrinogen	_____
10/7 Post treatment fibrinogen	_____
After 1st cycle Rx fibrinogen	_____
1st clinic visit fibrinogen	_____
Completion of chemo fibrinogen	_____
Last seen fibrinogen	_____
BMA	_____
BMT	_____
t(15;17)	○ Yes ○ No

Other variant	_____
CD34	○ Yes ○ No
CD33	○ Yes ○ No
CD13	○ Yes ○ No
CD11b	○ Yes ○ No
CD15	○ Yes ○ No
CD117	○ Yes ○ No
CD2	○ Yes ○ No
CD56	○ Yes ○ No
HLA DR	○ Yes ○ No
Other immunophenotype	_____
flt 3 itd	○ Yes ○ No
pml rara	○ Yes ○ No
Other molecular / cytogenetics	_____
QRT PCR (Date, number)	_____
QRT PCR (Date, number)	_____
QRT PCR (Date, number)	_____
QRT PCR (Date, number)	_____

QRT PCR (Date, number)	_____
ATRA	○ Yes ○ No
Date	_____
Phase, dose, duration	_____
ATO	○ Yes ○ No
Date	_____
Phase, dose, duration	_____
Chemo 1	○ Yes ○ No
Date	_____
Phase, dose, duration	_____
Chemo 2	○ Yes ○ No
Date	_____
Phase, dose, duration	_____
Chemo 3 date phase dose duration	_____
Chemo 4 date phase dose duration	_____
Chemo 5 date phase dose duration	_____
Chemo 6 date phase dose duration	_____
Chemo 7 date phase dose duration	_____

Chemo 8 date phase dose duration

_____

Chemo 9 date phase dose duration

_____

Chemo 10 date phase dose duration

_____

Supportive Treatment (Presentation)

	Yes	No
Blood Transfusion	○	○
Platelet Transfusion	○	○
FFP Transfusion	○	○
Allopurinol	○	○
Thromboprophylaxis	○	○
Hydroxyurea	○	○
Tranexamic Acid	○	○
Analgesia	○	○
Psychology / Social Work	○	○
Physiotherapy	○	○

Blood Number of Units

_____

Platelets Number of Units

_____

FFPs Number of Units

_____

Blood Number of Units

_____

Platelets Number of Units

_____

FFPs Number of Units

_____

Supportive Treatment (Late)

	Yes	No
Blood Transfusion	○	○
Platelet Transfusion	○	○
FFPs Transfusion	○	○

Allopurinol	○	○
Thromboprophylaxis	○	○
Hydroxyurea	○	○
Tranexamic Acid	○	○
Analgesia	○	○
Psychology / Social Work	○	○
Physiotherapy	○	○

Early Complications

	Yes	No
Haemorrhage	○	○
Infection	○	○
Differentiation Syndrome	○	○
TED	○	○
Renal failure	○	○
Tumour Lysis	○	○
Other	○	○

Haemorrhage site, amount

_____

Infection site, organism

_____

Other description

_____

Late Complications

	Yes	No
Haemorrhage	○	○
Infection	○	○
Differentiation Syndrome	○	○
TED	○	○
Renal Failure	○	○
Tumour Lysis	○	○
Other	○	○

Haemorrhage late site amount

_____

Infection late site organism

_____

Other late description

_____

Death		
	Yes	No
Pretreatment	○	○
Immediate	○	○
3 - 30 days post induction	○	○
Late > 30 days	○	○

Cause of death

_____

Survival time

_____

Response to treatment (Remission)		
	Yes	No
Post Induction	○	○
Post Consolidation	○	○
Post Maintenance	○	○
Relapse	○	○

Relapse when during course, treament

_____

Response at last date seen		
	Yes	No
Complete response	○	○
Partial response	○	○
Remission	○	○

Survival time in months

_____

Lost to Follow Up

○ Yes
○ No

Last date seen

_____

Status of disease at last visit

_____

Other Comments

_____

Appendix B: Plagiarism 'Turn-it-in' report

turnitin

Digital Receipt

This receipt acknowledges that Turnitin received your paper. Below you will find the receipt information regarding your submission.

The first page of your submissions is displayed below.

Submission author:	Brigid McMillan
Assignment title:	Final Similarity Report
Submission title:	Final MMed-1.docx
File name:	Final_MMed.docx
File size:	2.85M
Page count:	113
Word count:	22,365
Character count:	119,283
Submission date:	12-Apr-2022 10:26PM (UTC+0200)
Submission ID:	1809096459

ACUTE PROMYELOCYTIC LEUKAEMIA IN ADULTS
AT CHRIS HANI BARAGWANATH ACADEMIC HOSPITAL

Appendix C: Ethics Committee clearance report

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

R14/49 Dr Brigid McMillan

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M191010

NAME: (Principal Investigator)	Dr Brigid McMillan
DEPARTMENT:	Internal Medicine Chris Hani Baragwanath Academic Hospital
PROJECT TITLE:	Acute promyelocytic Leukemia (APL) in adults at Chris Hani Baragwanath Academic Hospital
DATE CONSIDERED:	25/10/2019
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof Moosa Patel and Dr Farah Rahman
APPROVED BY:	Dr. C Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	01/11/2019

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 **October** and will therefore be due in the month of **October** 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

Appendix D: Ethics Committee extension clearance report

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

2020/10/15

Dr B McMillan
School of Clinical Medicine
Department of Medicine
Division of Internal Medicine
Medical School
University

Sent by e-mail to: brigidmc2101@gmail.com

Dear Dr McMillan

Re: **Protocol Ref No: M191010**
Protocol Title: *Acute promyelocytic leukemia (APL) in adults at Chris Hani Baragwanath Academic Hospital*
Principal Investigator: Dr B McMillan

Thank you for your letter of 2020/10/02.

I confirm that we have noted and approve of your proposal to extend the study period by 12 months, to 31/12/2019, in order to include an additional 10 patients diagnosed with APL in that year.

Thank you for keeping us informed.

Yours Sincerely

.....
Mr I Burns
For the Human Research Ethics Committee (Medical)

.....
Dr CB Penny, Chairperson, Human Research Ethics Committee (Medical)

Works2000/lain0007/Acknowledge.docx