Journal of Hematopathology
https://doi.org/10.1007/s12308-022-00492-w

ORIGINAL ARTICLE

Surface CD22 is a highly sensitive and specific B lineage marker and can replace cytoplasmic CD79a/cytoplasmic CD22 in flow cytometric reagent panels for the diagnosis of B-acute lymphoblastic leukemia

Amar Dasgupta[1,2] · **Manisha N. Daruwalla**[1] · **Ravikiran Pawar**[1]

Received: 3 January 2022 / Accepted: 11 April 2022
© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2022

Abstract
World Health Organization (WHO) guidelines, 2008 and 2016, have laid down strict criteria for assigning the lineage to blast cells in the diagnosis and classification of acute leukemia. While cytoplasmic (c) CD22 has been recommended by WHO as a strong B lymphoid lineage-associated marker along with surface (s) CD19, cCD79a, and sCD10, there is no reference to sCD22 as a diagnostic marker in these guidelines. In view of the above fact and the technological advantage surface immunophenotyping assays provide over the cytoplasmic assays, we examined the sensitivity and specificity of sCD22 for B lymphoid lineage. Blast cells in 232 cases of B-acute lymphoblastic leukemia (B-ALL) were examined by flow cytometric immunophenotyping for expression of sCD10, sCD19, sCD20, sCD22, and cCD79a in addition to a host of other lineage-associated CD markers as a part of an antibody reagent panel. In 124/126 (98%) cases of B-ALL, the blast cells expressed sCD22, thereby confirming its high sensitivity as a B lymphoid marker. In 52/54 (96%) of these cases wherein cCD79a (another B lineage marker recommended by the WHO) expression was examined in parallel, both markers were positive suggesting a very high degree of correlation between sCD22 and c79a expression. sCD22 also showed 100% specificity for the B lymphoid lineage. Our data show that sCD22 can be used as a reliable and preferred marker for the diagnosis of B-ALL in place of cCD22 and cCD79a in view of its associated technological advantages. These findings also provide the basis for an increasing use of anti-CD22 antibody and/or CD22 CAR T cell therapy in B-ALL.

Keywords Surface CD22 · Cytoplasmic CD22 · B lineage markers · Cytoplasmic CD79a · CD22 CAR T

Introduction

In World Health Organization (WHO) 2008 guidelines for diagnosis and classification of acute leukemia, B lineage assignment of blast cells is based on strong expression of CD19 and at least one of the remaining B lymphoid markers, namely, CD10, cytoplasmic (c) CD22, and cCD79a, or weak CD19 expression along with strong expression of at least two other B markers — CD10, cCD22, and cCD79a [1]. There is no mention of surface (s) CD22 in this context. The reason for this is not mentioned in the guidelines but could be the result of the reported low incidence of sCD22 expression by the leukemic B blasts in some older publications [2, 3]. Since performance and interpretation of cytoplasmic markers is technically more challenging, and consensual cut offs for positive reactivity of cytoplasmic markers are not available [4], the availability of an additional and reliable B lineage-associated surface marker is highly welcome. The potential clinical use of the prior information on the intensity of surface CD22 expression by blast cells in planning anti-CD22 antibody and CAR T cell therapy in B-ALL cases is an added advantage that is not available with cytoplasmic CD22 [5]. As our data show, sCD22 is ideally suited to meet both the requirements.

✉ Amar Dasgupta
amar_dsgpt@yahoo.co.in

[1] Sections of Hematology and Flow Cytometry, SRL Diagnostics, 1 Prime Square Bldg., Gaiwadi Industrial Estate, S. V. Road, Goregaon (Wast), Mumbai 400 062, India

[2] Suburban Diagnostics, Aston Bldg., 2nd Floor, Shastri Nagar, Andheri (West), Mumbai 400 053, India

Published online: 23 April 2022

Materials and methods

Peripheral blood and bone marrow (BM) aspiration smears were air dried and stained with Leishman stain. Cytochemical myeloperoxidase staining of BM and/or blood smears was performed according to standard procedures [6].

In a retrospective analysis of 232 newly diagnosed cases of B-ALL examined at our laboratory during a 12-month period, the immunophenotype of blast cells was analyzed for expression of B lymphoid markers CD10, CD19, CD20, sCD22, and cCD79a. The diagnosis of all cases was based on the WHO guidelines of 2008 and 2016 (revised) [1, 7].

Flow cytometric immunophenotyping

Flow cytometric immunophenotyping was performed in all cases on anticoagulated (heparin or EDTA) BM and/or peripheral blood samples. Six color flow cytometric immunophenotyping was performed on Navios or FC500 flow cytometers (Beckman Coulter, Florida, USA). Blast cells were classified into B, T, and myeloid lineages on the basis of expression of the following CD markers — CD45, CD10, CD19, CD20, sCD22, cCD79a, CD1a, CD2, CD3, CD4, CD5, CD7, CD8, CD56, CD11b, CD13, CD14, CD15, CD16, CD33, CD117, CD34, HLA-DR, CD235a, CD41, and CD61. Additionally, the expression of cytoplasmic markers cCD3 and cMPO was examined as guided by the results of the surface marker studies. sCD22 and cCD79a were tested consecutively in 126 and 54 B-ALL cases respectively in the later part of the study. Nuclear TdT was checked in 97/105 CD34 negative cases. Fluorochromes used included fluorescein isothiocyanate, phycoerythrin (PE), PerCP5.5, allophycocyanin (APC), PE-Cy7, and APCH7. Isotype matched negative controls were used in appropriate fluorochrome combinations for each multicolor tube. Standard "lyse-wash" protocol was used to enrich the leucocytes followed by incubation of cells with fluorochrome-labelled antibodies at room temperature (22–25 °C) for 20 min. For cytoplasmic labelling, cells were permeabilized with ready-to-use ammonium chloride solution ("*Perm 2*"; Beckman Coulter, FL, USA). Blasts were selected for analysis on the basis of their low side scatter (SSC) and reduced to absent CD45 expression in CD45/SSC two dimensional dot plots, complemented by their forward and side scatter properties and CD34/TdT positivity. In the small number of CD34/TdT negative cases, B lymphoid blast cell populations were identified by back gating of populations co-expressing other relevant B lineage-associated CD marker combinations such as CD19, CD20, sCD22, and CD10 to CD45/SSC dot plots. Cases of CD10 positive mature B cell lymphomas were excluded from analysis by looking *simultaneously* at all the antigenic features (composite immunophenotype) of the neoplastic mature B cells, namely, bright CD45 expression, CD34/TdT negativity, CD20 positivity and surface Ig, and/or light chain expression. For interpretation of positivity of blast cells for a particular marker, we used a cut off of 20% for surface markers and 10% for cytoplasmic markers as recommended by the EGIL criteria [8]. The expression of CD markers was further characterized into dim, normal, intermediate, bright, partial, heterogeneous, etc. depending on the number and distribution of the fluorescent events in the positive quadrant and with respect to the quadrant markers placed as per the reactivity of the cells of interest with isotype matched negative controls. Mean fluorescence intensity (MFI) was recorded for assessment of the intensity of expression of a particular marker by the cells.

The study was approved by the institutional ethics committee.

Results

Most of the B-ALL cases belonged to the pediatric population — around 35% cases were in the age group of 0 to 10 years followed by 19% in the age group 11 to 20 years. Male to female ratio was 1.5:1. CD19 expression was seen in 100% of cases since this property of blast cells formed the basis for diagnosis of their B lineage. CD10 and CD20 expression was seen in 94% and 53% cases, respectively (Table 1). Stem cell/immaturity markers CD34 and HLA-DR were tested in all cases and were expressed in 54% and 97% cases, respectively. TdT was tested in 97 of 105 CD34 negative cases (Table 1). TdT positivity was observed in 90 (93%) of these 97 cases. Aberrant expression of myeloid markers CD33 and CD13 was seen in 12% and 18.5% of B-ALL cases, respectively, while expression of one or more T markers (CD7, CD5, and CD2) was seen in 3.8% cases (Table 1).

Out of the 232 cases of B-ALL, expression of sCD22 was examined in 126 cases and 124 of these cases were positive for this marker, yielding a sensitivity of 98%. The intensity of fluorescence reflecting the expression of sCD22 by the blast cells in these 124 cases varied widely — from dim to bright with heterogeneous distribution of the events. We did not observe any difference in the immunophenotypic profiles/patterns of cases in which CD22 was tested compared to those in whom this marker was not tested. Fifty four of the 126 cases were tested simultaneously and in parallel for sCD22 and cCD79a. Expression of both the markers was seen in 52 (96%) of these 54 cases. One of the 2 cases wherein the correlation between sCD22 and cCD79a was

Table 1 The incidence of relevant CD marker positivity in B-ALL cases ($N=232$). Note the high level of correlation between sCD22 and cCD79a in B-ALL cases

Lineage/CD marker	Number tested	Positive (percentage)
B lymphoid		
CD10	All cases	219 (94%)
CD19	All cases	232 (100%)
CD20	All cases	123 (53%)
sCD22*	***126 cases***	***124 (98.4%)***
cCD79a*	***54 cases***	***52 (96.2%)***
Myeloid		
CD11b	All cases	5 (2.3%)
CD13	All cases	28 (12%)
CD33	All cases	43 (18.5%)
CD117	All cases	4 (1.7%)
T-lymphoid		
CD2	All cases	1 (0.5%)
CD3	All cases	0
CD5	All cases	3 (1.2%)
CD7	All cases	6 (2.5%)
Stem cell/immaturity marker		
HLA-DR	All cases	226 (97.4%)
CD34	All cases	127 (54.7%)
Nuclear TdT	97 CD 34 negative cases	90 (93%)

*In 54 cases, both c79a and sCD22 were tested in parallel. In 52 (96%) of these cases, both markers were positive indicating a high degree of correlation and therefore, high sensitivity of sCD22 as a B lineage-associated marker

Cytoplasmic markers cCD3 and cMPO were applied, as required, on the basis of results of surface immunophenotype of blasts. Nuclear TdT was tested in 97/105 CD34 negative B-ALL cases

None of the 193 cases of different types of AML and 26 T-ALL cases showed sCD22 positivity indicating 100% specificity of this marker for B lymphoid lineage

The boldface entries are significant and are accordingly marked by astrix

missing, was positive for sCD22 and negative for cCD79a while in the other case, the situation was reverse. This indicates a very high degree of concordance in expression of these two B lymphoid markers by the blast cells.

sCD22 positivity was not seen in 193 cases of different types of acute myelogenous leukemia (AML) and in 26 cases of T-ALL during the same study period, using the same antibody reagent panel. This indicates 100% specificity of sCD22 for B lymphoid lineage.

Discussion and conclusions

CD22 is a B cell specific marker [1, 7, 9]. Intracellular expression of CD22 is first seen very early in B cell ontogeny. Its expression on the surface of immature B lymphoid cells follows soon and continues throughout all stages of B cell maturation until its total disappearance at the level of plasma cells — the terminally mature B cells [9]. Initial publications recommended the use of cytoplasmic CD22 instead of surface CD22 in the diagnosis of B ALL on the basis of the observed low incidence of surface CD22 positivity in very early stage of maturation of B precursor cells [2, 3]. WHO 2008 guidelines too recommended the use of cCD22 and not sCD22 as a B cell lineage marker for the diagnosis of B ALL along with cytoplasmic CD79a [1]. This recommendation was reiterated in the 2016 guidelines [7].

In our study, all the 232 newly diagnosed cases of B-ALL were positive for CD19. The immaturity markers such as CD34, TdT, HLA-DR, and CD10 (for B lineage) were expressed in 54%, 90%, 97%, and 94% of cases, respectively. Other studies [10, 11] have shown a wide range of expression of CD10 (60.5 to 95.6%) and CD34 (68.7 to 77.5%) in B-ALL cases. In contrast, TdT (80.5 to 98%) [12] and HLA-DR (97.4 to 100%) expression by B lymphoblasts is reported to be consistently high in most publications. Notably, and as observed in this study, CD34 expression by B-ALL blasts seems to be the lowest among the immaturity markers in all reported series [11]. This highlights the need for including TdT in the immunophenotyping reagent panels used for acute leukemia diagnosis.

We excluded the cases of CD10 positive mature B cell lymphoma from analysis by looking *simultaneously* at all the antigenic features (composite immunophenotype) of the

neoplastic B cells, namely, bright CD45, CD34/TdT negativity, CD20 expression and surface Ig, and/or light chain expression that suggested their "mature" phenotype. Only cases lacking these phenotypic features were analyzed in this study. The incidence and intensity of sCD22 expression in mature B cell lymphoma was also much lower (even absent in many cases) than those of CD20 (data not shown). In cases of "hairy cell" leukemia, however, sCD22 expression was bright as expected.

Although CD20 was a constituent of the primary reagent panel used by us for the diagnosis of acute leukemia, as in the case of most other laboratories, we found it to be expressed only in 53% (123/232) B-ALL cases. Bachir et al. [13] documented a similar incidence (54.2%) of CD20 expression in their B-ALL cases whereas Salem et al. showed a much lower incidence of expression (26.3%) [14]. These findings highlight the limited role of CD20 as a marker of B cell lineage of blast cells in acute leukemia diagnosis. However, the absence of CD20 in leukemic blast cells along with leukemia-associated changes in the other CD markers is useful in distinguishing them from hematogones during quantification and monitoring of measurable residual disease in B-ALL [15]. CD20 is also useful in the differential diagnosis of mature B cell lymphomas versus lymphoblastic lymphomas [1, 7, 14]. Hence, the majority of laboratories continue to use CD20 in their immunophenotyping panels for acute leukemia [16].

In contrast to the low incidence of CD20 expression in B-ALL, sCD22 was positive in 124/126 (98%) of our cases in whom this marker was tested, thereby clearly demonstrating a high degree of sensitivity of this marker for B lineage of blast cells in ALL. Furthermore, a very high degree of concordance (96%) was observed between sCD22 and cCD79a expression by blast cells (in 52/54 cases) in whom both the markers were simultaneously tested. In one of the two cases wherein this correlation was absent, blasts were positive for sCD22 but negative for CD79a and in the other case, the situation was reverse. Lahjouji et al. [17] too reported expression of sCD22 and cCD79a in all their cases of B-ALL. Similar high incidence of sCD22 positivity in B-ALL cases has been observed by other leading laboratories (Brent Wood, Children's Hospital Los Angeles, University of Southern California, USA; personal communication). It is somewhat intriguing to note against this background that the WHO guidelines for diagnosis and classification of acute leukemia [1, 7] recommended use of cCD22 and not sCD22 as a B lymphoid lineage marker. Although the reasons for this recommendation are not clear, this decision could have been influenced by the older published reports that found higher incidence of cCD22 in B-ALL cases compared to sCD22 [2, 3]. The methodologies used in these studies were manual immunofluorescence (IF) assays complemented by flow cytometric immunophenotyping (using home grown antibodies) and SDS PAGE electrophoresis. It is possible that the low sensitivity of manual IF assay and other methodological limitations and reagent-related issues could have resulted in lower sCD22 positivity in their B-ALL cases. Our data also show 100% specificity of sCD22 for the lymphoid lineage in that none of the AML and T-ALL cases tested positive for sCD22.

On the basis of the data referred to above and those shared by us in this article, we believe that sCD22 can be used (in conjunction with CD19 and CD10) as a highly reliable and independent marker for assigning B lineage to blast cells in acute leukemia. This will obviate the need to perform technically more complex cytoplasmic assays for CD22 and/or CD79a in acute leukemia cases. Accordingly, we also believe that only cases that are CD20 and surface CD22 negative need to be tested for cCD79a and/or cCD22 expression for establishing B lineage of the blast cells as per WHO recommendation [1, 7]. Furthermore, this will reduce the cost of immunophenotyping of B-ALL cases, an important consideration in resource poor countries.

The other important advantage of assessing sCD22 expression in ALL cases at presentation is the potential significance of this information in planning future CD22 CAR T cell therapy in these cases, especially those showing poor response or resistance to CD19 CAR T therapy [5, 18].

Therefore, we conclude that *surface* CD22 is as reliable as cytoplasmic CD79a and is an "easier-to-test" marker in the diagnosis of B ALL. Hence, we recommend inclusion of *surface* CD22 as the marker of choice in place of cytoplasmic CD22 (and even cCD79a) in the primary reagent panel for the diagnosis of B ALL. Some of the leading laboratories too follow this practice (Brent Wood, Children's Hospital Los Angeles, University of Southern California, USA; personal communication). Furthermore, this approach also provides important information for the future use of surface CD22 as the target molecule for immunotherapy of these cases.

Author contribution All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by all authors. The first draft of the manuscript was written by AD and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Declarations

Ethics approval The study was approved by the appropriate institutional research ethics committee and the committee certifies that the study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Conflict of interest The authors declare no competing interests.

References

1. Borowitz M, Bene MC, Harris NL, Porwit A, Matutes E (2008) Acute leukemia of ambiguous lineage. In: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiel J, Vardiman JW (eds) World Health Organization classification of tumours. Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues, 4th edn. IARC Press, Lyon, pp 150–155
2. Campana D, Janossy G, Bofill M, Trejdosiewicz LK, Ma D, Hoffbrand AV, Mason DY, Lebacq AM, Forster HK (1985) Human B cell development. I. Phenotypic differences of B lymphocytes in the bone marrow and peripheral lymphoid tissue. J Immunol 134:1524–1530
3. Boué DR, LeBien TW (1988) Expression and structure of CD22 in acute leukemia. Blood 71:1480–1486
4. Gujral S, Tembhare P, Badrinath Y, Subramanian PG, Kumar A, Sehgal K (2009) Intracytoplasmic antigen study by flow cytometry in hematolymphoid neoplasm. Indian J Pathol Microbiol 52:135–144
5. Fry TJ, Shah NN, Orentas RJ, Stetler-Stevenson M, Yuan CM, Ramakrishna S et al (2018) CD22-targeted CAR T cells induce remission in B-ALL that is naive or resistant to CD19-targeted CAR immunotherapy. Nature Med 24:20–28
6. Bain B (2017) Erythrocyte and leucocyte cytochemistry. In: Bain B, Bates I, Laffan M (eds) Dacie and Lewis practical hematology, 12th edn. Churchill Livingstone, London, pp 312–329
7. Arber AD, Orazi A, Hasserjian R, Thiele J, Borowitz MJ (2016) The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood 127:2391–2405
8. Bene MC, Castoldi G, Knapp W, Ludwig WD, Matutes E, Orfao A et al (1995) Proposals for the immunological classification of acute leukemias. Euro Group Immunol Char Leukemias (EGIL) Leuk 9:1783–1786
9. Tedder TF, Tuscano J, Sato S, Kehrl JH (1997) CD22, a B lymphocyte-specific adhesion molecule that regulates antigen receptor signalling. Annual Rev Immunol 15:481–504
10. Jennings CD, Foon KA (1997) Recent advances in flow cytometry: application to the diagnosis of hematologic malignancy. Blood 90:2863–2892
11. Cascavilla N, Musto P, d'arena G, Ladogana S, Matera R, Carotenuto M (1997) Adult and childhood acute lymphoblastic leukemia: clinicobiological differences based on CD34 antigen expression. Haematologica 82:31–37
12. Dexler HG, Menon M, Minowada J (1986) Incidence of TdT positivity in cases of leukemia and lymphoma. Acta Hematol 75:12–17
13 Bachir F, Bennani S, Lahjouji A, Cherkaoui S, Harif M, Khattab M et al (2009) Characterization of acute lymphoblastic leukemia subtypes in Moroccan children. Int J Pediat 2009:674801. https://doi.org/10.1155/2009/674801
14. Salem DA, Abd El-Aziz SM (2012) Flow cytometric immunophenotypic profile of acute leukemia: mansoura experience. Indian J Hematol Blood Transfus 28:89–96
15. SeRdek Ł, Bulsa J, Sonsala A, Twardoch M, Wieczorek M, Malinowska I, Derwich K, Niedzwiecki M, Sobol-Milejska G, Kowalczyk JR, Mazur B, Szczepanski T (2014) The immunophenotypes of blast cells in B-cell precursor acute lymphoblastic leukemia: how different are they from their normal counterparts? Cytometry Part B (Clin Cytometry) 86B:329–339
16. Gujral S, Subramanian PG, Patkar N, Badrinath Y, Kumar A, Tembhare P, Vzifdar A, Khodaji S, Madkaikar M, Ghosh K, Yargop M, Dasgupta A (2008) Report of proceedings of the national meeting on Guidelines for Immunophenotyping of Hematolymphoid Neoplasms by Flow Cytometry. Indian J Pathol Microbiol 51:161–163
17. Lahjouji A, Bachir F, Bennani S, Quessar A, Amzazi S (2015) The immunophenotype of adult T acute lymphoblastic leukemia in Morocco. Exp Oncol 37:64–69
18. Tan Y, Cai H, Li C, Deng B, Song W et al (2021) A novel full-human CD22-CAR T cell therapy with potent activity against CD22 low B-ALL. Blood Can J 11:71

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.