Analysis of Immunophenotypic Criteria by Flow Cytometry to Define B-Cell Chronic Lymphoproliferative Diseases

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Análise dos Critérios Imunofenotípicos por Citometria de Fluxo para Definição das Doenças Linfoproliferativas Crônicas de Células B

Análisis de Criterios Inmunofenotípicos por Citometría de Flujo para Definir Enfermedades Linfoproliferativas Crónicas de Células B

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ABSTRACT

Introduction: Flow cytometry is an important methodology for the diagnosis of chronic B-cell lymphoproliferative diseases (B-CLPD), however, sometimes the cytometrist does not find sufficient elements for the exact definition of the pathological entity involved. **Objective:** To analyze the medical reports issued to patients with chronic lymphoproliferative diseases (CLPD) tested at a private laboratory in Belém-PA, according to the classification criteria established by the studies by Matutes et al. and Craig and Foon. **Method:** Retrospective study with medical reports of patients who underwent immunophenotyping by flow cytometry for the diagnosis of B-CLPD from September 2015 to December 2019. **Results:** After applying the criteria by Matutes et al. and Craig and Foon to the reports analyzed, agreement was reached for 45.24% of the cases of chronic b-cell lymphocytic leukemia/small B-cell lymphocytic lymphoma, 14.29% of the cases of follicular lymphoma, 4.76% of the cases of hairy cell leukemia and 21.43% of the cases defined as "other B-CLPDs not classifiable by flow cytometry". However, Hotelling's hypothesis test (p = 0.049) showed a statistical difference for the definition of B-CLPD according to the criteria adopted. **Conclusion:** The results emphasize that even though flow cytometry is important for the characterization of B-CLPD, sometimes the cytometrist needs to include the category "other chronic B-cell lymphoproliferative diseases not classified by flow cytometry" in the report to induce the prescriber to request additional complementary exams.

Key words: lymphoproliferative disorders/diagnosis; immunophenotyping; flow cytometry.

RESUMO

Introdução: A citometria de fluxo é uma metodologia importante para o diagnóstico das doenças linfoproliferativas crônicas de células B (DLPCB), contudo, por vezes, o citometrista não encontra subsídios suficientes para a definição exata da entidade patológica envolvida. **Objetivo:** Analisar os laudos emitidos a pacientes com doenças linfoproliferativas crônicas (DLPC) atendidos em um laboratório particular de Belém-PA, segundo os critérios de classificação estabelecidos pelos estudos de Matutes et al. e Craig e Foon. **Método:** Estudo retrospectivo com laudos de pacientes que realizaram imunofenotipagem por citometria de fluxo para diagnóstico de DLPCB no período entre setembro de 2015 a dezembro de 2019. **Resultados:** Depois de aplicados os critérios de Matutes et al. e Craig e Foon para os laudos analisados, observou-se concordância em: 45,24% casos de leucemia linfoide crônica de células B/linfoma linfocítico de pequenas células B; 14,29% casos de linfoma folicular; 4,76% casos de leucemia de células pilosas; e 21,43% de casos definidos como "outras DLPCB não classificáveis por citometria de fluxo". Entretanto, o teste de hipóteses de Hotelling (p=0,0409) mostrou haver diferença estatística para a definição das DLPCB segundo os critérios aplicados. **Conclusão:** Os resultados ressaltam que, mesmo sendo a citometria de fluxo importante para a caracterização das DLPCB, por vezes, o citometrista necessita incluir no laudo a categoria "outras doenças linfoproliferativas crônicas de células B não classificadas por citometria de fluxo" para induzir o prescritor a solicitar mais exames complementares.

Palavras-chave: transtornos linfoproliferativos/diagnóstico; imunofenotipagem; citometria de fluxo.

RESUMEN

Introducción. La citometría de flujo es una metodología importante para el diagnóstico de enfermedades linfoproliferativas crónicas de células B (ELPCB), sin embargo, en ocasiones el citometrista no encuentra suficientes subsidios para la definición exacta de la entidad patológica involucrada. **Objetivo:** Analizar los informes emitidos a pacientes con enfermedades linfoproliferativas crónicas (ELPC) tratados en un laboratorio privado en Belém-PA, de acuerdo con los criterios de clasificación establecidos por los estudios de Matutes et al. y Craig y Foon. **Método:** Retrospectivo con relatos de pacientes que se sometieron a inmunofenotipificación por citometría de flujo para el diagnóstico de ELPC de septiembre de 2015 a diciembre de 2019. **Resultados:** Tras aplicar los criterios de Matutes et al. y Craig y Foon a los informes analizados, se observó concordancia en: 45,24% de los casos de leucemia linfocítica crónica de células B/linfoma linfocítico de células B pequeñas; 14,29% casos de linfoma folicular; 4,76% casos de leucemia de células peludas; y 21,43% de los casos definidos como "otros ELPCB no clasificables por citometría de flujo". Sin embargo, la prueba de hipótesis de Hotelling (p=0,0409) mostró diferencia estadística para la definición de ELPCB según los criterios aplicados. **Conclusión:** Nuestros resultados enfatizan que si bien la citometría de flujo es importante para la caracterización de ELPCB, en ocasiones el citometrista necesita incluir en el informe la categoría "otras enfermedades linfoproliferativas crónicas de células B no clasificadas por citometría de flujo" para inducir al prescriptor a solicitar más exámenes complementarios.

Palabras clave: trastornos linfoproliferativos/diagnóstico; inmunofenotipificación; citometría de flujo.

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INTRODUCTION

Chronic lymphoproliferative diseases (CLPD) are a heterogeneous group of cells B, T or NK malignant lymphoid neoplasms with epidemiological clinical behavior, pathological factors and epidemiological characteristics[1,2] quite different among themselves. The most frequent are B-cell chronic lymphoproliferative diseases (B-CLPD) most often diagnosed by flow cytometry with peripheral blood samples, mainly B cell chronic lymphoid leukemia/small cells lymphoma (CLL/SCL), hairy cell leukemia (HCL), mantel cells lymphoma (MCL), follicular lymphoma (FL) and prolymphocytic leukemia (PLL)[1-4].

According to the World Health Organization (WHO)[1,2], the diagnosis of B-CLPD with repercussion in the peripheral blood, is made in patients who present persistent lymphocytosis, more than 5 x 10^9 lymphocytes/μL of mature aspect on the blood count followed or not by bone marrow infiltration, adenomegaly and hepatosplenomegaly.

Based in these findings, several diagnostic methods should be applied in this investigation as, for instance, biopsy and immunohistochemistry of the tumor mass when present, classic or molecular cytogenetics and, mainly, immunophenotyping by flow cytometry of the patient's lymphocytes of the peripheral blood or bone marrow. Occasionally, the cytometrist does not find satisfactory materials to define exactly the pathologic entity involved, even with this simple, fast, efficient, less invasive methodology for using peripheral blood samples and allow the primary identification of the pathological entity involved according to cellular ontogeny, the stage of maturation and expression of aberrant antigens[3-9].

In that sense, several studies found in the literature[5,7,9,10-12] have been suggesting that the panels for the identification of mature clonal B cells should contain a large quantity of combination of monoclonal antibodies as, for instance, the antibodies CD19, CD20, CD22, CD79a, CD79b, CD43, Bcl2, Bcl6, CD10, CD5, CD38, CD25, CD23, IgM, CD200, CD103, CD11c, in addition to antibodies for the kappa and lambda light chains of the immunoglobulin, however, only after the immunophenotypic classification criteria are applied for B-CLPD as those determined by the studies of Matutes et al.[13] and Craig and Foon[14], it is possible to reach a safer definition of the disease in question.

The criteria of classification of the B-CLPD established by Matutes et al.[13], for instance, consider that B cell CLL/SCL is the most frequent B-CLPD of peripheral blood sample and as such, these authors evaluate the expression of five immunophenotypic markers (CD5, CD23, FMC7, CD22 or CD79b) for the differential diagnosis between B-cell CLL/SCL and other B-CLPD. They attribute scores from 0 to 1 according to the intensity and expression or not of the B-cell antigens and also that can be typical B cell CLL/SCL when the score is between 4 and 5, atypical B cell CLL/SCL when the score is 3 and another type of B cell CLL/SCL when the score is within the 0-2 range.

According to Craig and Foon[14], the criteria that can be utilized for immunophenotypic classification of the B-CLPD consider the variation in the expression of antigens CD5 and CD10 in mature B cells, that is, CD5+CD10-, CD5-CD10+, CD5+CD10+ or CD5-CD10- in association with other molecular and cytogenetic markers.

The objective of the present study was to analyze the medical reports issued to patients with B-CLPD consulted at a private laboratory of Belém-PA, according to the classification criteria determined by Matutes et al.[13] and Craig and Foon[14].

METHOD

Retrospective and analytical study based on medical reports of patients by spontaneous demand or after consultation at one of several public, private oncologic hospitals in the cities of Belém and Santarém, with immunophenotyping by flow cytometry for initial diagnosis of B-CLPD at a private laboratory, a reference for acute leukemias and CLPD in Belém-PA from September 2015 to December 2019.

The investigators signed a Term of Use, Anonymity and Data Protection requested by the institution which provided the data in compliance with Resolution 466/2012 of the National Health Council[15], because only deidentified data, except sex and age were utilized. They had no direct contact with the study participants.

Medical reports of patients with initial diagnosis of B-CLPD were included in the study. Patients with diagnosis of T cell lymphoproliferative disease and/or submitted to monitoring of minimal residual disease for B-CLPD were excluded.

The antibodies CD19, CD10, CD20, CD5, CD23, CD79b, CD22, CD38, FMC7, CD103, CD25, CD200, CD43, IgM, anti-kappa and antilambda and the criteria determined by Matutes et al.[13] were utilized to classify, define and issue lab reports of B-CLPD by the reference laboratory. Later, these lab reports were reclassified according to the criteria established by Craig and Foon[14].

Descriptive statistics to determine the frequency of the cases and Hotelling's hypothesis test were adopted to compare the data analytically; the null hypothesis (H0) was adopted if the definition criteria was able to classify the

diseases evenly and the alternative hypothesis (H1) when the definition criteria were unable to classify the diseases evenly and $p < 0.05$ were considered significant results.

RESULTS

During the period investigated, 54 medical reports of CLPD diagnosed utilizing immunophenotyping by flow cytometry of the lymphocytes present in peripheral blood sample were found. Of these, 12 medical reports (22.2%) were excluded because they were based in samples of patients in follow-up for B-CLPD or diagnosed with T cell CLPD. Eventually, only 42 medical reports (77.8%) of B-CLPD diagnosed according to the criteria of Matutes et al.[13] were included.

Of the total sample, 24 (57.14%) were classified as B-CLL/SCL, six (14.29%) as FL and ten cases (23.81%) were classified as other B-CLPD except B-CLL/SCL without specifying which B-CLPD (Table 1).

With the initial results of the 42 cases of B-CLPD classified by the criteria of Matutes et al.[13], the classification of these diseases according to the criteria of Craig and Foon[14] was made, with concurrence between the two methods for 19 cases (45.24%) of B-CLL/SCL, six cases of FL (14.29%), two cases of HCL (4.76%) and nine cases (21.43%) as "other B-CLPD not classified by flow cytometry".

The medical reports of B-CLPD defined by the criteria of Matutes et al.[13] (Table 2), total scores of 3 and 2 (10 of 42), initially classified as "other B-CLPD except B-CLL/SCL", were analyzed according to the criteria of Craig and Foon[14] and one of them was reclassified as diffuse large B cell lymphoma (DLBCL). Other five cases initially classified as atypical B-CLL/SCL by the criteria of Matutes et al.[13], and then applied the criteria of Craig and Foon[14], were reclassified as FL.

The Hotelling's hypothesis test (T2) was applied to the data of Table 1 which revealed statistical difference (p = 0.0409) to define B-CLPD when the criteria of Matutes et al.[13] and Craig and Foon[14] are applied, that is, if isolate, these criteria are unable to classify several entities that form the B-CLPD similarly.

Table 2. Distribution of cases of B-CLL/SCL, FL and other B-CLPD according to the criteria of Matutes et al.[13], obtained from a private laboratory of Belém-PA from September 2015 to December 2019

Classification/ Criteria	Matutes et al. 4 - 5	Matutes et al. 2 - 3
B-CLL/SCL	19	5
FL	3	3
HCL	0	2
Other B-CLPD	0	10

Captions: B-CLL/SCL = B-cell chronic lymphocytic leukemia/small cells lymphocytic lymphoma; FL = follicular lymphoma; HCL = hairy cell leukemia; Other B-CLPD = B-cell chronic lymphoproliferative disease.

DISCUSSION

According to WHO[1,2], the diagnosis of B-CLPD with repercussion in peripheral blood should be determined by clinical, morphologic, immunophenotype or immunohistochemical, molecular and cytogenetic data of the patient together. In some cases of initial suspected B-CLPD, the first diagnostic confirmation test is immunophenotyping by flow cytometry of the lymphocytes of peripheral blood[7,8,10-12].

Many technological advances as new flow cytometers and inclusion of novel antibodies to form the panels of immunophenotype definition of B-CLPD have been implemented in the last years to increase the accuracy of the diagnosis. But occasionally, the cytometrist is unable to find satisfactory materials at the phase of the diagnosis to define all B-CLPDs exactly.

In 1994, attempting to reduce these uncertainties, Matutes et al.[13] were the first to propose a scoring system based in the evaluation of five immunophenotyping parameters for the differential diagnosis of B-CLL/SCL

Table 1. Total distribution of cases of B-CLPD diagnoses according to the criteria of Matutes et al.[13], of Craig and Foon[14], or according to concurrence between them at a private laboratory in Belém-PA, September 2015-December 2019

Classification/ Criteria	Matutes et al. (A)	Craig and Foon (C)	Concurrence of criteria (B)	TOTAL (A + B)	TOTAL (C + B)
B-CLL/SCL	4	0	20	24	20
FL	0	5	6	6	11
HCL	0	0	2	2	2
DLBCL	0	1	0	0	1
Other B-CLPD	10	8	0	10	8
TOTAL (A + B)				42	42

Captions: B-CLL/SCL = B-cell chronic lymphocytic leukemia/small lymphocytic lymphoma; FL = follicular lymphoma; HCL = hairy cell leukemia; DLBCL = diffuse large B cell lymphoma; Other B-CLPD = B-cell chronic lymphoproliferative disease.

from other B-CLPD. They utilized the intensity of the expression in lymphocytes B as base for the antigens CD5, CD23, FMC7, CD22 or CD79b and the intensity of markers for light chains of immunoglobulin. For them, scores from 4 to 5 characterized typical B-CLL/SCL, up to 3, atypical B-CLL/SCL and from 0 to 2, the diagnosis of B-CLL/SCL would be rejected.

This classification is satisfactory to define 4-5 scores B-CLL/SCL, however, for score 3 B-cells which do not express the antigens IgM, CD5 and CD23 or that present strong and/or moderate expression for antigens CD20, CD22 and CD79b[5-7,10,12] this method fails. Thus, Craig and Foon[14] proposed a new immunophenotype classification system for various B and T-cells CLPD where for B-CLPD the criteria to be adopted considered the variation of the expression of the antigens CD5 and CD10 in mature B cells.

The criteria of Matutes et al.[13] and Craig and Foon[14] were effective to define B-CLL/SCL in the present study. However, for score 3 B-CLPD according to Matutes et al.[13] and in 21.43% of the cases (9 of 42) of B-CLPD reclassified by the criteria of de Craig and Foon[14], these classifications failed or were poorly accurate, requiring complementary results as molecular biology tests and cytogenetics not always available when the cytometrist is preparing the medical report.

Böttcher et al.[9] suggested in their study that, due to this difficulty, the cytometrist needs to include in its medical report, when necessary, the category "not-classified" for B-CLPD, which is clinically relevant because the prescriber is informed of the necessity of auxiliary histology, cytogenetics and/or biology molecular tests to include in the final medical report of the B-CLPD in question.

Bezerra et al.[16] and Boyd et al.[4] discussed that in many situations, only with the association of the results of flow cytometry with histopathological, immunohistochemical and molecular findings it is possible to diagnose and differentiate reaction processes of neoplasms and further, to subclassify many B-CLPD.

It was clear that the term "other chronic lymphoproliferative diseases" except B-cell chronic lymphoid leukemia/small cells lymphocytic lymphoma" utilized in the medical reports prompted the attending-physician to request complementary lab tests to conclude the diagnosis. And even if the criteria of Matutes et al.[13] can be adopted to diagnose scores 4-5 B-CLL/SCL, for score equal or lower than 3, the criteria of Craig and Foon[14] are recommended according to the current literature[3,8,9,17].

CONCLUSION

The results indicate that even flow cytometry being an important methodology for immunophenotyping characterization of B-CLPD, still it is not sufficient to define accurately all the pathological entities inherent to these diseases. Therefore, the cytometrist, whenever necessary, should include in its medical report another diagnostic characteristic described as: "other B-cell chronic lymphoproliferative diseases not classified by flow cytometry".

CONTRIBUTIONS

Lacy Cardoso de Brito Junior contributed substantially to the study design, acquisition, analysis and interpretation of the data, wording and critical review. Maria Beatriz da Silva Fonseca, Ana Paula Silveira Paixão, Nilmara Suellen Lopes Castro Mendes, Jessica Sabrina Cordeiro Parente, Matheus Holanda Nascimento (*in memoriam*) contributed substantially to the study design, acquisition, analysis and interpretation of the data. All the authors approved the final version to be published.

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DECLARATION OF CONFLICT OF INTERESTS

There is no conflict of interests to declare.

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