A FIFTEEN YEAR REVIEW OF CHRONIC LYMPHOCYTIC LEUKAEMIA IN ADULTS, AT CHRIS HANI BARAGWANATH ACADEMIC HOSPITAL

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ETHICS COMMITTEE APPROVAL

This study was approved by the Human Research Ethics Committee (HREC) (Medical), University of the Witwatersrand: Clearance certificate number: M200748 MED20-05-084 (see Appendix A).

DECLARATION

I, Cain Mikateko Khosa, declare that this research report is my own, unaided work. It is being submitted for the degree of Master of Medicine (in the speciality of Internal Medicine) to the University of the Witwatersrand. This research report has not been submitted before for any degree or examination at this or any other University.

I am aware that plagiarism is a serious offence and is tentamount to fraud. I have not attempted to pass off another author or entity's work, words or ideas as my own. I have not misrepresented my findings as novel or original if there is an existing source. All the work has been referenced appropriately.

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Signature of candidate

-18th---------- day of June----------, 2024, at Tobias Building----------

DEDICATION

This research report has been dedicated to my parents and family, who have always served as a pillar of strength and an enormous sense of inspiration and support throughout my life.

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1. The Clinical Haematology Unit (team) and the Department of Medicine at Chris Hani Baragwanath Academic Hospital (CHBAH), including the medical, nursing and allied staff,
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ABSTRACT

a. Background:

Chronic Lymphocytic Leukaemia (CLL) is one of the four common types of leukaemia encountered in adults. CLL is characterized by the clonal proliferation and accumulation of small, mature, neoplastic, CD-5 positive, B-lymphocytes in the blood, bone marrow and lymphoid tissues. There are geographical variations in the incidence of CLL worldwide, with CLL being the commonest form of leukaemia in some parts of Europe and the Western World. The median age at diagnosis is approximately 70 years, with less than 10% of patients presenting under 45 years of age. Most studies show a male predominance of 1.5-1.9:1. While the incidence in Europe is similar to that reported in the United States, the incidence is lower in Asia and Africa. Moreover, in Africa, the disease tends to present in individuals who are 5-10 years younger, primarily because of the younger age structure of the African population.

At Chris Hani Baragwanath Academic Hospital (CHBAH), CLL ranks 5th in order of frequency, amongst the haematological malignancies that are encountered in adult patients. Based on a small study done at CHBAH in 1994, the disease presents at a younger median age of 63 years, with a male predominance of 1.5:1.

Although CLL is generally a stable disease at CHBAH, there has been a noticeable increase in the number of patients by 1.5 fold, from 2015 to 2019. This study was undertaken to better characterize and describe the demographics, clinical and laboratory features, staging and treatment outcome of adult patients with CLL, seen at our centre over a 15 year period.

b. Patients and Methods:

This was a retrospective study of all adult patients with a confirmed diagnosis of CLL, seen over a 15 year period (01/01/2005 to 31/12/2019), at the Clinical Haematology Unit, Department of Medicine, CHBAH (15 years). Demographic, clinical, and therapeutic data was retrieved from the patient files and laboratory data from the NHLS data base. Data was obtained retrospectively from patient files, captured onto a data sheet and entered onto an Excel spread sheet prior to statistical analysis, using a programme such as Stata/Statistica (and/with the assistance of a statistician). The patient demographics were summarized using descriptive statistics for dependent variables that are normally distributed, including means and standard deviations. For comparisons between normally distributed variables, a Student t-test was be used. For comparing the different staging systems, a Chi squared test was used. Where a comparison was required in more than two groups, the Anova test was used.

When data was not normally distributed, the Mann-Whitney or Kruskal-Willis test was used for correlation between variables. For the purpose of statistical analysis, a 95% confidence interval, with a p-value ($p<0.05$) was considered significant.

c. Results and Discussion:

The key findings in this study were:

1. A stable number of patients in the first ten years of the study (01/01/2005 to 31/12/2014), with a 1.5 fold increase in the latter 5 years of this study (2015-2019).
2. A younger median age of 64 years, with a male predominance of 1.87:1.
3. Most of the patients were symptomatic, with an ECOG PS ≥1 in 92.8% of the patients.
4. Fatigue (49.2%), loss of weight (47%) and fever (43%) were the most common symptoms at presentation.
5. Lymphadenopathy was the dominant physical sign (91.2%). Hepatosplenomegaly was evident in 49.2% of the patients.
6. The vast majority of patients had anaemia (82.9%), with a mean haemoglobin of 9.44 g/dl. The mean white cell count and lymphocyte counts were 173 $x10^9$/l and 158.7 x 10^9/l, respectively. The mean platelet count was 155 x 10^9/l. Clinical thrombocytopenia was present in 37% of the patients.
7. More than half the patients presented with advanced stage/high risk disease, with a Rai stage III and IV accounting for 62.5% and Binet C for 56.4% of the patients at presentation.
8. A diffuse pattern of bone marrow infiltration, indicating adverse prognosis was evident in 89.5% of the patients.
9. Cytogenetics showed a favourable genotype (13q) in 43%, an intermediate phenotyope (trisomy 12) in 31.7% and an unfavourable phenotype (11q and 17p) in 14% and 11%, of the patients, respectively.
10. HIV sero-positivity was present in 8.8% of the patients, with sero-positive patients showing a number of differences, including a younger median age at presentation, a more marked male predominance, similar clinical presentation and staging of the disease, a higher proportion of TB, hepatitis B and C, and a lower mean survival, with a similar median survival.
11. Supportive care only was offered to 22.7%, while chemotherapy was administered to 69% of the patients.
12. The outcomes of the patients at the end of study were: i) Lost to follow up (65.2%), ii) Deceased (31.5%) and Alive (3.3%).

13. The mean survival for the whole group was 32 months, with a median survival of 6 months, while for the HIV sero-positive group the median survival was 20 months, with a median survival of 6 months.

d. Conclusions and future recommendations:

Based on the findings of this study, the following conclusions and future recommendations are suggested:

Education with regard to the key clinical manifestations of CLL, early suspicion of the possible diagnosis and timeous referral to a tertiary or specialized centre, so that the diagnosis can be confirmed and appropriate treatment (where indicated), can be initiated as soon as possible.

Every effort should be made to improve compliance and attendance at follow up visits. This is vital in order to assess response to treatment and to detect early relapse or progression of the disease.

Efforts to improve accessibility of ‘state of the art’ and novel therapies for public sector patients should be prioritised and ongoing.

Prospective, randomised, multi-centre studies should be performed to assess the benefits of various therapeutic options and to compare existing therapies with novel treatment options in our local South African patient population (including both the private and public sector).

Although HIV sero-positivity is not a major problem, the numbers of sero-positive patients is steadily increasing, with a doubling of the number in the latter 5 years, compared to the first 10 years of the study. In principle, these patients should be offered the same treatment options as HIV-1 sero-negative individuals, with the proviso that every attempt is made to achieve optimal virological suppression and immune reconstitution, with combination anti-retroviral therapy.

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LIST OF ABBREVIATIONS

A-Dex:	Acalabrutinib, Dexamethasone
AIDS:	Acquired Immunodeficiency Syndrome
AIHA:	Autoimmune haemolytic anemia
AIT:	Auto-immune thrombocytopenia
ALL:	Acute lymphoblastic leukaemia
ATM:	Ataxia-Telangiesctasia Mutated gene
Bcl-2:	B-cell lymphoma 2
B2M:	Beta-2 microglobulin
BR:	Bendamustine, Rituximab
BTK:	Brutan Tyrosine Kinase
CAR:	Chimeric antigen receptor
cART:	Combination Anti-retroviral Therapy
CC:	Cyclophosphamide, Cladribine
CCM/CMC:	Cyclophosphamide, Cladribine, Mitoxantrone
CD:	Cluster of differentiation/designation
CHBAH:	Chris Hani Baragwanath Academic Hospital
CHOP:	Cyclophosphamide, Hydroxydaunorubicin/Adriamycin, Oncovin, Vincristine, Prednisone
CK:	Complex karyotype
CLL:	Chronic lymphocytic leukemia
CLL-IPI:	Chronic lymphocytic leukaemia International Prognostic Index
CML:	Chronic myeloid leukaemia
CVP:	Cyclophosphamide, Vincristine, Prednisone
ECOG:	Eastern Cooperative Oncology Group
FA:	Fludarabine, Alemtuzumab
FBC:	Full Blood Count
FC:	Fludarabine, Cyclophosphamide
FCR:	Fludarabine, Cyclophosphamide, Rituximab
FISH:	Fluorescent-in-situ-hybridization
Hb:	Haemoglobin
HIV:	Human immunodeficiency virus
HL:	Hodgkin lymphoma
HREC:	Human Research Ethics Committee

HSC: Haematopoietic stem cell
IGHV: Immunoglobulin gene heavy-chain variable-region genes
IKZF3: Ikaros family zinc finger 3 gene
ITP: Immune Thrombocytopenia
iwCLL: International workshop on CLL
LDT: Lymphocyte doubling time
Len-R: Lenalidomide, Rituximab
MBL: Monoclonal B-cell lymphocytosis
MCL: Mantle cell lymphoma
MRD: Minimal residual disease
MYD-88: Myeloid differentiation primary response 88
MMed: Master of Medicine
NHL: Non-Hodgkin lymphoma
NMA: Non myeloablative
NOTCH-1: Neurogenic locus notch homolog protein 1
O-CLB: Obinutuzumab, Chlorambucil
OS: Overall survival
PCR: Polymerase chain reaction
PD: Progession of disease
PI3K: Phosphatidyl inositol 3 kinase
PLL: Prolymphocytic leukaemia
Plt: Platelets
PR: Partial response
PS: Performance status
PRCA: Pure red cell aplasia
R-CHOP: Rituximab plus CHOP
R-CLB: Rituximab, Chlorambucil
SCT: Stem cell transplantation
SD: Stable disease
SEER: Surveillance E and End Results
SLL: Small lymphocytic lymphoma
TLS: Tumour lysis syndrome
TP-53: Tumour protein 53
TB: Tuberculosis
ZAP-70: Zeta-associated protein 70

CHAPTER 1: LITERATURE REVIEW

1.1 Introduction

Chronic lymphocytic leukaemia (CLL) is one of the four common types of leukaemia, and is usually encountered in the middle aged and elderly population. CLL is characterized by the clonal proliferation and accumulation of small, mature, neoplastic, CD-5 positive, B-lymphocytes in the blood, bone marrow and lymphoid tissues (1,2,3).

Although CLL is classified as one of the chronic lymphoid leukaemias (of which there are a number of B-cell and T-cell varieties), for the purposes of this review, only CLL will be discussed.

1.2 Historical perspective

The earliest descriptions of CLL dates back to the mid 1800's (4). In 1846, Virchow referred to the disease as a leukaemia, having two components: 'splenic' and 'lymphocytic', and he was able to differentiate morphologically between these two aspects of the disease (5).

1.3 Epidemiology

There are geographical variations in the incidence of CLL, in the different regions of the world. In some parts of Europe and the Western world, CLL is the most common form of leukaemia. Based on the SEER (Surveillance E and End Results) database, the age-adjusted incidence of CLL is 4.9/100 000 per year (6). The median age at diagnosis is approximately 70 years, with less than 10% of patients presenting under 45 years of age (6). Most studies show a male predominance of 1.5-1.9:1 (6-9). While the incidence in Europe is similar to that reported in the United States, the incidence is lower in Asia and Africa (10-13). Moreover, in Africa, the disease tends to present in individuals who are 5-10 years younger, primarily because of the younger age structure of the African population (11-13).

1.4 Aetiopathogenesis

While the exact aetiology of CLL is unknown, a number of potential risk factors and mechanisms have been postulated and incriminated in the aetiopathogenesis of the disease. These include hereditary, genetic and environmental factors (2,3,14).

The primary event is likely to have occurred at the haematopoietic stem cell (HSC) stage, involving multipotent, self-renewing HSC's and evolving into a stepwise process of leukaemogenic transformation (15).

The majority of patients with CLL harbour at least one of four characteristic chromosomal abnormalities, including a deletion (del) in chromosome 13q14.3 (del 13q), del 11q, del 17p

and trisomy 12 (16), which implies that the process of leukaemogenesis is likely to be initiated by the loss or addition of chromosomal material (as indicated above), followed by clonal proliferation characterized by additional mutations, which in some patients, may impart a more aggressive nature to the disease (17). In addition to the classical chromosomal aberrations described above, the development and use of whole-exome sequencing has allowed the characterization of the genomic landscape of CLL, with the additional description of a number of mutations, including NOTCH1, MYD-88, TP53, ATM, IKZF3 etc. (16-18).

Moreover, epigenetics with changes in DNA methylation referred to as epimutations, also play a role in the aetiopathogenesis of CLL. Thus, the evolution and course of CLL is premised on the integration of genetic, epigenetic and transcriptional information (19).

Finally, survival of CLL leukaemic cells depends on an appropriate microenvironment, which consists of cytokines, chemokines and angiogenic factors that interact with CLL cells via surface receptors or adhesion molecules to provide this permissive microenvironment to support the survival of the leukaemic cells (20).

1.5 Clinical features

The clinical profile of patients with CLL is variable, with some patients displaying very stable disease and others generally manifesting with slowly progressive disease. In addition, a small subset of patients develop aggressive disease, often culminating in transformation to a high grade non-Hodgkin lymphoma (NHL) or prolymphocytic leukaemia (PLL) (see complications, discussed later) (2,3,21).

Patients who harbor the disease may be asymptomatic or symptomatic (with active disease). Symptomatic patients may present with constitutional symptoms (such as night sweats, fever, loss of weight and fatique). Lymphadenopathy is the most frequent physical sign, present in peripheral sites, including the cervical, axillary, inguinal, femoral and epitrochlear regions, as well as central sites, such as Waldeyer's ring (tonsillar/oro-naso pharynx), mediastinal, hilar, abdominal and testicular sites of lymphoid involvement. Other manifestations include hepatomegaly, splenomegaly, anaemia and thrombocytopenia. The cytopenias may occur on the basis of bone marrow infiltration, may be a consequence of hypersplenism or form part of the immune related cytopenias, such as auto-immune haemolytic anaemia (AIHA), auto-immune thrombocytopenia (AIT), pure red cell aplasia (PRCA), and secondary Evan's syndrome (i.e. combination of AIHA and AIT, ± auto-immune leucopenia/neutropenia). Therapy, bleeding and other comorbidities may also contribute to the cytopenias. Other features include infection (which is usually secondary to immunosuppression from hypogammaglobulinaemia, cellular immune dysfunction, neutropenia, therapy related

myelosuppression, comorbidities such as diabetes, advanced age and corticosteroid use). Metabolic features such as hyperuricaemia and pseudohyperkalaemia may also complicate the disease (2,3,9-13,21).

1.6 Laboratory investigations

1.6.1 Full blood count

The Full blood count (FBC) typically shows a lymphocytosis ≥5x 10^9/l, ± anaemia and ± thrombocytopenia. The haemoglobin (Hb) level and platelet count are intergral staging systems in CLL. A reticulocytosis may be indicative of autoimmune hemolysis or bleeding, while a reticulocytopenia may accompany bone marrow failure or pure red cell aplasia. The morphology of the lymphocytes are consistent with being small and mature, with a narrow border/rim of cytoplasm, a dense nucleus lacking discernable nucleoli, and having partially aggregated or clumped chromatin. Additionally, a small number of prolymphocytes (<15%) and smudge cells (Gumprecht nuclear shadows) may be seen. The finding of prolymphocytes in excess of 55% favours a diagnosis of PLL (2,22).

1.6.2 Flow cytometry

Flow cytometry of CLL lymphocytes typically show co-expression of the T-cell surface antigen – CD5, together with the B-cell antigens CD19, CD20 and CD23. The expression of CD20 is characteristically dim or low in CLL. CD200 may also be expressed. CD79b and FMC7 are usually negative. The presence of light chain restriction (either kappa or lambda), indicates clonality (23,24). Mantle cell lymphoma (MCL) may also exhibit CD-5 co-expression with B-cell antigens. However, other differentiating features in MCL include small to intermediate sized lymphocytes which are morphologically different from CLL lymphocytes, bright surface membrane expression, CD-79b, FMC7and cyclin-D1 positivity as well as the presence of chromosomal translocation t(11,14) (21).

1.6.3 Bone marrow

The bone marrow is an important baseline investigation for patients who are considered for treatment. It is used in addition to the FBC and flow cytometry to confirm the diagnosis, to characterize the cytopenias as being of central or peripheral origin, to exclude transformation to PLL and as a vehicle for cytogenetic analysis. Together with the trephine biopsy, one may gain an impression of the bone marrow reserve, exclude concomitant pathologies like tuberculosis (TB) and importantly, define the pattern of infiltration, whether diffuse or focal/interstitial. A diffuse pattern of infiltration indicates adverse prognosis (2,3,21,23).

1.6.4 Cytogenetics and Molecular studies

Somatic hypermutation occurs when B-cells recognize antigen in the germinal centre of secondary lymphoid tissues. Somatic hypermutation is characterized by random mutations that occur in the immunoglobulin heavy-chain gene. Molecular tests determine the IGHV (immunoglobulin gene heavy-chain variable-region-genes) mutational status (with an unmutated IGHV status having an unfavourable prognosis).

Additionally, the following cytogenetic abnormalities detected on karyotype analysis, FISH (fluorescent-in-situ-hybridization) or PCR (polymerase chain reaction) indicate an adverse prognosis – 17p/TP53 deletion, 11q23 deletion and trisomy 12 (2,3, 9,25).

1.6.5 Diagnosis

The diagnosis is based on a combination of typical clinical features (where relevant and appropriate) and confirmed by the characteristic laboratory findings on the full blood count (including the differential count and blood smear) and flow cytometry (immunophenotyping) (2).

The diagnosis requires the presence of an absolute lymphocytosis of >5 x 10^9/l in the peripheral blood for a duration of at least 3 months, together with the characteristic immunophenotypic findings (as detailed in 1.6.2 above) (2,3,23,24).

Small lymphocytic lymphoma (SLL) is the solid tumour or tissue equivalent of CLL (classically presenting with lymphadenopathy), and having the same immunophenotype and cytogenetics as CLL, but with an absolute lymphocyte count of <5 x 10^9/l in the peripheral blood (3). In SLL, the diagnosis must be confirmed following the evaluation of a lymph node biopsy (3).

Importantly, monoclonal B-lymphocytosis (MBL) should be excluded. MBL refers to the presence of <5 x 10^9/l of B-lymphocytes in the peripheral blood (usually 4-5 x 10^9/l), in the absence of disease related symptoms and signs, and other peripheral blood cytopenias. MBL may progress to CLL at a rate of 1-2% per year (26).

1.6.6 Classification systems

A number of classification systems are used in CLL. This includes clinical staging systems such as the Rai (0-IV) and Binet (A-C) systems (27,28). These staging classifications are simple and can be readily applied throughout the world. They are based on clinical and laboratory parameters such as lymphocytosis, lymphadenopathy, hepatosplenomegaly, anaemia and thrombocytopenia (27,28).

Table 1.1 shows the Rai staging system, while Table 1.2 denotes the revised Rai staging system (29). The Binet staging system is indicated in Table 1.3 below (28).

Table 1.1: Rai staging system (27)

Stage 0	Absolute lymphocytosis >5 x 10^9/l
Stage I	Lymphocytosis + lymphadenopathy
Stage II	Lymphocytosis + hepatomegaly and/or splenomegaly ± lymphadenopathy
Stage III	Lymphocytosis + anaemia (Hb <10g/dl) ± lymphadenopathy ± hepato-splenomegaly
Stage IV	Lymphocytosis + thrombocytopenia (platelets <100 x 10^9/l) ± lymphadenopathy ± hepatosplenomegaly

N.B: Anaemia and thrombocytopenia is on the basis of bone marrow infiltration by CLL and not due to other causes such as iron deficiency anaemia, autoimmune haemolytic anaemia or auto-immune thrombocytopenia

Table 1.2: Revised Rai staging system (29)

Low risk (formerly stage 0)	Lymphocytosis >15 x 10^9/l, and >40% lymphocytes in the bone marrow
Intermediate risk (formerly stages I and II)	Lymphocytosis, with lymphadenopathy, or splenomegaly/hepatomegaly or both
High risk (formerly stages III and IV)	Lymphocytosis, with lymphadenopathy, or splenomegaly/hepatomegaly or both + disease related anaemia (Hb <11g/dl or haematocrit <33%) or platelets <100 x 10^9/l

Table 1.3: Binet staging system (28)

Stage A	Fewer than 3 areas of lymphoid tissue are enlarged, with no anaemia or thrombocytopenia
Stage B	3 or more areas of lymphoid tissue are enlarged, with no anaemia or thrombocytopenia
Stage C	Anaemia (Hb <10g/dl) and/or thrombocytopenia (platelets <100 x 10^9/l) are present and any number of lymphoid tissue areas may be enlarged.

N.B: Lymphoid areas include cervical, axillary and inguinal lymphadenopathy >1cm, splenomegaly and lymphadenopathy (5 areas = 3 lymph nodal + hepatomegaly + splenomegaly)

1.7 Prognostic factors

Adverse prognostic factors include the following:

i) Advanced clinical disease, i.e. Rai III and IV and Binet C,

ii) Biological prognostic markers such as the mutational status of the immunoglobin-IGHV genes (unmutated, implying adverse prognosis),

iii) Biomarkers, including a raised Beta-2 microglobulin, soluble CD23, thymidine kinase and LDH level,

iv) Pattern of bone marrow infiltration (with a diffuse pattern of bone marrow infiltration indicating adverse prognosis),

v) High proportion of CLL cells containing ZAP-70 (zeta-associated protein-70, ≥20%) or bright CD38 (≥30%) expression on flow cytometry,

vi) Cytogenetic abnormalities, including trisomy 12, 17p deletion and/or TP53 mutation, 11q23 abnormality as well as a complex karyotype (CK), which refers to the presence of ≥3 structural or numerical chromosomal abnormalities,

vii) Lymphocyte doubling time (LDT) < 6 months, and

viii) Other e.g. significant co-morbidities, etc. (2,3,21,25,30).

Due to advances in CLL biology and therapy, the two clinical staging systems as indicated in 1.6.5 above, is insufficient to adequately distinguish prognostic groups. In addition, there are a number of other prognostic factors (see 1.7 above), that need to be considered and factored into relevant prognostic scoring systems. One such scoring system is the CLL International Prognostic Index (CLL-IPI), which incorporates a weighted grading of five independent prognostic factors: TP 53 deletion and/or mutation (collectively called TP53 dysfunction), immunoglobulin heavy chain variable (IGHV) mutational status, serum B-2 microglobulin, clinical stage and age. The CLL-IPI separates four groups with different survival at 5 years (see Table 1.4 below) (31,32).

Table 1.4: CLL International Prognostic Index (CLL-IPI) categories (31)

CLL-IPI category	Overall survival (OS) at 5 years	Potential clinical consequences
Low-risk	93.2%	Do not treat
Intermediate-risk	79.3%	Do not treat except if the disease is really symptomatic
High-risk	63.3%	Treatment indicated except if the disease is asymptomatic
Very high-risk	23.3%	If you need to treat, do not use chemotherapy but rather targeted agents or treatment in clinical trials

1.8 Complications

Complications of CLL include the following:

i) Cytopenias (anaemia, leucopaenia, thrombocytopenia), whether on an immune or non-immune basis, including Evan's syndrome and pure red cell aplasia (PRCA),

ii) Infection (primarily due to immunosuppression from hypogammaglobulinaemia and cellular immune dysfunction). Other contributors to infection include advanced age, and the immunosuppressive and myelosuppressive complications of therapy, including the use of corticosteroids, chemotherapy and targeted therapy such as the monoclonal antibodies (e.g. rituximab - anti-CD20 monoclonal antibody). Early in the course of disease, bacterial infections predominate (e.g. pneumonia, sinusitis). With advanced disease, viral infections (e.g. herpes zoster) and fungal infections are also encountered.

ii) Features related to enlarging splenomegaly (abdominal pain/discomfort, early satiety etc.) and lymphadenopathy (compression and cosmetic),

iii) Metabolic, such as hyperuricaemia, pseudohyperkalaemia and rarely TLS (tumour lysis syndrome),

iv) Bleeding (usually secondary to thrombocytopenia and less commonly due to other causes such as acquired coagulation factor deficiencies),

vi) Transformation (including transformation to a high-grade NHL – Richter's syndrome, prolymphocytic leukaemia (PLL), acute lymphoblastic leukaemia (ALL) and Hodgkin lymphoma (HL),

vii) Hyperviscosity syndrome (rare, compared to chronic myeloid leukaemia – CML)

viii) Second malignancies (due to advanced age, chemotherapy with alkylating agents and anthracyclines and loss of immune surveillance due to B-cell immunodeficiency).
ix) Therapy related (myelosuppression and immunosuppression, including an increased risk of infection), AIHA secondary to fludarabine, and adverse effects of corticosteroids, rituximab and other monoclonal antibodies, chemotherapy, radio-immunotherapy, targeted therapy, allopurinol and blood products) (2,3,21).

1.9 Treatment

The treatment of CLL includes both supportive measures and specific modalities of therapy. The indications for treatment, the treatment modality and choice of treatment are based on whether the patient is asymptomatic or symptomatic, the extent/stage of the disease, the risk profile as indicated by the prognostic factors (such as the IGHV mutational status and del 17p or TP53 mutation), age, fitness and concomitant diseases or co-morbidities of the patient (with particular regard to the potential organ toxicity of the newer, targeted agents), cost and availability of the treatment as well as expertise/experience of the treating clinician and the treatment situation (first versus second line, response versus non-response to the last treatment, duration of response etc.) (2,3,21).

1.9.1 Supportive care

Supportive measures include: (2,3,21)
i) Psychosocial and educational support,
ii) Appropriate treatment of infections (including preventative measures, antibiotics, growth factors, intravenous immunoglobulin, vaccination),
iii) Adequate pain management, where indicated,
iv) Correction of metabolic derangements and electrolyte abnormalities,
v) Blood and blood product support, and
vi) Treatment of co-morbidities.

1.9.2 Specific modalities of treatment

Specific modalities of treatment include: (2,3,21,36-49)
i) Watch and wait approach (e.g. for patients with asymptomatic, early stage disease - e.g. Rai 0, Binet A)

ii)Chemotherapy

a) Single agent: alkylating agents (chlorambucil); purine analogs (fludarabine, cladribine, pentostatin); bendamustine

b) Combination chemotherapy: CVP (cyclophosphamide, vincristine/oncovin, prednisone), CHOP (CVP, hydroxydaunorubicin/adriamycin); FC (fludarabine, cyclophosphamide); CC (cyclophosphamide, cladribine); CMC (cyclophosphamide, cladribine, mitoxantrone); FCP (fludarabine, chlorambucil, prednisone) etc.

Prednisone is useful for the treatment of auto-immune complications of CLL, either alone or in combination with chemotherapy or chemo-immunotherapy. PRCA may additionally respond to cyclosporine.

iii) Monoclonal antibodies

a) Anti-CD20 monoclonal antibodies: rituximab, ofatumumab, obinutuzumab

b) Other monoclonal antibodies: alemtuzumab (anti-CD52 monoclonal antibody)

iv)Targeted therapy

a) PI3K (phosphatidyl inositol-3 kinase) inhibitors: idelalisib, duvelisib, copanlisib, umbralisib

b) BTK (Bruton tyrosine kinase) inhibitors: ibrutinib, acalabrutinib, pirtobrutinib

c) Immunomodulatory agents: lenalidomide

d) Bcl-2 (B-cell lymphoma 2) inhibitors or inhibitors of apoptosis: venetoclax

e) Checkpoint inhibitors/PD-1 (programmed death 1) inhibitors: pembrolizumab

v) Chemo-immunotherapy - FCR (fludarabine, cyclophosphamide, rituximab), BR (bendamustine, rituximab), R-CLB (rituximab, chlorambucil), FA (fludarabine, alemtuzumab), O-CLB (obinutuzumab, chlorambucil)

vi) Combination treatments

a) PI3K inhibitors and monoclonal antibodies: idelalisib, rituximab; idelalisib, ofatumumab

b) BTK inhibitors and monoclonal antibodies: ibrutinib, rituximab; ibrutinib, obinutuzumab; acalabrutinib, obinutuzumab, ibrutinib, ofatumumab

c) Bcl-2 antagonists ± monoclonal antibodies ± BTK inhibitors: venetoclax, rituximab; venetoclax, obinutuzumab; venetoclax, ibrutinib; venetoclax, acalabrutinib, obinutuzumab; venetoclax, ibrutinib , obinutuzumab

d) Len-R (lenalidomide, rituximab); bendamustine, obinutuzumab, venetoclax; bendamustine, ibrutinib, rituximab

vii) Radiotherapy and radioimmunotherapy

Radiotherapy is seldom used for bulky disease (splenomegaly and lymphadenopathy). Radioimmunotherapy with agents such as Yittrium-90 Ibritumomab Tiuxetan, has fallen out of favour in most current treatment protocols, as there is limited efficacy and significant and prolonged myelosuppression. However, Iodine I 131 Tositumomab has been used in one study as consolidation therapy after first remission, providing feasible benefits of converting patients in PR to CR and eliminating MRD. The references are quoted after specific modalities of treatment, above.

viii) CAR (chimeric antigen receptor) T-cells

Still largely experimental. Used for relapsed, refractory disease.

ix) Stem cell transplantation

Allogeneic stem cell transplantation (SCT) may be indicated in selected, younger (<60 years) patients, without co-morbidities, with high-risk CLL, at first or second relapse.

Non-myeloablative (NMA) allogeneic SCT and Autologous SCT is not generally recommended outside of a clinical trial.

Not all patients with CLL require immediate therapy. As such, there are suggested criteria for initiation of therapy as proposed in the iwCLL (International Workshop on CLL) guidelines (2). These criteria are shown in Table 1.5 below.

Table1.5: Criteria to define symptomatic or active disease, (iwCLL) guidelines (2)

1	Evidence of progressive marrow failure as manifested by the development of, or worsening of, anaemia and/or thrombocytopenia. Cut-off levels of Hb <10g/dl or platelet counts <100 X 10^9/l are generally regarded as indication for treatment. However, it should be pointed out that in some patients, platelet counts of < 100 x 10^9/l may remain stable over a long period of time; this situation does not automatically require therapeutic intervention
2	Massive (i.e. ≥6 cm below the left costal margin), or progressive or symptomatic splenomegaly
3	Massive nodes (i.e. ≥10 cm in longest diameter), or progressive or symptomatic lymphadenopathy
4	Progressive lymphocytosis with an increase of ≥ 50% over a 2-month period, or lymphocyte doubling time (LDT) of less than 6 months. LDT can be obtained by linear regression extrapolation of absolute lymphocyte count (ALC) obtained at intervals of 2 weeks over an observation period of 2-3 months; patients with initial blood lymphocyte counts of <30 x 10^9/l may require a longer observation period to determine the LDT. Factors contributing to lymphocytosis other than chronic lymphocytic leukaemia (e.g.infections) should be excluded
5	Auto-immune complications including anaemia or thrombocytopenia poorly responsive to corticosteroids
6	Symptomatic or functional extranodal involvement (e.g. skin, kidney, lung, spine)
7	Disease-related symptoms as defined by any of the following: a) Unintentional weight loss ≥10% within the previous 6 months., b) Significant fatigue (i.e. ECOG-PS 2 or worse; cannot work or unable to perform usual activities)., c) Fevers ≥100.5˚F or 38˚C for 2 or more weeks without evidence of infection, and d) Night sweats for ≥1 month without evidence of infection

Assessment of response (response criteria) is based on the consensus guidelines of the International Workshop on Chronic Lymphocytic Leukemia (iwCLL) (see appendix D) (2). For details and response assessment definitions, refer to the guidelines (2). However, in summary, the following response categories have been defined: CR – complete remission, PR – partial remission, SD – stable disease, PD – progressive disease. Patients may also relapse or have refractory disease.

The last two decades have witnessed significant advances in the therapeutic landscape of CLL, with improvements in response assessment (deep responses resulting in high CR rates, and importantly, high rates of undetectable MRD (minimal residual disease). With MRD becoming a realistic therapeutic goal, there are four additional response assessment categories, which incorporate MRD, including: i) CR, MRD+, ii) CR, MRD-, iii) PR, MRD+, and iv) PR, MRD- (33,34). The techniques for assessing MRD include six-colour flow cytometry (MRD flow) and allele specific oligonucleotide PCR (or high throughput immunosequencing), with a sensitivity of $<10^{-4}$(35). Furthermore, the progress with new combination therapies is providing an opportunity for fixed-duration therapy and an MRD-guided approach to discontinuation of treatment for patients with CLL (36).

A suggested algorithm is included, with regard to patients receiving first line treatment (see Table 1.6) and for patients with relapsed or refractory disease (see Table 1.7). It is not within the scope of this research report to include the various doses of the drugs used in the different CLL treatment regimens, as well as a list of the potential side effects of these drugs. However, where relevant, some of the adverse effects of the relevant drugs have been mentioned.

Table 1.6: Updated treatment algorithm for patients with CLL in first line indications (3)

Stage	Del (17p) or TP53 mutation	Fitness	IGHV	Therapy
Inactive disease, Binet A-B, Rai 0-II	Irrelevant	Irrelevant	Irrelevant	None
Active disease or Binet C or Rai III-IV	Yes	Irrelevant	Irrelevant	Ibrutinib/Acalabrutinib[1] or Venetoclax, Obinutuzumab or Idelalisib-Rituximab (if contraindications for other options)
	No	Go-go	Mutated	FCR (BR above 65 years) or Ibrutinib/Acalabrutinib[1] or Venetoclax, Obinutuzumab[2]
	No	Go-go	Unmutated	FCR (BR above 65 years) or Ibrutinib/Acalabrutinib[1] or Venetoclax, Obinutuzumab[2]
	No	Slow-go	Mutated	Ibrutinib/Acalabrutinib[1,2] or Venetoclax , Obinutuzumab or Chlorambucil-Obinutuzumab
	No	Slow-go	Unmutated	Ibrutinib/Acalabrutinib[1,2] or Venetoclax , Obinutuzumab or Chlorambucil-Obinutuzumab

1. Addition of obinutuzumab to acalabrutinib may be considered

2. Consider and discuss with patient: Continuous versus fixed-duration therapy, specific side effects of drug classes - myelosuppression, infections, second malignancy for chemo-immunotherapy; cardiac toxicity and bleeding for Bruton-tyrosine-kinase inhibitors (Acalabrutinib < Ibrutinib); tumour lysis syndrome (TLS) for Ven-Obi; autoimmune disease and infections for idelalisib. M=mutated; U=unmutated

Table 1.7: Updated treatment algorithm for patients with CLL in second-line indications (3)

Response to 1st line therapy	Fitness	Therapy
Refractory or progress within 3 years	Go go	Change to: venetoclax, rituximab, ibrutinib or acalabrutinib. Other options include: idelalisib, rituximab, FA, FCR (after BR), venetoclax, A-Dex (acalabrutinib, dexamethasone), lenalidomide, rituximab, BR (after FCR). Discuss consolidation with allogeneic SCT
	Slow go	Change to: venetoclax, rituximab, ibrutinib or acalabrutinib. Other options include: idelalisib, rituximab, acalabrutinib, FCR-lite, BR, lenalidomide, rituximab, ofatumumab, HD-R
Progress after 3 years	All	Repetition of 1st line therapy could be considered, but change to targeted therapy if chemotherapy previously given

A-Dex: alemtuzumab-dexamethasone, B: bendamustine, FA: fludarabine-alemtuzumab, FCR: fludarabine-cyclophosphamide-rituximab, HD: high dose, R: rituximab, SCT: stem cell transplant

At Chris Hani Baragwanath Academic Hospital (CHBAH), CLL ranks 5th in order of frequency, amongst the haematological malignancies that are encountered in adult patients. Based on a small study done at CHBAH in 1994, the disease presents at a younger median age of 63 years, with a male predominance of 1.5:1. The vast majority of patients were found to be symptomatic, with a significant proportion of patients manifesting with advanced stage disease. Infections were noted to be a common complication and a significant contributor to morbidity and mortality (12).

Other studies from South Africa and Africa demonstrate similar features. A study by Nel et al., 1998, from Bloemfontein (South Africa), showed that of the 185 patients reviewed between 1975 and 1996, the mean age of the patients was 63 years, with a male predominance of 1.6:1. In their study, 'black' patients presented with more advanced stage disease and higher lymphocyte counts. A complete or partial response was noted in 32.2% of all the patients treated in the study (13). A further study by Musaigwa et al., 2021, from Stellenbosch, Cape Town (South Africa), showed a higher mean age of 67 years, in the 80 patients studied between 2011 and 2016, with a male to female ratio of 1.05:1. The HIV seroprevalence rate was noted to be 6.98% in this study (50).

A smaller study published by Sall et al., 2016, from Senegal, showed the following: A mean age of 61 years in the 40 patients studied between 2011 and 2015, with a more marked male predominance of 3.44:1. The vast majority of patients (82,5%) presented with advanced stage disease. However, there was no comment on response to treatment and treatment outcome in this study (14). Another study by Salawu et al., 2010, from Nigeria showed that in the 79 patients reviewed between September 1986 and March 2007, there were 34 males and 45 females, with a male to female ratio of 0.8:1. The median age of the patients was 60 years (range of 30-81 years). Massive splenomegaly (>10 cm below the costal margin) and hepatomegaly were present in 70.9% and 29.1% of the patients, respectively. More than 63% of the patients presented with advanced stage disease (Binet C). The overall survival at 2 years was 70.2% (51).

Over the last 2 decades, there has been significant advances in the treatment of CLL, particularly with the use of novel agents, targeting tumour cells expressing specific B-cell antigens and B-cell receptor signaling pathways. This has resulted in better outcomes for patients with CLL. Unfortunately, access to these agents for our public sector patients has been limited to a few patients who have been enrolled into clinical trials, with the exception of the anti-CD20 monoclonal antibody rituximab. Thus, the impact of these novel agents has been somewhat limited in our patient population.

Nevertheless, in view of the paucity of studies locally (particularly from South Africa), it is now timely to revisit CLL, as it presents and manifests in a tertiary, academic, Clinical Haematology centre in southern Africa, and to compare the findings in our study with the information available on CLL in the published literature. The limitations of the study should serve as a basis to improve the gaps in our documentation, work up and management of patients with CLL and to continue to motivate for improved access to the novel, therapeutic agents that are available, but not accessible to our patients.In addition, there has also been a small contribution from Human Immunodeficiency Virus infection to CLL at our centre, as has been noted in the Stellenbosch University, Tygerberg, Cape Town, study (50), and this will also be highlighted.

CHAPTER 2: PATIENTS AND METHODS

2.1 Aim of the study

The aim of the study was to describe the profile of adult patients with chronic lymphocytic leukaemia (CLL) who were seen and managed at the Clinical Haematology Unit, Department of Medicine, Chris Hani Baragwanath Academic Hospital (CHBAH), during the period 01/01/2005 to 31/12/2019 (15 years).

2.2 Objectives of the study

i) To describe the demographic profile of CLL patients (including age, gender, ethnic group)
ii) To describe the clinical and laboratory parameters at presentation
iii) To categorize patients based on the clinical staging systems, such as the Rai and Binet system,
iv) To describe the treatment and response to treatment in patients with CLL
v) To describe the complications and outcome of the patients
vi) To compare the findings of this study with other studies published in the literature

2.3 Methodology

2.3.1 Study design

This study is a retrospective review of CLL patients, with descriptive and comparative elements.

2.3.2 Study population

Data was collected from patient files, on patients who had a confirmed diagnosis of CLL and who were seen and managed at the Clinical Haematology Unit, Department of Medicine, CHBAH, over a fifteen year period (01/01/2005 – 31/12/2019). Anonymity was ensured with the use of study numbers, rather than other patient identifiers.

A sample size of 181 patients who fulfilled the inclusion and exclusion criteria were selected for review, from a total of 211 patients with CLL, who were seen during the time period of the study.

2.3.2.1 Inclusion criteria

- Adults ≥ 18 years of age
- Confirmed diagnosis of CLL, based on a combination of clinical features, as well as laboratory features, including morphology, flow cytometry, cytogenetics and molecular studies where available
- Patients diagnosed and managed during the time period 01/01/2005 to 31/12/2019

2.3.2.2 Exclusion criteria

- Patients with inadequate and insufficient data, as well as missing records (including clinical and laboratory information, as well as therapeutic and follow up outcome information/details).Such patients were regarded as being in-evaluable
- Patients with unclear and inconclusive diagnoses, or with 'CLL-like' disorders

2.3.3 Data collection and statistical analysis

Data was obtained retrospectively from patient files, captured onto a data sheet (see appendices B and C), and entered onto an Excel spread sheet prior to statistical analysis, using a programme such as Stata/Statistica (and/with the assistance of a statistician). The patient demographics were summarized using descriptive statistics for dependent variables that are normally distributed, including means and standard deviations. For comparisons between normally distributed variables, a Student t-test was be used. For comparing the different staging systems, a Chi squared test was used. Where a comparison was required in more than two groups, the Anova test was used. When data was not normally distributed, the Mann-Whitney or Kruskal-Willis test was used for correlation between variables. For the purpose of statistical analysis, a 95% confidence interval, with a p-value ($p<0.05$) was considered significant.

2.3.4 Ethics approval

Based on the retrospective nature of the study, the following ethical principles were followed:

2.3.4.1 Approval

Ethics approval was obtained from the Human Research Ethics Committee (HREC), University of the Witwatersrand, Johannesburg (see Appendix A). The study commenced once permission and approval was obtained from the HREC, as well as from all the relevant authorities, as indicated below.

2.3.4.2 Consent

Consent for the use of the patient's records was obtained from the Head of the Clinical Haematology Unit and Academic Head of the Department of Medicine, as well as the CEO of CHBAH. In addition, consent was obtained from the National Health Laboratory Service (NHLS), to assess appropriate laboratory data from the patients.

2.3.4.3 Confidentiality

In order to maintain confidentiality and ensure anonymity, a study number was given to each patient, rather than using patient identifiers. Patient information and raw data was only accessible to the investigator, supervisors and statistician.

CHAPTER 3: RESULTS

3.1 Demographic Findings

During the study period (01/01/2005 to 31/12/2019 – 15 years), a total of 211 patients were diagnosed with CLL (average of 14 new patients per year). Of these 211 patients, 181 were evaluable (181/211= 86%), and were included in the current study. 30 patients were excluded (inevaluable), as the data in their files was inadequate or the files were missing (30/121=14%).

Of the 181 study patients, there were 118 males and 63 females, with a M:F ratio of 1.87:1. The median age at presentation was 64 years, with a mean age of 64.1 years (range 28-91 years). The peak age frequency was in the 7th decade (60-69 years).

HIV-seropositive patients accounted for 16/181 (8.8%). There were 11 males and 4 females, with a M:F ratio of 3:1. The median age at presentation of the HIV sero-positive group was younger, at 53 years, with a mean age of 50.3 years (range 28-68 years). The peak age frequency was in the 6th decade (50-59 years) (see Table 3.1 below).

Regarding the 181 evaluable patients, the CLL numbers were stable in the first five years of the study - (2005-2009) (52/181; 10.4 new patients per year), and second five years of the study - (2010-2014) (51/181; 10.2 new patients per year). However, there was a 1.51 fold increase in the patient numbers in the latter five years of the study (2015-2019) (78/181; 15.2 new patients per year). Similarly, the HIV sero-positive numbers more than doubled in the latter five years (2015-2019) (9/16 – 56%), compared to the first ten years of the study (2005-2014) (7/15 – 44%) (see Table 3.1 below).

Table 3.1: Demographic findings in patients with CLL

Parameter	HIV sero-negative	HIV sero-positive	Total (whole group)
Gender: Males	106	12	118
Gender: Females	59	4	63
M:F ratio	1.8:1	3:1	1.87:1
Mean age in years	65	50.3	64.1
Median age in years		53	64
Range (years)	38-91	28-68	28-91
Peak age frequency	61-70 (6th decade)	51-60 (6th decade)	61-70 (7th decade)
HIV status	165/181=91.2%	16/181=8.8%	
Patient numbers: 2005-2009	48	4	52/181=28.7%
Patient numbers: 2010-2014	48	3	51/181=28.2%
Patient numbers: 2015-2019	69	9	78/179=43.1%

Total number	165	16	100%

3.2 Clinical Findings

The clinical findings (symptoms and signs) at presentation are shown in Tables 3.2 and 3.3 below.

Table 3.2: Presenting symptoms in CLL patients

Symptom	Frequency	Percentage
Fatigue	89/181	49.2%
Loss of weight	85/181	47%
Fever	78/181	43%
Night sweats	76/181	42.5%
Acute Infection	38/181	21%
Bleeding	11/181	6.1%

The most common presenting symptoms in our patients included fatigue (49.2%), loss of weight (47%), fever (43%), night sweats (42.5%) and infection (21%). Bleeding was less commonly encountered (6.1%)(see Table 3.2).

Table 3.3: Presenting signs in CLL patients

Sign	Frequency	Percentage
Lymphadenopathy	165/181	91.2%
Hepatomegaly	107/181	59.1%
Splenomegaly	107/181	59.1%
Hepatosplenomegaly	89/181	49.2%
Pallor	66/181	36.5%
Jaundice	11/181	6.1%

Lymphadenopathy was the dominant clinical sign in our patient population, occurring in the vast majority of the patients (91.2%), followed by an equal number of patients with splenomegaly (59.1%) and hepatomegaly (59.1%). As indicated in Table 3.4 below, the lymphadenopathy was mainly generalised and typically seen in peripheral sites. Other details of the lymphadenopathy are shown in Table 3.4. Details of the hepatomegaly and splenomegaly are shown in Tables 3.5 and 3.6, respectively. Moderate (32.7%) and moderate to massive (31.8%) hepatomegaly were more common than mild (25.2%) or

massive (5.6%) hepatomegaly. However, mild (34.6%) and moderate (28%) splenomegaly, were more common than moderate to massive (17.8%) and massive (15.9%) splenomegaly. Interestingly, massive splenomegaly was approximately three times more frequently encountered than massive hepatomegaly (see Tables 3.5 and 3.6 below), in our patient population. As a clinical sign, overt pallor (indicating anaemia) was evident in 36.5% of the patients. However, based on the full blood count, anaemia was present in a much larger proportion of patients (i.e. 82.8 %) (see Table 3.12).

Table 3.4: Details of lymphadenopathy

Details of the lymphadenopathy	Frequency	Percentage
Presence of lymphadenopathy	165/181	91.2%
Generalised lymphadenopathy	157/165	95.2%
Localised lymphadenopathy	8/165	4.8%
Peripheral lymphadenopathy	159/165	96.4%
Central lymphadenopathy	93/165	56.4%
Lymphadenopathy together with hepatosplenomegaly	87/165	52.7%
Lymphadenopathy without hepatosplenomegaly	43/165	26.1%
Lymphadenopathy with splenomegaly only	13/165	7.8%
Lymphadenopathy with hepatomegaly only	12/165	7.3%
3 most common sites of lymphadenopathy: a. Axillary b. Cervical c. Inguinal	 146/165 139/165 127/165	 88.5% 84.2% 76.9%

Table 3.5: Details of hepatomegaly

Characteristic	Frequency	Percentage
Mild hepatomegaly (<4 cm)	27/107	25.2%
Moderate hepatomegaly (4-8 cm)	35/107	32.7%
Moderate to massive hepatomegaly (8-12 cm)	34/107	31.8%
Massive hepatomegaly (>12 cm)	6/107	5.6%
Unknown	5/107	4.7%
Total	107	100%

Table 3.6: Details of splenomegaly

Characteristic	Frequency	Percentage
Mild splenomegaly (<4 cm)	37/107	34.6%
Moderate splenomegaly (4-8 cm)	30/107	28%
Moderate to massive splenomegaly (8-12 cm)	19/107	17.8%
Massive splenomegaly (>12 cm)	17/107	15.9%
Unknown	4/107	3.7%
Total	107	100%

As CLL is typically a disease of the 'elderly', a number of co-morbidities were found in our patients with CLL. Some of the more common associated co-morbidities are shown in Table 3.7.

Table 3.7: Common co-morbidities in CLL patients

Co-morbidity	Frequency	Percentage
Hypertension	56/181	30.9%
Acute infection	38/181	21%
Diabetes Mellitus	23/181	12.8%
Second malignancy	23/181	12.8%
Cardiovascular disease	18/181	9.9%
Human immunodeficiency virus positivity	16/181	8.8%
Tuberculosis	16/181	8.8%
Vitamin B12 deficiency	12/181	6.6%
Chronic Kidney disease	11/181	6%
Hepatitis B or C positivity	11/181	6%
Chronic Obstructive Pulmonary disease	10/181	5.5%
Neurological disease	8/181	4.4%
Dermatological disease	8/181	4.4%
Hypothyroidism	5/181	2.8%
Benign prostatic hypertrophy	5/181	2.8%
Neurological disease	5/181	2.8%

The performance status (PS), based on the Eastern Cooperative Oncology Group (ECOG) grading, is shown in Table 3.8 below. Approximately half of the patients had a PS of 1 (95/181 – 52.5%), followed by about one third of the patients having a PS of 2 (56/181 – 30.9%), with

a smaller proportion having a PS of 3 (15/181 – 8.3%), a PS of 0 (13/181 – 7.2%) and a PS of 4 (2/181 – 1.1%), respectively.

Table 3.8: Eastern Cooperative Oncology Group (ECOG) performance status (PS)

Performance Status	Frequency	Percent
0	13/181	7.2%
1	95/181	52.5%
2	56/181	30.9%
3	15/181	8.3%
4	2/181	1.1%
TOTAL	181	100%

Both the Rai stage and the Binet stage was documented in our patients. Based on the Rai stage, stages 0 and I (low risk) was present in 39/181 (21.5%) of the patients. Stage II (intermediate risk) was present in 29/181 – 16%, while stage III and IV (high risk), was evident in the majority of the patients (113/181 – 62.5%). This is shown in Table 3.9 below.

Table 3.9: Rai stage in CLL patients

Stage	Frequency	Percentage
0	10/181	5.5%
I	29/181	16%
II	28/181	16%
III	48/181	27.1%
IV	64/181	35.4%
TOTAL	181	100%

Binet stage A generally corresponds to Rai stage 0 and 1 (low risk), while Binet stage B corresponds to Rai stage II (intermediate risk) and Binet stage C corresponds to Rai stage III and IV (high risk). More than half of the patients had Binet stage C (56.4%), as shown in Table 3.10 below, correlating with Rai stage III and IV, as indicated above.

Table 3.10: Binet stage in CLL patients

Stage	Frequency	Percentage
A	37/181	20.4%
B	41/181	23.2%
C	102/181	56.4%
TOTAL	181	100%

3.3 Laboratory Results

The relevant blood results are shown in Table 3.11 below.

Table 3.11: Laboratory results

Characteristic	Normal value	Mean (range)
Haemoglobin	Males 14 -18 g/dl Females 12- 16 g/dl	9.44 (2.8 – 17.7)
Mean cell volume	80 -100 fl	97.5 (68 -145)
White cell count	4-11 x 10^9/l	173.05 (6.05-985)
Lymphocyte count	1-4 x 10^9/l	158.75 (5.44-985)
Urea	2.6 – 7 mmol/l	6 (2.1 – 65)
Creatinine	60 -120 umol/l	103 (48 – 680)
LDH	Up to 200 U/L	454 (70 -2096)
Calcium	2.05 – 2.56 mmol/l	2.2 (1.79 – 3)
Beta-2 microglobulin	0.7 – 1.8 mg/L	8.3 (0.4 – 457)
Hypogammaglobulinaemia		37/109 (33.9%)

Table 3.12: Details of the full blood count

Parameter	Mean (Range)	Number	Percentage
Haemoglobin (Hb) g/dl	9.44 (2.8 – 17.7)	150/181	82.9%
No anaemia	(males Hb ≥ 13 g/dl; females Hb ≥ 12 g/dl)	31/181	17.1%
Borderline (grade 0 anaemia – males)	11 - ≤ 13 g/dl	22/150	14.7%
Borderline (grade 0 anaemia – females)	11 - ≤ 12 g/dl	6/150	4%
Grade 1 (mild anaemia)	9.5 – 11 g/dl	31/150	20.6%
Grade 2 (moderate anaemia)	8.0 – 9.4 g/dl	28/150	18.7%
Grade 3 (severe anaemia)	6.5 – 7.9 g/dl	23/150	15.3%
Grade 4 (very severe anaemia)	< 6.5 g/dl	40/150	26.7%
Auto-immune haemolytic anaemia		14/101	13.8%
False positive Coomb's test		37/101	36.7%

Negative Coomb's test		50/101	49.5%
White cell count x 10^9/l Leucocytosis	173.05 (6.05 – 985)	181/181 178/181	100% 98.3%
Lymphocyte count x 10^9/l	158.75 (5.44 – 985)	181/181	100%
Platelet count x 10^9/l	155.09 (7 – 521)	181/181	100%
Clinical thrombocytopenia	Platelets < 100 x 10^9/l	67/181	37%

Table 3.11 and 3.12 shows the important blood results in the patients with CLL. The mean haemoglobin was 9.44 g/dl, with anaemia being present in 82.9% of the patients. Grade 3 and 4 anaemia was evident in 42% of the patients (15.3% and 26.7%, respectively). Similarly, the more advanced nature of the disease was indicated by a mean white cell count of 173.05 x 10^9/l and a mean lymphocyte count of 158.75 x 10^9/l. Moreover, the lymphocyte count was > 300 x 10^9/l in 28/181 (15.5%) of the patients. The mean platelet count was 155.09, with 37% of the patients presenting with clinical thrombocytopenia (platelet count < 100 x 10^9/l). A high mean LDH level of 454 U/L, as well as a high mean Beta-2 microglobulin level of 8.3 mg/L, also serve as evidence of more advanced disease and an adverse prognosis.

3.4 Bone Marrow Biopsy and Cytogenetics

Bone marrow biopsies were available for review in 152/181 (84%) of the patients studied. In the remaining 29 patients, the bone marrow biopsy was most likely performed at a referral hospital, prior to transferring the patients to CHBAH. However, the diagnosis of CLL was clear in all 181 patients studied, and was based on a constellation of clinical features, bone marrow biopsy findings where available, flow cytometry, immunophenotyping and cytogenetics.

The morphological features of CLL was typical and characteristic in 150/152 (98.7%) of the bone marrow aspirates, while two patients had atypical features. However, as indicated above, in the two patients with atypical morphological features, other findings were present to confirm the diagnosis of CLL, and exclude other 'CLL-like' disorders. The trephine biopsy was important from a prognostic point of view, where 136/152 (89.5%) of the patients had a diffuse pattern of infiltration (indicating an adverse prognosis), 10/152 (6.6%) had a focal/interstitial pattern of infiltration, and in 6/152 (3.9%) of the patients, the pattern was unknown or not clearly defined.

One of the limitations of this retrospective study, is that cytogenetics was performed in a smaller proportion of patients than was expected. In total, cytogenetics was attempted in 89/181 (49%) of the patients. It was not done (or the results were missing/not available) in 92/181 (51%) of the patients. A breakdown of the results obtained is shown in Table 3.13 below.

Table 3.13: Cytogenetic findings in CLL patients

Finding	Number where relevant	Percentage
Normal Karyotype	2	
Complex Karyotype	3	
13q abnormality positive	27/63	43%
13q abnormality negative	36/63	57%
Trisomy 12 abnormality positive	20/63	31.7%
Trisomy 12 abnormality negative	43/63	68.3%
11q abnormality positive	8/57	14%
11q abnormality negative	49/57	86%
17p abnormality positive	7/64	11%
17q abnormality negative	57/64	89%
Monosomy 13 positive	1	
t(4;17) positive	1	
t(14;18) positive	1	

Based on the cytogenetic findings as indicated above, the four most common abnormalities found in descending order were: i) 13q positivity (43%), ii) Trisomy 12 positivity (31.7%), iii) 11q positivity (14%) and 17q positivity (11%).

In our public centre setting, the IGHV mutational status was unfortunately not performed routinely. As such we are unable to comment on this important prognostic factor, which is regarded as a further limitation of this retrospective study.

3.5 Treatment

Treatment included supportive care and specific modalities of treatment. The details of supportive care and specific modalities of treatment are mentioned in the literature review (see Chapter 1).

When patients were seen initially, an attempt was made to stabilize them, correct any electrolyte abnormalities, transfuse blood or blood products where necessary, administer allopurinol, treat pain with appropriate analgesia, use haematinics where indicated and treat co-existent co-morbidities. The use of combination anti-retroviral therapy will be mentioned under the discussion of HIV sero-positive CLL.

The decision to treat with specific modalities of treatment was based on a number of factors, as indicated in the literature review. These factors included: age, performance status,

symptomatic disease, stage of disease, prognostic factors, availability and accessibility of the therapy (including cost), need for specific modalities of treatment, efficacy of the treatment, ability to tolerate the treatment, adverse effects of the treatment, experience of the treating physician etc.

In general, less aggressive treatments were used initially, with a need to up scale the treatment as required during the course of the disease, and whether the initial treatment was deemed adequate, based on tolerability, efficacy and adverse effects.

Some patients only received supportive care (41/181 – 22.7%), while in a proportion of patients, a 'wait and watch' approach was used initially (28/181 – 15.5%), prior to commencing chemotherapy. Chemotherapy was the mainstay of definitive and specific treatment, and included single agents such as prednisone or chlorambucil or combinations such as chlorambucil and prednisone; cyclophosphamide, vincristine/oncovin and prednisone (COP); COP plus hydroxydaunorubicin (CHOP); fludarabine and cyclophosphamide (FC) and FC plus rituximab (FCR). Bendamustine was not available in our state-sector patients, for use with rituximab. Additionally, in the state sector, we had no access to the novel agents for routine use (primarily because of cost constraints), and agents such as the BTK (Bruton tyrosine kinase) inhibitors, including ibrutinib and acalabrutinib, BCL-2 (B-cell lymphoma 2) inhibitors such as venetoclax, newer anti-CD20 monoclonal antibodies such as ofatumumab and obinutuzumab, and the PI3-K (phosphatidyl inositol 3 kinase) inhibitors such as copanlisib and idelalisib, were only available to us, and used in a select few patients who were given access to and enrolled in clinical trials.

Because of these limitations in the state sector and other factors which will be discussed later, the outcome in our patient population was inferior than that which is described in the literature.

A smaller proportion of patients were treated with surgery or radiotherapy (see Table 3.14 below). No patients received radio-immunotherapy or an autologous or allogeneic stem cell transplant.

Table 3.14: Treatment of patients with CLL

Modality	Number	Percentage
Supportive care	41/181	22.7%
Chemotherapy	125/181	69%
Surgery	13/181	7.2%
Radiotherapy	2/181	1.1%

Total	181	100%

Furthermore, most patients received multiple lines of therapy during the course of their disease. For example: 1st line – chlorambucil; 2nd line – COP; 3rd line – FC; 4th line – FCR. The percentage of patients who received at least a partial response (PR) or more (CR – complete response), is indicated in Table 3.15 below. Unfortunately, over time some patients lost this response, developed progression of disease or other complications resulting in death, default or became non-compliant and were lost to follow up, or may have died of a completely unrelated cause.

Table 3.15: Percentage of patients who achieved a PR or CR

Type of therapy	PR or CR
Chlorambucil with or without prednisone	12%
Cyclophosphamide, vincristine, prednisone (CVP/COP)	31%
CVP plus hydroxydaunorubicin (CHOP)	4%
Fludarabine and cyclophosphamide (FC)	20%
FC plus rituximab	13%

PR - Partial Response; CR – Complete Response

3.6 HIV sero-positivity

As HIV is endemic in South Africa for the last 3 decades, it has impacted on all the haematological disorders with a variable effect (minimal to significant) and AIDS (Acquired Immunodeficiency Syndrome) defining, AIDS associated or co-incidental. With CLL, the association with HIV is non-significant, and likely to be co-incidental.

As indicated earlier, of the 181 evaluable patients in this 15 year study, 16 were HIV sero-positive (8.8%). This percentage is lower than the approximate 20-30% background HIV sero-positive rate in adults at CHBAH. However, despite the fact that the number is <10%, there are notable differences in the HIV sero-positive group, compared to the whole CLL group. These differences are shown in Table 3.16 below.

Table 3.16: Characteristics of HIV sero-positive patients

Characteristic	Frequency	Percentage	Comparison with whole group
Gender: Males Females M:F ratio:	12/16 4/16 3:1	75% 25%	118/181 (65.2%) 63/181 (34.8%) 1.87:1
Mean age (range): 50.3 (28-68)			64.1 (28-91)
Performance status: 0 (1/16 - 6.25%); 1 (8/16 – 50%); 2 (7/16 - 43.75%)			0 (13/181-7.2%); 1 (95/181-52.5%); 2 (56/181-30.9%)
Lymphadenopathy	16/16	100%	165/181 (91.2%)
Hepatosplenomegaly	12/16	75%	89/181 (49.2%)
Splenomegaly only	3/16	18.75%	13/181 (7.2%)
No Hepatosplenomegaly	1/16	6.25%	92/181 (50.8%)
Mean Hb (range): 8.84 g/dl (2.8-14.3)			9.44 (2.8-17.7)
Mean Platelet count (range): 144.56 x 10^9/l (7-501)			155.09 (7-521)
Mean WCC count (range): 136.4 x10^9/l (11.1-913.2)			173.05 (6.05-985)
Mean Lymphocyte count (range): 131.3 x 10^9/l (8.9-885.6)			158.75 (5.44-985)
Mean CD4 count (range): 2228.3 (93-7651) cells/ul			
HIV diagnosis: Known 14/16 (87.5%); Newly diagnosed 1/16 (6.25%); Unknown 1/16 (6.25%)			
On antiretroviral therapy: On ARV's (12/16 - 75%)			
Rai stage: 1 (1/16 - 6.25%); 2 (4/16-25%); 3 (4/16 - 25%); 4 (7/16 - 43.75%)			1 (29/181 - 16%); 2 (28/181 - 15.5%); 3 (48/181 - 27.1%); 4 (64/181 - 35.4%)

Binet stage: A (1/16 - 6.25%); B (4/16 - 25%); C (11/16 - 68.75%)			A (37/181 - 20.4%); B (41/181 - 23.2%); C(102/181 - 56.4%)
Tuberculosis: Past Active Total	3/16 5/16 8/16	18.75% 31.25% 50%	 16/181 (8.8%)
Hepatitis B and C	5/16	31.25%	11/181 (6%)
Cytogenetics: Trisomy 12; 13q; 11q; 17p	2/7; 2/7; 1/6; 0/6	28.6%; 28.6%; 16.7%; 0%	20/63 (31.7%); 27/63 (43%); 8/57 (14%); 7/64 (11%)
Outcome: Alive/Died/Lost to follow up	1/16 5/16 10/16	6.25%; 31.25%; 62.5%	6/181(3.3%); 59/181 (32.5%); 118/181 (65.2%)
Survival (mean/median) in months	Mean 32 months Median 6 months		Mean 32 months Median 6 months

The HIV sero-positive patients were treated in a similar way to the HIV sero-negative cohort, with the addition of cART to the chemotherapy, as required. Similar chemotherapy regimens were used, including chlorambucil, CVP/COP, CHOP, FC and FCR. CLL patients who were HIV sero-positive were excluded from CLL clinical trials and were therefore not offered any of the novel therapeutic agents, including the BTK inhibitors, PI3 kinase inhibitors and BCL-2 antagonists. The best response to the therapies used in HIV sero-positive CLL patients, included the following: CR (5/16 - 31.25%), PR (3/16 - 18.75%); PD (7/16 - 43.75%) and Unknown (1/16 - 6,25%). The final outcome was 6.25% alive, 31.25% deceased and 62.5% lost to follow up.

3.7 Survival

In this 15 year period, the outcome of the whole group of patients was as follows: i) Alive 6/181 = 3.3%; ii) Lost to follow up 118/181= 65.2%, and iii) Deceased 57/181 = 31.5%. One of the limitations of this study is its retrospective nature, and unfortunately, almost two thirds of the patients were non-compliant and were lost to follow up. The survival in the whole group of CLL was a mean of 32 months (range of 0-128 months) and a median of 6 months,

compared to the HIV sero-positive group, with a mean of 20 months (range 1-58 months), and a median survival of 6 months. Survival was not influenced by PS, Rai stage or Binet stage. Direct comparisons between the HIV sero-positive and sero-negative patients was not feasible from a statistical point of view, due to the small number of patients in the HIV sero-positive group (i.e. 10% versus 90%). The Kaplan Meier Survival curve for the whole group shows that the patients have at least a 40% chance of surviving 2.85 years and a 20% chance of surviving for 10 years (see Figure 4.1 below). Patients who were treated have a 50% chance of survival for 3.7 years (see Figure 4.2 below).

With regard to the deceased patients, the main cause of death was infection. The causes of death are shown in Table 3.17 below.

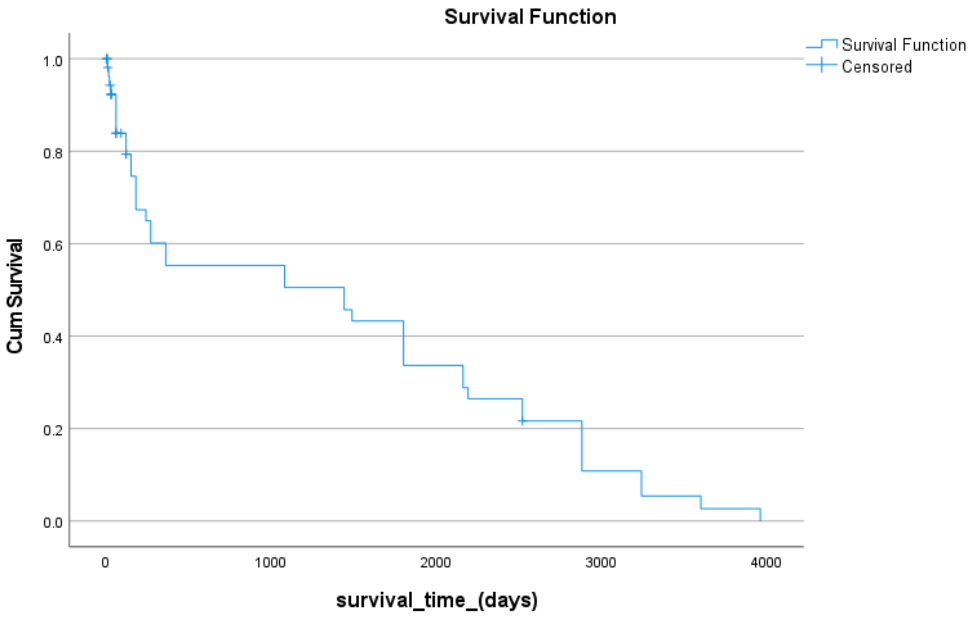

Figure 3.1: Kaplan Meier Survival curve for the whole group

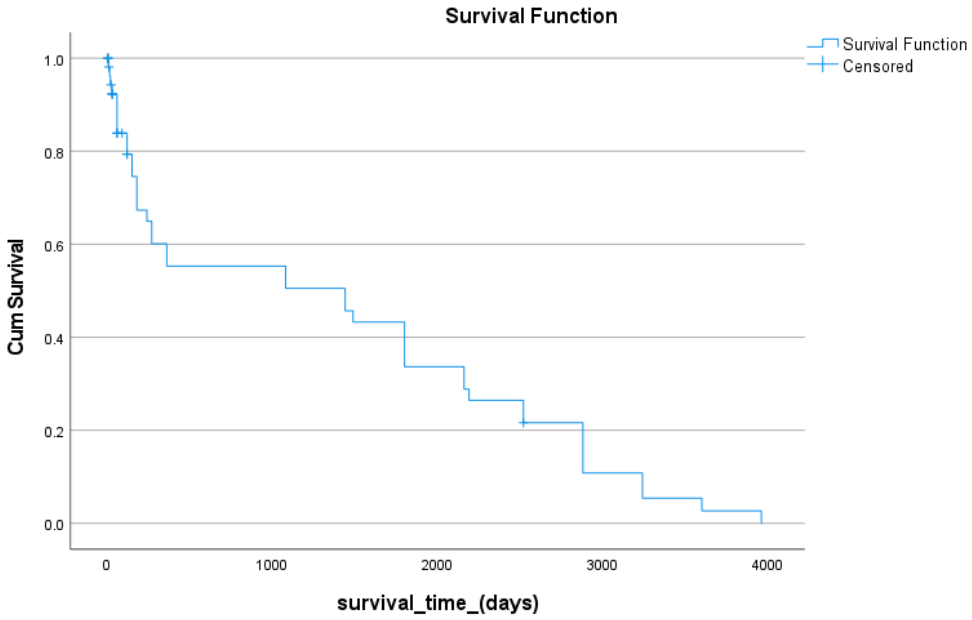

Figure 3.2: Kaplan Meier Survival curve for the chemotherapy treatment group

Table 3.17: Causes of death in CLL patients

Cause of death	Number	Percentage
Infection/Sepsis (acute, chronic (TB), covid)	27	47.4%
Cardiac disease (IHD, CMO, Hypertensive CCF)	7	12.3%
Progression of disease	4	7%
Pulmonary Thromboembolic disease	3	5.3%
Malignancy (carcinoma, sarcoma, melanoma)	3	5.3%
Major bleed (Intracerebral, GIT)	2	3.5%
Transformation (PLL, Richter's syndrome)	3	5.3%
Diabetes Mellitus (ketoacidosis)	1	1.7%
Liver Failure	1	1.7%
Other	6	10.5%
Total	57	100%

CHAPTER 4: DISCUSSION

CLL is characterized by the clonal proliferation and accumulation of small, mature, neoplastic, CD-5 positive, B-lymphocytes in the blood, bone marrow and lymphoid tissues (1,2,3).

4.1 Epidemiology and Demographic Features

There are geographical variations in the incidence of CLL worldwide, with CLL being the commonest form of leukaemia in some parts of Europe and the Western World (6). The median age at diagnosis is approximately 70 years, with less than 10% of patients presenting under 45 years of age (6). Most studies show a male predominance of 1.5-1.9:1 (6-9). While the incidence in Europe is similar to that reported in the United States, the incidence is lower in Asia and Africa (10-13). Moreover, in Africa, the disease tends to present in individuals who are 5-10 years younger, primarily because of the younger age structure of the African population (11-13).

At Chris Hani Baragwanath Academic Hospital (CHBAH), CLL ranks 5th in order of frequency, amongst the haematological malignancies that are encountered in adult patients. Based on a small study done at CHBAH in 1994, the disease presents at a younger median age of 63 years, with a male predominance of 1.5:1 (12). In the current study, there were 211 patients with CLL diagnosed over a period of 15 years (01/01/2005 to 31/12/2019), at CHBAH. Although CLL is generally a stable disease, the number of patients increased by 1.5 fold in the latter 5 years of this study (2015-2019). This may be a reflection of the increased number of patients referred to CHBAH during this period and additionally an increasing number of older patients seen at CHBAH. Of the 211 patients seen, 181 (86%) were evaluable for review in this study. Of the evaluable patients, there were 118 males and 63 females, with a M:F ratio of 1.87:1. The median age at presentation was 64 years, with a mean age of 64.1 years (range 28-91 years). This younger age and male predominance is similar to what has been noted previously at CHBAH and concurs with the younger age at presentation as shown in other South African studies (Bloemfontein – mean age 63years, M:F ratio 1.25:1; Cape Town – mean age 66 years, M:F ratio 1:1.05) and African studies (Nigeria – median age 60 years, M:F ratio 0.75:1; Senegal – 61 years, M:F ratio 3.44:1). However, the male predominance is not uniform, as in two of the four studies mentioned above, there is a female predominance (11-13,50,51).

4.2 Clinical Features and Laboratory Findings

As indicated previously, the vast majority of patients with CLL in a previous study at CHBAH were found to be symptomatic, with a significant proportion of patients manifesting with advanced stage disease. Infections were noted to be a common complication and a significant contributor to morbidity and mortality (12). This is true of the current study, where the performance status (PS) of the patients was ≥1 in 92.8%, of the patients at presentation (see Table 3.8). Although the presenting symptoms are somewhat non-specific (fatique-49.2%; loss of weight-47%; fever-43%; night sweats-42.5%), lymphadenopathy was the dominant clinical sign, occurring in over 90% (91.2%) of the patients (see Tables 3.2 and 3.3). The lymphadenopathy was generalized (95.2%), and manifested predominantly in peripheral sites (axilla, cervical and inguinal regions being the most common) (96.4%), and occurred in association with hepatosplenomegaly in approximately half of the patients (52.7%) (see Table 3.4 for details). Hepatomegaly and splenomegaly were present in an equal number of patients (59.1%). A similar number of patients (approximately 30% each), presented with mild, moderate, and moderate-massive hepatomegaly, while massive hepatomegaly was encountered in only 5.6% of the patients (see Table 3.5). Although, approximately two-thirds of the patients had mild and moderate splenomegaly (62.6%), massive splenomegaly was present in 15.9% of the patients at presentation (see Table 3.6). Pallor was evident clinically in 36.5% of the patients (see Table 3.3). This is much lower than the laboratory confirmation of anaemia, which was present in 82.9% of the patients (see Table 3.11). Documented infection as a presenting feature was present in about one-fifth of the patients (21%) (see Table 3.2). As CLL is typically a disease of the 'elderly', a number of co-morbidities were noted in the patients at presentation, including hypertension (30.9%), acute infection (21%), diabetes mellitus (12.8%) and second malignancies: most commonly prostate carcinoma (9/23 – 39%), basal/squamous cell carcinoma (4/23 – 17%), neuroendocrine tumours and adenocarcinoma of the colon (2/23 -9% each, respectively) and 1/23 each, of the following tumours – lung carcinoma, Kaposi's sarcoma, anal cell carcinoma, carcinoma of the cervix, fibrosarcoma and melanoma (see Table 3.7).

The mean haemoglobin was 9.44 g/dl, with a range of 2.8-17.2 g/dl. As a measure of the more advanced nature of the disease, a grade 3 and 4 anaemia (i.e. <8 g/dl and <6.5 g/dl, was present in 15.3% and 26.7 of the patients, respectively) (see Tables 3.11 and 3.12). A positive Coomb's test (IgG and C3d positivity) was present in 36.7%, although clinical and further biochemical evidence of an auto-immune haemolytic anaemia was only evident in 13.8% of the patients, implying that a 'false positive' test result (22.9%) is not uncommon in CLL. The mean white cell count (WCC) was 173.05 x 10^9/l, with a range of 6.05-985 x 10^9/l).

A leucocytosis was present in 98.3%, while a lymphocytosis (which is a defining criterion of CLL) was seen in all the patients (100%). The mean lymphocyte count was 158.75 X 10^9/l, with a range of 5.44-985 x 10^9/l). The mean platelet count was 155.09 x 10^9/l, with a range of 7-521 x 10^9/l. Clinical thrombocytopenia (platelet count <100 x 10^9/l) was present in 37% of the patients at presentation. The more severe cytopenias and the marked lymphocytosis encountered in this study is an indication of the more advanced nature of presentation of CLL, in our patient population. A further indication of the bulky and more advanced biochemical nature (adverse prognosis) of the disease was the high mean LDH of 454 U/L and Beta-2 microglobulin of 8.3, respectively. Hypogammaglobulinaemia, an important factor predisposing patients to infection in CLL, was present in one third of the patients (33.9%), at presentation. The clinical and blood picture concurs with the other South African and African studies mentioned previously, which is in keeping with symptomatic disease, presenting at a more advanced stage and with overt clinical manifestations (11-13,50,51).

4.3 Bone Marrow and Cytogenetic Analysis

Bone marrow biopsies were available for review in 152/181 (84%) of the patients studied. In the remaining 29 patients, the bone marrow biopsy was most likely performed at a referral hospital, prior to transferring the patients to CHBAH. However, the diagnosis of CLL was clear in all 181 patients studied, and was based on a constellation of clinical features, bone marrow biopsy findings where available, flow cytometry (peripheral blood and/or bone marrow), immunophenotyping and cytogenetics.

The morphological features of CLL was typical and characteristic in 150/152 (98.7%) of the bone marrow aspirates, while two patients had atypical features. However, as indicated above, in the two patients with atypical morphological features, other findings were present to confirm the diagnosis of CLL. The trephine biopsy was important from a prognostic point of view, where 136/152 (89.5%) of the patients had a diffuse pattern of infiltration (indicating an adverse prognosis), 10/152 (6.6%) had a focal/interstitial pattern of infiltration, and in 6/152 (3.9%) of patients, the pattern was unknown or not clearly defined.

One of the limitations of this retrospective study, is that cytogenetics was performed in a smaller proportion of patients than was expected. In total, cytogenetics was attempted in 89/181 (49%) of the patients. It was not done (or the results were missing/not available) in 92/181 (51%) of the patients. A breakdown of the results obtained is shown in Table 3.13 below. Based on the cytogenetic findings as indicated above, the four most common abnormalities found in descending order were: i) 13q positivity (43%), ii) Trisomy 12 positivity (31.7%), iii) 11q positivity (14%) and 17p/TP53 positivity (11%). A clearly adverse prognosis,

as indicated by the presence of 11q positivity and 17p/TP53 positivity, was present in 14% and 11% of the patients, respectively. Trisomy 12 is associated with an intermediate prognosis and 13q with a better/favourable prognosis (54). While 13q deletions account for 36-64% of CLL, trisomy 12 for 16-25%, 11q deletions for 11-18% and 17p deletions for 5-7%, and the percentages were similar in our patients, there was statistically significant association with outcome (survival) in our study, with p values >0.05
(54). Interestingly, in the Senegal cohort, 13q was present in 44%, which is similar to our study, but the percentage of trisomy 12 (17.5%), and 11q and 17p (7.5% each), was lower than in our patients (11).

4.4 Staging Classifications

Both the Rai stage and the Binet stage was documented in our patients. Based on the Rai stage, stages 0 and I (low risk) was present in 39/181 (21.5%) of the patients. Stage II (intermediate risk) was present in 29/181 – 16%, while stage III and IV (high risk), was evident in the majority of the patients (113/181 – 62.5%). This is shown in Table 3.9. Binet stage A generally corresponds to Rai stage 0 and 1 (low risk), while Binet stage B corresponds to Rai stage II (intermediate risk) and Binet stage C corresponds to Rai stage III and IV (high risk). More than half of the patients had Binet stage C (56.4%), as shown in Table 3.10. There is a good correlation between the two staging systems in this study. Both staging systems indicate that our patients present more commonly with high risk/more advanced stage disease. However, there was no correlation between survival outcome and either of the staging systems (Rai, p=0.747 and Binet, p=0.589), likely because of the large number of patients who were lost to follow up.

4.5 Treatment

The treatment of CLL includes both supportive measures and specific modalities of therapy. The need for initiating treatment, the indications for treatment, the treatment modality and choice of treatment are based on whether the patient is asymptomatic or symptomatic, the extent/stage of the disease, the risk profile as indicated by the prognostic factors (such as the IGHV mutational status and del 17p or TP53 mutation), age, fitness and concomitant diseases or co-morbidities of the patient (including the potential organ toxicity and complications of the newer, targeted agents – examples include i) BTK inhibitors resulting in cardiac toxicity including atrial fibrillation, bleeding, infection and arthralgias; ii) BCL-2 antagonists resulting in tumour lysis syndrome and iii) PI3K inhibitors, with side effects such as pneumonitis, transaminitis, colitis and diabetes mellitus (3,53), cost and availability of the

treatment as well as expertise/experience of the treating clinician and the treatment situation (first versus second line, response versus non-response to the last treatment (including relapsed and refractory disease), duration of response etc. (2,3,21,53).

4.5.1 Supportive care

Supportive measures include: (2,3,21)

i) Psychosocial and educational support,

ii) Appropriate treatment of infections (including preventative measures, antibiotics, growth factors, intravenous immunoglobulin, vaccination,combination anti-retroviral therapy (cART) and prophylaxis of opportunistic infections in the HIV sero-positive patient),

iii) Adequate pain management, where indicated,

iv) Correction of metabolic derangements and electrolyte abnormalities,

v) Blood and blood product support, and

vi) Treatment of co-morbidities.

4.5.2 Specific modalities of treatment

Specific modalities of treatment include (see details in the literature review section above) (2,3,21,36-49):

i) Watch and wait approach (e.g. for patients with asymptomatic, early stage disease - e.g. Rai 0, Binet A)

ii) Chemotherapy

a) Single agent chemotherapy, and

b) Combination chemotherapy

iii) Monoclonal antibodies

a) Anti-CD20 and other monoclonal antibodies

iv) Targeted therapy

a) PI3K (phosphatidyl inositol-3 kinase) inhibitors

b) BTK (Bruton tyrosine kinase) inhibitors

c) Immunomodulatory agents

d) Bcl-2 (B-cell lymphoma 2) inhibitors or inhibitors of apoptosis

e) Checkpoint inhibitors/PD-1 (programmed death 1) inhibitors

v) Chemo-immunotherapy

vi) Other combination treatments

a) PI3K inhibitors and monoclonal antibodies

b) BTK inhibitors and monoclonal antibodies

c) Bcl-2 antagonists ± monoclonal antibodies ± BTK inhibitors
d) Other therapies not included in the list above
vii) Radiotherapy and radioimmunotherapy
viii) CAR (chimeric antigen receptor) T-cells, and
ix) Stem cell transplantation.

In general, less aggressive treatments were used initially, with a need to up scale the treatment as required during the course of the disease, and whether the initial treatment was deemed adequate, based on tolerability, efficacy and adverse effects. Also, patients were 'risk-stratified' into low risk, intermediate or high risk (based on the Rai and Binet stage and other prognostic factors).

The need for chemotherapy, use of single agent chemotherapy (e.g. chlorambucil) versus combination chemotherapy and the need for fludarabine based treatment (FC – fludrabine and cyclophosphamide versus FCR – fludarabine, cyclophosphamide and rituximab), was also based on the risk profile of the patient and the other parameters as described in the introduction section in Chapter 1, above.

Some patients only received supportive therapy (41/181 – 22.7%), while in a proportion of patients, a 'wait and watch' approach was used (28/181 – 15.5%), prior to initiating chemotherapy. Chemotherapy was the mainstay of treatment (125/181 –69%) (see Table 3.14), and included single agents such as prednisone or chlorambucil or combinations such as chlorambucil and prednisone, cyclophosphamide, vincristine/oncovin and prednisone (COP), COP plus hydroxydaunorubicin (CHOP), fludarabine and cyclophosphamide (FC) and FC plus rituximab (FCR). Bendamustine was not available for use with rituximab. Additionally, in the state sector, we had no access to the novel agents for routine use (primarily because of cost constraints), and these agents such as the BTK (Bruton tyrosine kinase) inhibitors, including ibrutinib and acalabrutinib, BCL-2 (B-cell lymphoma 2) inhibitors such as venetoclax, newer anti-CD20 monoclonal antibodies such as ofatumumab and obinutuzumab, and the PI3-K (phosphatidyl inositol 3 kinase) inhibitors such as copanlisib and idelalisib, were only available and used in a select few patients who were given access to, and enrolled in clinical trials.

Because of these limitations in the state sector and other factors which will be discussed later, the outcome in our patient population was inferior compared to that generally described in the literature.

A smaller proportion of patients were treated with surgery or radiotherapy (see Table 3.14 below). No patients received radio-immunotherapy or an autologous or allogeneic stem cell transplant.

Furthermore, most patients received multiple lines of therapy during the course of their disease. For example: 1st line – chlorambucil; 2nd line – COP; 3rd line – FC; 4th line – FCR. The percentage of patients who achieved at least a partial response (PR) or more (CR – complete response), is indicated in Table 3.15 (see in Results section). Unfortunately, over time some patients lost this response, developed progression of disease or other complications resulting in death, default and/or became non-compliant and we
re lost to follow, or died of a completely unrelated cause.

4.6 Survival

In this 15 year period, the outcome of the whole group of patients was as follows: i) Alive 6/181 = 3.3%; ii) Lost to follow up 118/181= 65.2%, and iii) Deceased 57/181 = 31.5%. The survival in the whole group of CLL was a mean of 32 months (range of 0-128 months) and a median of 6 months. With regard to the deceased patients, the three main documented causes of death was infection (27/47 – 47.4%), cardiac disease (7/57 – 12.3%) and progression of disease (4/57 – 7%). Other causes of death are shown in Table 3.17. below.

4.7 HIV sero-positivity

As HIV is endemic in South Africa for the past 3 decades, it has impacted on all the haematological malignancies with a variable effect (minimal to significant) and terminologies such as AIDS (Acquired Immunodeficiency Syndrome) ‘defining’, AIDS ‘associated’ and ‘co-incidental’ have been used. Because of the lack of a clear association between CLL and HIV worldwide and the paucity of information in the literature on the combined occurrence of the two entities, the association is best regarded as coincidental (52).

Sixteen of the 181 evaluable patients in our study were HIV sero-positive (8.8%). However, despite the low number (<10%) and the fact that the percentage sero-positivity is lower than the approximate 20-30% background HIV sero-positive rate in adults at CHBAH, the information regarding HIV-CLL is still highly meaningful, as this number represents the largest cohort in the world to be described from a single centre (and that will hopefully be described and published in the literature).

In Table 3.16, the characteristics of the HIV-seropositive group is shown and comparisons are made with the whole CLL group.However, statistical significance was not documented, as the 2 groups are not entirely comparable, based on the small number in the HIV-

seropositive group. In summary, the following was noted in the HIV-seropositive group:The median age is younger (64 versus 53 years), with a more marked male predominance (3:1 versus 1.87), similar PS, higher percentage of lymphadenopathy (100 versus 91.25%), higher percentage of hepatosplenomegaly (75 versus 49.2%), a lower mean haemoglobin (8.84 versus 9.44 g./dl), lower mean platelet count (144.5 versus 155 x 10^9/l), lower mean white cell count (136.4 versus 173 x 10^9/l) and lower mean lymphocyte count (131.3 versus 158.7 x10^9/l, similar Rai and Binet stage values, a higher proportion of past and current Tuberculosis (50 versus 8.8%), a higher association with hepatitis B and C infection (31.25 versus 6%), a different cytogenetic profile, with lower percentages of 13q (28.6 versus 43%) and 17 p (0 versus 11%) deletions. Similar supportive care (but, with the addition of cART) and chemotherapy were used in the HIV sero-positive group. The outcome was similar to the whole group, with 6.25% of the patients being alive, 31.25% deceased and 62.5% lost to follow up. However, the mean survival was inferior, i.e. 20 months compared to 32 months, with an equal median survival of 6 months.

There is a paucity of information in the literature on HIV-CLL. In the only other South African study at Tygerberg Hospital, Cape Town, where HIV sero-positivity was documented in association with CLL, the findings were as follows: 3/43 patients (6.98%) were HIV sero-positive, with a younger age range of 43 to 50 years. However, the clinical, morphological, immunophenotypic and cytogenetic picture was similar to the HIV sero-negative patients, suggesting a likely coincidental association between the two conditions (50). An earlier brief communication on CLL occurring with HIV from the USA, explored possible mechanisms for this rare association, implicating T-cell dysfunction as a potential key role player, but once again emphasized the rarity of the association (52). In a further report of 6 patients with HIV-CLL presented at the 2009 ASCO (American Society of Clinical Oncology) Annual Meeting, the following was noted: Median age of 60 years (range 50-76 years), male predominance (5/6 patients), median CD4 count of 930 cells/ul (likely a reflection of the high lymphocyte count). Two of the patients were not treated and four patients required treatment for either haemolytic anaemia, CLL-induced renal failure, pancytopenia, or hypogammaglobulinaemia with recurrent infections (one died of renal failure and two from infectious complications) (55).

4.8 Limitations of the study

This study has a number of limitations, which are discussed below:

1. Retrospective nature of the study, hampered by incomplete and inadequate data in some of the patients,
2. Single centre study, although the long time period allowed a sufficient number of patients to be included,
3. Direct comparisons with smaller subsets of patients, i.e. those who were HIV sero-positive was not possible, as the numbers in the HIV sero-positive group was less than 10% of the total patient cohort. However, the data in this subset of patients is relevant, as there is very little that has been published in the global literature to date, on HIV sero-positive CLL.
4. The high number of patients lost to follow up, primarily because of the poorer socio-economic status of the patients in this study, the chronic nature of the study, the relatively older age of the patients (with an increasing number of co-morbidities as they age further),
5. Delays in presentation to hospital, diagnostic delays and delays in initiation of specific treatment (this is a global problem at tertiary, specialized, public healthcare facilities in South Africa, where large numbers of patients are treated, where the resources are limited and where patients lack the appropriate education and empowerment related to their illness),
6. Late presentations with advanced stage disease, with a number of patients dying of complications during the work up or shortly after a definitive diagnosis is made, and without receiving any specific therapy or an adequate number of courses of specific therapy,
7. Limitations regarding an optimal work-up (such as an inability to do IGHV testing); lack of access to ‘state of the art’ therapy or optimal therapy in the public sector setting (such as a combination of a BTK inhibitor, BCL-2 antagonist and anti-CD20 monoclonal antibody), due mainly to cost constraints and other management and accessibility issues.

All these challenges need to be looked at, in order to provide realistic and potential solutions, as we move forward to improve the healthcare of our society and our nation.

CHAPTER 5: CONCLUSION

Chronic Lymphocytic Leukaemia (CLL) is one of the four common types of leukaemia encountered in adults. This study was undertaken to better characterize the demographics, clinical presentation, laboratory findings, staging, treatment and outcome of patients seen with a confirmed diagnosis of CLL, over a 15 year period (01/01/2005 to 31/12/2019), at the Clinical Haematology Unit, CHBAH. The key findings in this study were:

1. A stable number of patients in the first ten years of the study (01/01/2005 to 31/12/2014), with a 1.5 fold increase in the latter 5 years of this study (2015-2019).
2. A younger median age of 64 years, with a male predominance of 1.87:1.
3. Most of the patients were symptomatic, with an ECOG PS ≥1 in 92.8% of the patients.
4. Fatique (49.2%), loss of weight (47%) and fever (43%) were the most common symptoms at presentation.
5. Lymphadenopathy was the dominant physical sign (91.2%). Hepatosplenomegaly was evident in 49.2% of the patients.
6. The vast majority of patients had anaemia (82.9%), with a mean haemoglobin of 9.44 g/dl. The mean white cell count and lymphocyte counts were 173 $x10^9$/l and 158.7 x 10^9/l, respectively. The mean platelet count was 155 x 10^9/l. Clinical thrombocytopenia was present in 37% of the patients.
7. More than half the patients presented with advanced stage/high risk disease, with a Rai stage III and IV accounting for 62.5% and Binet C for 56.4% of the patients at presentation.
8. A diffuse pattern of bone marrow infiltration, indicating adverse prognosis was evident in 89.5% of the patients.
9. Cytogenetics showed a favourable genotype (13q) in 43%, an intermediate phenotyope (trisomy 12) in 31.7% and an unfavourable phenotype (11q and 17p) in 14% and 11%, of the patients, respectively.
10. HIV sero-positivity was present in 8.8% of the patients, with sero-positive patients showing a number of differences, including a younger median age at presentation, a more marked male predominance, similar clinical presentation and staging of the disease, a higher proportion of TB, hepatitis B and C, and a lower mean survival, with a similar median survival.
11. Supportive care only was offered to 22.7%, while chemotherapy was administered to 69% of the patients.
12. The outcomes of the patients at the end of study were: i) Lost to follow up (65.2%), ii) Deceased (31.5%) and Alive (3.3%).
13. The mean survival for the whole group was 32 months, with a median survival of 6 months, while for the HIV sero-positive group the median survival was 20 months, with a median survival of 6 months.

5.1 Future Recommendations

Future recommendations include:

Education with regard to the key clinical manifestations of CLL, early suspicion of the diagnosis and timeous referral to a tertiary or specialized centre, so that appropriate treatment (where indicated), can be initiated as soon as possible.

Every effort should be made to improve compliance and attendance at follow up visits. This is vital in order to assess response to treatment and to detect early relapse or progression of the disease.

Efforts to improve accessibility of 'state of the art' and novel therapies for public sector patients.

Prospective, randomised, multi-centre studies should be performed to assess the benefits of various therapeutic options and to compare existing therapies with novel treatment options in our local South African patient population (including both the private and public sector).

Although HIV sero-positivity is not a major problem, the numbers of sero-positive patients is steadily increasing, with a doubling of the number in the latter 5 years, compared to the first 10 years of the study. In principle, these patients should be offered the same treatment options as HIV-1 sero-negative individuals, with the proviso that every attempt is made to achieve optimal virological suppression and immune reconstitution, with combination anti-retroviral therapy.

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APPENDICES

APPENDIX A: HUMAN RESEARCH ETHICS COMMITTEE CLEARANCE CERTIFICATE

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R14/49 Dr Cain Khosa

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M200748 MED20-05-084

NAME: (Principal Investigator)	Dr Cain Khosa
DEPARTMENT:	Internal Medicine Chris Hani Baragwanath Academic Hospital
PROJECT TITLE:	A fifteen year review of chronic lymphocytic leukaemia in adults, at Chris Hani Baragwanath Academic Hospital
DATE CONSIDERED:	31/07/2020
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof Moosa Patel
APPROVED BY:	Dr C Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	03/12/2020

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 in Room 301, 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 July and will therefore be due in the month of July each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature _____ Date _____

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

APPENDIX B: DATA COLLECTION SHEET

Demographics:

Study Number	
Gender	Male ☐ Female ☐
Date of birth	DD: MM: YYYY: Age: _____
Date of diagnosis (first high WCC)	DD: MM: YYYY:
Date of haematology referral	DD: MM: YYYY:
Performance status (initial)	0 ☐ 1☐ 2☐ 3☐ 4☐
Ethnic group	B ☐ W ☐ C ☐ A ☐ Other: _____
Residence	Province: _____ Country: _____
Occupation	

History:

Symptoms	**Yes**	**No**	
Fever	☐	☐	
Loss of weight	☐	☐	Amount (kg): _____ Time (weeks-months):____
Night sweats	☐	☐	
Fatigue	☐	☐	
Bleeding	☐	☐	Site/s: _____
Bone pain	☐	☐	Site/s:_____
Comorbidities	☐	☐	HIV ☐ TB☐ Other ☐ (see below)
Hypertension	☐	☐	Duration: _____
Diabetes	☐	☐	Duration: _____ Type: _____
Cardiac / Pulmonary / Renal disease	☐	☐	Duration: _____ Type: _____

If HIV seropositive	☐	☐	CD4 count:_____ Date diagnosed: _____
If positive, is patient on cART?	☐	☐	Date Commenced: _____ Type: _____
If prior or current TB	☐	☐	Pulmonary/Extrapulmonary TB?_____ When was TB diagnosed?_____ Duration of treatment?_____

Examination:

Signs	**Yes**	**No**	**Details / Size (cm):**
Pallor	☐	☐	
Jaundice	☐	☐	
Lymphadenopathy (peripheral): I – Cervical	Submental ☐ Submandibular ☐ Anterior triangle ☐ Posterior triangle ☐ Pre-auricular ☐ Post-auricular ☐ Supraclavicular ☐ Occipital ☐	☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐	
II – Axillary	☐	☐	
III – Epitrochlear	☐	☐	
IV – Inguinal	☐	☐	
V – Femoral	☐	☐	
Lymphadenopathy (Central): I –Waldeyer's ring/ Tonsillar involvement	☐	☐	

II – Intra-thoracic	SVC syndrome D'espines sign Pemberton's sign	☐ ☐ ☐	☐ ☐ ☐	
III – Intra-abdominal	Masses Ascites Leg swelling DVT Scrotum / testes	☐ ☐ ☐ ☐ ☐	☐ ☐ ☐ ☐	
Other lesions / masses (i.e. extra-nodal disease/extranodal sites		☐	☐	Site/s: _____ _____ _____
Hepatomegaly		☐	☐	Size below costal margin _____cm
Splenomegaly		☐	☐	Size below costal margin _____cm
Bleeding	Petechiae Purpura Ecchymoses Mucosal	☐ ☐ ☐ ☐	☐ ☐ ☐ ☐	Oral cavity:_____ Gums: _____ Genitourinary:_____
Neurological deficit	☐		☐	
Other relevant clinical findings, if present	☐		☐	

Chest X Ray:

Normal ☐ Abnormal ☐

If abnormal, comment on the presence of:

i. Nodal disease _____

ii. Lung parenchymal disease_____

iii. Pleural disease _____

iv. Bony disease _____

v. Cardiac disease (CTR/ chamber enlargement) _____

CT Scan (where relevant) or ultrasonography:

Normal ☐ Abnormal ☐

If abnormal, detail all the abnormalities (organomegaly, adenopathy etc.).

Bone Marrow:

Morphology: Classical CLL Yes ☐ No ☐

Atypical CLL Yes ☐ No ☐

If atypical, why _____

Prolymphocytes: % peripheral blood _____and bone marrow _____

Flow cytometry and cytogenetics on bone marrow (or peripheral blood if no bone marrow done):

Pattern of infiltration	Diffuse ☐ Focal/nodular ☐ Interstitial ☐
Predominant cell size (tick all blocks that apply)	Small ☐ Intermediate ☐Large ☐ Mixed ☐
CD 5	Positive ☐ Negative ☐
CD 20	Positive ☐ Negative ☐
CD 19	Positive ☐ Negative ☐
CD 22	Positive ☐ Negative ☐
CD 23	Positive ☐ Negative ☐
CD 38	Positive ☐ Negative ☐ Bright ☐ Dim ☐
CD 200	Positive ☐ Negative ☐
Cyclin D1	Positive ☐ Negative ☐
CD 10	Positive ☐ Negative ☐
Other	

Light chain restriction – (kappa or lambda)	Yes ☐ No ☐
Ki – 67	Positive ☐ Negative ☐ % positivity _____
Mutational status of immunoglobulin genes	Mutated Yes ☐ No ☐ Unmutated Yes ☐ No ☐
ZAP 70 positivity	
Elevated B_2M	Yes ☐ No ☐Level_____
Elevated LDH	Yes ☐ No ☐Level_____
Cytogenetics (specify translocation)	Normal Yes☐ No ☐ Trisomy 12 ☐ ☐ 17p/p53 ☐ ☐ 13q deletion ☐ ☐ 11q deletion ☐ ☐ Other ☐ ☐Details___ _____

Summary:

_____

_____

_____

Rai stage:

_____

Binet stage:

Other comments:

Treatment:

i) Watch and wait Yes ☐ No ☐

Duration _____

ii) Specific treatment

A. Specific	#cycles/doses/date and duration/other
Chemotherapy	Yes ☐ No ☐
1. Chlorambucil only	
2. Prednisone	
3. Fludarabine only	
4. CVP	
5. FC	
6. FCR	
7. CHOP	
8. R-CHOP	
9. BR	
10. Other combinations	
Radiation therapy	Yes ☐ No ☐ Details _____
Surgery	Yes ☐ No ☐ Details_____
Other treatment	
B. Supportive	
Allopurinol	Yes ☐ No ☐ Dose_____
Infection/s	Yes ☐ No ☐
i. Type of infection	
ii. How treated?	
iii. Hospitalization required for infection?	Yes ☐ No ☐ Duration How treated _____ No. of times hospitalized. Comment on each visit (e.g. 3 visits, received IVI AB) _____
iv. WasIVIg used at any stage?	Details (dose/duration/no of times/response _____ _____
v. Use of prophylactic antibodies?	Yes ☐ No ☐

	Duration_____Type of prophylaxis _____
vi. Use of growth factors?	Yes ☐ No ☐ Details (dose/duration/no of times used/response) _____
Thrombo-prophylaxis	Yes ☐ No ☐ Details_____
Blood transfusions	Yes ☐ No ☐ Details _____
Platelet transfusions	Yes ☐ No ☐ Details _____
Erythropoiesis stimulating agents	Yes ☐ No ☐ Details (dose/duration/response) _____
Other	_____ _____

Complications (list all complications if not discussed/reviewed already):

i. Cytopenias (including immune manifestations)
ii. Metabolic (pseudohyperkalaemia/hyperuricaemia)
iii. Infection
iv. Pressure/comprehensive symptomatology
v. Transformation
vi. Second malignancy
vii. Hyperviscosity
viii. Thrombosis
ix. Bleeding
x. Other

Date	Complication	Management

Response:

	After initial therapy	After relapse and re-treatment (where indicated)	Atlast date seen
Date			
Complete response			
Partial response			
Disease progression (provide details)			
Stable disease			

Relapse disease (no of relapses/duration)_____

_____

_____

Refractory disease (details)_____

_____

_____

Outcome:

Alive ☐ Deceased ☐ Lost to follow up ☐

If deceased:

Cause/s of death _____

Survival time (from date of diagnosis to date of death): in months _____

If lost to follow up:

Date last seen_____

Status of disease at last visit (and type of remission, active disease):

PR Yes ☐ No☐

CRYes ☐ No☐

PD Yes ☐ No☐

SD Yes ☐ No☐

APPENDIX C: FLOW SHEET OF BLOOD RESULTS

	Normal Values	**Initial**	**At end of initial therapy**	**Relapse (if occurs)**	**As at last date seen**
Date					
WCC	4-11 x 10^9/l				
Hb	♀ 14-18g/dl ♂ 12-16g/dl				
Hct	♀ 0.4-0.52 l\l ♀ 0.35-0.48 l/l				
MCV	80-100 fl				
PLT	150-400 x 10^9/l				
Neutro	2-7.5 x 10^9/l				
Lympho	1-4 x 10^9/l				
Mono	0.2-0.8 x 10^9/l				
Baso	0-0.1 x 10^9/l				
Eosino	0-0.5 x 10^9/l				
Smear					
Smudge cells					
Retics	0.2-2%				
RPI	1-2				
Na	135 – 147 mmol/				
K	3.3 – 5.3 mmol/l				
CI	99 – 113 mmol/l				
CO2	18 – 29 mmol/l				
Urea	2.6 – 7.0 mmol/l				
Creat	60 – 120 μmol/l				
Glucose	4.1 – 11.1 mmol/l				
Ca	2.05 – 2.56 mmol/l				
Mg	0.65 – 1.1 mmol/l				
Po4	0.8 – 1.4 mmol/l				
LDH	100 - 200 U/l				
B2 Microglobulin	0.7 – 1.8 mg/l				
Uric acid	0.22 – 0.45 mmol/l				
HIV status					
HIVVL					
CD4	0.2 – 1.0 g/l				
Hep A					
Heb B					
Hep C					
Iron	10 – 30 μmol/l				
Transferrin	2.0 – 3.6 g/l				
%sat	25 – 50%				

Ferritin	30 – 400 µg/ml				
Vitamin B12	145 – 637 pmol/l				
Serum Folate	5 - 20µg/l				
Red cell folate	924 – 3337 mmol/l				
Glucose	2.2 – 3.9 mmol/l				
Bilirubin	0 – 21 µmol/l				
Direct bilirubin	0 – 6 µmol/l				
Total protein	60 – 86 g/l				
Albumin	35 – 52 g/l				
Globulin	25 – 30 g/l				
ALP	40 – 120 U/l				
GGT	0 – 60 U/l				
ALT	5 – 40 U/l				
AST	5 – 40 U/l				
Immunoglobulins					
IgG	7.0 – 16.0 g/l				
IgA	0.7 – 4 g/l				
IgM	0.4 – 2.3 g/l				
Paraprotein					
SFLC					
Ƙ	3.3 – 19.4 mg/L				
ƛ	5.7 – 26.3 mg/L				
Ratio	0.25– 1.65				

APPENDIX D: CONFIDENTIAL DETAILS OF THE PATIENT

Study No/ Patient ID	Name	Hospital No	File Number	Contact No	Physical Address

APPENDIX E: RESPONSE DEFINITIONS AFTER TREATMENT OF CLL PATIENTS (Hallek M, et al. 2018)

Group	Parameter	CR	PR	PD	SD
A	Lymph nodes	None ≥1.5 cm	Decrease ≥50% (from baseline)*	Increase ≥50% from baseline or from response	Change of 249% to 149%
	Liver and/or spleen size†	Spleensize<13cm; liver size normal	Decrease ≥50% (from baseline)	Increase ≥50% from baseline or from response	Change of 249% to 149%
	Constitutional symptoms	None	Any	Any	Any
	Circulating lymphocyte count	Normal	Decrease ≥50% from baseline	Increase ≥50% over baseline	Change of 249% to 149%
B	Platelet count	≥100 3 10^9/L	≥100 3 10^9/L or increase ≥50% over baseline	Decrease of ≥50% from baseline secondary to CLL	Change of 249 to 149%
	Hemoglobin	≥11.0 g/dL (untransfused and without erythropoietin)	≥11 g/dL or increase ≥50% over baseline	Decrease of ≥2 g/dL from baseline secondary to CLL	Increase<11.0g/dL or<50%overbaseline,ordecrease< 2g/dL
	Marrow	Normocellular, no CLL cells, no B-lymphoid nodules	Presence of CLL cells, or of B-lymphoid nodules, or not done	Increase of CLL cells by ≥50% on successive biopsies	No change in marrow infiltrate

*Sum of the products of 6 or fewer lymph nodes (as evaluated by CT scans and physical examination in clinical trials or by physical examination in general practice).

†Spleen size is considered normal if, 13 cm. There is not firmly established international consensus of the size of a normal liver; therefore, liver size should be evaluated by imaging and manual palpation in clinical trials and be recorded according to the definition used in a study protocol.

CR, complete remission (all of the criteria have to be met); PD, progressive disease (at least 1 of the criteria of group A or group B has to be met); PR, partial remission (for a PR, at least 2 of the parameters of group A and 1 parameter of group B need to improve if previously abnormal; if only 1 parameter of both groups A and B is abnormal before therapy, only 1 needs to improve); SD, stable disease (all of the criteria have to be met; constitutional symptoms alone do not define PD).

APPENDIX F: TURN-IT-IN REPORT