Aberrant Phenotypes in Acute Myeloid Leukemia and Its Relationship with Prognosis and Survival: A Systematic Review and Meta-Analysis

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ABSTRACT

Background: The aim of this review was to evaluate the influence of aberrant phenotypes in prognosis and survival in acute myeloid leukemia (AML) patients by multiparametric flow cytometry.

Materials and Methods: Following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, a review of PubMed, Scopus, Science Direct and Web of Science was carried out through 1998 to 2016, conducted by two reviewers independently, evaluating titles, abstracts and full-texts of the selected studies.

Results: Ten studies were included on this review, in which the aberrant phenotype expression of 17 markers were detected in AML patients. From these, 11 aberrant phenotypes were associated with prognosis, which eight had shown negative impact on prognosis: CD7, CD56, CD15, CD2, CD3, CD90low, CD123high, CD117high, and three others were associated with good prognosis: CD19, CD98high and CD117+/CD15+. Meta-analysis showed that aberrant expression of CD56 as a poor prognostic marker with unfavorable outcomes is implicated in decreased overall survival in AML patients in 28 months (95% CI: 0.62 to 0.92).

Conclusion: This was observed when there was association between CD56 expression and other prognostic factors, influencing on patients’ management care and treatment.

Keywords: Aberrant phenotype; Acute myeloid leukemia; Immunophenotyping; Prognosis; Survival

INTRODUCTION

Acute myeloid leukemia is a hematological malignancy characterized by abnormal proliferation and impaired differentiation of myeloid cells ¹,². This group of clonal hematologic disorders presents a huge heterogeneity that makes diagnosis difficult leading to negative impacts on treatment³,⁴. Although AML affects people of all ages, it is more...
prevalent in elderly patients. The 5-year relative survival rate for patients over 65 years-old has been reported to be 6.0%, and complete remission is achieved in just 40% of these patients. Hemopoietic cells present immunophenotypic markers or specific antigen expression patterns on their surface; however leukemic cells often display unusual expression called aberrant phenotypes. The aberrant phenotypes can be classified into four categories according to their expression pattern: lineage infidelity, in which there is co-expression of lymphoid markers in myeloid cells; asynchronous expression wherein early markers are co-expressed with markers that usually are expressed in more mature cells; overexpression, underexpression, or loss of marker generally expressed by myeloid cells, and abnormal light scatter patterns. Aberrant phenotypes have been reported as adverse prognostic factors in AML, and they have been associated to survival. Besides, aberrant phenotypes have demonstrated strong association with cytogenetics abnormalities that lead patients to a worse clinical course of the disease, consequently being associated to poor prognosis. Multiparameter flow cytometry immunophenotyping provides an accurate analysis of patients’ samples providing relevant data which make possible the diagnosis and classification of AML subtype. This analysis allows the detection of changes in the expression patterns of markers being capable to differentiate normal hematopoietic cells from malignant cells. Then, it is possible to identify the immunophenotypic profile of leukemic blasts in patients with AML. Patients under treatment often have demonstrated unpredictable outcomes, then it necessitates the consolidation of additional prognostic immunophenotypic markers to improve the stratification risk helping clinicians to choose the best therapeutic approach to patients with the purpose to increase overall survival (OS). Nevertheless, there are few studies relating aberrant phenotypes with poor prognosis and patients’ survival. Consequently, by adding this prognostic factor into risk stratification, it would be possible to manage the best care for these patients. Therefore, the aim of this systematic review was to evaluate the influence of aberrant phenotypes expression in prognosis and survival of patients with AML.

MATERIALS AND METHODS

A systematic review was performed based on a scientific research protocol describing the aims and methods used. This synthesis was performed according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA), and it was registered on PROSPERO under the following number: CRD42018099708. This systematic review aimed to answer the following question: What are the aberrant phenotypes in acute myeloid leukemia and their influence in prognosis and survival?

Search strategy

The literature search was conducted using PubMed, Science Direct, Web of Science, Scopus and Cochrane Library databases looking for articles published from 1998 to 2016. The timeline was established according to the beginning of the use of eight-color flow cytometry. To this search were used the following terms: Aberrant phenotype (MeSH) OR Aberrant immunophenotype (MeSH) OR Aberrant expression (MeSH) OR Aberrant marker (MeSH), AND Prognosis (MeSH) OR Survival rate (MeSH) OR Survival analysis (MeSH), AND Leukemia, Myeloid, Acute (MeSH) OR Acute leukemia (MeSH). Also, it was used their equivalents in Portuguese and Spanish.

Study selection

Inclusion criteria was used on selected articles to determine the study relevance: (1) articles published from 1998 to 2016; (2) articles published in English, Spanish and Portuguese; (3) articles that used immunophenotyping in their methodologies; (4) articles about acute myeloid leukemia; (5) articles with available abstracts and full text. Case studies, systematic and literature reviews, meta-analysis, editorials, conference proceedings and books were excluded from the study. Two reviewers independently evaluated the titles and abstracts from the articles applying the inclusion criteria. Articles that seem to be relevant were fully analyzed, and the articles that were included in this systematic review were based in agreement between the two reviewers. Disagreements between the two reviewers were inspected by a
third reviewer that fully analyzed the articles and made the final decision whether or not to use the article.

**Rating quality of individual studies**
The methodological quality of each individual study was evaluated using the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement, which consisted of 22 items. STROBE checklist consists of: (1) title and abstract; into introduction section: (2) background/rationale; (3) objectives; into methods section: (4) study design; (5) setting; (6) participants; (7) variables; (8) data sources/measurement; (9) bias; (10) study size; (11) quantitative variables; (12) statistical methods; into results section: (13) participants; (14) descriptive data; (15) outcome data; (16) main results; (17) other analyzes; into discussion section: (18) key results; (19) limitations; (20) interpretation; (21) generalizability; into other information section: (22) funding. Points in checklist are summing up to achieve a score, in which high scores meant that studies had enough information and good design.

**Data extraction and management**
From the selected studies, information regarding the following parameters was obtained: (1) journal of publication; (2) The Journal Citation Reports (JCR) impact factor; (3) location; (4) study design; (5) aim of the study; (6) number of samples analyzed; (7) acute myeloid leukemia classification; (8) most incident subtype; (9) aberrant immunophenotypic marker; (10) prognostic value; (11) first induction treatment protocol; (12) follow-up; (13) survival; (14) limitations; and (15) STROBE scores.

**Statistical analysis**
A statistical meta-analysis was performed of the relative risks related to the probability of survival at 28 months. For analysis purposes, low expression of the immunophenotypic marker was considered as absent, and high expression was considered as present.

Survival analysis was analyzed by dichotomous data using the knowledge of the situation of all patients in the study at 28 months. A contingency table was constructed (Table 1) to analyze every connection and then the relative risks were calculated using this equation:

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\frac{\text{Risk of the event on the group with positive expression}}{\text{Risk of the event on the group with negative expression}} = \frac{d}{\frac{d}{a+b}} \quad \frac{e}{\frac{e}{c+d}}
\]

| Outcome          | Alive | Dead | Total       |
|------------------|-------|------|-------------|
| Positive Marker  | A     | b    | a + b       |
| Negative Marker  | C     | d    | c + d       |

Table 1. Contingency table (2 x 2). Correlation between the presence or absence of aberrant phenotype (CD56) and the survival outcome or not

A correction value of 0.5 was used in order to enable the statistical analysis using the absence of death and survival data in the absence of one of the aberrant phenotypes at 28 months follow-up as effect measures.

The heterogeneity of the meta-analysis was assessed using the Cochran Q and Higgins I² tests. We used the relative risk as an effect measure after taking into consideration the number of people who would be alive in the absence and presence of the aberrant phenotype at 28 months. This period of follow-up was chosen in a way that could be possible to include the four studies in this meta-analysis, once one of the studies only presented the follow-up of 28 months. Meta-effect estimates were reported, and relative risks summarized with their 95% confidence intervals. The funnel graph was used to assess potential publication bias related to survival at 28 months. The bias was considered significant at p = 0.05. All analyses were performed with the program R version 3.3.1 and the “Metafor” package.
RESULTS
The literature search
Findings on databases (PubMed, Scopus, Science Direct, Web of Science and Cochrane Library) reached 20,660 articles. After primary readings of titles and abstracts, 31 potentially articles remained. Final analysis of full-texts resulted in a total of 10 studies\textsuperscript{23-32} that were included into this systematic review as illustrated in the Figure 1. Four of them were used on the meta-analysis for 28-month survival analysis\textsuperscript{24,28-30}.

![Flowchart of the study selection process.](image-url)
Study characteristics
An overview was conducted from the 10 studies to extract relevant characteristics (Tables 2 and 3). A range of 12–789 patients with a total of 1,333 hematological samples were studied in all articles. One study used the World Health Organization classification for AML and nine studies used the French-American-British (FAB) classification. In the articles, the most reported AML subtype was FAB M2 followed by FAB M1. Most of the studies were conducted in developed countries, such as, United States of America, Japan, Saudi Arabia, and Italy. The expression of 17 different aberrant phenotypes was evaluated in all 10 articles. Although only 11 aberrant phenotypes were correlated with prognosis, and only four performed survival analysis. The studies showed that the most common treatment protocol was anthracycline-based induction therapy, as a chemotherapy regimen. For AML-M3 the therapeutic choice was based on all-trans-retinoic acid (ATRA) protocol.

The articles were submitted to methodological quality evaluation based on STROBE tool, and they ranged from 77.8% to 91%. The highest methodological quality found in the articles scored >80%.
### Table 2: Description of articles included on the systematic review regarding to each aberrant phenotype related to studies location, aims, design, and quality assessment

| Marker | Article | JCR | Location | Design | Aim of the Study | Strobe |
|--------|---------|-----|----------|--------|------------------|--------|
| CD7    | Chen et al. (Int J Lab Hematol, 2007) | 2.401 | Taiwan | Cross-sectional observational | Retrospective study to characterize the frequency and significance of aberrant antigen expression of AML in Taiwan | 18 (81.8%) |
| CD56   | Rausei-Mills et al. (Am J Clin Pathol, 2008) | 0.138 | USA | Cross-sectional observational | Analyze the clinical and pathologic features of 15 cases of de novo AML with normal cytogenetics and with FLT3/ITD mutation. | 17 (77.8%) |
| CD19   | Bahia et al. (Haematologica, 2001) | 6.671 | Brazil | Cohort | Analyze 35 cases of AML, examining them for aberrant phenotypes by multiparametric flow cytometry. | 19 (86.4%) |
| CD117+/CD15+ | Chávez-González et al. (Arch Med Res, 2014) | 6.671 | Mexico | Case-control | Determine the prevalence of aberrant antigen expression in acute leukemia, to assess clinical relevance, and to demonstrate immunophenotype-karyotypic correlations | 18 (81.8%) |
| CD15   | Breccia et al. (Leuk Res, 2014) | 2.606 | Italy | Cross-sectional observational | Assess the frequency of CD15 and CD56 expression, and their prognostic value in a large series of APL patients, uniformly diagnosed and treated according to the AIDA schedule | 20 (91%) |
| CD56   | Iriyama et al. (Leuk Res, 2013) | 2.606 | Japan | Cross-sectional observational | Investigation of the clinical significance for the prognosis of surface antigen expression in patients with AML t(8; 21) | 19 (86.4%) |
| CD19   | Abdulateef et al. (Asia Pac J Cancer P, 2014) | 2.190 | Saudi Arabia | Cohort | Analyze the expression of four cell surface antigens relevant to human hematopoiesis — CD90, CD96, CD117, and CD123 — in bone marrow from pediatric AML patients and normal control subjects | 18 (81.8%) |
| CD7    | Cui et al. (Int J Lab Hematol, 2014) | 2.401 | USA | Cross-sectional observational | Changes in leukemia-associated aberrant immunophenotype (LAIP) in patient with refractory and relapsed acute myeloid leukemia (AML) | 19 (86.4%) |
| CD56   | Nikolova et al. (Leuk Res, 1998) | 2.606 | Bulgaria | Cross-sectional observational | Evaluation of CD98 expression levels in patients with leukemia | 17 (77.8%) |

AML: acute myeloid leukemia; JCR: journal citation reports; FLT3/ITD: fms-like tyrosine kinase-3/internal tandem duplication; APL: acute promyelocytic leukemia; AIDA: ATRA+IDA or all-trans-retinoic acid + idarubicin; LAIP: leukemia-associated immunophenotypes.
### Table 3. Subtypes of AML, number of samples and treatment features of the individual studies associated with each aberrant phenotype expression and its prognosis included on the systematic review

| ARTICLE                        | MARKER | PATIENTS (N) | CLASSIFICATION | PROGNOSIS | TREATMENT | CUT-OFF |
|--------------------------------|--------|--------------|----------------|-----------|-----------|---------|
| Chen et al. (Int J Lab Hematol, 2007) | CD7    | 111          | M0: 10; M1: 15; M2: 36 | POOR      | ARA-C     | NR      |
|                                | CD56   |              | M3: 15; M4: 21; M5: 8; M7: 6 | POOR      | ATRA (M3) |         |
|                                | CD19   |              | M4: 8; M6: 2    | GOOD      |           |         |
| Rausei-Mills et al. (Am J Clin Pathol, 2008) | CD7    | 31           | M0: 2; M1: 12; M2: 7 | POOR      | CHEMOTHERAPY |         |
|                                | CD2    |              | M4: 8; M6: 2    | GOOD      | HSCT      | NR      |
|                                | CD3    |              | M0: 10; M1: 27; M2: 9 | POOR      |           | >20%    |
|                                | CD7    | 56           | M3: 4; M5: 4; M7: 3 | POOR      | NR        | >20%    |
|                                | CD19   |              | M0: 1; M1: 7; M2: 8; M3: 4; M4: 6; M5: 6; M6: 1; M7: 2 | POOR      | NR        | >20%    |
|                                | CD7    | 54           | M0: 1; M1: 7; M2: 8; M3: 4; M4: 6; M5: 6; M6: 1; M7: 2 | POOR      | NR        | >20%    |
|                                | CD1117+/CD15+ |          | 6; M5: 6; M6: 1; M7: 2 | GOOD      |           |         |
| Breccia et al. (Leuk Res, 2014) | CD15   | 116          | M3: 116         | POOR      | AIDA 0496 | >20%    |
|                                | CD56   |              |                |           | AIDA 2000 |         |
| Irinyama et al. (Leuk Res, 2013) | CD56   | 789          | FAB            | POOR      | NR        | >20%    |
|                                | CD19   |              |                | GOOD      |           |         |
| Abdulateef et al. (Asia Pac J Cancer P, 2014) | CD7    | 79           | M1: 10; M2:9; M3:3; M4: 12; M5: 4; M6:1; M7: 1 | POOR      | NR        | 20%     |
|                                | CD90 + |              |                | POOR      |           |         |
|                                | CD117 + |             |                | POOR      |           |         |
|                                | CD123 + |             |                | POOR      |           |         |
|                                | CD7    | 12           | M1: 2; M2:4; M4: 1; M5: 2; M7: 3 | POOR      | ATEDox    | NR      |
| Cui et al. (Int J Lab Hematol, 2014) | CD7    | 47           |                | POOR      |           |         |
|                                | CD56   |              |                |          |           |         |
|                                | CD98 + |              |                |          |           |         |
| Nikolova et al. (Leuk Res, 1998) | CD98 + | 38           | M0:4; M1:8; M2:12; M3:1; M4: 7; M5: 5; M6: 1 | GOOD      | Farmarubicine, cytosine arabinoside and 6-thioguanine | 20%    |

AML: acute myeloid leukemia; NR: not related; ARA-C: cytosine arabinoside; ATRA: all-trans-retinoic acid; HSCT: Hematopoietic stem cell transplantation; FLT3/ITD: fms-like tyrosine kinase-3/internal tandem duplication; AIDA: ATRA+IDA or all-trans-retinoic acid + idarubicin; PML/RARA: promyelocytic leukemia/retinoic acid receptor alpha; ATEDox: cytarabine, mercaptopurine, doxorubicin; MLL: mixed-lineage leukemia; NPM1: nucleophosmin 1; NOS: not otherwise specified.
Prognostic value and survival
Most articles showed that the expression of the evaluated eight aberrant phenotypes had negative impact on AML prognosis: CD7, CD56, CD2, CD3, CD90\textsuperscript{low}, CD123\textsuperscript{high}, CD117\textsuperscript{high}, CD15 [23-31] and only three had positive value on prognosis: CD19, CD117\textsuperscript{-}/CD15\textsuperscript{+}, and CD98\textsuperscript{high}\textsuperscript{25-27,29,32}.

Regarding to poor prognostic markers, Rausei-Mills \textit{et al.} \textsuperscript{23}, Abdulateef \textit{et al.} \textsuperscript{24} Bahia \textit{et al.} \textsuperscript{25}, Jahedi \textit{et al.} \textsuperscript{26}, Chen \textit{et al.} \textsuperscript{27}, and Cui \textit{et al.} \textsuperscript{28} showed that CD7 is an indicator of poor prognosis due to its association with poor clinical outcomes and low remission rates. Aberrant expression of CD56 was evaluated by Abdulateef \textit{et al.} \textsuperscript{24}, Chen \textit{et al.} \textsuperscript{27}, Cui \textit{et al.} \textsuperscript{28}, Iriyama \textit{et al.} \textsuperscript{29}, and Breccia \textit{et al.} \textsuperscript{30}, and it was observed its expression was significantly related to negative outcomes in AML patients. Jahedi \textit{et al.} \textsuperscript{26} discoursed and analyzed CD2 and CD3 aberrant expression in AML patients, and their findings revealed these markers have implication on prognosis, leading to unfavorable outcomes being associated to low remission rates. Chávez-González \textit{et al.} \textsuperscript{31} conducted a study wherein was observed the aberrant expression of CD90, CD123 and CD117. They reported that low expression of CD90 was associated with increased relapse rate in AML patients, and overexpression of both markers, CD117 and CD123, were associated with adverse outcomes and high incidence of relapse. Finally, Breccia \textit{et al.} \textsuperscript{30} showed that in acute promyelocytic leukemia (APL) cases, the expression of CD15 was associated to an increased cumulative incidence of relapse, being associated to poor prognosis.

Aberrant immunophenotypic markers related to good prognosis also were found: CD19 and CD98. Studies conducted by Bahia \textit{et al.} \textsuperscript{25}, Jahedi \textit{et al.} \textsuperscript{26}, Chen \textit{et al.} \textsuperscript{27}, and Iriyama \textit{et al.} \textsuperscript{29}, showed results related to the aberrant expression of CD19, and their findings showed that CD19 expression in AML patients was associated with favorable prognosis since patients reached complete remission. Nikolova \textit{et al.} \textsuperscript{32} showed that AML patients expressing high levels of CD98 reached complete remission and had increased survival duration, concluding that this aberrant marker is associated with good prognosis. Bahia \textit{et al.} \textsuperscript{25} observed that CD117\textsuperscript{-}/CD15\textsuperscript{+} phenotype is associated with favorable prognosis since patients expressing it reached complete remission.

Meta-analysis
Four studies \textsuperscript{24,28-30} were analyzed regarding to the presence of aberrant CD56 phenotype expression in patients with acute myeloid leukemia and its influence in survival. CD56 was the only aberrant phenotype in which survival analysis was performed in different studies making possible to group them into this statistical approach. The results were combined in order to increase statistical power and were summarized using the meta-analysis of relative risks related to the probability of survival. The technique was then used to evaluate the influence on prognosis concerning to the expression of CD56 aberrant phenotype. Survival analysis showed great homogeneity with significant Cochran Q test (Q (df = 1) = 0.8888, p = 0.8281) and the Higgins I\textsuperscript{2} test showed a result of 0.00%. Therefore, the fixed effects model was used. The meta-analysis (Figure 2) presented a meta-analytic estimate of significant risk of 0.76 (95% CI: 0.62 to 0.92). The funnel graph (Figure 3) used to evaluate publication bias did not show asymmetries.
Figure 2. Forest plot with relative risks and confidence intervals of the survivals related to the detection/non-detection of the aberrant CD56 phenotype and its meta-analytic measurement.

Figure 3. Funnel plot of 28-month survival studies related to detection/non-detection of aberrant CD56 phenotype for evaluation of publication bias.
DISCUSSION

Based on antigen expression in different subtypes of hematologic cell lineages, multiparametric flow cytometry of high resolution had been used to identify the leukemic cells characteristics. Studies related to positive or negative and high or low antigen expression, as well as translineage of antigens on malignant cells, had obtained information about the immunophenotype of different leukemia subtypes. The wide application of immunophenotyping in acute myeloid leukemia has become essential to understand the relationship between aberrant phenotypes, cytogenetic features, survival and disease progression.

During this systematic review, it was possible to notice that prognosis implication of aberrant phenotypes was associated with worse outcomes. In some cases, this relationship remains controversy and new studies are essential for a better understanding of leukemia features. From the 11 aberrant phenotypes cited, eight were associated with poor prognosis, CD7, CD56, CD15, CD2, CD3, CD90\textsuperscript{low}, CD123\textsuperscript{high}, and CD117\textsuperscript{high}. Nonetheless, three others CD19, CD98\textsuperscript{high} and CD117+/CD15\textsuperscript{+} were associated with favorable prognosis.

Lineage infidelity

AML cells can display particular immunophenotypic features, wherein there is co-expression of a specific lymphoid marker in their surface showing lineage infidelity \cite{8}. CD7 is a cell surface glycoprotein that belongs to the immunoglobulin superfamily, and it is expressed on most thymocytes, peripheral blood T cells, and natural killer (NK) cells \cite{33}. Further, CD7 plays a pivotal costimulatory role in T-cell activation and development \cite{34}. Rausei-Mills et al. \cite{23} showed in their study that CD7 aberrant expression in AML patients with FLT3/ITD\textsuperscript{+} mutation is present in high frequency, and Abdulateef et al. \cite{24} results demonstrated that aberrant expression of this marker was associated with cytogenetic abnormalities, conferring both unfavorable prognosis. Bahia et al. \cite{25} showed that association between CD7 and markers from more immature cells, such as CD34, drives patients to a more aggressive clinical course of the disease, being associated with multiple drug resistance protein (MDR). In addition, patients who aberrantly expressed CD7 achieved poor clinical outcomes and had low remission rates as observed by Jahedi et al. \cite{26}, Chen et al. \cite{27}, and Cui et al. \cite{28}.

Other immunophenotyping marker, CD56, also known as neural cell adhesion molecule (NCAM), a type I cell surface glycoprotein expressed on NK cells, activated T cells, neurons and glial cells. It is involved in cell-cell and cell-matrix adhesion, playing an important role by providing adhesion of immature cells on bone marrow, maintaining long-term hematopoiesis \cite{35,36}. Abdulateef et al. \cite{24} showed that CD56 is associated with poor treatment outcomes, and Chen et al. \cite{27} found that patients who were CD56-positive achieved low remission rates. Cui et al. \cite{28} observed there was association among the high expression of CD56 and the aggressive clinical behavior of the disease and poor clinical outcomes in patients with AML. Iriyama et al. \cite{29} reported that CD56 positivity was associated with increased white blood cell (WBC) count at diagnosis which suggests that CD56 is an independent prognostic factor for relapse although its expression is associated with t(8;21), a favorable chromosome abnormality. Breccia et al. \cite{30} findings demonstrated that CD56 positivity in APL resulted in higher frequency of relapse (34%) than CD56 negativity (20%). Thus, patients who expressed aberrant CD56 had lower overall survival at 5-years when compared with those who were negative to CD56 (58\% vs 85\%). Jahedi et al. \cite{26} investigated the aberrant expression of CD2 and CD3 in AML. CD2 is a T lymphocyte cell-adhesion molecule found on the surface of T cells and NK cells. It belongs to the immunoglobulin superfamily that mediates adhesion of T cells to antigen-presenting cells and target cells, playing important roles in immune recognition \cite{37}. CD3 is a transmembrane protein complex of four subunits that belongs to the immunoglobulin superfamily also expressed on T cells, assembled to T cell receptor (TCR), and it is involved in T cell activation, proliferation, and survival \cite{38}. In the study conducted by Jahedi et al. \cite{26}, from the 15 patients that expressed CD2, 10 did not reach complete remission. The same result was observed in those patients who expressed CD3, where five of the eight patients that
were CD3-positive did not achieved complete remission. Thereby, CD2 and CD3 aberrant expression were associated with poor prognosis since their expression were related to low remission rates.

Different from the aberrant phenotypes cited above, the expression of CD19 in AML patients is correlated with good prognosis. CD19, a type I transmembrane glycoprotein and member of the immunoglobulin superfamily that is expressed in B lymphocytes. It participates in B lymphocyte activation and differentiation through modulation of B cell receptor (BCR) signaling. It is a major B cell marker that plays an important role in B cell activation and its biologic functions in immune response. It is highly associated with t(8;21), and the combination of these two features leads to high complete remission rates in AML. Similar conclusions were found by Iriyama et al., considering that complete remission was achieved by patients expressing this marker. Controversially, Jahedi et al. demonstrated in their results that CD19 expression was associated with poor prognosis, as long as just one patient from six achieved complete remission, but these results maybe can be explained due to the small sample size.

Antigens expression patterns
Leukemic cells can present aberrant antigen expression patterns involving overexpression wherein myeloid markers are highly expressed, underexpression which is characterized by low expression of specific markers, or antigen loss often expressed by myeloid blasts.

CD90, also known as Thy-1, a glycosylphosphatidylinositol (GPI)-anchored glycoprotein expressed in hematopoietic stem cells (HSC), thymocytes and T cells, plays an important role in cell proliferation, differentiation, migration, and survival. Regard to underexpression, Chávez-González et al. reported that levels of CD90 were lower in leukemic cells than in normal cells at diagnosis. On the other hand, patients that expressed slightly increased levels of CD90 did not relapse. This suggests that CD90 is involved in hematopoietic cell growth and can mediate a negative signal that results in inhibition of proliferation of primitive hematopoietic progenitors. Thus, CD90 can modulate negatively the cell proliferation, and when it is underexpressed this action is downregulated. These findings suggest that low levels of CD90 can be associated to poor prognosis.

The same authors also investigated CD123 and CD117 overexpression. CD123 is the alpha subunit of the interleukin-3 (IL-3) receptor, a single-pass type-I membrane protein. This marker is expressed on hematopoietic stem and progenitor cells that regulates the cell growth and promotes cell proliferation. It is seen that high levels of CD123 may contribute to leukemia cell proliferation and survival due to its increased resistance to apoptosis. Chávez-González et al. noticed that CD123 levels were higher in AML cells, and patients with this aberrant expression relapsed after treatment, concluding that CD123 overexpression is associated with unfavorable prognosis and adverse outcomes.

CD117, also known as c-Kit, is a type III tyrosine kinase receptor expressed on normal stem cells. It is expressed in HSC, and it is fundamental for several biologic cell functions, such as cell survival, metabolism, cell growth, proliferation, apoptosis, cell migration, and cell differentiation. Similarly, CD117 overexpression may have prognostic value, where it is associated with increased relapse rates. It is considered a stem cell factor, playing an important role in stem cell maintenance and differentiation. Deregulation of CD117 can occur in different ways such as gain of function, loss of function, overexpression, and point mutations. Overexpression of CD117 has demonstrated crucial function in leukemogenesis since it stimulates cell proliferation. In their study, Chávez-González et al. verified that CD117 were significantly higher in patients who relapse after treatment, in contrast with those who expressed this marker in a very small proportion.

CD98, an integral membrane protein classified as a type II membrane glycoprotein, is expressed on leukemic stem cells (LSC). CD98 is implicated in cell adhesion, thereby controlling cell proliferation, survival and migration. Additionally, it plays a role in
regulation of amino acid transport. Concerning favorable prognosis, Nikolova et al. demonstrated that CD98 expression in LSC has impact on prognosis, whereas patients with high levels of CD98 had demonstrated increased complete remission rates and longer survival duration. High expression of CD98 might have an independent prognostic significance in adult AML since there was no correlation of its expression with other prognostic factors, for instance age, sex, percentage of blasts, leukocytes and platelet count, hemoglobin level, organomegaly, expression of lymphoid markers, and expression of CD34.

Asynchronous expression
AML can present asynchronous antigen expression, where early hematopoietic markers are co-expressed with more mature ones. Bahia et al. observed that mature markers were often seen associated with CD117+/CD34+ phenotype, and the most frequent phenotype was CD117+ and/or CD34+ associated to CD11c, followed by CD15 and CD65. In this study, it was observed that asynchronous expression of CD117+/CD15+ was correlated to favorable karyotype, for instance, t(8;21) and t(15;17). Despite no statistical significance, the mean age was lower in favorable karyotype group, and it could be explained due to the small number of cases of this study. Then, CD117+/CD15+ phenotype is associated with favorable prognosis since patients expressing it reached complete remission. Regard to AML-M3, the expression of the aberrant phenotypes, CD117+/CD34+/CD15+ and CD117+/CD34+/CD65+, were also associated with t(15;17), a good prognostic factor since patients presenting this chromosome abnormality are treated with ATRA protocol and good outcomes are reached.

CD15, also called Lewis X, is a carbohydrate antigen expressed on monocytes, neutrophils and eosinophils. It is recognized as an adhesion molecule or ligands for adhesive structures that mediates neutrophil adhesion through selectin ligands, favoring the rolling E-selectin dependent, being implicated in migration function. The study conducted by Breccia et al. involved CD15 aberrant expression in APL patients with PML/RARα-positive, treated by AIDA protocol. Classical morphology subtype was observed in 92% of patients who expressed CD15 associated to bcr1 expression, wherein the majority was classified as low relapse risk at baseline. This group was associated with 5-years cumulative incidence of relapse of 45%, when compared to 11.3% in the group that was CD15-negative. Sialylated CD15 (CD15s) is present on more immature cell, and it is converted in CD15 through cleavage of α(2,3)-linked sialic acid by sialidase inducing maturation of myeloid cells. At diagnosis, APL promyelocytes are CD15-negative; however, the expression of CD15 has been shown to be upregulated during ATRA treatment. The aberrant expression of CD15 may represent an activation antigen that is associated with an increased adhesion property of the blast cells at baseline, which is implicated in the high rates of thrombosis and relapses observed in APL patients. Then, CD15 aberrant expression in APL is associated with poor prognosis.

Meta-analysis
Meta-analysis showed that CD56 aberrant expression was implicated with unfavorable outcomes in AML patients, leading to a decreased overall survival, becoming CD56 a poor prognostic marker. Data showed that even when CD56 was expressed in association with t(8;21), a favorable chromosome abnormality that usually confers good prognosis, patients still demonstrated low survival rates. The same is not observed when this karyotype is present with other aberrant phenotype, such as CD19 and CD117+/CD15-, which lead patients to a better prognosis.

In meta-analysis, two points are fundamental, homogeneity and relative risk of the studies. This summary estimate reached total homogeneity and reasonable relative risk. Summary estimate is the measure that results from the compilation between the studies, not being a simple average of the studies’ results. Therefore, the more accurate is the study the more weight it will have on the meta-analysis result. Although some studies presented smaller sample size with large confidence intervals, when applied this statistical tool, it was possible to merge the
results adequately. This combination enabled to reduce the confidence interval, making possible conclusions regard to the influence of the aberrant expression of CD56 in AML, when associated with other prognostic factors such as age, increased WBC count, AML subtype and chromosome abnormalities.

**Strengths and limitations**

Our analysis has showed strengths regarding an expansive search with more than 20 thousand articles, in which severe criteria was applied resulting in few relevant articles. The selected articles comprised population from different parts of the world, including Europe, Asia, North America, and South America. Almost articles scored >80% revealing a high methodological quality. The study has limitations regarding the criteria to determine aberrant phenotype expression that showed variation across the included studies, and some studies had a low number of cases. Survival information was extracted from survival curves and not from a mortality table. The search was conducted looking for articles published in English, Spanish, and Portuguese and may have missed relevant publications in other languages.

**CONCLUSIONS**

This study demonstrated that most of aberrant phenotypes have negative influence on prognosis and survival in AML patients, mostly those markers classified as lineage infidelity and expression patterns. The meta-analytical measurement reaffirmed aberrant expression of CD56 as a poor prognostic marker with unfavorable outcomes is implicated in decreased overall survival in AML patients, even when they are associated with t(8;21), a favorable karyotype. Then, prognosis shows a meaningful influence in patients’ survival since depending of the risk stratification that the patient is included, it is possible to achieve different treatment outcomes. Besides, it helps to provide new insights into investigation of new therapeutic targets in AML.

**Authors’ contributions**

LHS Pinheiro and LD Trindade performed the systematic research, selected the studies, extracted and analyzed data and wrote the article; AF Sandes analyzed data, wrote and reviewed the article; MAP Nunes did the meta-analytical analysis and wrote the article; AFO Costa, NL Silva, CB Correa, CAC Almeida, GS Cruz and DP Lyra Júnior analyzed data and reviewed the article. DM Schimieguel selected the studies, analyzed data, wrote and reviewed the article. All authors agreed with the article publication.

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**CONFLICT OF INTEREST**

The authors reported no potential conflict of interest.

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**Abbreviations**

AIDA – ATRA+IDA or all-trans-retinoicacid + idarrubicin
AML – Acute myeloid leukemia
APL – Acute promyelocytic leukemia
ARA-C – Cytosine arabinoside
ATEDox – Cytarabine, mercaptopurine, doxorubicin
ATRA – All-trans-retinoic acid
FAB – French-American-British
FLT3-ITD – FMS-like tyrosine kinase 3 internal tandem duplication
HSC – Hematopoietic stem cell
HSCT – Hematopoietic stem cell transplantation
IL-3 – Interleukin-3
JCR – Journal citation reports
LAIP – Leukemia-associated immunophenotypes
LSC – Leukemic stem cell
MeSH – Medical Subject Headings
MLL – Mixed-lineage leukemia
NCAM – Neural cell adhesion molecule
NK – Natural killer
NOS – Not otherwise specified
NPM1 – Nucleophosmin 1
NR – Not related
OS – Overall survival
PML/RARA – Promyelocytic leukemia/retinoic acid receptor alpha
PRISMA – Preferred Reporting Items for Systematic Reviews and Meta-Analyses
STROBE – Strengthening the Reporting of Observational Studies in Epidemiology
WBC – White blood cell

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