

## Aberrant immunophenotypes in acute lymphoblastic leukemia

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### Abstract

Acute lymphoblastic leukemia (ALL) is the most prevalent hematologic neoplasia worldwide. To classify leukemia, we analyzed the immunophenotypic characteristics in the neoplastic cells obtained with antibodies by cell flow cytometry or immunohistochemistry. The aberrant immunophenotypes are antigen expression patterns that differ from the normal hematopoietic maturation process, which can include some different lineage antigens such as myeloid antigens in ALL or asynchronous expression of antigens. These aberrant immunophenotypes have been studied as prognostic factors and residual disease markers. In this review, some aspects of aberrant immunophenotypes are addressed, including definition, epidemiology, and potential uses.

**Key words:** Immunophenotypes. Leukemia. Lymphoblastic. Hematologic. Review.

### Inmunofenotipos aberrantes en la leucemia linfoblástica aguda

#### Resumen

La leucemia linfoblástica aguda es la neoplasia hematológica más prevalente en el mundo. Para clasificar la leucemia se utilizan características inmunofenotípicas en las células neoplásicas que se pueden observar con anticuerpos en la citometría de flujo o mediante inmunohistoquímica. Los inmunofenotipos aberrantes son los patrones de expresión de los antígenos que difieren del proceso normal de maduración hematopoyética, y pueden incluir antígenos de linajes diferentes, como antígenos mieloides en la leucemia linfoblástica aguda, o la expresión asincrónica de antígenos. Estos inmunofenotipos aberrantes se han estudiado como factores de pronóstico y como marcadores de enfermedad mínima residual. En esta revisión se abordan algunos aspectos de los inmunofenotipos aberrantes, incluyendo su definición, epidemiología y usos potenciales.

**Palabras clave:** Inmunofenotipos. Leucemia. Linfoblástica. Hematología. Revisión.

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## Introduction

Acute lymphoblastic leukemia (ALL) is a malignant transformation of hematopoietic progenitor cells in the blood marrow, blood, and extramedullary sites, characterized by the accumulation of altered hematopoietic progenitors in those sites<sup>1</sup>. ALL is the most common malignancy in childhood<sup>2,3</sup>. In the United States, it has a global incidence of 1.7 cases/100,000 inhabitants<sup>4</sup>, while in middle-income countries like Mexico, the incidence is higher: 7.98/100,000 inhabitants<sup>5</sup>. The first classification of ALL was based on the morphological criteria of the French-American-British classification, which includes the nucleus/cytoplasm ratio, nucleoli, size of the cell, vacuoles<sup>6</sup>, and the fact that the bone marrow must contain at least 20% of blast cells (Table 1)<sup>7</sup>. After the introduction of flow cytometry in clinical diagnosis, lineage markers to classify leukemia as B cell origin or T cell origin have been widely used. This method revealed that some abnormal antigens can appear in the neoplastic cells.

## Common immunophenotype markers in ALL

At present, leukemia can be classified into B cell or T cell types, depending on the markers that the neoplastic cells express in a flow cytometry assay. The most common cellular markers are immunoglobulins (Ig): CD19 or CD22 for B cell neoplasia, and CD3 and T cell receptor (TCR) for T cell neoplasia (Table 2)<sup>8,9</sup>. These markers are present in the non-neoplastic counterpart and represent well-characterized molecules that are typical for these lineages. Even in the European Group for Immunological Characterization of Acute Leukemia (EGIL) first proposal for the classification of acute leukemia, it was recognized that some of the ALL express myeloid antigens<sup>9</sup>, which are not present in their normal counterpart cells. Further studies defined biphenotypic and bilineal leukemia. At present, in Mexico, some efforts have been made to establish an immunological classification for acute leukemia (Table 3).

Khalidi et al. identified non-significant differences between primary and relapsed ALL patients<sup>10</sup>.

## Biphenotypic and bilineal leukemia

EGIL defines biphenotypic leukemia as a group in which blasts express simultaneously myeloid and lymphoid antigens, with a score of  $\geq 2$  points present in two different lineages (Table 4)<sup>11</sup>.

**Table 1.** French-American-British classification for acute lymphoblastic leukemia<sup>46</sup>

Type	Morphology	Immunophenotype
L1	Homogeneous blasts, regular nucleus, homogenous chromatin, small or inexistent nucleoli, mild basophilia	B cell: CD19, CD22, CD79a, CD10, CD20, Ig cytoplasmic, or superficial T cell: CD3, CD7, CD5, CD2, or CD4
L2	Irregular nucleus, heterogeneous chromatin, and large nucleoli	B cell: CD19, CD22, CD79a, CD10, CD20, Ig cytoplasmic, or superficial T cell: CD3, CD7, CD5, CD2, or CD4
L3	Large blasts, prominent nucleoli, abundant cytoplasm, vacuolation covering the nucleus	B cell: CD19, CD22, CD79a, CD10, CD20, Ig cytoplasmic, or superficial T cell: CD3, CD7, CD5, CD2, or CD4

Ig: immunoglobulin.

**Table 2.** Markers used for lineage classification of acute lymphoblastic leukemia

Lineage	Markers
B cell	Surface immunoglobulins, cytoplasmic light chains, CD19, CD22, CD79
T cell	CD3 (cytoplasmic or membrane), CD2, CD5, CD8, T cell receptor

Bilineal leukemia is defined as a heterogeneous group of hematopoietic malignancies, with blasts that cannot be classified as myeloid or lymphoid, or blasts from both lineages<sup>12</sup>.

Not only EGIL, but the World Health Organization also proposed criteria for mixed-phenotype blasts in the Classification Tumors of Hematopoietic and Lymphoid Tissues 2016 update (Table 5)<sup>11-13</sup>. This classification comprises bilineal and biphenotypic leukemia into this entity and includes 2-5% of all leukemia diagnosis. No threshold level of antigen expression is defined for the diagnosis<sup>14</sup>.

## Aberrant immunophenotypes in ALL

Some leukemias show antigen expression of two cellular lineages but not all the biphenotypic, bilineal, or mixed phenotype criteria. The expression of these antigens has been termed anomalous or aberrant<sup>15</sup>. In B cell ALL, myeloid antigens are the best-studied antigens. Another definition of aberrant immunophenotypes is the antigen expression patterns in neoplastic

**Table 3.** Markers proposed in the National Consensus of Acute Leukemia Phenotyping in Mexico<sup>47</sup>

Type of ALL	Lineage	Differentiation/Maturation	Subclassification	Optional
B cell precursor	CD19 Cytoplasmic CD79	CD34, CD45, CD20, CD38	CD10, Ig superficial, $\mu$ cytoplasmic chains	TdT, CD13, CD33
T cell precursor	CD3, CD7	CD34, CD45, TdT, CD1a, CD99	—	CD2, CD4, CD5, CD8, CD13, CD33

ALL: acute lymphoblastic leukemia; TdT: terminal deoxynucleotidyl transferase; Ig: immunoglobulin.

**Table 4.** EGIL criteria for biphenotypic leukemia

Points	B cell lineage	T cell lineage	Myeloid lineage
2	CD7, CD22, IgM	CD3, TCR	MPO
1	CD19, CD20, CD10	CD2, CD5, CD8, CD10	CD117, CD13, CD33
0.5	TdT $\gamma$ CD24	TdT, CD7, CD1a	CD14, CD15, CD64

\*For the diagnostic of biphenotypic leukemia, myeloperoxidase (a major myeloid antigen), and another major antigen of B or T cell origin should be present. The diagnostic of aberrant immunophenotypes is determined with one point in two different categories.

EGIL: European Group for Immunological Characterization of Acute Leukemia; MPO: myeloperoxidase; TCR: T cell receptor; TdT: terminal deoxynucleotidyl transferase.

cells that differ from the normal antigens in the maturation process of hematopoietic cells. In essence, this includes other lineage antigens: myeloid antigens in ALL, B cell antigens in T cell ALL or vice versa, or antigen asynchrony (when earlier phase antigens are expressed in mature cells). The threshold for positivity<sup>10,16</sup> is 20-30% of the isolated leukemic cells<sup>17</sup>.

Conflicting information on the prevalence of aberrant antigens has been reported. These differences can be explained by the variation of antibodies against antigens, diverse cutoff levels, or the samples used in the analysis<sup>18</sup>. Seegmiller et al. reported 86.5% of myeloid antigens in 200 cases of B lineage ALL and 9% of T cell antigens in their series<sup>19</sup>. The Brazilian study by Lopes et al. showed only 49.2% of myeloid expression in T and B cell ALL<sup>20</sup>. In turn, Bachir et al. studied a population of Moroccan children and found myeloid expression in 46.3% of B cell ALL and 22% of T cell ALL<sup>21</sup>; other studies mentioned higher prevalence in T cell ALL<sup>22</sup>. Ibrahim et al. mentioned a percentage as low as 5% of myeloid expression<sup>18</sup>. From these reports, it is assumed that incidence varies depending on the population studied. The gathered information is consistent in the fact that the expression of myeloid antigens is higher in B cell ALL. Furthermore, Khalidi et al. reported T cell markers in B cell ALL, CD3, CD4, and CD7, were expressed in < 5% of the cases<sup>10</sup>. B cell

**Table 5.** WHO criteria for mixed-phenotype blasts

Lineage	Markers
Myeloid	Myeloperoxidase or two of the following: CD11c, non-specific esterase, CD14, CD64, lysozyme
T lineage	Cytoplasmic or surface CD3
B lineage	Strong CD19 plus at least one of the following: CD79a, cytoplasmic CD22 or CD10; or weak CD19 and two of the following: CD79a, cytoplasmic CD22, or CD10

WHO: World Health Organization.

markers in T cell ALL were expressed in 15% of the cases in the Sharma study, with CD79a and CD19 being the most commonly found<sup>23</sup>. Bhushan et al. reported a higher incidence of myeloid antigens in adults<sup>24</sup>. However, these findings were not observed in other studies<sup>10,19</sup>. In Argentinian patients, Novoa et al. characterized the presence of antigen asynchrony in B cell ALL, with CD34+ CD20+ and CD34+ CD10- being the most frequently expressed phenotypes<sup>25</sup>. The differences found in these studies reported during the past 20 years were the main reason to do a review on this topic. Table 6 shows the studies and results from different authors<sup>15,16,18,26-28</sup>.

One of the most frequent aberrant myeloid markers is CD13, a glycoprotein with peptidase activity<sup>18</sup> expressed in normal hematopoietic cells in the processes of maturation, from myeloblasts to granulocytes. In the Seegmiller study, CD13 was the most common myeloid antigen present in ALL (54.5%)<sup>19</sup>. Another antigen, CD33, is a transmembrane protein present in myeloblast with inhibitory functions, expressed in promyelocytes, monocytes, and erythrocyte<sup>29</sup>. CD33 was found in 43% in the Seegmiller study. Other myeloid markers such as CD117 are not frequently expressed in ALL<sup>30,31</sup>. HLA-DR is more frequently found in B cell precursors ALL than in T cell ALL. NK markers, like CD56, have been found on B cell ALL in low percentages<sup>10</sup>.

**Table 6.** Several studies of myeloid antigens as possible prognostic factors in patients with acute lymphoblastic leukemia

Author	Year	Type of leukemia (B, T or both)	Results
Taberero et al. <sup>26</sup>	2001	B	13 cases with BCR/ABL (Abelson) translocation were positive for CD13
Yenerel et al. <sup>16</sup>	2002	Both	The patients positive to CD13 had better overall survival and disease-free survival (85 vs. 50% and 67 vs. 43%, respectively)
Vitale et al. <sup>27</sup>	2006	Both	No correlation was found between the expression of CD13 and the disease-free survival or overall survival
Noronha et al. <sup>15</sup>	2011	Both	The positive myeloid group achieved more remission cases
Supriyadi et al. <sup>28</sup>	2012	Both	The negative myeloid group got a better disease-free survival compared with the positive myeloid group (80 vs. 67%)
Ibrahim et al. <sup>18</sup>	2017	Both	CD13 positive group had complete remission and fewer blasts in bone marrow, compared to the negative group

BCR: B cell receptor.

### Aberrant immunophenotypes as a prognostic factor

At present, several contradictory results from different studies on this topic have emerged. In 2017, Kavianpour et al.<sup>30</sup> discussed some studies that found a lower death rate in the positive myeloid group. However, the results of other authors are not consistent with this study<sup>16,24,27,31</sup>. Copeland and McGuire addressed the same problem<sup>32</sup>.

The Children’s Cancer Group reported that the aberrant immunophenotype expression was not an adverse prognostic factor: 1557 children enrolled with B or T cell ALL, but only 13.9% and 2.8%, respectively, expressed positive myeloid antigens<sup>17</sup>. The research of the St Jude Children’s Research Hospital in acute mixed lineage leukemia stated that lineage infidelity does not have prognostic significance<sup>33</sup>. Moreover, The Medical Research Council, in the United Kingdom, showed no prognostic significance of the expression of CD13 and CD33<sup>34</sup>. Lopez et al. found a significantly higher platelet value in those patients with positive myeloid markers<sup>20</sup>. In adult patients, Yenerel et al. found differences in overall survival between the myeloid positive and negative groups (85 vs. 50%); also, the disease-free survival was different (67 vs. 43%), which was not consistent with previous studies<sup>16</sup>. In Indonesian patients treated with the ALL 2006 protocol, Supriyadi et al. found that leukemia-free survival analyses showed worse prognosis in the positive myeloid group: disease-free survival was 67% in the positive myeloid group versus 80% in negative myeloid patients<sup>28</sup>. Sharma et al. reported a lower number of peripheral blasts and lower white blood cell

count in the myeloid positive group<sup>23</sup>, while Amirghofran et al. reported less significance in survival time in CD13 positive cases versus negative cases<sup>35</sup>. Similar results were found in the Mexican study by Rodriguez et al., as well as a lower disease-free survival period<sup>36</sup>. Craddock et al. showed an increased relapse risk in CD13 positive cases<sup>37</sup>, while Dalal et al. proposed a more aggressive treatment for ALL with this immunophenotype<sup>38</sup>.

Myeloid expression was observed in Philadelphia chromosome-positive ALL, both in children and adults. This chromosomal aberration is considered as bad prognostic in adults. Other chromosomal abnormalities with an expression of myeloid antigens reported by Khalidi et al. were the deletion of band 11q23 and translocations involving this chromosome<sup>10</sup>. The expression of CD5 in B cell ALL has been reported in association with small supernumerary marker chromosomes<sup>39</sup>.

### Immunophenotypic detection of minimal residual disease

Over the past decades, a considerable effort has been made in developing methods to detect residual leukemia in patients that had achieved clinical remission. Minimal residual disease (MRD) is defined as the detection of at least 0.01% leukemic blasts per mononuclear or total nucleated cells<sup>40</sup>. The methods for MRD detection are the polymerase chain reaction with fusion gene transcripts or Ig/TCR gene rearrangements by flow cytometry<sup>41,42</sup>.

In 1990, Campana et al. studied the asynchronous expression and lineage infidelity and detected minimal disease (35%) and operationally leukemia-specific.

Only in two of 35 cases, the characteristics in the cells changed<sup>43</sup>. In 1997, San Miguel et al. reported that immunophenotypic aberrancies were useful in predicting relapse and MRD in acute myeloid leukemia<sup>44</sup>.

García-Vela et al. characterized four different types of aberrant immunophenotypes or leukemia-associated immunophenotypes that can be detected by flow cytometry, which include lineage infidelity, asynchronous antigen expression, ectopic antigen expression (expression of markers outside the thymus), and antigen overexpression. Therefore, the study of these antigens was suggested for MRD, as they found a high frequency of aberrancies<sup>45</sup>.

In 1999, Lucio et al. performed multicentric studies to detect MRD with flow cytometry, with positive results detecting malignant lymphocytes from normal lymphoblasts<sup>46</sup>. Several subsequent studies have demonstrated the same results<sup>47,48</sup>. Gaipa et al. mentioned that leukemia-associated immunophenotypes are widely applicable, rapid, and direct but operator-dependent and require standardization<sup>42</sup>. Campana emphasizes the use of large antibody panels and interpretation expertise<sup>41</sup>. At present, six to eight-color flow cytometry methods are used for leukemia immunophenotyping in MRD<sup>42</sup>. This approach was useful in the Dhahran Health Center study, where they identified six MRD positive cases in B ALL<sup>40</sup>. The Euroflow group is recently standardizing new leukemia immunophenotyping and MRD protocols, with a more significant number of immunological markers.

Aberrant immunophenotypes are antigens that differ from the typical expression pattern in hematolymphoid neoplastic cells. The incidence of this phenomenon varies from 5% to 86.5%. This difference could be explained by several reasons. For example, in the study that showed an incidence of 86.5%, several antigens that are generally not measured were considered. Conversely, in the series that considered the two most common myeloid antigens (CD13 and CD33), the percentage was much lower (30-50%). Furthermore, the different laboratories or dilutions used for the antibodies, as well as the positivity threshold that some of the antigens showed in different studies, ranging from 20 to 30%, should be considered. Another crucial variable is the different populations in the studies.

The potential role of aberrant immunophenotypes as a prognostic factor is still controversial. Some studies in low- and medium-income countries reveal that prognosis is different between myeloid positive and myeloid negative ALL. Contrastingly, in more prominent studies from the USA and UK, no significant differences were

reported. In an Indonesian study, a different treatment protocol from the St. Jude protocol was used, which could explain the positive results in the evaluation of the disease remission. In patients with aggressive protocols, no evidence that the aberrant immunophenotypes change the prognostic of patients has been found. Other factors that need to be considered are the controversies between different populations and between low- and high-income countries.

In MRD, the detection of different antigens in the cell and the introduction of flow cytometry have been useful for diagnosis and treatment. However, as the panels are extensive, standardization is essential because they are operator-dependent.

## Ethical disclosures

**Protection of human and animal subjects.** The authors declare that no experiments were performed on humans or animals for this study.

**Confidentiality of data.** The authors declare that they have followed the protocols of their work center on the publication of patient data.

**Right to privacy and informed consent.** The authors declare that no patient identifying information appears in this article.

## Conflicts of interest

The authors declare no conflicts of interest.

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