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Analysis of CD200 expression in patients with Chronic-B Lymphocytic Leukemia and other chronic-B lymphoproliferative malignancies: the clinical and laboratory context

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Abstract: ***Objective: To determine the clinical and epidemiological and laboratory characteristics of Chronic-B Lymphocytic Leukemia (B-CLL) and other chronic-B lymphoproliferative malignancies (B-CLM), including the expression of CD200. Methods: A retrospective and descriptive study of the case series type conducted at the Foundation HEMOPE with adults of both sexes diagnosed with B-CLL and other B-CLMs. Results: 95 patients were assessed, of whom 64 had B-CLL and 31 other B-CLMs. The average age was 70.40 years in B-CLL and 61.80 years in the other B-CLMs. Adenomegaly was more frequent in patients with B-CLL, whereas splenomegaly and B symptoms were more frequently observed in other B-CLMs. In patients with B-CLL, the average of leukocytes was 82,607/ul (80.26% lymphocytes and 13.33% neutrophils), whereas in other B-CLMs, the average of leukocytes was 63,614 / ul (70.32% lymphocytes and14.67% neutrophils) but this was statistically significant only in the observation of greater lymphocytosis in B-CLL (p=0.008). The CD200 was positive in all patients with B-CLL with an average value of 79.15, while in patients with other B-CLMs, CD200 was negative in 25.80% of the cases, there being an average value of 46.52. Thus, CD200 presented a higher expression in B-CLL relative to other B-CLMs (p=0.03). Conclusions: The clinical, epidemiological and laboratory characteristics traditionally described in B-CLM were observed in this study and the difference of expression of the CD200 in B-CLL compared to other B-CLMs was shown to be useful in the differential diagnosis of these diseases.***

Keywords: B-cell ChronicLymphocytic Leukemia; Non-Hodgkin's lymphoma; B-cell lymphoma; CD200**.**

1. Introduction

Chronic-B lymphoproliferative malignancies (B-CLM) are a heterogeneous group of neoplasms, which have in common their origin from mature lymphoid B-cells[1,2]. The World Health Organization (WHO) proposed a classification for mature B-cell neoplasms by separating entities according to their clinical, morphological, immunophenotypic, cytogenetic characteristics and molecular alterations in: Chronic Leukemia (pro-lymphocytic leukemia, hairy cell leukemia and chronic lymphocyticleukemia); Non-Hodgkin lymphoma; Multiple Myeloma and Plasma Cell Leukemia[3].

Chronic-B Lymphocitic Leukemia (B-CLL) is the most common of the B-CLMs and represents about 90% of cases, and is the most common leukemia in adults with an increased incidence for both sexes with age [2,3]. The median age of patients at diagnosis of B-CLL is 65 years, with a life expectancy of eight years on average, there also being observed a higher incidence in men (M:F=1.9:1)[2,4].

In the Western world the incidence of B-CLL is five cases per 100,000 inhabitants/year and in individuals aged >65 years, the annual incidence is 20.6 cases/100.000³. In Brazil the incidence of B-CLL is approximately 1,500 cases/year[5].

The classical view of B-CLL has undergone major changes in recent years, this disease which was once perceived as a homogeneously indolent disease, of slow and progressive accumulation of lymphocytes in the organism, is currently evidenced with a heterogeneous character and a variable clinical course[12]. Many studies have been conducted to evaluate prognostic factors, staging and to try to predict survival from CLL. Binet staging is the one most used, and is characterized by three clinical stages: Stage A when there is the presence of one or two enlarged areas of lymphoid tissue, stage B when there are three or more areas and stage C when there is anemia or thrombocytopenia[10]. On the other hand, the differential diagnosis between B-CLL and other B-CLMs is important and the leukemic phase of non-Hodgkin's lymphomas (NHL) should be considered[6]. To do so, the clinical picture, cytomorphological and histological characteristics, chromosomal abnormalities and the immunophenotypic profile of leukemia cells must be evaluated[7].

CD200 (formerly called OX-2) is a membrane glycoprotein which has its expression in various cell lineages, including B lymphocytes[8]. The presence of CD200 in neoplastic cells decreases the Th1 immune response, thereby suppressing the antitumoralimmune response[9]. Different studies have demonstrated that the expression of CD200 is usually high in B-CLL when compared with normal B cells or present in other B-BLMs and is therefore useful in the differential diagnosis of these diseases[7,9].

2. Methodology

This is a descriptive, observational, retrospective study of the case series type conducted at the Hematology and Hemotherapy Foundation of Pernambuco (HEMOPE) - Recife / PE. A search was performed in the files of adult patients of both sexes diagnosed with B-CLL and other B-CLMs between September 2011 and July 2013. The data of interest (age, sex, color/race, adenomeglay, visceromegaly, B symptoms, staging, the number of leukocytes and lymphocytes, the value of CD200 and deaths) were collected from medical records and submitted to statistical analysis and the study was approved by the Committee for Ethics in Research of the HEMOPE Foundation.

For statistical analysis, the adhesion test used was Kolmogorov-Smirnov in order to assess if the distribution was or was not normal, considering a significance level of$\alpha = 0.05$ (confidence level 95%). When one of the samples did not follow a normal distribution, the nonparametric Wilcoxon Rank Sum Test was used. Both with a 95% confidence level.

This research was approved by the Committee for Ethics in Research of the HEMOPE Foundation on 25/05/2012, report No. 007/2012, and satisfies the proposal by the Helsinki Declaration revised in 2008. Since this is a retrospective study, based on a survey of records, the free and informed consent form was dispensed with.

3. Results

95 patients were analyzed, of whom 64 had B-CLL and 31 patients suffered from other B-CLMs. Regarding age, in patients with B-CLL, the overall mean age was 70.04 years (range 38-92 years), while in other B-BLMs overall average age is 61, 80 years (ranging from 49 to 82 years).

Regarding gender, in patients with B-CLL, 57.81% were male and 42.18% female, while in other B-CLMs 51.61% were male and 48.38% were female. Regarding race/color, those who predominated self-declared themselves to be brown in both groups of patients: 84.37% in B-BLL and 80.64% in other B-CLMs.

The Binet staging in patients with B-CLL, at diagnosis was 54.68%, at the moment of staging, in stage A, 15.62% in stage B and 29.68% in stage C. In the group of the other B-CLMs, all patients were diagnosed with B cell non-Hodgkin lymphoma in stage IV.

Among patients with B-CLL, the number of deaths was five (7.8%) patients and in the group with other B-CLMs, there were two (6.45%) deaths. Table 1 shows the relationship between Binet staging and death.

Table 1: Relationship between deaths and Binet staging of the patients with B-CLL.

BINET STAGING	DEATHS YES	NO
A	0	35(100%)
B	1(10%)	9(90%)
C	4(21.05%)	15(78.95%)

Adenogemaly (in any lymphatic chain) was found in 40.62% of patients with B-CLL and 38.71% in patients with other B-CLMs. Splenomegaly was observed in 37.5% in cases of B-CLL, whereas in other B-CLMs it was observed in 61.29% of patients. B symptoms occurred in 12.5% of patients with B-CLL and 35.48% of patients with other B-CLMs. Table 2 shows the relationship between adenopathy, splenomegaly and B symptoms in patients with B-CLL and other B-CLMs.

Table 2: Clinical characterístics: adenomegaly, esplenomegaly and B symptoms.

Signs/ symptoms B-CLL	B-CLMs
Adenomegaly40.62%	38.71%
Splenomegaly37.5%	61.29%
B Symptoms12.5%	35.48%

In the group of patients with B-CLL, the average number of leukocytes in peripheral blood at diagnosis was 82,607/ ul with the relative average of 80.26% lymphocytes and 13.33% neutrophils. In the group of other B-CLMs, the average number of leukocytes in peripheral blood at diagnosis was 63,614/ul with a relative average of 70.32% lymphocytes and 14.67% neutrophils. Despite leukocytosis being seen to be higher in B-CLL, there is no statistically significant difference (P=0.06). This was also observed for the relative number of neutrophils (P=0.54) when compared with B-CLL and other B-CLMs. As to lymphocytosis, there is an increase in lymphocytes in B-CLL compared with other B-CLMs (P=0.008).

Table 3: Laboratory data and statistical tests.

Laboratory data	B-CLL	B-CLM	Statistcal test
CD 200	79.15%	46.52%	P=0.0000003
Leucocytes	82,607/ul	63,614/ul	P=0.068
Lymphocytes	80.26%	70.32%	P=0.008
Neutrophils	13.33%	14.67%	P=0.294

As for the expression of CD200 expression, it was positive in all patients with B-CLL, and was quantified at a mean value of 79.15%. However, in patients with other B-CLMs, the CD200 was negative in 25.8% of cases, with an average value of 46.52 in the cases where it was positive and with low fluorescence intensity. Therefore, there is a greater expression of CD200 (%) in B-CLL when compared to the other B-CLMs (P=0.0000003).

4. Discussion

Our findings corroborate what is described in the literature with greater involvement of men in B-CLL, but the mean age

was slightly higher than that found in other séries[2,3,4,13].

Although Binetstaging remains fundamental for predicting prognosis and targeting therapeutic measures, it is known today that about half of the patients who present with an early stage disease will evolve aggressively, with rapid progression and premature death because of B-CLL[12]. Thus, it was observed that at the moment of diagnosis, 54.68% of patients were at stage A, which is consistent with other reports which also described the greater frequency of stage A and there were no deaths in this group in our study. When the other B-CLMs are evaluated, all patients in our study were (NHL-B) in stage IV, but with reports of only two deaths, probably because they were seen to be low-grade lymphomas.

The strong positivity of CD200, using multiparametric flow cytometry, was observed in all patients with B-CLL, unlike what was observed in B-CLMs, which is consistent with the results of other studies[11,16].

5. Conclusions

The epidemiological, clinical and laboratory characteristics of B-CLL found in this study are consistent with what is described in other surveys. However, the recent use of evaluating CD200 by multiparametric flow cytometry is highlighted, as an aid in the differential diagnosis of B-CLL and other B-CLMs, should therefore be incorporated into the panels of immunophenotypic analysis.

References

[1] J.M. Bennett, D. Catovsky, et al. Proposal for the classification of chronic B lymphoid leukaemias. FAB Cooperative Group, J.Clin.Pathol (42), pp.567-584, 1989.

[2] M. Yamamoto, V.L.P. Figueiredo VLP. Epidemiologia da leucemia linfocítica crônica e leucemia linfocítica crônica familiar, Rev. Bras. Hematol. Hemoter, 27(4), pp.229-232, 2005.

[3] S. Grzegorz, M.D. Nowakowski. Using Smudge Cells on Routine Blood Smears to Predict Clinical Outcome in Chronic Lymphocytic Leukemia: A Universally Available Prognostic Test, Mayo Clin Proc, 82(4), pp.449-453, 2007.

[4] R.P. Gonçalves, D.P. Maia, R.K.A. Custódio, R.P.G.M. Machado, F.B. Duarte, L.B. Silva. Avaliação do perfil hematológico de pacientes com leucemia linfocítica crônica (LLC-B) em um hemocentro estadual, Rev. Bras. Hematol. Hemoter, 31(4), pp.228-234, 2009.

[5] A. Redaelli, B.L. Laskin, J.M. Stephens, et al. The clinical and epidemiological burden of chronic lymphocytic leukemia, Eur J Cancer Care (13), pp.279-287, 2004.

[6] R.M. Rowan, B.J. Bain, J.M. England, K. Hyde. Immunophenotyping in the diagnosis of chronic lymphoproliferative disorders, J.Clin.Pathol (47), pp.871-875, 1994.

[7] G.A. Palumbo, N. Parrinello, G. Fargione, et al. CD200 expression may help in differential diagnosis between mantle cell lymphoma and B-cell chronic lymphocytic leukemia, Leuk Res (33), pp.1212-1216, 2009.

[8] K. Kotwica, M.B. Cioch, A. Damoszynka. Expression of surface molecules on multiple myeloma cells and their potential role in pathogenesis, prognosis and treatment, J MolBiomarkDiagn (2), pp.103, 2011.

[9] D.M. Dorfman, et al. CD200 (OX-2menbraneglycoprotein) expression in B-cell derived neoplasms. Am J ClinPathol (134), pp.726-733, 2010.

[10] J.R. Faria, J.S.R. Oliveira, R.M.D. Faria, M.R.R. Silva, S. Goihman, et al. Prognosis related to staging systems forchronic lymphocytic leukemia, Rev Paul Med, 118(4), pp.83-88, 2008.

[11] D. Alapat, et al. Diagnostic Usefulness and Prognostic Impact of CD200Expression in Lymphoid Malignancies and Plasma Cell Myeloma, Am J ClinPathol, 137(1), pp.93–100, 2012.

[12] C.S. Chiattone, R.P. Falcão. Leucemia linfóide crônica: nova visão de uma velha doença, Rev. bras. hematol. Hemoter, 27(4), pp.227-228, 2005.

[13] P.J. Shenoy, et al. Racial Differences in the Presentation and Outcomes of Chronic Lymphocytic Leukemia and Variants in the United States, Clin Lymphoma Myeloma Leuk, 11(6), pp.498-506, 2011.

[14] M. Hallek, B.D. Cheson, D. Catovsky, F. Caligaris-Cappio, G. Dighiero, H. Dohner, et. al. Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: a report from the International Workshop on Chronic Lymphocytic Leukemia updating the National Cancer Institute–Working Group 1996 guidelines, BLOOD, 111(12), pp.5446-5456, 2008.

[15] P.H. Ramírez. Leucemia linfoide crônica: aspectos clínicos y biológicos, Rev Cubana Hematol Inmunol Hemoter, 15(1), pp.7-20, 1999.

[16] V. Pillai, et al. CD200 Flow Cytometric Assessment and SemiquantitativeImmunohistochemical Staining Distinguishes Hairy Cell Leukemia From Hairy Cell Leukemia-Variant and Other B-Cell Lymphoproliferative Disorders, Am J ClinPathol (140), pp.536-543, 2013.