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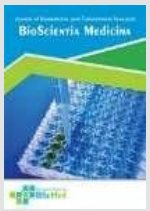

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The Role of Immunophenotyping in the Diagnosis of Acute Leukemia: A Narrative Literature Review

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A B S T R A C T

Leukemia is the expansion of leukocyte cells in the bone marrow, which results in an increase in the number of leukocyte cells in the blood circulation and abnormal cell proliferation in the lymphatic tissue. The diagnosis of leukemia is established based on anamnesis, physical examination, and laboratory and confirmed by supporting examinations such as bone marrow aspiration to immunophenotyping. This literature review aimed to describe the role of immunophenotyping in the diagnosis of acute leukemia. The working process of immunophenotyping consists of a group of cells stained with a fluorochrome-conjugated antibody as a dye that is targeted to antigens on the cell surface. Most of these antigens are assigned a cluster of differentiation (CD) numbers. In conclusion, immunophenotyping analysis using multiparameter flow cytometry is an essential tool in detecting leukemia. Immunophenotyping examination is very useful for determining the diagnosis of leukemia. Targeted therapy is one of the modalities of leukemia therapy that is selective for certain cells that can be given based on the results of immunophenotyping.

1. Introduction

Leukemia is a type of cancer that occurs when the proliferation of blood cells is out of control and interferes with normal blood cell division.[1] The incidence of leukemia, according to GLOBOCAN in 2020, found 14,979 cases of leukemia, with a percentage of 3.8% of the total cases.[2] The number of new cases of leukemia in 2022 in America is 60,650 cases, with a death rate of 24,000 cases.[3] Nonetheless, the use of chemotherapy has resulted in remission of acute leukemia as much as 90%-100% compared to the previous 4 decades.[2,3]

Based on the speed of proliferation, leukemia can be classified as acute or chronic, and based on the originating cells, it is classified as myeloid and lymphoid.[4] The predominant subtypes are acute myeloid leukemia (AML) and chronic myeloid leukaemia (CML), which involve the myeloid chain,

and acute lymphoblastic leukemia (ALL) and chronic lymphoid leukemia (CLL), involving the lymphoid chain. Other less common variants include B-cell and T-cell leukemia, natural killer cell leukemia, and other types.[4]

The cause of leukemia is still not known with certainty. However, environmental factors and family history are thought to increase the risk of developing leukemia. Exposure to high doses of radiation and viral infections is also thought to play a role in the occurrence of leukemia. Cell abnormalities that characterize leukaemic cells include clonal abnormalities, proliferative abnormalities, cytogenetic and morphological abnormalities, failure of differentiation, and biochemical differences from normal cells. From cytogenetic and phenotypic analyzes it can be concluded that cell transformation in leukemia can occur at various sites in the stem cell developmental pathway.[1,4]

The diagnosis of leukemia is established based on anamnesis, physical examination, and laboratory and confirmed by supporting examinations such as bone marrow aspiration to immunophenotyping. Bone marrow aspiration is a frequent investigation for the diagnosis of acute leukemia, where >20% of lymphoblasts or myeloblasts must be found to confirm the diagnosis.[4] However, bone marrow aspiration is considered to have several problems in diagnosing leukemia, so repeated sampling is required, which ultimately causes a delay in the diagnosis of leukemia. Immunophenotyping tests can determine the characteristics of blood cell malignancies with antigen differentiation that appear at various stages of hematopoietic development and describe the lineage that is seen through the expression of specific markers on the cell surface with a system called cluster of differentiation (CD).[5] This literature review aimed to describe the role of immunophenotyping in the diagnosis of acute leukemia.

Definition of acute leukemia

Leukemia is the expansion of leukocyte cells in the bone marrow, which results in an increase in the number of leukocyte cells in the blood circulation and abnormal cell proliferation in the lymphatic tissue. Abnormalities in leukemia lie in the process of hematopoiesis. Hematopoiesis is the process by which stem cells differentiate and mature into erythrocytes, megakaryocytes, and immune cells of myeloid, lymphoid, or monocytic lineages in bone marrow or lymphatic tissue.[6] Genetic errors, such as chromosomal translocations, chromosomal deletions, point mutations, and epigenetic changes, can stop stem cell maturation at various stages of hematopoiesis, giving rise to the uncontrolled proliferation of immature cells.

Leukemias are generally classified into subtypes determined by the type of cell differentiation (lymphoid or myeloid) and the stage of the arrest of maturation (acute or chronic).[6] Based on the speed of proliferation, leukemia can be classified into acute or chronic, and based on the originating cells, it is classified into myeloid and lymphoid. The predominant subtypes are acute myeloid leukemia (AML) and chronic myeloid leukaemia (CML), which involve the myeloid chain, acute lymphoblastic leukemia (ALL), and chronic lymphoid leukemia (CLL) involving the lymphoid chain. Other less common variants include B-cell and T-cell leukemia, natural killer cell leukemia, and other types.[4,5]

ALL is the most common type of leukemia in children, where the prevalence of ALL in children accounts for up to 80% of cases, while as many as 20% of cases occur in adults. Acute lymphoid leukemia (ALL) frequently occurs in children aged 0 to 14 years, with a peak prevalence in children aged 2-5 years and then decreases with age.

Meanwhile, AML is the most aggressive cancer with a prognostic that varies depending on the molecular subtype.[5] The American Cancer Society estimates that 19,520 new cases of AML, namely 10,380 in men and 9,140 in women, will occur in the United States in 2018.[4] The prevalence of AML increases with age. The average age of onset is around 70 years. However, AML affects all age groups. AML is more common in men

than in women (60% versus 40%, and especially in older patients).[5]

A cluster of differentiation (CD)

A cluster of differentiation (CD) is a protocol used for the identification and investigation of cell surface antigens of individual cells that are recognized by antibodies. All mature cells express different receptor proteins on the cell surface, which can help determine the type and maturation stage of the cells examined. The CD molecule can act in a variety of roles, often acting as a receptor or a ligand, a molecule that activates receptors that are important to cells. Some CD proteins play no role in cell signaling but have other functions, such as cell adhesion. To date, there are about 250 different proteins used as markers for individual cells.[7,8]

Immunophenotyping in acute leukemia

Immunophenotyping is one of the features of a flow cytometry tool that is used to analyze groups of cells and test multiple parameters.[9] Flow cytometry with the work technique immunophenotyping works by measuring cell characteristics using the help of laser light which will be captured by side scatter (SSC) and forward scatter (FSC) detectors, which eventually produce fluorescence which is filtered and collected, then converted into digital data that can be read and stored using the software.

Immunophenotyping adds fluorochrome monoclonal antibodies, which will identify individual cells so that multiparameter information is obtained. In a simple explanation, the working process of immunophenotyping consists of a group of cells being stained with a fluorochrome-conjugated antibody as a dye that is targeted to an antigen on the cell surface. Most of these antigens are assigned a cluster of differential (CD) numbers.[10,11] Thus, it created a nomenclature that is generally agreed to be used to define monoclonal antibodies directed against specific cellular antigens. For example, CD3 is a cluster of differential number 3 which refers to the T cell co-receptor present in all T cells.[12] Table 1 presents the commonly used antibodies in flow cytometry leukemia immunophenotyping.

Usage of immunophenotyping in leukemia

Immunophenotyping examination is very useful for determining the diagnosis, therapy, and prognosis of leukemia. Prompt and precise diagnosis of leukemia is essential so that proper treatment can be started as soon as possible. With the development of new treatment modalities, accurate prognostic factors are also important to recognize.[11] Immunophenotyping is examined by flow cytometry which is the detection of leukemia cell markers and is more a confirmation of the diagnosis. Immunophenotyping examination is often used to determine the type of leukaemic cells based on surface antigens. The criteria used in the diagnosis of AML were finding ≥20% of leukemic cells expressing abnormal cell markers (Table 2).[12]

Immunophenotyping examination can be used to differentiate myeloid or lymphoid leukemia cell differentiation, determine the classification of ALL B cells or T cells, identify undifferentiated blasts that appear morphologically or cytochemical as ALL, detect expression of aberrant antigens, and detect minimal residual disease (Table 3).[13]

Aside from being a diagnostic, immunophenotyping can also be used as a determinant of whether leukemia patients can be given targeted therapy. Targeted therapy is aimed at certain target cells that are specific to some types of leukemia.[14] The working principle of target therapy is to selectively kill cancer cells and save normal cells. Targeted therapy is primarily aimed at patients who have a poor functional status, such as elderly patients, and are contraindicated from chemotherapy. Currently, the available target therapy is anti-CD 33, namely Gemtuzumab Ozogamicin. According to several studies, acute leukemia patients with positive CD 34 have a poor prognosis, so this immunophenotyping can also play a role in seeing the prognosis of leukemia patients.[14]

Table 1. Immunophenotyping in CD

Antibody (CD)	Reactivity
CD1a	Thymocytes and immature T cells
CD2	T cells, Large granular lymphocytes (LGL), NK cells, some APL, neoplastic mast cells
CD3	T cells, primary effusion lymphoma
CD4	T cells (helper/inducer), monocytes, myeloblasts, NK cell lymphoma
CD5	T cells, B-CLL/SLL, MCL
CD7	T cells, some myeloblasts
CD8	T cells (suppressor/cytotoxic), large granular lymphocytes (LGL)
CD10	Follicle center cells, FL, some DLBCL, pre-B-ALL, pre-T-ALL, thymocytes, BL
CD11b	Granulocytes, monocytes
CD11c	Monocytes, HCL, LGL, activated T cells, MZL
CD13	Myeloid cells, rare pre-B-ALL
CD14	Monocytes
CD15	Granulocytes, Hodgkin's lymphoma
CD16	Granulocytes, NK cells, LGL
CD19	B cells, pre B-ALL, subset of AML (AML1/ETO with t(8;21))
CD20	B cells, rare plasma cell myelomas
CD22	B cells
CD23	B-CLL/SLL, plasma cells, follicular dendridic cells
CD25	HCL, subset of B and T cell lymphomas
CD30	Hodgkin's lymphoma, anaplastic large cell lymphoma, subset of DLBCL, subset of B cell lymphoma
CD33	Myeloid cells, rare pre-B-ALL, rare blastic NK lymphoma
CD34	myeloblasts, lymphoblasts, endothelial cells
CD38	Plasma cells, activated T and B cells, subset B-CLL/SLL, epithelial cells
CD41	Megakaryocytes
CD43	Myeloid cells, T-cell lymphoma, pre-B ALL, pre-T ALL, B-cell lymphoma (subset), plasma cells
CD56	NK cells, LGL
CD57	NK cells, LGL
CD61	Megakaryocytes
CD79a	B cells, plasma cells, megakaryocytes
CD103	HCL, rare T cell lymphomas
CD117	AML, mast cells, stromal tumors (GIST), plasma cells
bcl-2	Mature B cells (except benign GCC), T cells, and FL
Heavy chains (IgG, IgA, IgM, IgD)	B cells, plasma cells, DLBCL with ALK expression
HLA Dr	AML (except APL), B cells monocytes
Light chains (κ or λ)	B cells (surface), plasma cells (cytoplasmic)
TdT	pre-B-ALL, pre-T-ALL, some AML and hematogones

Table 2. Immunophenotyping in AML.

Expression of markers for diagnoses	
Diagnosis of acute myeloid leukemia (AML)*	
Precursor stage	CD34, CD38, CD117, CD133, HLA-DR
Granulocytic markers	CD13, CD15, CD16, CD33, CD65, cytoplasmic myeloperoxidase (cMPO)
Monocytic markers	Nonspecific esterase (NSE), CD11c, CD14, CD64, lysozyme, CD4, CD11b, CD36, NG2 homologue‡
Megakaryocytic markers	CD41, (glycoprotein IIb/IIIa), CD61 (glycoprotein IIIa), CD42 (glycoprotein 1b)
Erythroid markers	CD235a (glycophorin A)
Diagnosis of mixed phenotype acute leukemia (MPAL)†	
Myeloid lineage	MPO or evidence of monocytic differentiation (at least 2 of the following: NSE, CD11c, CD14, CD64, lysozyme)
B-lineage	CD19 (strong), with at least one of the following: CD79a, cCD22, CD10, or CD19 (weak) with at least 2 of the following: CD79a, cCD22, CD10
T-lineage	cCD3, or surface CD3

Table 3. Classification of ALL based on EGIL.

Immunological grouping	**Immunophenotyping profile**
ALL B lineage	CD19+ and or CD79a+ and or CD22+
Pro-B	No special differentiation antigens
Common B	CD10+
Pre-B	cIgM+
Mature B	cIgM+ or sIgk+ or λ+
ALL T lineage	CD3+ cytoplasmic or membrane
Pro-T	CD7+
Pre-T	CD2+ and or CD5+ and or CD8+
Cortical T	CD1a+
Mature B	CD3+ membrane, CD1a-

2. Conclusion

Immunophenotyping analysis using multiparameter flow cytometry is an essential tool in detecting leukemia. Immunophenotyping examination is very useful for determining the diagnosis of leukemia. Targeted therapy is one of the modalities of leukemia therapy that is selective for certain cells that can be given based on the results of immunophenotyping.

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