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Role of CD200, CD43 and LAIR-1 in diagnosis and prognosis of CLL and NHL patients

A thesis
Submitted to the Council of the College of Medicine and the Committee of Postgraduate Studies of Babylon University in Partial Fulfillment of the Requirements for the Degree of Master in Pathology

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وزارة التعليم العالي والبحث العلمي
جامعة بابل
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بِسم اللّهِ الرَّحمنِ الرَّحِيم

"وَيَسْأَلُونَكَ عَنِ الرُّوحِ قُلِ الرُّوحُ مِنْ

أَمْرِ رَبِّي وَمَا أُوتِيتُمْ مِنَ الْعِلْمِ إِلَّا قَلِيلًا"

صَدَقَ اللّهُ العلي العَظِيم

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Certification of Supervision

We certify that this thesis was prepared under our supervision at the **College of Medicine / University of Babylon** as a partial requirement for the master degree in pathology.

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Sinan Yahya

Dedication

To those who helped me and stood beside me along my life,

My Mother,

Father,

Wife and amazing lovely children

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List of Abbreviations

Abbreviation	Key
BCR	B cell receptor
B2 MCG	B2- microglobuline
BM	Bone marrow
CBC	Complete blood count
CD	Cluster of differentiation
CCND1	Cyclin D1 gen
CLL	chronic lymphocytic leukaemia
CNS	Central nervous system
DLBCL	Diffuse large B cell lymphoma
DNA	Dineuclic acid
EDTA	Ethylene-diamine-tetra-acetic acid
EMZL	Extra nodal Marginal zone lymphoma
ERIC	European receptor initiative on CLL
ESCCA	European society for clinical cell analysis
FACS	Flow cytometry activated cell sorting
FC	Flow cytometry
FISH	Flourecent in situ hybridization
FL	Follicular lymphoma
HCL	Hairy cell leukaemia
HCV	Hepatitis C virus
Ig VH	Immunoglobulin heavy chain variable region
ICH	Immune histochemistry
IWCLL	International work chronic lymphocytic leukaemia
ISMCN	In situ mantle cell neoplasia
LAIR-1	Leukocyte association immunoglobulin receptor inhibitor 1
LPD	Lymphoproliferetive disorders
LDH	Lactate dehydrogenase
g/dL	Gram per deciliter
MALT	Mucosa associated lymphoma
MZL	Marginal zone lymphoma
MS	Matutes score
MCL	Mantle cell lymphoma

MRD	Minimal residual disease
MIPI	Mantle international prognostic index
NHL	NON-HODGKIN lymphoma
NK	Natural killar
NMZL	Nodal Marginal zone lymphoma
OS	Overall survival
PCR	Polymerase chain reaction
PLL	Prolymphocytic leukaemia
RNA	Ribonucleic acid
LFT	Liver function test
SEER	Surveillance epidemiology and end results
SLL	Small lymphocytic lymphoma
SMZL	Splenic Marginal zone lymphoma
TH1	T helper 1
TLR	Tall like receptor
TNF	Tumor necrosis factors
WHO	World health organization
ZAP 70	Zeta association protein 70

Abstract

Background

Chronic lymphoproliferative disorder (LPD), is a malignant disorder of lymphocytes, that affects lymph cells or lymphocytes that make lymphatic tissue. Chronic lymphocytic leukaemia (CLL) is the most common chronic lymphoproliferative disorder (LPD).

The Matutes score (MS) immunophenotypic scoring system is used to separate B-cell chronic lymphocytic leukemia (B-CLL) from other B-NHLs.

CD200 (OX2) is a membrane glycoprotein and belongs to the type I immunoglobulin superfamily.

CD43 (Sialophorin) or (leukosialin) is a major sialoglycoprotein on the surface of human T lymphocytes, monocytes, granulocytes, and some B lymphocytes.

Leukocyte-associated immunoglobulin-like receptor-1 (LAIR1), also known as CD305, is a transmembrane glycoprotein that acts as an inhibitory receptor and is expressed by most immune cells

Aim of study

-To evaluate the role of CD200, CD43 and CD305 (LAIR-1) expression in diagnosis and prognosis of CLL and NHL.

Materials and Methods: This cross sectional study conducted on one hundred forty five patients with chronic lymphoprliferative disorders who were attending Baghdad teaching hospital at medical city from beginning of January 2020 to end of December 2020, patients divided in to two groups;

chronic lymphocytic leukemia (CLL) and non-Hodgkin lymphoma (NHL) patients At diagnosis, there was assessment of CD200, CD43 and LAIR-1 expression in addition to CD38. Clinical and laboratory assessment were done including staging by modified Rai, and Ann arbor staging system (for CLL and NHL respectively), then follow up for about 6-12 months.

Results: There is significant statistical correlation between CD 200 and CD 43 and often co-expression of both in differentiation between CLL and NHL (p value < 0.001), while LAIR-1 have no role.

Almost all patients of CD 200 positive expression show moderate to bright pattern of expression in CLL in apposite to NHL patients, who showed dim to moderate pattern of expression, while the greatest majority of CD43 expression was dim to moderate pattern in both CLL and NHL patients.

In CLL patients there was no significant correlation between prognostic markers (age, Hb, platelets count, lymphocytes count and CD 38 expression) and CD200, CD 43 and LAIR-1 apart of significant inverse correlation between the LAIR-1 and CD38 (p value 0.037).

For NHL patients, all markers show no significant correlation except that CD 43 show significant correlation with expression of CD 38; LAIR-1 with Hb level.

There is significant correlation between LAIR-1 expression and clinical staging system for both CLL and NHL patients, negative expression correlate with advanced stage (p value 0.009, 0.003) repectively.

In our series, LAIR-1 show significant correlation with early need of treatment and remission state in CLL patients, meanwhile those with NHL, both CD43 and LAIR-1 show significant correlation with these variables.

Conclusion: CD200, CD43 and its co-expression of both have a significant value in diagnosis and facilitate the separation of CLL from other B-NHLs particularly in ambiguous cases.

LAIR1 expression is a dependable and inexpensive marker capable of independently expecting those with poor prognostic features and predict the time to first treatment in newly diagnosed unselected patients with chronic lymphocytic leukemia and non-Hodgkin lymphoma.

1.1 Introduction:

Chronic Lymphoproliferative Disorder (LPDs), is a malignant disorder of lymphocytes, that affects lymph cells or lymphocytes that make lymphatic tissue. Chronic Lymphcytic Leukaemia (CLL) is the most communal chronic lymphoproliferative disorder (LPD) (1). It is frequently diagnosed by a matching clinical, cytogenetic and immunophenotypic picture (2). Meanwhile, the boundaries of CLL still unclear because the cytological and immunophenotypic findings can overlay with those of other LPDs that present with leukemic phase (3). The relationship between CLL and doubtful LPDs, defined as disorders with CLL-like phenotypic features but with occasioinal differences, remains unclear. Although efforts have been made to find immunophenotypic markers or scores that would help to make this line more clearly, at present, there is no single, specific, well-established laboratory test that can clearly differentiate between them (4).

The Matutes score (MS) immunophenotypic scoring system is used to separate B-cell chronic lymphocytic leukemia (B-CLL) from other B-NHLs. Strong expression of CD23, CD5 and weak or absent expression of CD79b (or CD22), FMC7, and surface IgM translate into 0–5. However, patient samples with the diagnosis of a B-NHL and an intermediate MS score (2–3 points) are often difficult to classify according to the conventional WHO lymphoma classification (3). Flow cytometry cell sorting (FACS) is an important tool in the diagnostics of B-cell non-Hodgkin lymphomas (B-NHLs). The European Research Initiative on CLL (ERIC) recommendations for flow cytometry include additional markers like CD43, CD79b, CD81, CD200, CD10 and ROR1 for differential diagnosis of CLL and low grade B cell lymphoma (5).

CD200 (OX2) is a membrane glycoprotein and belongs to the type I immunoglobulin superfamily. It is expressed on a wide variety of human cells including B-cells and a subset of T-cells (6).

CD43 (Sialophorin) or (leukosialin) is a major sialoglycoprotein on the surface of human T lymphocytes, monocytes, granulocytes, and some B lymphocytes, which appears to be important for immune function and may be part of a physiologic ligand-receptor complex involved in T-cell activation (7).

Leukocyte-associated immunoglobulin-like receptor-1 (LAIR1), also known as CD305, is a transmembrane glycoprotein that acts as an inhibitory receptor and is expressed by most immune cells. LAIR1 expression varies during B-cell differentiation and has recently been demonstrated in patients with CLL. The in-vivo role of LAIR1 in B cells consists in its inhibiting B-cell receptor (BCR)-mediated signaling and in controlling kinase pathways involved in cell proliferation (8).

1.2 The aims of study:

1. Evaluate the role of CD200, CD43 and CD305 (LAIR-1) expression in diagnosis of CLL and NHL.
2. Evaluate the significance of CD200, CD43 and CD305 in prognosis of both CLL and NHL.

2.1 Lymphoproliferative disorders:

2.1.1. Definition:

B- Cell lymphoproliferative disorders; there are a number of disease entities arising from mature B lymphocytes and which involve primarily the blood, bone marrow and other lymphoid organs such as the lymph nodes and spleen. these disorders are classified by the World Health Organization (WHO) on the basis of their histopathological features. Their clinical course is often chronic and they affect mainly adults, a constant finding in all these entities is the presence in peripheral blood of leukaemic cells in various degrees. Some of these conditions could be considered as primary leukaemias. Others represent the leukaemic phase of indolent non - Hodgkin lymphomas (NHL) and their recognition is important for differential diagnosis. The study of lymphoid leukaemias has been enriched with the presence of monoclonal antibodies that define antigenic determinants specific for the B- and T- cell lineages. Characterization of these malignancies is not possible without the use of these reagents, which help to define the cell lineage and the maturation stage of the leukaemic cell (9).

DNA analysis for the detection of immunoglobulin and T- cell receptor gene rearrangements. Chromosome abnormalities that characterize some of the genetic changes in the lymphoid leukaemias are also important for diagnostic and prognostic purposes and are routinely studied by fluorescence in situ hybridization (FISH) as it is not easy to obtain metaphases in slowly dividing lymphocytes (9).

The primary B- cell leukaemias include chronic lymphocytic leukaemia (CLL), which is by far the most common, the rare B -prolymphocytic

leukemia (B - PLL) and hairy cell leukemia (HCL). The B-cell NHLs that most frequently affect the blood and bone marrow include splenic marginal zone lymphoma (SMZL), mantle cell lymphoma (MCL), in particularly cases with splenomegaly and follicular lymphoma which, in its generalized or systemic form, regularly involves the bone marrow and may spill over to the peripheral blood(9).

Table 2.1 2016 WHO classification of mature lymphoid, histocytic, and dendritic neoplasms [17]

Mature B-cell neoplasms
Chronic lymphocytic leukaemia/small lymphocytic lymphoma
B-cell prolymphocytic leukaemia
Splenic B-cell marginal zone lymphoma
Hairy-cell leukaemia
Splenic lymphoma/leukaemia unclassifiable
Splenic diffuse red pulp small B-cell lymphoma
Hairy-cell leukaemia variant
Lymphoplasmacytic lymphoma/Waldenstrom¨ macroglobulinaemia
Heavy-chain diseases
Plasma-cell myeloma
Solitary plasmacytoma of bone
Extraosseous plasmacytoma
Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma)
Nodal marginal zone lymphoma
Paediatric nodal marginal zone lymphoma
Follicular lymphoma
Paediatric follicular lymphoma
Primary cutaneous follicle centre lymphoma
Mantle-cell lymphoma
Diffuse large B-cell lymphoma (DLBCL), not otherwise specified
T-cell/histiocyte-rich large B-cell lymphoma
Primary DLBCL of the CNS
Primary cutaneous DLBCL, leg type
EBV-positive DLBCL of the elderly
DLBCL associated with chronic inflammation

Lymphomatoid granulomatosis
Primary mediastinal (thymic) large B-cell lymphoma
Intravascular large B-cell lymphoma
ALK-positive large B-cell lymphoma
Plasmablastic lymphoma
Large B-cell lymphomas arising in HHV8-associated multicentric Castleman disease
Primary effusion lymphoma
Burkitt lymphoma
B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and Burkitt lymphoma
B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classical Hodgkin lymphoma

2.2. Chronic Lymphocytic Leukemia

Chronic lymphocytic leukemia (CLL) is "a monoclonal hematopoietic disorder characterized by progressive expansion of B lymphocytes. These small, mature-appearing lymphocytes accumulate in the blood, bone marrow, lymph nodes, liver, and spleen. CLL is a common leukemia in the Western world, accounting for 25% to 30% of all adult leukemias (10).

The 2003-2007 annual age-adjusted incidence of CLL in the United States was 4.2 per 100,000 per year; it was 5.7 per 100,000 for men and 3.0 per 100,000 for women.The median age at diagnosis was 72 years, and the incidence increases with increasing age. For 2010, there were 14,990 estimated new cases, 8,870 men and 6,120 women. During the same year there were 4,390 estimated deaths, 2,650 men and 1,740 women, with a median age at death of 79. The majority of patients have significant comorbidities owing to their advanced age. As a result, they tend to have health, geographic, and access limitations. Patients with CLL enrolled in clinical trials tend to be

younger, with a median age of 58 to 62 years, thereby limiting the ability to generalize results from such trials to practice (10).

In Asian countries, CLL represents only 5% of leukemias, with T-cell phenotype predominating. Geographic and ethnic differences in incidence are most likely the result of genetic factors, since Japanese who settled in Hawaii do not have a higher incidence of CLL than native Japanese. Population studies have not linked the development of CLL to known occupational or environmental risk factors. CLL has a strong familial aggregation, with a two- to sevenfold higher prevalence among family clusters than in the general population.(10)

In Iraq the incidence of CLL from all type of leukaemia during 2018-2019 was 5.2 in male and 2.42 in female(11).

2.2.1. Etiology

It was hypothesized that the B-cell receptor (BCR)- mediated stimulation with putative antigen plays an important role in the natural history of CLL, this hypothesis was supported by studies that revealed highly restricted immunoglobulin heavy chain variable region (IgHV) gene repertoire of CLL cells compared to normal adult B-cell repertoire (12).

Patients with CLL expressing IgHV1-69 gene were found to share closely homologous complementarity determining region 3 (CDR3) sequences on IgH and light (L) chains. Overexpression of IgHV1-69 was associated with unmutated CLL and IgHV4-34 in mutated CLL subtypes, respectively. Although physiological aging was associated with increased B-cell populations expressing IgHV4-34, this was not confirmed for IgHV1-69 expression which seems to be CLL-specific. It was reported that more than

20% of unrelated CLL cases carried stereotyped receptors. This data supports a role of putative environmental antigen or autoantigen in etiopathogenesis of this disease.(12)

2.2.2 Clinical Findings

Most CLL patients in the general population are elderly (median age at diagnosis, 71.5 years). In contrast, the median age at diagnosis of patients seen in CLL clinics is younger, varying from 58 to 68 years, with 20% to 25% of patients younger than 55 years. However, the presenting features are similar, regardless of age. In current practice, 70% to 80% of patients are diagnosed incidentally when they have a routine blood count and will have early-stage (Rai 0 or I) disease. Alternatively, lymphadenopathy, splenomegaly, or both may be detected during a regular physical examination. The most frequent complaint is fatigue or a vague sense of being unwell. Less frequently, enlarged nodes or infections are the initial complaint. Fever and weight loss are uncommon at presentation but may occur with advanced and drug-resistant disease (13).

Most symptomatic patients have enlarged lymph nodes and/or splenomegaly. Enlargement of the cervical and supraclavicular nodes occurs more frequently than axillary or inguinal lymphadenopathy. The lymph nodes are usually discrete, freely movable, and nontender. Painful enlarged nodes usually indicate a superimposed bacterial or viral infection, or possibly a Richter transformation. There is usually only mild to moderate enlargement of the spleen, and splenic infarction is uncommon. Less common manifestations are enlargement of the tonsils, abdominal masses because of mesenteric or retroperitoneal lymphadenopathy, and skin infiltration. Direct involvement of the skin by CLL typically affects the face and the features can be quite

variable, from macules or papules (which may be vascular) to more extensive involvement that may simulate rhinophyma (13).

The patient may also present with skin cancers, shingles, and recalcitrant warts related to immunosuppression. Symptomatic anemia can also be a presenting feature, which may be related to marrow replacement or, more rarely, to autoimmune hemolysis or red cell aplasia. Alternatively, patients may have bruising or bleeding, most commonly related to thrombocytopenia and infrequently to acquired von Willebrand disease. Rarely, patients present with a paraneoplastic syndrome, such as nephrotic syndrome, paraneoplastic pemphigus, or angioedema.(13)

2.2.3. Diagnosis

According to the International Workshop on CLL (IWCLL), the diagnosis of CLL is sustained by the following parameters:

Presence in peripheral blood of $>5\times10^9$/L monoclonal B lymphocytes persisting for at least 3 months, demonstration of the clonality of the population (κ/λ analysis), characteristic immunophenotype: SmIg weak, CD5+, CD19+, CD20weak, CD23+. Regarding the immunophenotype, FMC7 (a CD20 epitope) and CD79b are usually absent or weakly expressed. Based on immunophenotypic characteristics and giving one point to each one of the following: CD5+, CD23+, FMC7weak, SmIg (κ/λ staining) weak and CD79b weak Matutes and Catovsky showed that in patients with a score of 4–5, the diagnosis is virtually always CLL, while in those cases with a score <3, the diagnosis of CLL is extremely unlikely. The typical immunophenotype for CLL as accepted in current guidelines should be revisited because of the

availability of markers highly characteristic of CLL, such as CD200 and ROR1(14).

Great effort has been undertaken to determine appropriate antigens that may enhance the diagnostic accuracy in B-NHL immunophenotyping. , but the optimal strategy and antigen combination remain controversal.The European Research Initiative on CLL (ERIC) & European Society for Clinical Cell Analysis (ESCCA) defined “required” and “recommended” antigens for the flow cytometric diagnosis of the B-CLL. The “required” antigens proposed in this harmonization approach almost matched the conventional MS antigens except for kappa/lambda instead of IgM and FMC7. Among the six “recommended” antigens used for borderline cases were CD200 and CD43 (13).

2.2.4. Bone marrow finding

Bone marrow aspirate will usually show greater than 30% lymphocytes, with flow cytometry confirming monoclonality in the CD19/CD20/CD23/CD5+ population; however, bone marrow evaluation is not required for diagnosis. Other B-cell malignancies may also present with increased circulating lymphoid cells and should be differentiated from CLL. The diseases that may be confused with CLL are prolymphocytic leukemia (PLL), the leukemic phase of non-Hodgkin's lymphoma (mantle cell lymphoma, follicular lymphoma, or splenic lymphoma with circulating villous lymphocytes), and hairy cell leukemia (HCL). Immunophenotyping is helpful in differentiating these disorders (10).

2.2.5. Cytogenetics/FISH and molecular study

Cytogenetic and FISH testing is important in this disease. FISH has the proficiency of recognizing over 80% of the clonal cytogenetic abnormalities present in CLL cells. Solo deletion 13q and normal karyotypes are good prognostic profiles in CLL, whereas deletion 11q and deletion 17p has un favorable prognosis (14). Of these clonal aberrations, the most common is deletion 13q14.3, occurring in approximately 50% of patients, and trisomy 12 in about 20%. The other frequent cytogenetic abnormalities (such as deletion 11q22-23, deletion 17p13 and deletion 6q21), constitute the majority of the cytogenetic abnormalities exhibited in the remaining 30% of CLL patients. Calin et al. reported that miR15 and miR16 are located at chromosome 13q14 region, which is deleted in about 50% of patients with CLL. The same group described a unique miRNA expression signature composed of 13 genes that differentiated cases of CLL with low versus high expression of ZAP-70, and cases with unmutated IgVH-region gene from those with mutated IgVH. This signature also correlated with disease progression (15). Patients with a 17p deletion (17p-) are considered to have a very poor prognosis mainly due to their refractoriness to conventional therapy and alternative more effective but also more toxic therapeutic approaches, such as alemtuzumab or allogeneic haematopoietic cell transplantation, are generally recommended. The 17p deletion is found in 2–4% of cases at diagnosis (de novo deletions), but can also be acquired during the evolution of the disease, particularly in patients who have received chemotherapy (14).

2.2.6. Assessment of remission

The recommendation was made that patients treated in a clinical trial have evaluation of the blood or bone marrow by sensitive tests for residual disease

such as multicolor flow cytometry or allele-specific polymerase chain reaction (PCR) for the IGHV gene. In some patients who achieve complete remission by IWCLL criteria, one or both of these methods can demonstrate residual disease, referred to as minimal residual disease (MRD). Patients free of MRD following treatment have a longer remission duration and longer survival (10).

Therefore, in addition to improving complete remission rates, investigators are focusing on eliminating MRD. A sensitive four-color flow cytometry assay was developed to differentiate CLL cells from normal B cells (CD5/CD19 with CD20/CD38, CD8 1/CD22, and CD79b/CD43). The assay can detect one CLL cell in 1 of 10000 leukocytes. PCR techniques can also be used to assess MRD (10).

2.2.7. Staging and prognostic factors:

The natural history of CLL is variable, with survival times ranging from 2 to over 20 years from diagnosis. In 1975, Rai et al. developed a staging system consisting of five stages (Rai 0 to IV) based on Dameshek's model of orderly disease progression in CLL. The Rai staging system is modify into a three-stage system: low risk (Rai 0), intermediate risk (Rai I, II), and high risk (Rai III, IV) (10).

A similar staging system was developed in Europe by Binet et al.; Binet stages A, B, and C generally correspond to low-risk, intermediate-risk, and high-risk disease in Rai staging. Both classifications show bulk of disease and extent of marrow failure (i.e., anemia, thrombocytopenia). Both staging systems have predict the overall survival. Although most patients in the high-risk group (both staging group) have a unfavorable clinical course and short survival, the course of the disease is not classical. Patients in the low- and intermediate-risk

groups may have a hidden disease course that occur in years or even decades, or the course may be rapidly deteriorating and associated with a shortened survival. Thus, it is very important to have prognostic factors associated with clinical outcomes in CLL in the low-risk groups. Several prognostic factors have been associated with shortened survival in CLL. These include a short lymphocyte doubling time (less than 6 months), a diffuse pattern of bone marrow infiltration, advanced age and male gender, abnormal karyotype, high serum levels of B2-microglobulin and soluble CD23, and a CLL-PLL category (11 % to 54% prolymphocytes in the blood), newer prognostic factors in CLL include IGHV mutation status, expression of CD38 and ZAP-70(10).

2.3. Non Hodgkin lymphoma

It has been known for more than 35 years that some patients with non-Hodgkin lymphoma (NHL) can be cured using chemotherapy. In the past decade, advances in molecular medicine have provided exciting insights into the biology of NHL. The viral and bacterial etiology of certain lymphomas has now been well documented. Cell surface antigens have been defined that provide targets for therapy with monoclonal antibodies and radio immunotherapy. Moreover, knowledge of cell signaling pathways and the results of gene expression analyses have revealed the evidence of the malignant microenvironment in the neoplastic process and have provided good way for targeted therapy with novel small molecules. With these advances, improved survival has been observed in patients with both indolent and aggressive B-cell NHLs (10).

2.3.1 Epidemiology

Registration of data from the Surveillance Epidemiology and End Results (SEER) database in the United States conclude that there was a marked increase in the number of cases of NHL at the end of the twentieth century, but the increment in cases appears to have slowed since 2000. It is not clear why there was a rise in incidence, it could be that of changes in medical practice, reporting conventions, and changes to the classification of lymphomas have had an impact. several studies investigated possible links to environmental agents such as pesticides or industrial exposures, but not in definite pattern have been identified. The increasing incidence of low-grade lymphomas is less associated with AIDS epidemic as high-grade lymphoma. There is marked geographical variation in incidence of some lymphoma subtypes, such as an infectious aetiology. Although low grade NHLs can present at any age they are all more common with increasing age (9). In Iraq non-Hodgkin lymphoma showed a significant increase in its rate from 2000 to 2016 affecting both genders equally, in 2000 the incidence rate was 3.2 while in 2008, 2.24 reaching 2016 was 3.16, so the Percentage change from 2000- 2016 was 1.25(16).

2.3.2 Mantle Cell Lymphoma

Mantle cell lymphoma (MCL) classically has been recognized as an aggressive but curable small B-cell lymphoma that developed in a linear fashion from naıve B cells. Two types of clinically indolent variants which are Classical MCL, is usually composed of IGHV-unmutated or minimally mutated B cells that usually express SOX11 and typically involves lymph nodes and other extranodal sites. the molecular/cytogenetic abnormalities can lead to even more aggressive blastoid or pleomorphic MCL (17).

Other MCL develop from IGHV-mutated SOX11-(nagitive) B cells which leads to leukemic non-nodal MCL, usually involving the peripheral blood, bone marrow, and often spleen. These cases are frequently clinically indolent; however, secondary abnormalities, often involving TP53, may occur and lead to very aggressive disease. In situ MCL is now to be called in situ mantle cell neoplasia (ISMCN), again emphasizing a more conservative approach for lymphoid neoplasms with a low rate of progression. It is characterized by the presence of cyclin D1 cells, most typically in the inner mantle zones of follicles, in lymphoid tissues that do not otherwise suggest the diagnosis of a MCL, and is found incidentally, sometimes in association with other lymphomas.They are much less common than ISFN and although they may be disseminated, appear to have a low rate of progression. ISMCN should be distinguished from overt MCL with a mantle zone growth pattern.

Nevertheless, these latter cases in addition to other classical MCL with a low proliferative fraction may also be relatively indolent (17).

2.3.2.1 Clinical Features

Mantle cell lymphoma accounts for approximately 7% of adult cases of NHL in the United States and Europe. In a review of 376 cases of disseminated low-grade lymphoma (Working Formulation categories A through E), mantle cell lymphoma made up 10%. It is a tumor of older adults, with a marked male predominance (7S%). The majority (70%) of patients are in stage IV at diagnosis; sites involved are lymph nodes, spleen, Waldeyer's ring, bone marrow (>60%), blood (up to 50%), and extranodal sites, especially the gastrointestinal tract (lymphomatous polyposis) . The course is moderately aggressive. The median OS in most series is 3 years, with no plateau in the

curve, but OS may be improved in recent series. The blastoid variant is reported in some studies to be more aggressive (10).

2.3.2.2 Diagnosis

Mantle cell lymphoma is a B-cell lymphoma that is composed of small lymphocytes with irregular nuclear outlines that have a CD5+ and CD23− phenotype with overexpress cyclin D1 (18).

These lymphomas are usually widespread at time of diagnosis with generalized involvement of lymph nodes and extensive bone marrow involvement. They may involve extranodal sites, such as the Waldeyer ring, or present as lymphomatous polyposis of the lower gastrointestinal tract (18).

A few have a leukemic phase mimicking CLL, prolymphocytic leukemia, or acute leukemia (blastoid variants of mantle cell lymphoma). Recognition of mantle cell lymphoma is clinically important, as these lymphomas have a more aggressive clinical course than other small B-cell lymphomas. In This type of lymphomas the Mitotic activity is often brisk, and large lymphocytes are usually few in number except in the pleomorphic form of the blastoid variant, in which they predominate (18).

2.3.2.3 Lymph node histopathology

Mantle cell lymphomas usually have a diffuse growth pattern or surround reactive germinal centers in a mantle zone pattern. Extension of the lymphoma into the capsule and perinodal fat is common. The blastoid variant of mantle cell lymphoma is characterized by nuclei with increase size, dispersed chromatin, small nucleoli, frequent mitoses, that have an aggressive clinical course. There is now molecular genetic evidence that at least some blastoid variants of mantle cell lymphoma are morphologic transformations of typical

mantle cell lymphoma. The differential diagnosis of blastoid transformation of mantle cell lymphoma includes: acute leukemia involving lymph nodes and lymphoblastic lymphoma (19).

2.3.2.4 Immunophenotypic features

The immunophenotypic finding of mantle cell lymphoma including moderate amounts of surface IgM, usually with IgD. Neoplastic cells are usually CD5+ and CD10−, but a subset of mantle cell lymphomas has CD5− cells. Mantle cell lymphomas are marked with antibodies to pan–B-cell antigens CD19, CD20, and CD22. CD23 is negative or sometimes partially expressed, and FMC7 is positive in contrast to the tumor cells of CLL/SLL (37).

Overexpression of cyclin D1 is universal in mantle cell lymphom is not seen in follicular lymphoid hyperplasia, and is uncommon in other small B-cell malignancies, with the exception of plasmacytic neoplasms and hairy cell leukemia (37).

2.3.2.5 Cytogenetics/FISH and molecular study

High Ki-67 expression is associated with a poor prognosis. On classic cytogenetics, majorty of mantle cell lymphoma show evidence of t(11;14) (q13;q32) involving the cyclin D1 gene (CCND1) (19). FISH analysis of paraffin-embedded tissue extends the sensitivity of t(11;14) detection to greater than 90% and does not require fresh or frozen tissue. Rarely cyclin D1-negative mantle cell lymphomas exhibit a gene expression profile similar to cyclin D1-positive tumors including expression of SOX11. The former cases show overexpression of cyclin D2 or cyclin D3 without translocations involving these genes. nearly one-third of patients with mantle cell lymphoma have somatic mutation of their Ig heavy chain genes. The presence of somatic

mutation is associated with nonnodal disease, lack of SOX11 expression, and a more indolent clinical features. CNS involvement is rare at presentation, but may occur at relapse. MCL is typically FDG-avid and therefore FDG-PET scanning is increasingly used for staging and response assessment (19).

2.3.2.6 Prognosis in MCL

MCL has a worse prognosis than the other 'low-grade' NHLs; the median survival is only 3–5 years, but in approximately 15% of patients the disease has a more indolent course. Patients with indolent disease mostly have lymphocytosis and splenomegaly, with occasional nodal involvement. These cases are also associated with hypermutated immunoglobulin heavy chain variable gene and the absence of SOX11. Patients with MCL can be classified into three main prognostic groups according to the MCL International Prognostic Index (MIPI), which depends on age, performance status, LDH and leucocytosis. The low-risk MIPI group has a median survival in excess of 5 years, while the intermediate- and highrisk MIPI groups is only 51 months and 29 months, respectively. Other factors that could affect prognosis include Ki67 score, β2MCG and CNS involvement. Ki67 has now been incorporated into the MIPI score to give a 'biological MIPI' (MIPIb) (9).

2.3.3 Marginal zone lymphoma

Mucosa-associated lymphoid tissue (MALT) or marginal zone B-cell lymphomas account for about 7–8% of all NHLs in the Western world and occure in an indolent course. Marginal zone lymphomas can be classified into

three distinct entities: extranodal marginal zone lymphoma (EMZL), splenic marginal zone lymphoma (SMZL), and nodal marginal zone lymphoma (NMZL). Upon immunophenotypical features, neoplastic cells from all types express surface immunoglobulin, CD19, CD20, and CD79a, and the usual lack of expression of CD5, CD10, CD23, CD25, and CD103, distinguishes them from other B-cell lymphoproliferative disorders. No marker is specific for marginal zone lymphoma. The diagnosis is often done by the appearance of typical morphology in association with clinical findings and by exclusion of other disorders. Recurrent cytogenetic abnormalities present in gastric MALT lymphoma including trisomy 3, trisomy 18, t(11;18), t(1;14), t(14;18), and t(3;14) (20).

2.3.3.1 Splenic marginal zone lymphoma

Splenic B - cell marginal zone lymphoma (splenic lymphoma) has often been confused with other low - grade B - cell conditions including chronic lymphocytic leukaemia (CLL), immunocytoma and hairy cell leukaemia. It was only identified as a lymphoma in its own right (21).

2.3.3.1.1. Clinical features

Patients are usually older adults who present with splenomegaly or associated conditions such as hypersplenism and autoimmune haemolytic anaemias or thrombocytopenias. Patients with SMZL are usually over the age of 50 years with there being either no gender difference or an excess of females. Some patients may have hepatitis C virus. There may be a low level paraprotein or autoimmune disease. Acquired C1 inhibitor deficiency is associated particularly with this type of lymphoma. SMZL is an indolent lymphoma with prominent splenomegaly and often no lymphadenopathy.

Transformation to diffuse large B- cell lymphoma could occur in up to 18% of patients in 10 years (21).

2.3.3.1.2. Diagnosis

The WHO classification recognizes splenic marginal zone lymphoma (SMZL) as a distinct entity. The diagnosis could be after splenectomy or from blood and bone marrow features (38).

2.3.3.1.3. Peripheral blood findings

The WBC ranges from normal to moderately elevated. In patients diagnosed by splenectomy, approaching 70% have peripheral blood involvement. Circulating neoplastic cells have a pleomorphic features, small lymphocytes, which can include 'villous' lymphocytes and some plasmacytoid lymphocytes. Villous lymphocytes are somewhat bigger than CLL cells and have a round or oval nucleus with moderately condensed chromatin and, in some cases, a small nucleolus. Their cytoplasm is moderately basophilic and have short, fine cytoplasmic projections, often at one pole of the cell. Occasional patients have autoimmune thrombocytopenia or anaemia. Some have a paraproteinmia in low concentration (9).

2.3.3.1.4 Bone marrow findings

The bone marrow aspirate is normal in around 50% of patients with SMZL, even though some of those patients are found to have a nodular infiltrate on trephine biopsy. The bone marrow show inflteration in those patients who present with SLVL. Flow cytometric immunophenotyping There is strong expression of SmIg (IgM and oftenly IgD) and expression of B- cell markers,

such as CD19, CD20, CD22, CD79a and CD79b. CD25 is positive in one third of cases, CD10 in a third and CD11c in a half. The antigen recognized by FMC7 is expressed in most cases. CD5 has usually been found to be negative; in the minority of patients with CD5 expression there could be a discrepancy between CD5- positive cells in the peripheral blood and bone marrow and CD5- negative cells in the spleen. CD43, CD103, annexin A1, BCL6 and cyclin D1 are characteristically negative (9).

2.3.3.1.5. Cytogenetic and molecular study analysis

Trisomy 3 can demonstrated by FISH in approximately 18% of patients. Trisomies 7 and 12 are present in smaller numbers of cases. Approximately 1/5 of cases were previously reported to have t(11;14)(q13;q32) but these cases are now considered to represent mantle cell lymphoma. In contrast to MALT lymphoma, t(11;18), t(14;18) and t(1;14) are not detected. Loss or inactivation of TP53 at 17p13 is present in 10% of patients. In about half of cases the cell of origin is a post- germinal centre B cell with somatic hypermutation of immunoglobulin variable region genes and other are intraclonal variation. One patient has been reported in whom cells initially had unmutated genes but somatic hypermutation was present when disease evolution occurred. Complex rearrangements, rearrangements of 14q and TP53 deletion are prognostically worse, but not when the age and anaemia are taken into account (38).

2.3.3.1.6 Bone marrow histology

In patients diagnosed by splenectomy, around 80% have bone marrow infiltration. This is usually mixed, often with interstitial, nodular,

intrasinusoidal and paratrabecular components. Multiple nodules and intrasinusoidal infiltration are the most characteristic. Nodules occasionally have germinal centres with a marginal zone, the germinal centres being reactive with encircling neoplastic cells. An intrasinusoidal pattern is not unique to SMZL but, when present as the predominant pattern, this is highly suggestive of this diagnosis. Meanwhile, a pure intrasinusoidal pattern is uncommon. In about 40% of patients there is an infiltration of monotypic plasma cells and these patients are more likely to have a paraprotein. In a similar percent of patients, there are polytypic, mostly reactive, plasma cells (38).

2.3.3.1.7 Immunohistochemistry

The neoplastic cells express the B- cell markers, CD20 and CD79a. Staining for CD5, CD10, CD43, cyclin D1, annexin A1 and LEF1 is negative. DBA.44 staining was positive in one third of patients. Immunohistochemical staining for CD20 or CD79a can be crucial in demonstrating intrasinusoidal infiltration. This pattern of infiltration can also be shown by using CD34 to foucus on endothelial cells. Reactive germinal centres in infiltrated areas can be highlighted by the lack of BCL2 expression by germinal centre B cells, whereas the surrounding lymphoma cells show expression of this marker.(37)

2.3.3.1.8 Differential diagnosis

In the spleen the differential diagnosis is generally with the other ‘low - grade ’B - cell lymphomas. The imunophenotyping, allied with a careful clinicopathological correlation is the good way to make a confident diagnosis. Cyclin D1 immunostaining is the crucial marker in distinguishing it from mantle cell lymphoma (21).

Early reports of t(11;14) and cyclin D1 positivity in splenic lymphoma are now generally believed to be misdiagnose mantle cell lymphomas as more detailed studies show splenic lymphomas to be cyclin D1 negative . A note of caution has been raised by a study of six cases of splenic lymphoma with increased numbers of blasts and an aggressive clinical picture, of which two expressed cyclin D1, although again these may be unusual examples of mantle cell lymphoma. Although the distinction from hairy cell leukaemia is rarely problematic any doubts may be dispelled by showing negativity for annexin A1 (21).

2.3.3.1.9 Staging and prognostic factors

Staging of splenic marginal zone lymphoma is same as the mantle cell lymphoma that depend on ann-arbor staging system to make evidence about poor prognostic group that has a sequels on treatment and overall survival.

Prognosis for SMZL is favorable with a median survival of 10 years; only a small number of patients display an unfavorable course. Low albumin levels, high LDH levels, and lower hemoglobin levels seems to be higher risk disease. Symptoms and/or obvious cytopenias prompt initial therapy, usually splenectomy, which can yield a prolonged treatment-free benefit. There is no proven advantage for adjuvant chemotherapy after splenectomy. If patients are unable to have splenectomy or require treatment afterwards, chemotherapy with alklyating agents, purine analogs, or rituximab produces significant rates of response. Lastly, if HCV infection is diagnosed, antiviral treatment must be considered (20).

2.3.3.2 Splenic diffuse red pulp small B - cell lymphoma:

This is a very rare B - cell lymphoma in which there is red pulp sinusoidal infi ltration of the spleen by unremarkable, small, basophilic, lymphoid cells with little or no evidence of residual white pulp. A similar infiltration pattern is seen in the bone marrow. The immunophenotype is virtually identical to SMZL apart from usually being IgD negative (21).

2.3.3.3 Nodal Marginal Zone Lymphomas

2.3.3.3.1 Epidemiology and etiology

NMZL is a rare lymphoma, accounting for 1.5-1.8% of lymphoid neoplasms. The incidence of NMZL seem to be increasing, which is most likely due to a 'real' increase, rather than better recognition. Most studies report a median age around 60 years with a wide age distribution ranging from adolescence to patients over 90 years old. Both sexes are affected with approximately equal frequency. Morphologically, NMZL resembles EMZL. EMZL is known for its association with conditions that provide a chronic stimulation to the immune system, in which chronic infections (e.g. Helicobacter pylori infection in gastric EMZL) and autoimmune conditions (e.g. salivary gland EMZL in Sjögren's syndrome) (22).

From this, one could hypothesize that NMZL, or a subset of NMZLs, might also be caused by specific chronic inflammatory conditions. Infections and autoimmune disorders have been reported in association with NMZL, but this evidence remains away from sufficient to establish a definitive role for these stimulation in lymphomagenesis. Recently, the detectection of H. pylori by PCR analysis and a gastric biopsy revealed EMZL. This case implies that

without extensive workup, including gastric biopsies, one cannot be sure that a case of NMZL really represents this entity. Hepatitis C virus (HCV) has been reported in a subset of NMZL patients. The monocytoid B-cell lymphoma had the highest prevalence of HCV in comparison with other lymphoma types. The difference in HCV prevalence in NMZL between studies might be caused by geographical variation, which also appears to hold true for HCV prevalence in lymphoplasmacytic lymphoma, an entity closely related to NMZL. NMZL has also rarely been described in human immunodeficiency virus-infected patients (22).

2.3.3.3.2 Clinical and laboratory features of patients with NMZL

Most patients with NMZL present with peripheral lymphadenopathy; a small minority of patients have only central lymphadenopathy at presentation. The head and neck lymph nodes are most frequently involved. Bulky tumors (> 5 cm) are notced in 11-31% of patients. Roughly half the patients present with stage III or IV disease and 10-20% of patients experience B symptoms. Anemia is present with varying frequency (11-36%).

Thrombocytopenia has been present in one-tenth of patients. Involvement of the peripheral blood is reported in 10-24% of patients. The incidence of bone marrow involvement varies from 0-62% (22).

2.3.3.3.3 Cell of origin

Marginal zone lymphoma receives its name from the resemblance to the physiological marginal zone. The marginal zone surrounds the mantle zone of the germinal center and is usually not recognized morphologically in lymph nodes. However, the spleen and some mesenteric lymph nodes do show a marginal zone in normal situations, and, occasionally, other lymph nodes also

have marginal zone development. NMZL arises from mature B cells that have rearranged (22).

2.3.3.3.4 Histopathology

NMZL has great variability in growth pattern and cellular morphology and is, therefore, almost never a ‘spot diagnosis’. Rather, a diagnosis of NMZL requires careful integration of morphology, immunohistochemistry, molecular studies, and clinical features (22).

On low power, multiple growth patterns, a diffuse pattern of infiltration was most frequent, followed by interfollicular and nodular patterns of infiltration in 14% and 10%, respectively. On high power, NMZL cells show heterogeneous morphology, varying from centrocyte-like cells to monocytoid cells to plasmacytoid cells and plasma cells with varying numbers of interspersed centroblasts and immunoblasts. Monocytoid cells have a central nucleus with condensed chromatin and indistinct nucleoli, surrounded by ample pale cytoplasm. Centrocyte-like cells, resembling the centrocytes of the germinal center, have nuclei with slightly irregular nuclear membranes and a coarser chromatin structure. Lymphoplasmacytoid cells have some, but not all features of plasma cells; in comparison to plasma cells they have less cytoplasm that is basophilic. They are smaller than typical ‘Marschalko-type’ plasma cells and have a finer chromatin structure. Monocytoid cells were reported in one-third of cases in one study, but predominance of monocytoid cells is rare and should prompt consideration of secondary lymph node involvement by MALT lymphoma. Plasmacytic differentiation has been reported in 22-47% and can be extensive (22).

2.3.3.3.5 Immunophenotype

NMZL cells express pan B-cell markers including CD20, CD79a, and PAX5. The majority of cases are BCL2 positive, although numbers vary from 43% to 100% of cases. Most studies report no expression of BCL6, although one study describes BCL6 staining in a proportion of cells or large cells only in 43% of cases. CD10 positivity has been reported only rarely. CD5 and CD23 are usually negative, being reported in 0-17% and 0-29% of cases, respectively. Although studied in only few patients, the majority of NMZLs appear to express MUM1. CD43 expression varies between studies from 5-75% (33). Splenic, nodal and extranodal marginal zone lymphomas have no distinctive immunophenotypic features. The expression of CD200 is weaker than that of normal B cells, but in about a third of patients with nodal or extranodal marginal zone lymphoma, there is moderate to strong expression overlapping with that of CLL. CD274 (PD-1) is not expressed (23).

2.3.3.3.6 Cytogenetics

Multiple studies have investigated the cytogenetic features of NMZL using classical cytogenetics, comparative genomic hybridization, and fluorescence in situ hybridization (FISH). Although numerous cytogenetic abnormalities have been reported, no specific alterations have been identified so far. Gains of chromosome 1q, 2p, 3p, 3q, 6p, and 6q are most frequent, as are losses of 1q and 6q. Chromosomes 3, 12, and 18 most often show trisomy. Monosomy is more rarely observed and most frequently involves chromosomes 9, 13, and 14. Multiple translocations have been reported in NMZL, but they do not share a common breakpoint region (22).

2.3.3.3.7 Molecular features

Gene expression studies in NMZL have generated different results. In a study of 16 NMZL and 8 FL cases identified MNDA as a gene that is differentially expressed between FL and NMZL, with a rather low ranking of genes that are known to be expressed more often in FL than NMZL (e.g. CD10, BCL6). Another study of 15 NMZLs and 16 FLs reported a rather homogeneous gene expression profile in NMZLs resembling marginal zone and memory B cells. Compared to FL, NMZL showed overexpression of NF-κB-related and -binding genes (TRAF4, CD82), IL-32, histones, members of the TNF family (TACI, TNFRSF14), and genes involved in lymphocyte activation (TGFB1). FLs showed higher expression of germinal center markers (CD10, BCL6, GCET1, LMO2) in comparison to NMZL (24).

2.3.3.3.8 Differential diagnosis

Reactive conditions Toxoplasmosis and human immunodeficiency virus (HIV)-associated lymphadenopathy can show hyperplasia of monocytoid B cells. Morphological differentiation from NMZL can be difficult in some cases. Immunohistochemistry can be helpful; normal monocytoid B cells are BCL2 negative whereas NMZL cells are usually BCL2 positive. If a final diagnosis cannot be made by morphology and immunohistochemistry, clonality testing can provide important additional information (24).

2.3.3.3.9 Staging and prognostic factors

Staging of splenic marginal zone lymphoma is same as the mantle cell lymphoma that depend on ann-arbor staging system to make evidence about poor prognostic group that has a sequels on treatment and overall survival (29).the prognosis appear to be relatively good with median survival times in years. The indications of treatment are cytopenias and symptoms, and

recommendations for therapy include local radiation for low or early-stage disease and either single-agent or combination chemotherapy (alkylating agents, purine analogs, rituximab) for late or advanced disease states (20).

Table 2.2: Ann arbor staging system (9)

Ann Arbor stage	Criteria
Stage I	Involvement of a single lymph node region or lymphoid structure
Stage II	Involvement of two or more lymph node regions on the same side of diaphragm
Stage III	Involvement of lymph node regions or lymphoid structures on both sides of diaphragm
Stage IV	Diffuse or disseminated involvement of one or more extralymphatic organs or tissues with or without associated lymph node enlargement. Involvement of liver and bone marrow alwase consider stage IV
Additional classifiers	
B	Presence of B symptoms; unexplained fever, weight loss and recurrent drenching night sweat in last month
E	Limited extra nodal extention from adjacent nodal site or apparent discrete single extranodal deposit (excluding liver and bone marrow)
X	A node or nodal mass greater than 10 cm. maximum width is equal to or greater than one third of the internal transverse diameter of the thorax at the level of T 5/6 on the chest -ray

2.4 Flowcytometric markers

2.4.1 CD 200:

CD200 (OX-2 antigen) is a type I immunoglobulin superfamily membrane glycoprotein1 which expressed in multiple cell types, including B cells, a subset of T cells, dendritic cells, endothelial cells, and in the peripheral and central nervous system. CD200 interacts with it is receptor CD200R, an immunoglobulin superfamily inhibitory receptor expressed primarily on myeloid/monocyte lineage cells, and has a suppressive effect on T cell–mediated immune response (25).

CD200 has been reported to be expressed by plasma cells in multiple myeloma by flow cytometric immunophenotyping, in acute myeloid leukemias by gene expression analysis, and in a number of carcinomas and other malignant neoplasms, including malignant melanoma, by same procedure analysis. CD200 expression was found to be up-regulated in chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma (SLL) when compared with normal B cells, CD200 expression by neoplastic cells down-regulates the TH1 immune response and suppresses the antitumor immune response in an animal model of CLL (2).

CD200 is a marker of worse prognosis in multiple myeloma and acute myeloid leukemia and was responsible for tumor progression in a number of carcinomas, probably through its immunosuppression effect on the host immune system(25).

The difference in expression patterns of CD200 in BCLPDs, literature partially mentioned this to the different activation of the AKT and MEK/ERK pathways in these different disorders. It is important noting that ERK also

activated in B-CLL, in which expresses CD200. Remarkably , in MCL, the activated pathway is the AKT, which plays a major role in down-regulation of active ERK and this could contribute to the decrease CD200 expression in NHL especially MCL(4).

2.4.2 CD43

CD43 (leukosialin), also known as sialophorin, was first identified in 1981. CD43 is a heavily glycosylated transmembrane protein expressed on the surface of most hematopoietic cells including B lymphocytes of definite phase as well as some lymphomas, leukemias, and solid tumors. The physiologic role of CD43 has been extensively studied but still remains controversial. The 235 amino acid extracellular domain of CD43 usually contains mainly sialylated O-linked glycans and can regulate cell adhesion both positively and negatively. The intracellular C-terminal domain of CD43 is evolutionarily conserved and involved in signal transduction. CD43 also plays a role in locomotion, apoptosis modulation, differentiation, and immune homeostasis (26).

Abnormal expression of CD43 has been implicated in autoimmune diseases such as diabetes, systemic lupus erythematous, Wiskott-Aldrich syndrome, and human immunodeficiency virus infection. Recent studies have focused on the relationship between CD43 expression and tumorigenesis, particularly hematopoietic malignancies (3).

In the cases of B-cell malignancies, its negativity goes with the diagnosis of follicular lymphoma (FL) while its positivity can be seen in a variety of B-cell disorders, mostly in CLL and MCL. Other disorders such as marginal zone lymphoma (MZL) and lymphoplasmocytoid lymphoma also have CD43-

positive by immunohistochemistry (IHC), but its positivity rate varies and depend on the study population and the diagnostic criteria employed. Diffuse large B-cell lymphomas (DLBCL) can also be positive and, in fact, CD43-positivity has been correlated with a worse outcome in this population (7).

CD43 appears to be involved in the tumor metastasis and can serve as a marker of malignant transformation. Moreover, combined application of anti–CD43 antibodies and anti–CD5 antibodies can differentiate tumor cells from normal T cells and B cells, and coexpression of CD43 and CD20 on peripheral B cells is associated with malignancy. CD43 is expressed in approximately 25% of patients with DLBCL; however, its prognostic significance remains unclear(26).

2.4.3 LAIR-1 (CD305)

Leukocyte associated immunoglobulin-like receptor-1 (LAIR1), also known as CD305, is a transmembrane glycoprotein inhibitory receptor with a cytoplasmic tail containing two immunoreceptor tyrosine-based inhibitory motifs (ITIMs). LAIR-1 has previously been expressed on almost all immune cells, including NK cells, T cells, B cells and monocytes, monocyte derived dendritic cells (moDCs), eosinophils, basophils and mast cells, as well as on CD34+ hematopoietic progenitor cells, the majority of thymocytes(27).

LAIR1 expression varies during B-cell differentiation and has recently been demonstrated in patients with CLL. The in-vivo role of LAIR1 in B cells consists in its inhibiting B-cell receptor (BCR)-mediated signaling and in controlling kinase pathways involved in cell proliferation.(28)

Collagens are functional LAIR-1 ligands and directly inhibit immune cell activation in vitro. In addition, LAIR-1 also recognizes proteins of collagen

domains, such as surfactant protein D (10) and C1q, which are a component of the classical complement pathway. Activation of LAIR-1 in vitro potently inhibits diverse immune functions (8).

Crosslinking of LAIR-1 results in inhibition of T cell receptor-mediated signaling, immunoglobulin (Ig) G and IgE production by B cells and lysis of target cells by NK cells. Also, LAIR-1 crosslinking and C1q stimulation suppresses interferon alpha (IFN-α) release in plasmacytoid dendritic cells (pDC) and toll-like receptor (TLR) 9-stimulated cytokine production by monocytes. Aberrant LAIR-1 expression associated with autoimmune diseases, leukemia and viral infections. Moreover, LAIR-1 is absent in high–risk B cell chronic lymphocytic leukemia cells and LAIR-1 is down regulated on NK cells isolated from patients enduring a chronic active Epstein Barr virus infection (28).

More recently, it was shown that LAIR-1 is expressed on in vivo activated human neutrophils and that LAIR-1 suppresses neutrophil extracellular trap formation by airway-infiltrated neutrophils obtained from patients with respiratory syncytial virus bronchiolitis. LAIR-1 is a distinctive receptor in the immune inhibitory receptor family because of the broad expression pattern of both the receptor and the ligands. The regulation of LAIR-1-mediated inhibition might be dependent on different factors such as the strength of the activation signals, the levels of expression of the receptor, but also on soluble LAIR-1 molecules.(27).

2.5 Flow cytometry Immunophenotyping (FCI)

Flow cytometry is a technique for counting, examining, and sorting microscopic particles suspended in a stream of fluid. It allows simultaneous multiparametric analysis of the physical and/or chemical characteristics of 28 single cells flowing through an optical and/or electronic detection apparatus (29).

2.5.1 Historical view

The first fluorescence-based flow cytometry device was developed in 1968 by Wolfgang Göhde (University of Münster, Germany) and such instruments were first commercialized by Partec in Göttingen in 1968/69.

The original name of the flow cytometry technology was pulse cytophotometry (Impulszytophotometrie in German, ICP) and this was changed to flow cytometry at the Conference of the American Engineering Foundation in Pensacola, Florida in 1978. The ability to measure multiple parameters (volume, light scatter, fluorescence) using a single instrument was developed by Paul Mullaney, and the capacity to measure side scatter was developed by Gary Salzman. Whereas the sorting process was applied by Mack Fulwyler who was worked in Marvin van Dilla's laboratory at the Los Alamos National Laboratories, USA 1965 and Leonard Herzenberg at Stanford University, USA used the term, Fluorescence Activated Cell Sorter (FACS) in the mid1970s (29).

Flow cytometry is a very important diagnostic tool in modern clinical laboratories. It analyzes thousands of cells in less than a minute and classifies cell populations accurately. Flow Cytometric techniques are routinely used for the analysis of peripheral blood or bone marrow specimens in clinical

laboratories and research labs. Subtyping of different cell types is possible due to the availability of a large number of immune markers (25).

2.5.2 Immunofluorescence:

The Basics Immunofluorescence is a technique that permits visualization of cellular features or structures by linking these to a molecule that emits light when stimulated by light at a separate wavelength. Immunofluorescent techniques may use either dyes that bind directly to structures in or on the cell, or fluorochromes conjugated to a ligand such as monoclonal antibodies, lectins, or cytokines. The common feature of these dyes and fluorochromes is that they absorb light at one set of wavelengths (excitation wavelengths) and emit light at a different set of wavelengths (and emission wavelengths) (30).

The difference between the excitation and emission wavelengths is termed the Stoke's Shift. Because most flow cytometers use lasers as a light source and these emit light at specific wave lengths, the dyes and fluorochromes used for flow cytometry must be compatible with the laser in the cytometer. The fluorochromes must be excited at a wave length emitted by the cytometer and must fluoresce at a wave length distinct from the laser light (39).

2.5.3 Instrumentation

A flow cytometer is versatile in its capability of measuring multiple parameters simultaneously. These parameters include the physical properties of cells (e.g., cell size and cytoplasmic granularity), surface membrane, cytoplasmic and nuclear antigens, and DNA-RNA contents of individual cells in a cell suspension. Surface, cytoplasmic, and nuclear antigens are detected by means of fluorochromes conjugated antibodies. These antigens 30 are thus the extrinsic properties of the cell. The physical properties of the cell are the

intrinsic properties, because no exogenous reagents are added for their detection. These parameters are measured through an optical system, and the light signal thus generated is registered in an electronic system. The computer system is responsible for data storage, gating, and graphic display on a screen (31).

2.5.4 Flow Cytometer Design

Flow cytometers may be viewed as ‘‘organ systems.’’ Such systems would be: (1) optics (including the light source), (2) fluidics, (3) electronics, and (4) computer (30).

2.5.4.1 The optical system

The optical system may be broken into two types of components: those necessary to appropriately excite the cells and those necessary for collection of the light emitted from, or scattered by, the cells. The former components include the light source (usually a laser) and the lens and mirrors used to shape, focus, and direct the beam of light to the point where the cells are to be interrogated. The latter include an assortment of filters and mirrors needed to separate and direct emissions of different wave lengths to the corresponding detectors. Lasers do not produce broad spectrum light, rather they emit light at specific peak emission wavelengths. Thus the laser used in the cytometer needs to be matched with the fluorochromes used for Immunofluorescent staining analysed on the cytometer (31).

The most commonly used lasers, argon ion lasers, produce a strong emission at 488 nm that is capable of exciting several commonly used 31 fluorochromes and dyes. Many .cytometers are capable of using two or more lasers simultaneously to excite a wide array of fluorochromes (39).

In addition to detecting and quantitating immunofluorescence, virtually all flow cytometers are capable of detecting light scatter signals. These light scatter signals are the result of the laser light reflecting and refracting off the cells. Light scatter measured at a right angle to the direction of the laser beam is termed orthogonal (or side or 90°) light scatter and correlates with the granularity of the cell. Light scatter measured in the forward direction (180° from the point at which the laser beam intersects the cell) is called forward scatter and roughly correlates with cell size (31).

2.5.4.2 Fluid Transport System

The fluid transport system starts with a sample receiving area where test tubes containing patients' specimens and controls are placed in a carousel. The cell suspension is aspirated by means of differential air pressure or vacuum into a tubing system leading to the flow chamber. When in the flow chamber, which is a conical nozzle, the specimen is surrounded by a cell-free stream of sheath fluid, producing a laminar flow configuration. The outer sheath fluid forces the cells in the sample to line up single file. When the sample exits from the flow chamber through a narrow orifice, the flow velocity becomes markedly increased (about 1 to 10 m/s) (39). The cell stream then meets the light source (mercury arc lamp or laser) at the light interception point in the sensing area, and electric or optical signals are generated (31).

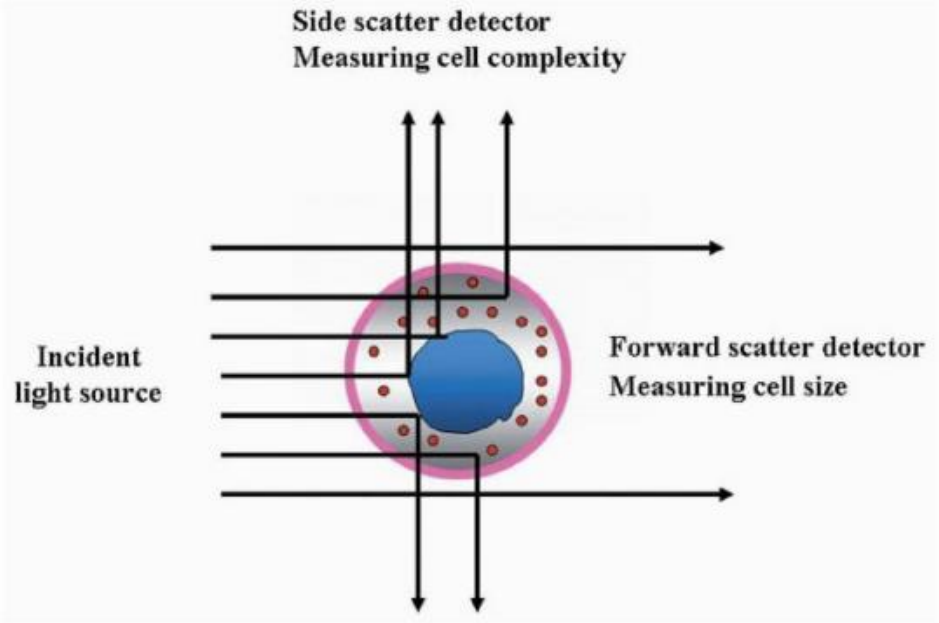

Figure 2.1 Schematic illustration shown the relationship of light scatter and cell (39)

2.5.4.3 Electronic System

After traveling through all the filters and lenses, the photons of light impinge on the detectors and are converted into electrons (photoelectric effect). The detectors incude photomultiplier tubes, which are commonly used for sidescatter and fluorescence signals, and photodiodes, which are used for absorption, extinction, and forward-scatter signals. The electronic signals or voltage pulses are analog signals in various magnitudes from 1 to 10 V. Analog signals may be processed as peak amplitude (height), integral (area), width (duration), or shape of the pulse and are expressed in either logarithmic or linear scales last component of the electronic system is the pulse height

analyser, which analyses the digital signals and quantifies them for computer display on an oscilloscope screen (41).

2.5.4.4 Computer System

The flow cytometer can be interfaced with an external computer system that performs three important functions. 1. List-mode storage: All parameters measured can be stored permanently on a floppy disk or temporarily on a hard disk. All data can be combined and analysed later and can be printed in graphic form as a permanent record. 2. Gating: A gate is an electronic window that can be set with a cursor on the screen in a rectilinear or amorphous form to circumscribe a group of cells with similar characteristics (e.g., size and cytoplasmic granularity). Gating is performed to isolate electronically this special group of cells for analysis, avoiding the difficult task of purifying the cell population by biologic means. Depending on the software capability, several gates can be set so that information can be gathered on several populations of cells. 3. Graphic display: The computer can provide graphic displays in several form (41).

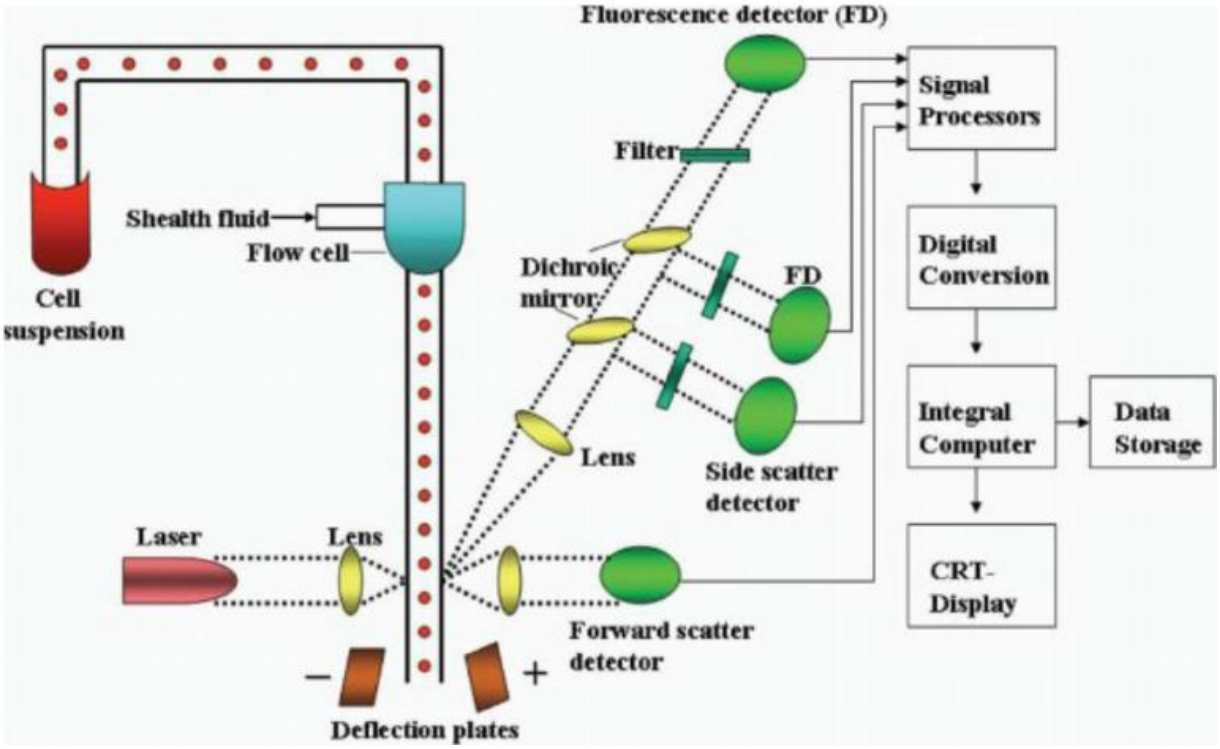

Figure 2.1 Show basic structure of a flow cytometer (39)

3.1: Patients and materials

3.1.1 Chemicals and Instruments

This section presents the chemicals and materials used in this study and their manufacturers are shown in Table 3.1, while Table 3.2 presents the all instruments and apparatus that have been used in this study.

Table 3.1 Chemicals and materials used in this study and their origin.

Chemical/material	Manufacturer (country)
Leishman stain	SYRBIO (SAR)
Methylene blue stain	J&K Scientific (China)
Oil	SYRBIO (SAR)
McAb combo or multicolor cocktail	Becton Dickinson, Bio
polyclonal antibodies (PcAb)	Becton Dickinson, Bio

Table 3.2: Instruments used in this study

Instrument	Manufacturer (country)
Hematology Analyzer Ruby	Abbott (Germany)
Incubator	Fisher Scientific (Germany)
Micropipette 10-1000	Slammed (Germany)
Micropipette 2- 20	Gilson (France)
Light microscope	Olympus (Japan)
EDTA tube (5 ml)	Plasmatic Laboratory (UK)
Eppendorf tube (1.5 ml)	IkEME (China)
Slides	SAIL BRAND (China)
Gloves	Kleinhans (Malaysia)
BD FACS Calibur™ flow cytometer	Becton Dickinson, Bio

3.1.2 Study subject

This study was conducted on 145 adult patients of newly diagnosed CLL and leukaemic phase of NHL through January to December 2020. Those patients were admitted to hematology department in Baghdad teaching hospital, the

immunophenoptyping using a BD FACS Calibur™ flow cytometer (Becton Dickinson, Bio) was done in the flowcytometery department, teaching laboratories in Baghdad, Iraq. The diagnosis depend on clinical suspicion, peripheral and bone marrow morphology which showed absolute mature lymphocytosis, then immunophenotypic analysis by flowcytometry with MS scoring was done for diagnosis of chronic lymphocytic leukaemia and non-Hodgkin lymphoma, CD38 expression was also measured in addition to CD200, CD43 and CD305 which are the main markers on the study.

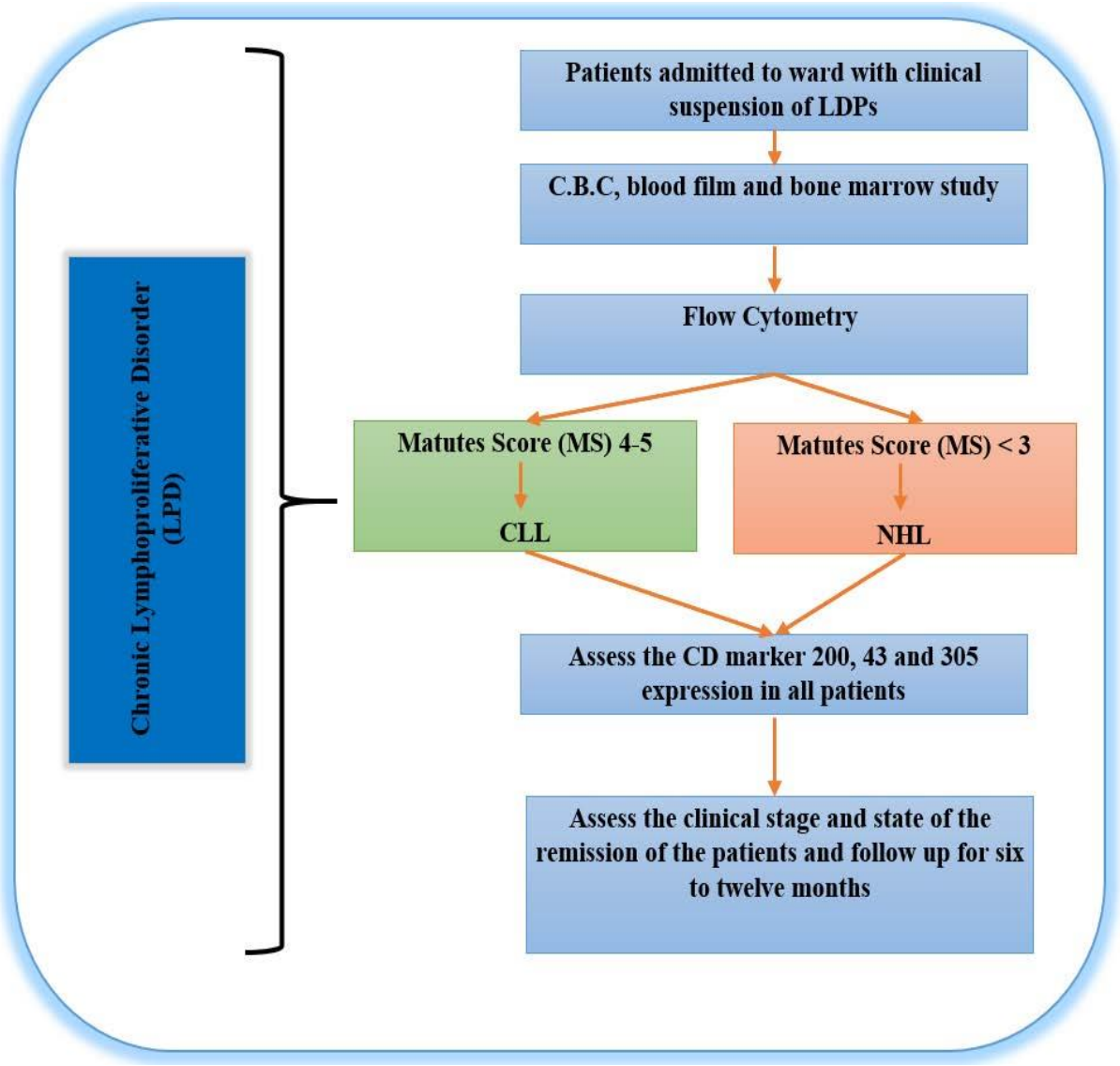

Figure 3.1 Flowchart showing the study stage

At diagnosis, clinical data include age, gender, physical sign, clinical presentation and examination such as organomegaly, generalize or localize lymphadenopathy were collected from patient laboratory request and case sheet of patient. Hematological data include WBC, absolute lymphocytes count, hemoglobin and platelets count were also collected.

According to hematological parameters, physical and clinical findings we made staging for each patient in order to make good categorization for patient stage and the future response to treatment.

So for CLL patients we use modified Rai staging system to differentiate low, intermediate and high risk group. For NHL patients we depend on Ann Arbor staging system, also to make categorization of patient in to four staging group in which there are four groups (I, II, III, IV).

All patient were evaluated (after having verbal and/or written consent) there after either by face to face collection of data on their attendance to hospital or by phoning, follow up program including all patients (on treatment or those whose physician prefer to not use treatment) for six to twelve months, evaluation of their hematological, clinical and physical response and assessment of their remission state depending on blood film, bone marrow finding and some patient with detection of minimal residual disease by flowcytometry was done.

3.1.3 Inclusion criteria

1-Newly diagnosed lymphoproleferative disorders.

2-The patient are randomly collected in relation to gender.

3-Adult group patients.

4-All patient were not receive any treatment before sample collection.

3.2 Laboratory tests

3.2.1 Blood and bone marrow sampling

A total venous blood sample of 2.5 ml and /or bone marrow aspirate sample of 0.5 ml were obtained from each patient included in this study by venipuncture from anticubital fossa or bone marrow aspirate from posterior superior iliac crest under aseptic technique respectively, and the samples were collected in EDTA K2 tubes.

Blood sample from suspected patient was examined for complete blood indices in the teaching laboratory department of the medical city, a blood film and bone marrow slides was made by taking a drop of blood and /or bone marrow sample spreads it on a clean dry slide, and staining it by Leishmen; the slides were examined by a specialist in the teaching laboratory department of medical city.

Accordingly sample of peripheral blood is prepared for analysis by automated device (Cell-DYN, RUBY list), and then in association with clinical suspicion, we recommend flowcytometric panel according to Euro Flow antibody panels(32)

3.2.2 Staining

It was prepared by adding 2 g of Leishman powder in 500 ml methanol, and then the container was put in water bath at 56c and shook every 15 minutes to make a homogenous mixture then the mixture filtered by filter paper. After the drop of blood sample was spread on a clean dry slide, it was covered with Leishman stain for about 4 min to fix, add the buffered solution (composed of

100 ml of 66 mmol/l buffer solution to 1000 ml of distal water at a PH of 6.8) leave for 6 min then washed by tap water then left to dry by air and examined under light microscope (33).

3.2.3 Flowcytometry

By Four color flow cytometric analysis was performed using a BD FACS Calibur™ flow cytometer (Becton Dickinson, Bio) and FACS Canto II flow cytometer (Becton Dickinson Immunocytometry Systems, San José, CA, USA). Cell Quest software (Becton Dickinson, San Jose, CA) and FACS Diva software were used to analyze the data (34).

During immunotyping, 100,000 events were collected. CD45+ lymphocytes were gated on CD45 and SSC dot plot, then B cells were isolated by differentiated on CD19+ cells. A cut-off percentage of positive value of CD markers was 20% (34).

3.2.3.1Methods

Preparation of samples; Flow cytometry can be used on any sample that has cells in suspension. Such suspensions can be seen in peripheral blood, bone marrow, CSF, ascitic fluid, pleural fluid, and FNAs, which simply require red cell lysis and labeling with appropriate antibody panels, as described below. Following tissue disaggregation to obtain a cell suspension, lymph node, spleen, liver, and bone marrow trephine biopsy specimens (not in fixative) can be processed for flow cytometry to obtain a cell suspension (35).

3.2.3.2 Detection of membrane antigens

To detect the antigens including CD200, CD43 and CD305 on the membrane we follow the procedure as below:

1. Stain–Lyse–Wash method:

• Pipette 100µl of the specimen into a round-bottom tube. Note: if the cell count of a specimen is known to be high, dilute this accordingly, aiming for a final cell concentration of 1 to 2×106 per tube.

• Mix in the desired amount of McAb combo or multicolor cocktail.

• Incubate in the dark at room temperature for 15min.

• Incubate for 10 minutes at room temperature in the dark with 1 mL of ammonium chloride-based lysing solution.

• Centrifuge for 5min at 1500 rpm and discard the supernatant. Repeat this step.

• Resuspend the cells in 0.2–0.5ml of sheath fluid solution and acquire data on the flow cytometer without delay.

2. Stain–Lyse–No Wash method: This other method uses the same procedure as the previous methodology, but after incubation with the lysing solution, the sample data are acquired on the flow cytometer. (This method is ideal for samples with few cells) because it reduces cell loss during the washing step's centrifugation.

3. Lyse–Stain–Wash method: This method of bulk specimen lysis is used to expedite and enrich the collection of leucocytes for minimal residual disease (MRD) monitoring (35).

3.2.3.3 Detection of surface immunoglobulin:

In LPDs there are two main phenotypic aberrations that distinguish mature B cells from their normal counterparts; surface immunoglobulin light chain restriction and aberrant B-cell antigen expression (35).

Staining for surface immunoglobulins requires some extra steps in the sample preparation. This is to avoid any nonspecific binding due either to cytophilic antibodies binding to Fc receptors (monocytes and some lymphocytes) or to the binding of antibodies to cell membranes of damaged or dying cells. Before staining for surface immunoglobulins, wash the sample with an isotonic solution to avoid nonspecific staining. Nonspecific staining can also be reduced by pre-soaking the cells in serum before staining. Finally, some B-cell LPDs such as chronic lymphocytic leukaemia (CLL) may express surface immunoglobulins very weakly and it is preferable to use polyclonal antibodies (PcAb) to detect light chain restriction in these cases. Depending on whether a PBS wash or a lysing procedure is employed as the initial step, two approaches are available for identifying surface membrane immunoglobulin (SmIg) of blood and bone marrow cells (35).

3.3 Data Analysis

Statistical analysis was carried out using SPSS version 25. Categorical variables were presented as frequencies and percentages. Continuous variables were presented as (Means ± SD). Student t-test was used to compare means between two groups. Mann-Whitney Test was used to compare means between two groups when variable was not normally distributed. Pearson chi-square and Fisher's exact test were used to find the association between categorical variables. A p-value of ≤ 0.05 was considered as significant.

4.1: The study groups

This is across sectional study including 145 LPD patients diagnosed by flowcytometry in to two main subtypes, 98 patients with CLL and 47 patients with leukaemic phase NHL have mean age of 62.73 and 62.1 years respectively. Male is more than female percentage as in table (1).

4.1.1 Age and sex distribution

Table 4.1: The association between socio-demographic characteristics and diagnosis (N=145)

Study variables	CLL (N=98)	NHL (N=47)	P-value
Age (years)	(62.73 ± 9.20)	(62.10 ± 10.25)	0.711
Gender			
Male	61 (62.2)	32 (68.1)	0.493
Female	37 (37.8)	15 (31.9)	
Total	98 (100.0)	47 (100.0)	

4.2 Role of CD200, CD43 and CD 305 in differentiation between CLL and NHL.

4.2.1 The relevance of each CD marker in diagnosis of CLL and NHL

There was significant association between study variables including (CD200 and CD43) and diagnosis, CD200 and CD43 are positive in high percentage of CLL patients, p value below 0.001, CD305 has no significant association with diagnosis of CLL and NHL, as shown in table (2)

Table 4.2 Association between study variables (CD200, CD43 and CD 305) and diagnosis (N=145)

Study variables	Diagnosis		Total	X^2	P-value
	CLL	Non Hodgkin Lymphoma			
CD 200					
Positive	98 (100.0)	29 (61.7)	127 (87.6)	**42.85**	**<0.001***
Negative	0 (0.0)	18 (38.3)	18 (12.4)		
Total	98 (100.0)	47 (100.0)	145 (100.0)		
CD 43					
Positive	82 (83.7)	15 (31.9)	97 (66.9)	**38.42**	**<0.001***
Negative	16 (16.3)	32 (68.1)	48 (33.1)		
Total	98 (100.0)	47 (100.0)	145 (100.0)		
CD 305					
Positive	22 (22.4)	16 (34.0)	38 (26.2)	2.208	0.137
Negative	76 (77.6)	31 (66.0)	107 (73.8)		
Total	98 (100.0)	47 (100.0)	145 (100.0)		

*P value ≤ 0.05 was significant.

4.2.2 The significance of co-expression CD 200 and CD 43 in CLL and NHL cases

There is significant positive co-expression of both CD200 and CD43 and diagnosis of CLL, p value below 0.001 as shown in table 3.

Table 4.3. Association between CD200 and CD43 co-expression and diagnosis (N=145)

Study variables	Diagnosis		Total	P-value
	CLL(98)	NHL(47)		
Positive CD 200 and CD 43 co-expression	82 (83.7)	9 (19.1)	91 (62.8)	**<0.001***

*P value ≤ 0.05 was significant. Fisher's Exact Test.

4.2.3 Association of pattern of expression and diagnosis

There is significant correlation between pattern (bright, moderate and dim) expression of CD200 and the provisional diagnosis with moderate to bright expression in CLL, p value below 0.001, as in figure 1

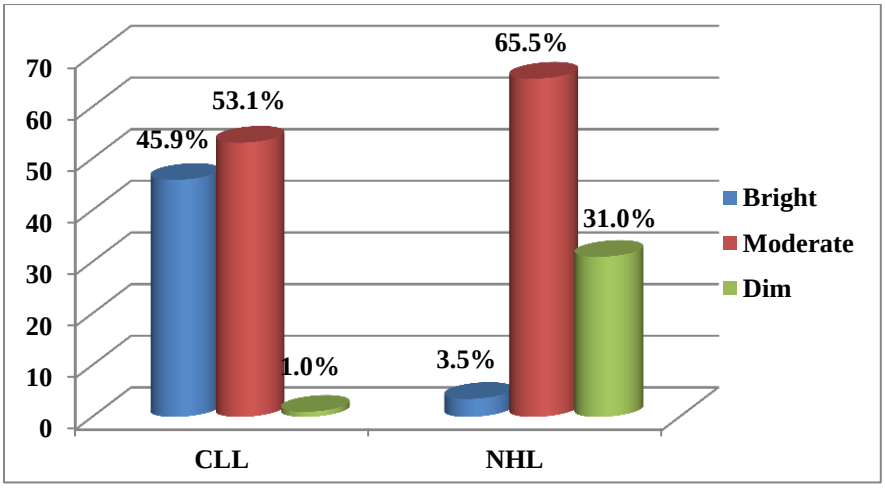

Figure 4.1: Distribution of patients with positive CD200 according to flow cytometry pattern of positive (N=127, P<0.001*)

There is no significant correlation between pattern (bright, moderate and dim) expression of CD43 and the provisional diagnosis, p value 1.000.as in figure 2.

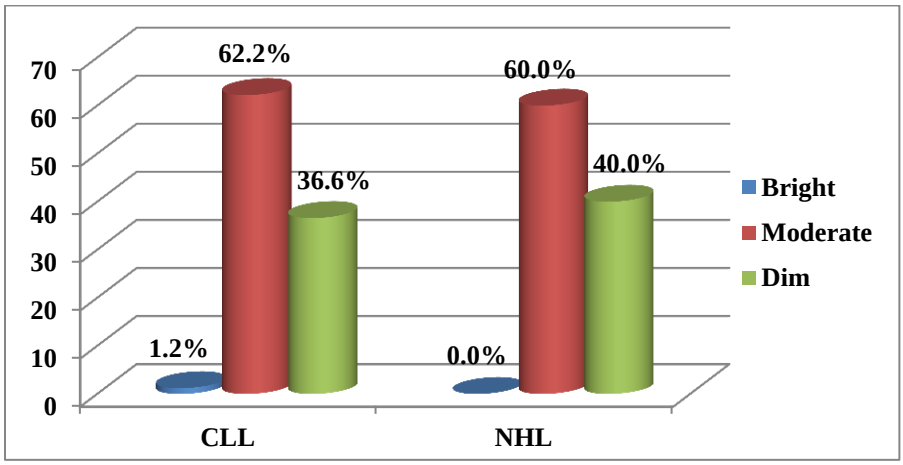

Figure 4.2: Distribution of patients with positive CD43 according to flow cytometry pattern of positive (N=97, P=1.000

There is no significant correlation between pattern (bright, moderate and dim) expression of CD305 and the provisional diagnosis, p value 0.409. as in figure 3.

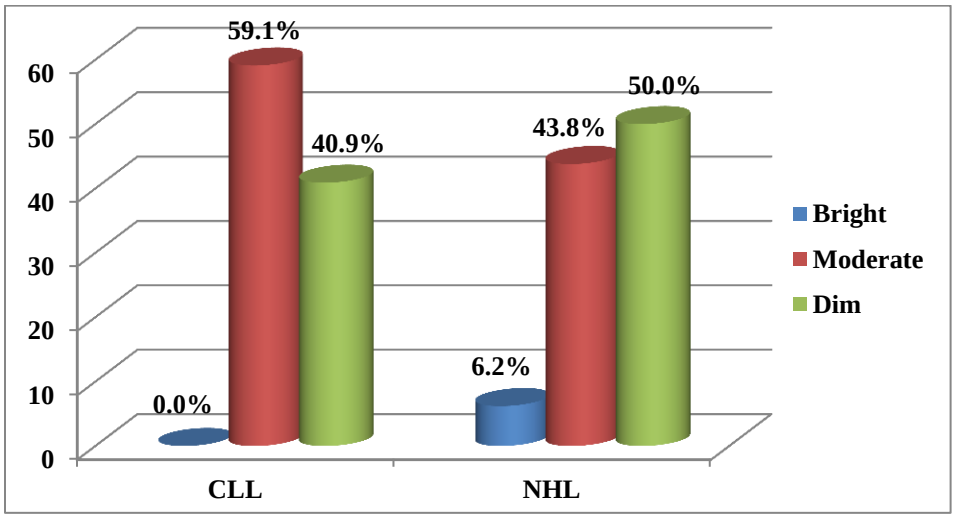

Figure 4.3: Distribution of patients with positive CD305 according to flow cytometry pattern of positive (N=38, P=0.409)

4.3 Role of CD 200, 43 and CD305 in prognosis of CLL patients

4.3.1 Association with laboratory factors

There is no significant association between CD200, CD43 and CD305 with age, Hb. Level, platelets, lymphocytes count and CD38 in CLL patients apart of inverse association of CD305 and CD38, p value 0.037 as in table (4)

Table 4.4. Association between study variables (CD200, CD43 and CD305) and laboratory prognostics factors in CLL patients (N=98)

Prognostic factors	**CD 200**			**CD43**			**CD 305**		
	+ (98)	- (0)	P	+ (82)	- (16)	P	+ (22)	- (76)	P
Age									
< 60	36 (36.7)	0 (0.0)	-	30 (36.6)	6 (37.5)	0.945	8 (36.4)	28 (36.8)	0.967
≥ 60	62 (63.3)	0 (0.0)		52 (63.4)	10 (62.5)		14 (63.6)	48 (63.2)	
Hb									
< 11	46 (46.9)	0 (0.0)	-	40 (48.8)	6 (37.5)	0.408	7 (31.8)	39 (51.3)	0.107
≥ 11	52 (53.1)	0 (0.0)		42 (51.2)	10 (62.5)		15 (68.2)	37 (48.7)	
Platelet count									
<100	11 (11.2)	0 (0.0)	-	8 (9.8)	3 (18.8)	0.381	0 (0.0)	11 (14.5)	0.066
≥ 100	87 (88.8)	0 (0.0)		74 (90.2)	13 (81.2)		22 (100.0)	65 (85.5)	
CD 38									
Positive	13 (13.3)	0 (0.0)	-	9 (11.0)	4 (25.0)	0.218	0 (0.0)	13 (17.1)	**0.037***
Negative	85 (86.7)	0 (0.0)		73 (89.0)	12 (75.0)		22 (100.0)	63 (82.9)	
Lymphocyte									
< 30	56 (57.1)	0 (0.0)	-	47 (57.3)	9 (56.2)	0.937	14 (63.6)	42 (55.3)	0.485
≥ 30	42 (42.9)	0 (0.0)		35 (42.7)	7 (43.8)		8 (36.4)	34 (44.7)	

*P value ≤ 0.05 was significant. Fisher's Exact Test.

4.3.2 Relation to clinical stage of disease

There was significant association between CD305 and clinical stage of disease (there is negative CD305 expression with those of high risk patients, p value 0.009. CD200, CD43 has no significant relation to clinical stage as shown in table (5).

Table 4.5. Association between study variables (CD200, CD43 and CD305) and clinical stage among CLL patients (N=98)

Study variables	**Clinical Stage**					**Total**	**X^2**	**P-value**
	Low risk (0) (8.2%)	**Intermediate risk**		**High risk**				
		I	**II (56%)**	**III**	**IV (35.7%)**			
CD 200								
Positive	8 (100.0)	31(100.0)	24 (100.0)	23 (100.0)	12 (100.0)	98 (100.0)	-	-
Negative	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)		
Total	8 (100.0)	31 (100.0)	24 (100.0)	23 (100.0)	12 (100.0)	98 (100.0)		
CD 43								
Positive	8 (100.0)	26 (83.9)	19 (79.2)	20 (87.0)	9 (75.0)	82 (83.7)		0.652 f
Negative	0 (0.0)	5 (16.1)	5 (20.8)	3 (13.0)	3 (25.0)	16 (16.3)		
Total	8 (100.0)	31 (100.0)	24 (100.0)	23 (100.0)	12 (100.0)	98 (100.0)		
CD 305								
Positive	4 (50.0)	12 (38.7)	3 (12.5)	2 (8.7)	1 (8.3)	22 (22.4)	**13.4**	**0.009***
Negative	4 (50.0)	19 (61.3)	21 (87.5)	21 (91.3)	11 (91.7)	76 (77.6)		
Total	8 (100)	31 (100.0)	24 (100.0)	23 (100.0)	12 (100.0)	98 (100.0)		

*P value ≤ 0.05 was significant. f: Fisher's Exact Test.

4.3.3 Relation to state of remission

There is clinical significant correlation between CD305 negative expression in CLL patients and early death while on treatment and patients on treatment (both with and without remission) in comparison to patients on no treatment, p value 0.02 as shown in table 4.6.

figure 4.4 show 35% of CLL patients not need treatment.

CD200 and CD43 had neither association to clinical stage of disease nor to remission state after treatment.

Table 4.6. Association between study variables (CD200, CD43 and CD405) and state of remission in CLL patients (N=98)

Study variables	**State of remission**				**Total**	**P-value**
	Patients die while on treatment	Patients have treatment without remission	Patients have treatment with remission	Patients not need treatment		
CD 200						
Positive	9 (100.0)	14 (100.0)	41 (100.0)	34 (100.0)	98 (100.0)	-
Negative	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Total	9 (100.0)	14 (100.0)	41 (100.0)	34 (100.0)	98 (100.0)	
CD 43						
Positive	7 (77.8)	11 (78.6)	33 (80.5)	31 (91.2)	82 (83.7)	0.468
Negative	2 (22.2)	3 (21.4)	8 (19.5)	3 (8.8)	16 (16.3)	
Total	9 (100.0)	14 (100.0)	41 (100.0)	34 (100.0)	98 (100.0)	
CD 305						
Positive	1 (11.1)	2 (14.3)	5 (12.2)	14 (41.2)	22 (22.4)	**0.02***
Negative	8 (88.9)	12 (85.7)	36 (87.8)	20 (58.8)	76 (77.6)	
Total	9 (100.0)	14 (100.0)	41 (100.0)	34 (100.0)	98 (100.0)	

*P value ≤ 0.05 was significant. Fisher's Exact Test.

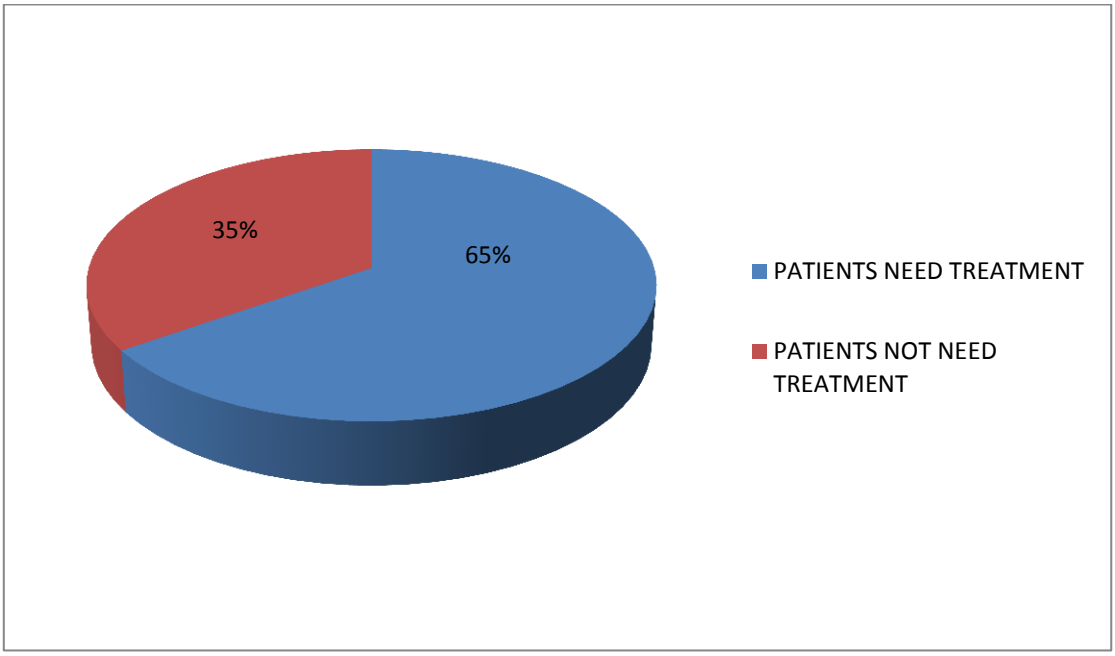

Figure 4.4. Show number of CLL patients who need treatment 64 (65%), and those who not need 34 (35%) total number 98 patients.

4.4 Role of CD 200, 43 and CD305 in prognosis of NHL patients

4.4.1 Association with laboratory factors

There is no significant association between CD200 , CD43 and CD305 with age, platelets, lymphocytes count in NHL patients apart of significant association with expression of CD305 in relation to hemoglobin level and expression of CD43 in relation to the presence of CD38, p value 0.03 and 0.009 respectively, as in table (7).

Table 4.7. Association between study variables (CD200, CD43 and CD405) and laboratory prognostics factors in NHL patients (N=47)

Prognostic factors	CD 200			CD43			CD 305		
	+ (29)	- (18)	P	+ (15)	- (32)	P	+ (16)	- (31)	P
Age < 60 ≥ 60	 12 (41.4) 17 (58.6)	 4 (22.2) 14 (77.8)	 0.178	 6 (40.0) 9 (60.0)	 10 (31.3) 22 (68.7)	 0.555	 6 (37.5) 10 (62.5)	 10 (32.3) 21 (67.7)	 0.719
Hb < 11 ≥ 11	 14 (48.3) 15 (51.7)	 11 (61.1) 7 (38.9)	 0.391	 9 (60.0) 6 (40.0)	 16(50.0) 16 (50.0)	 0.522	 5 (31.3) 11 (68.7)	 20 (64.5) 11 (35.5)	 **0.03***
Platelet count <100 ≥ 100	 8 (27.6) 21 (72.4)	 3 (16.7) 15 (83.3)	 0.492	 5 (33.3) 10 (66.7)	 6(18.8) 26 (81.2)	 0.292	 3 (18.8) 13 (81.2)	 8 (25.8) 23 (74.2)	 0.725
CD 38 Positive Negative	 6 (20.7) 23 (79.3)	 2 (11.1) 16 (88.9)	 0.692	 6 (40.0) 9 (60.0)	 2 (6.3) 30 (93.7)	 **0.009***	 1 (6.3) 15 (93.7)	 7 (22.6) 24 (77.4)	 0.234
Lymphocyte count	17.18	30.11	0.347	12.58	26.61	0.268	32.86	16.60	0.191

*P value ≤ 0.05 was significant. Fisher's Exact Test.

4.4.2 Relation to clinical stage of disease

There is significant association between CD305 and clinical stage of NHL patients, there is negative CD305 expression with those of high risk patients, p value 0.003.

CD200 and CD43 has no significant role as shown in table (8).

Table 4.8. Association between study variables (CD200, CD43 and CD305) and clinical stage among non-Hodgkin lymphoma patients (N=47)

Study variables	Clinical Stage				Total	P-value
	Low risk		High risk			
	I	II (27.7%)	III	IV (72.3%)		
CD 200 Positive Negative Total	 1 (100.0) 0 (0.0) 1 (100.0)	 10 (83.3) 2 (16.7) 12 (100.0)	 12 (50.0) 12 (50.0) 24 (100.0)	 6 (60.0) 4 (40.0) 10 (100.0)	 29 (61.7) 18 (38.3) 47 (100.0)	 0.215
CD 43 Positive Negative Total	 0 (0.0) 1 (100.0) 1 (100.0)	 3 (25.0) 9 (75.0) 12 (100.0)	 7 (29.2) 17 (70.8) 24 (100.0)	 5 (50.0) 5 (50.0) 10 (100.0)	 15 (31.9) 32 (68.1) 47 (100.0)	 0.577
CD 305 Positive Negative Total	 0 (0.0) 1 (100.0) 1 (100.0)	 9 (75.0) 3 (25.0) 12 (100.0)	 6 (25.0) 18 (75.0) 24 (100.0)	 1 (10.0) 9 (90.0) 10 (100.0)	 16 (34.0) 31 (66.0) 47 (100.0)	 **0.003***

*P value ≤ 0.05 was significant. Fisher's Exact Test.

4.4.3 Relation to state of remission

There is clinical significant correlation between CD305 negative expression in NHL patients and early death while on treatment and patient without remission; while CD43 negative expression correlated with achievement of remission state and with patients on no treatments, p value 0.003 and 0.019 respectively as shown in table (9) keeping in mind that 89% of NHL patients in this study were on treatment as in figure (5).

Table 4.9. Association between study variables (CD200, CD43 and CD405) and state of remission in NHL (N=47)

Study variables	**State of remission**				**Total**	**P-value**
	Patients die while on treatment	Patients have treatment without remission	Patients have treatment with remission	Patients not need treatment		
CD 200 Positive Negative Total	5 (55.6) 4 (44.4) 9 (100.0)	12 (54.5) 10 (45.5) 22 (100.0)	7 (63.6) 4 (36.4) 11 (100.0)	5 (100.0) 0 (0.0) 5 (100.0)	29 (61.7) 18 (38.3) 47 (100.0)	0.336
CD 43 Positive Negative Total	3 (33.3) 6 (66.7) 9 (100.0)	11 (50.0) 11 (50.0) 22 (100.0)	0 (0.0) 11 (100.0) 11 (100.0)	1 (20.0) 4 (80.0) 5 (100.0)	15 (31.9) 32 (68.1) 47 (100.0)	**0.019***
CD 305 Positive Negative Total	2 (22.2) 7 (77.8) 9 (100.0)	3 (13.6) 19 (86.4) 22 (100.0)	7 (63.6) 4 (36.4) 11 (100.0)	4 (80.0) 1 (20.0) 5 (100.0)	16 (34.0) 31 (66.0) 47 (100.0)	**0.003***

*P value ≤ 0.05 was significant. Fisher's Exact Test.

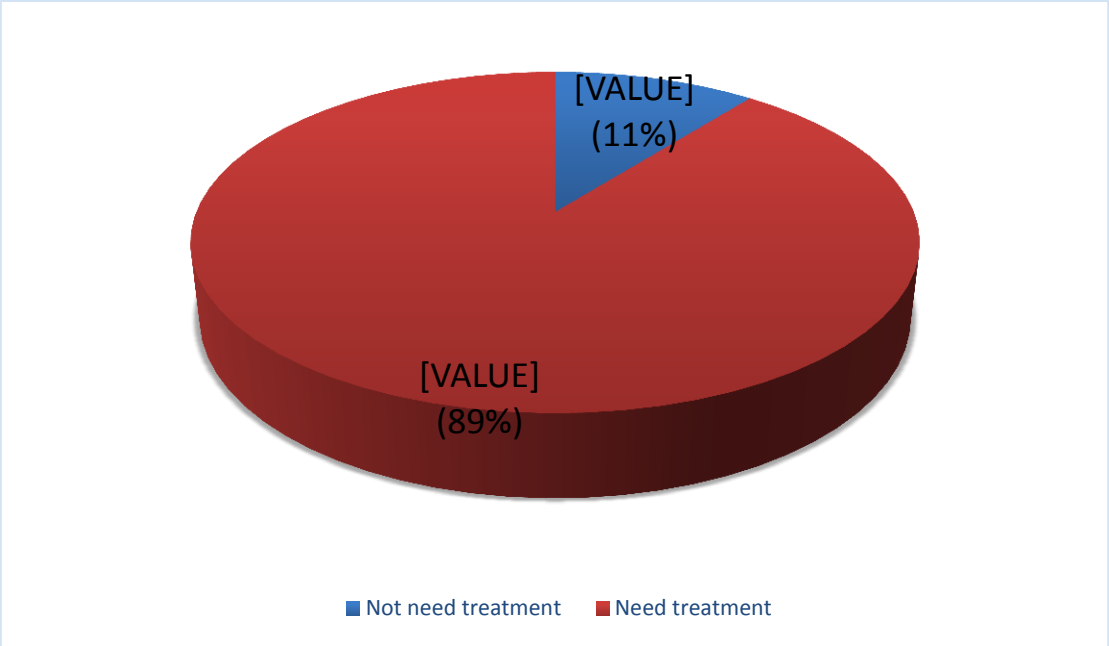

Figure 4.5. Show number of NHL patients who need treatment 42 (89%), and those who not need 5 (11%) total number 47 patients

4.4.4 frequency of subtype of NHL

Table 4.10. frequency of the incidence of subtype of NHL patients (N=47

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NHL SUBTYPE	FREQUENCY
Mantle Cell Lymphoma	27.6% (13)
Marginal zone lymphoma	40.4% (19)
Other	32 % (15)
total	100% (47)

5.1 Age and sex distribution in patients with CLL and NHL

Chronic Lymphoproliferative Disorder (LPD), is a malignant disorder of lymphocytes, that affects lymph cells or lymphocytes that make lymphatic tissue. Chronic lymphcytic leukaemia (CLL) is the most communal chronic lymphoproliferative disorder (LPD) (1). It is frequently diagnosed by a matching clinical, cytogenetic and immunophenotypic picture (2)

In this study, mean age of CLL patients is 62.73 and NHL is 62.1, this goes with the fact that LPDs can affect elderly age group and has clear age preference. LPD has no sex predilection, and in this study, no significant sex difference between CLL and NHL patients, although most were male that account for (62%) in CLL, (68%) for NHL patients. This may be attributed to the sample size and random chance.

5.2 Expression of CD200, CD43 and LAIR-1 in diagnosis of CLL and NHL

We investigate the value of expression of CD200, CD 43 and LAIR-1 in diagnosis of LPDs

- **CD200** was significantly positive in almost all patients in this study, with moderate to bright expression in all CLL (98/98) (100%), while in (NHL) patients CD 200 was mostly dim to moderate expression in 29 out of 47 patients (61.7%) (p value < 0.001) as in table 2, figure 1.

The explanation of the difference in expression patterns of CD200 in BCLPDs, literature partially mentioned this to the different activation of the AKT and MEK/ERK pathways in these different disorders (4).

- **CD43** was expressed in (82/98) (83.7%) in CLL patients with dim to moderate expression while it was almost always dim - moderate expression in

15 out of 47 NHL patients (31.9%) (p value < 0.001) as show in table 2, figure 2, these findings are consistent with El Desoukey NA et al, Sandes AF et al, Alapat D et al, Brunetti L et al, Pillai V et al, McWhirter JR et al and Kohnke T et al; all those series show that almost all CLL cases show moderate to bright expression of CD200 and CD 43 with lower expression in those with NHL patients.(4, 5,40,48, 49, 50,51).

In this series of chronic lymphoproliferative disorders with exclusive PB and/or BM affection, CD43-positivity increased with increasing Matutes score of CLL, suggesting that this CD marker could be of help in the distinctive role in diagnosis of complex or borderline cases. The frequent CD43-positivity in CLL has been well recognized both by FC and our results agree with Sorigue M et al, Quijano S et al, Kostopoulos IV et al, Jung G et al. (7,52,53,58).

Other studies show that CD43 improved the classification accuracy of the classical scoring system. However, the inclusion of CD43 offers a better performance that affect discriminant functions rather than the classical scoring system. So CD43, together with classical markers, is useful in differentiation between CLL and non-CLL, and should be systematically used in addition to well-known methods to improve the diagnosis and classification of the B-cell lymphoproliferative disorders (58).

-**Co-expression** of both 200 and 43 in differentiation between CLL and NHL; 82 with CLL out of 98(83.6%) of patients show co-expression of both CD200 and CD43 while 9 with NHL out of 47(19.1) of patients show both CD200 and CD43 markers expression, p value was significant (< 0.001) as show in table 3.

Some studies state that the combination of CD200 and CD43 offers a highest accuracy to differentiate between CLL and non-CLL LPDs. However borderline LPDs, which constitute a small proportion of cases, remain a diagnostic challenge and cannot be dependably identified or readily diagnosed with our proposed combination of CD200 and CD43. But further studies will be necessary to determine a precise score (36).

We confirmed that the introduction of CD200 and CD 43 to CLL matutes score system had a very high accuracy to distinguish between CLL and NHL patients (3).

- **LAIR-1** expression show no significant expression in differential diagnosis between CLL and NHL patients (the p value 0.137) in contrast to Chuksina et al show that a significant role for LAIR-1 in differentiation between CLL and some NHL patients and this could be due to sample size and relatively a high number of hairy cell lekaemia in later study in compare to our study (73).

5.3. Role of CD 200, 43 and LAIR-1 as a prognostic parameter in CLL patients:

In this study, also we evaluate whether the use of CD200, CD43 and LAIR-1 as an innovative routine marker could be helpful to improve the utility of flow cytometry in prognostic significance of CLL patients.

Prognostic factors can help us to sort CLL patients who need immediate therapy soon after diagnosis in which include certain clinical and laboratory features, cytogenetic, molecular, and biochemical characteristics of the neoplastic cell.

-CD 200 there is no significant statistical correlation between CD 200 expression and age, Hb, platelet count, lymphocytes count and CD38.

Meanwhile about 86.7% of same group have negative expression of CD 38, which indicate good prognostic group (p value 0.037) as in table 4. the above parameters consider a prognostic laboratory features, these finding is consistent with El Desoukey NA et al and D'Arena G et al in whom state that CD200 expression in B-CLL was not correlated with these laboratory features (4,19); while in contrast to our results, Dorfman DM et al, El Din Fouad et al, Wang et al., Zahedi M et al and Wong KK conclude that positive CD200 expression was associated with these prognostic variable.(19,55,56,59,72) .

In present study we also notify that correlation of CD200 positive expression to the clinical stage of the disease that 35 out of 98 patients (35.7%) have high risk group (stage III-IV) while 63patients (64%) of them low and intermediate group (0-I-II) according to RIA staging system (there is no p value because all CLL patients was CD200 positive as in table 5, similarly El Desoukey NA et al and D'Arena G et al that consider the CD200 expression in B-CLL was not correlated with the clinical staging system (RAI staging) (4,34) while in contrast to our results, Dorfman DM et al, El Din Fouad et al, Wang et al., Zahedi M et al and Wong KK conclude that positive CD200 expression was associated with advanced stage and earlier time to progression .(19,55,56,59,72) .

There is no significant clinical association between CD200 expression and the state of remission as in table 4.6, this finding is consistent with El Din Fouad et al that conclude that there is no correlation with response to treatment and overall survival (55).

-CD 43 there is no significant statistical correlation between CD 43 expression and age, Hb, platelet count, lymphocytes count and CD 38, as in table 4.

Also notify there is no significant correlation between CD43 expression and the clinical stage of the disease, as show in table 5.

There are no significant clinical association between CD43 expression and the state of remission (p value 0.468) as show in 6.

No other study correlate the expression of CD43 as a prognostic factor.

-LAIR-1 no significant correlation between LAIR-1 expression and age, hemoglobin level, platelets count and lymphocytes count of CLL patients as show in table 4, in contrast to Hammad R and Ahmedy et al (60,63) that report that there are significant correlation, which could be due to sample size .

On the other hand, all LAIR-1 positive patients 22 (100%) show negative expression for CD38, so there is a significant inversely correlation between LAIR-1 and CD38 expression (p value 0.037) as show in table 4, similarly as with Perbellini et al, Hammad R et al, and Ahmedy et al. (28, 60, 63,).

Interestingly LAIR-1 decrease significantly with advanced modified Rai staging which means that the positive expression are found in earlier clinical stages (mostly of low and intermediate stages) (p value 0.009) as show in table 5. Similarly Rawstron et al, Ahmedy et al. and Sales MM et al . Found that LAIR-1 positive patients were presented in early stages (stage 0-I-II) than in advanced stages (stage III and IV)(13, 63, and 74). Moreover Ahmedy et al. and Poggi et al, confirmed that its expression is lower in high risk patient (61,63).

As LAIR-1 constrain B-cell receptor (BCR)-mediated signaling and control kinase pathways of cell proliferation. It was reported that LAIR-1 inhibits the

activation of immunological cells using two immune receptor tyrosine-based inhibitory motifs located in the cytoplasmic tail of the receptor (64).

There is a clinically significant association between LAIR-1 expression and the state of remission and need to treatment (p value 0.02) as show in table 4.6, similarly with Perbellini et al. and Benedetti et al. that report LAIR1positive expression was significantly associated with longer time to first treatment (28,75).

5.4 Role of CD200, CD43 and LAIR-1 as a prognostic parameter in NHL patients

-CD200 there is no significant correlation between CD200 expression and the prognostic factors (age, Hb, platelet count, CD 38 expression and lymphocytes count) as in table 7, similarly El Desoukey NA et al, Sandes AF et al and Rodrigues et al (4, 5 and 68) .

Also there is no significant correlation between CD200 positive expression and clinical stage of the disease (p value 0.215) as in table 8.

Moreover there are no significant clinical association between CD200 expression and the state of remission (p value 0.336) as show in table 9, unfortunately there are no similar studies found to correlate these findings.

-CD43 there is no significant statistical correlation between CD43 expression and age, Hb, platelet count and lymphocytes count. While there is a significant correlation with presence of CD38 (which suggest poor prognostic value) (p value 0.009) as in table 7.

There is no significant association between CD43 and clinical stage of disease as in table 8, further studies are required to correlate CD43 with clinical stage.

There is significant correlation between CD 43 expression and state of remission, p value (0.019) so high percentage of patients 100%, 80% of patients achieved remission, not need treatment respectively show negative expression of CD43 as in table 9. so we conclude that CD 43 has negative expression in those with good prognostic history, this agreed with Ma XB et al that showed CD43 is an independent prognostic factor for DLBCL, NOS and other types of NHL that foresees poor prognosis, further studies are also recommended (26). Further studies are required to verify our conclusions in other subtypes of NHL.

- **LAIR-1** expression show significant correlation just with haemoglobin levele (p value 0.03) as in table 7 , while other prognostic variable (age, platelets, CD38 and lymphocytes count) show no significant association with LAIR-1 expression, further studies need to correlate this parameters .

LAIR-1 expression decrease significantly with advanced Ann arbor staging system which means that the positive expression is found in earlier clinical stages (low risk stages) (p value 0.003) as show in table 8. Further studies are mandatory to correlate with these results.

There is a clinical significant association between LAIR-1 expression and the state of remission and need to treatment (p value 0.003) as show in table 9, high percent of patients who died early after initiation of treatment and patients on treatment but without remission had negative expression of LAIR-1, no similar study found to correlate.

6.1 Conclusions:

From the present study, we may conclude the following:

1. CD 200, CD43 and co-expression of both has a significant role in diagnosis and discrimination between CLL and NHL.
2. CD200 has always moderate to bright expression in CLL while CD43 has dim to moderate expression.
3. LAIR-1 has no role in diagnosis of CLL and NHL.
4. CD200 and 43 expressions had no prognostic value for both CLL and NHL.
5. LAIR-1 has significant prognostic value for both CLL and NHL patients.
6. Negative LAIR-1 expression correlate with advanced stages of disease in both CLL and NHL patients.
7. LAIR-1expression has inverse correlation with CD38 in CLL patients.
8. About 34.6% of CLL patients not need treatment.
9. LAIR-1 expression has correlation with early death and early need of treatment in both CLL and NHL.

6.2 Recommendations:

1. Study correlation of LAIR-1 expression with cytogenetic and molecular prognostic factors.
2. Extended follow up period in order to detect relation of LAIR-1 expression with remission state and early relapse.
3. Study on large cohort

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خلاصة البحث

ابيضاض الدم الليمفاوي المزمن (CLL) ، هو اضطراب خبيث في الخلايا الليمفاوية ، يؤثر على الخلايا الليمفاوية التي تصنع الأنسجة اللمفاوية. وهو الاضطراب التكاثري الليمفاوي المزمن الأكثر شيوعًا (LPD). غالبًا ما يتم تشخيصه من خلال مطابقة الصورة السريرية والخلوية والنمطية المناعية. ومع ذلك ، لا تزال حدود CLL غير واضحة لأن النتائج الخلوية والنمطية المناعية يمكن أن تتداخل مع تلك الخاصة بـ LPDs الأخرى مع عرض اللوكيميا. في الواقع ، لا تزال العلاقة بين CLL و LPDs الحدودية ، التي تم تعريفها على أنها اضطرابات مع بعض السمات المظهرية الشبيهة بـ CLL ولكن مع بعض الاختلافات الرئيسية ، غير واضحة. على الرغم من بذل محاولات للعثور على علامات أو علامات نمطية مناعية من شأنها أن تساعد في رسم هذا الخط بشكل أكثر وضوحًا ، في الوقت الحالي ، لا يوجد اختبار معمل واحد محدد وراسخ يمكنه التمييز بوضوح بينها.

يتم استخدام نظام تسجيل النمط المناعي (MS) Matutes score لفصل سرطان الدم الليمفاوي المزمن للخلايا (B-CLL)) عن (B-NHLs) . يُترجم التعبير القوي عن CD23 و CD5 والتعبير الضعيف أو الغائب عن CD79b (أو CD22) و FMC7 و IgM السطحي إلى (0-5) نقاط . ومع ذلك ، غالبًا ما يصعب تصنيف عينات المرضى مع تشخيص B-NHL ودرجة MS المتوسطة (2-3 نقاط) وفقًا لتصنيف سرطان الغدد الليمفاوية التقليدي لمنظمة الصحة العالمية. يعد فرز خلايا قياس التدفق الخلوي (FACS) أداة مهمة في تشخيص الأورام اللمفاوية (B-NHLs). تتضمن توصيات مبادرة الأبحاث الأوروبية حول (CLL) لقياس التدفق الخلوي علامات إضافية مثل CD43 و CD79b و CD81 و CD200 و CD10 و ROR1 للتشخيص التفريقي لـ CLL وسرطان الغدد الليمفاوية للخلايا منخفضة الدرجة.

CD200 (OX2)هو بروتين سكري غشائي وينتمي إلى النوع الأول من عائلة الغلوبولين المناعي. يتم التعبير عنه في مجموعة متنوعة من الخلايا البشرية بما في ذلك الخلايا البائية (B-cells) ومجموعة فرعية من الخلايا التائية (T cell) .

(CD43)(Sialophorin)) أو (leukosialin) هو بروتين سيالوغليكوبروتين رئيسي على سطح الخلايا اللمفاوية التائية البشرية ، والوحيدات ، والخلايا المحببة ، وبعض الخلايا الليمفاوية نوع B ،

والتي يبدو أنها مهمة لوظيفة المناعة وقد تكون جزءًا من مركب فسيولوجي له دور مهم في تنشيط الخلايا التائية.

المستقبل الشبيه بالجلوبيولين المناعي المرتبط بالكريات البيض (LAIR1) ، المعروف أيضًا باسم(CD305) ، هو بروتين سكري عبر الغشاء يعمل كمستقبل مثبط ويتم التعبير عنه بواسطة معظم الخلايا المناعية. يختلف تعبير LAIR1 أثناء تمايز الخلايا البائية وقد ظهر مؤخرًا في مرضى CLL. يتمثل الدور الحي لـ LAIR1 في الخلايا البائية في تثبيط إشارات مستقبلات الخلايا البائية (BCR) والتحكم في مسارات kinaseالتي تشارك في تكاثر الخلايا.

الهدف من الدراسة

تقييم تعبير CD200 و CD43 و cd305في تشخيص CLLو NHL ومدى تأثير وجود هذه التعابير على مستقبل المريض العلاجي

المواد والطرق:

أجريت هذه الدراسة المقطعية على مائة وخمسة وأربعين مريضًا يعانون من اضطرابات زيادة الخلايا اللمفاويه المزمنة الذين كانوا يحضرون إلى مستشفى بغداد التعليمي في المدينة الطبية من بداية كانون الثاني (يناير) 2020 إلى نهاية كانون الأول (ديسمبر) 2020 ، تم تقسيم المرضى إلى مجموعتين ؛ ابيضاض الدم الليمفاوي المزمن (CLL) وسرطان الدم الليمفاوي الا هودجيني (سرطان الغدد الليمفاوية اللاهودجين) (NHL) وفقًا لملفها المستضدي أو نظام التسجيل (درجة Matutes) ، تم جمع عينة من الدم و / أو نخاع العظم من كل مريض ، ووفقًا لفيلم الدم المحيطي ، تم إجراء فحص نخاع العظم والنمط الظاهري المناعيبواسطة قياس التدفق وفي بعض الأحيان خزعة نخاع العظم والعقدة الليمفاوية.

تم قياس تقييم CD200 و CD43 وتعبير LAIR-1 على الخلايا نوع ب عن طريق قياس التدفق الخلوي عند التشخيص. تم إجراء التقييم السريري والمختبري بما في ذلك التدريج بواسطة نظام راي المعدل ، ونظام آن أربور (لـ CLL و NHL على التوالي) في بعض المجموعات الإنذارية ، وعدد الخلايا الليمفاوية المطلق بالإضافة إلى تقييم العمر ، وتقييم عدد الصفائح الدموية ، والمتابعة لمدة 6-

12 بما في ذلك جميع المرضى وما إذا كانوا بحاجة إلى علاج أم لا ، يتم تقييم حالة الشفاء عن طريق تعداد الدم الكامل وتشكل نخاع العظم وتقييم الحد الأدنى من الأمراض المتبقية وتقييم تعبير CD 38.

النتائج: هناك تعبير ذو دلالة إحصائية عن CD 200 و CD 43 في التمايز بين CLL و NHL ، بينما لم يحدث ذلك في LAIR-1

في مرضى CLL ، لا يوجد ارتباط كبير بين العلامات النذير (العمر ، Hb ، عدد الصفائح الدموية ، عدد الخلايا الليمفاوية وتعبير CD 38) و CD200 و CD 43 ، بينما يرتبط LAIR-1 بشكل كبير فقط بتعبير CD 38 وسلبي للعلامات الأخرى.

بالنسبة لمرضى NHL ، لا تظهر جميع العلامات ارتباطًا مهمًا باستثناء أن CD 43 تظهر ارتباطًا كبيرًا مع التعبير عن CD 38 ؛ LAIR-1 بمستوى خضاب.

يُظهر جميع مرضى التعبير الإيجابي لـ CD 200 نمطًا متوسطًا إلى مشرقًا للتعبير في مرضى CLL وفي جزء كبير من مرضى NHL ، بينما تظهر الغالبية العظمى من تعبير CD43 نمطًا باهتًا إلى معتدلًا في مرضى CLL و NHL ، علاوة على تعبير LAIR-1 النمط في الغالب متوسط إلى مشرق في مرضى CLL ، وفي جزء كبير من مرضى NHL.

يختلف تعبيرمجموعتا التمايز CD200 و CD43 المشتركه بشكل كبير بين مرضى CLL و NHL.

هناك ارتباط كبير بين تعبير LAIR-1 ونظام التدريج السريري لكل من مرضى CLL و NHL ، وفي الوقت نفسه ، فإن اثنين من العلامات الأخرى (CD200 و CD43) ليس لهما أهمية إلسريريه في كلا المرضين.

في سلسلتنا ، تُظهر LAIR-1 ارتباطًا كبيرًا مع حالة الحاجه المبكره للدواء وما إذا كنت بحاجة إلى علاج مع استجابتها أم لا في مرضى CLL ، في حين أن أولئك الذين يعانون من NHL ، يظهر كل من CD43 و LAIR-1 ارتباطًا كبيرًا مع هذه المتغيرات.

الإستنتاجات: CD200 و CD43 لهما قيمة تعبيريه عالية في التنميط المناعي التشخيصي ويسهلان فصل CLL عن B-NHLs الأخرى خاصة في الحالات الغامضة.

تعبير LAIR1 هو علامة يمكن الاعتماد عليها وغير مكلفة قادرة على توقع بشكل مستقل أولئك الذين يعانون من سمات عدم الشفاء والتنبؤ بوقت العلاج الأول في المرضى الذين تم تشخيصهم حديثًا غير المختارين والذين يعانون من سرطان الدم الليمفاوي المزمن وسرطان الغدد اللمفاوي الغيرهوجكيني.