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Brief Communication

Expression of immunohistochemical markers in patients with AIDS-related lymphoma

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AIDS-related lymphomas (ARL) present high biological heterogeneity. For better characterization of this type of lymphoma, the objectives of the present study were to evaluate the expression of immunohistochemical markers of cell differentiation (CD10, Bcl-6, MUM-1) and determine cell origin profile according to Hans' classification of diffuse large B-cell lymphoma in AIDS patients. This study included 72 consecutive patients with ARL diagnosed at the University Hospital, Universidade Federal do Rio de Janeiro (UFRJ) and at the Brazilian Instituto Nacional de Câncer (INCA) from 2000 to 2006. The morphologic distribution of the lymphomas was the following: 61% were diffuse large B-cell lymphomas (DLBCLs), 15% were Burkitt's lymphomas, 13% were plasmablastic lymphomas, 10% were high-grade lymphomas and 1% was follicular lymphoma. The positivity for each immunohistochemical marker in DLBCLs, Burkitt's lymphoma and plasmablastic lymphoma was respectively: CD20, 84%, 100%, and 0; CD10, 55%, 100%, and 0; Bcl-6, 45%, 80%, and 0; MUM-1, 41%, 20%, and 88%. A higher positivity of CD20 (84% x 56%, $p = 0.01$) was found in DLBCL compared to non-DLBCL; in Burkitt's lymphomas a higher positivity of CD10 (100% x 49%, $p = 0.04$) and Bcl-6 (80% x 39%, $p = 0.035$) were found compared to non-Burkitt's lymphomas. Germinal center (GC) profile was detected in 60% of DLBCLs. Our study suggests particular findings in ARL, as the most frequent phenotype was GC, different from HIV-negative patients.

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Introduction

Non-Hodgkin lymphoma is the second most common malignancy in HIV-infected patients and is an AIDS-defining condition. Diffuse large B-cell lymphoma (DLBCL) is the most common subtype of AIDS-related lymphoma (ARL).^{1,2}

Data from gene expression studies have indicated that markers of germinal center (GC) derivation are associated with clinical behavior in DLBCL in immunocompetent patients.³ Further work led to the confirmation that the expression of

immunohistochemical markers of cell differentiation (CD10, Bcl-6 and MUM1) can be used to determine the GC and non-GC subtypes of DLBCL, and predict survival similar to the cDNA microarray.⁴

Few studies have reported the use of immunohistochemical expression of CD10, Bcl-6, and/or MUM1 to classify cases of DLBCL into GC and non-GC subtypes in ARL patients.⁵⁻⁷ In two studies, a higher prevalence rate of GC subtypes was found.^{5,6} Also, co-expression of GC and activation markers was noted in ARL when compared to lymphomas in HIV-negative patients.⁸

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The aim of the present study was to evaluate the expression of immunohistochemical markers of cell differentiation (CD10, Bcl-6, MUM-1) and to determine the cell origin profile according to Hans' classification of DLBCL in AIDS patients.

Methods

This study included 72 consecutive patients with AIDS-related lymphoma treated on initial diagnosis at the Brazilian Instituto Nacional de Câncer (INCA) and at the Hospital of Universidade Federal do Rio de Janeiro from 2000 to 2006. Diagnosis of DLBCL, Burkitt's lymphoma, plasmablastic lymphoma and high-grade lymphoma were confirmed independently on review by two authors (DA and JCM) using morphologic and immunohistochemical criteria defined in the WHO classification.⁹ The diagnosis of "unclassifiable B-cell lymphoma", with intermediate features between DLBCL and Burkitt lymphoma LBCL and BL was not possible, because molecular analysis was not available. The same pathologists analyzed the immunohistochemical tissue sections.

Patients were selected based on the availability of histological material for tissue microarray (TMA) construction. The following baseline clinical characteristics were recorded: sex, age, stage, presence of bulky disease or B symptoms, performance status and blood counts. The international prognostic index (IPI) was computed; patients were categorized as low risk IPI if they presented with up to two risk factors; and as high risk IPI if three or more risk factors were present.¹⁰ The study was approved by the institutions' Ethics Committees.

Tissue microarrays (TMA) were constructed using a tissue arayer (Beecher Instruments, Silver Spring, MD). Duplicated cores of 1.0 mm were selected from areas of characteristic morphology typical of the case, based on examination of the hematoxylin and eosin-stained original whole tissue sections, without prior knowledge of immunohistologic stains of individual cases. These areas were circled on the glass slide of the whole section and superimposed on the corresponding paraffin block, which was then punched at the selected location to obtain the desired core. 4 mm-thick sections were cut from the TMA and placed on glass slides, which were then baked for 1 hour at 60°C. These slides were then subjected to immunohistochemistry.

Monoclonal antibodies to the following antigens were used: CD10 (56C6; Novocastra, dilution 1:900-Novolink amplification), Bcl-6 (PG-B6p; DAKO; dilution 1:

350-Novolink amplification), MUM-1 (MUM1p; DAKO; dilution 1:8000-Novolink amplification), CD20 (L26; DAKO; dilution 1:1500-Novolink amplification).

Cases were interpreted as positive when more than 30% of neoplastic cells were immunoreactive.

Cases were assigned to the GC group if CD10 alone was positive, or if both Bcl-6 and CD10 were positive. If both Bcl-6 and CD10 were negative, the case was assigned to the non-GCB subgroup. If Bcl-6 was positive and CD10 was negative, the expression of MUM1 determined the group: if MUM1 was negative, the case was assigned to the GCB group; if MUM1 was positive, the case was assigned to the non-GCB group.⁴

Results

The median age of the patients was 40 years (range: 8-69 years), and 52 (72%) were men. Patients' main characteristics at diagnosis were: the presence of B symptoms in 74% (25/34); bulky disease in 72% (18/25), extranodal disease in 53% (9/17), advanced disease (Ann Arbor stage III or IV) in 61% (27/44) and high IPI in 34% (13/38).

A total of 44 patients (61%) were classified as DLBCL, 11 (15%) were Burkitt's lymphomas, 9 (13%) were plasmablastic lymphomas and 7 (10%) were high grade lymphomas. One patient had the diagnosis of follicular lymphoma in the morphologic review.

The distribution of markers (described in Table 1) in DLBCLs, Burkitt's lymphoma and plasmablastic lymphoma are CD20: 84%, 100%, and 0; CD10: 55%, 100%, and 0; Bcl-6: 45%, 80%, and 0; MUM-1: 41%, 20%, and 88%. A higher positivity of CD20 (84% x 56%, $p = 0.01$) was found in DLBCL compared to non-DLBCL; in Burkitt's lymphomas there were higher CD10 (100% x 49%, $p = 0.04$) and Bcl-6 (80% x 39%, $p = 0.035$) positivity rates compared to non-Burkitt's lymphomas (Table 1).

Among DLBCL patients, cell origin assignment was verified using Hans' definitions.⁴ In one case, the subtype could not be determined. GC profile was detected in 60% (26/43) and non-GC profile in 40% (17/43).

Among cases in which the expression of all three antigens (CD10, BCL-6, and MUM-1) was successfully evaluated (42 patients) – positive examples are shown on Figs. 1, 2 and 3 – we compared the distribution of the various combinations with the reported data (Table 2).^{4,6}

Table 1 - Immunophenotypic analysis per morphologic group

Antigens	DLBCL % (n/total)	BL % (n/total)	PL % (n/total)	HGL % (n/total)	Total % (n/total)
CD20	84 (37/44)	100 (11/11)	0 (0/9)	57 (4/7)	74 (53/72)
CD10	55 (24/44)	100 (10/10)	0 (0/9)	71 (5/7)	56 (40/71)
Bcl-6	45 (19/42)	80 (8/10)	0 (0/9)	57 (4/7)	45 (31/69)
MUM-1	41 (17/42)	20 (2/10)	88 (7/8)	29 (2/7)	41 (28/68)

BL, Burkitt's lymphoma; PL, plasmablastic lymphoma; HGL, high grade lymphoma; Total, including one follicular lymphoma; % number of cases with positive immunomarkers/number of available cases for analysis.

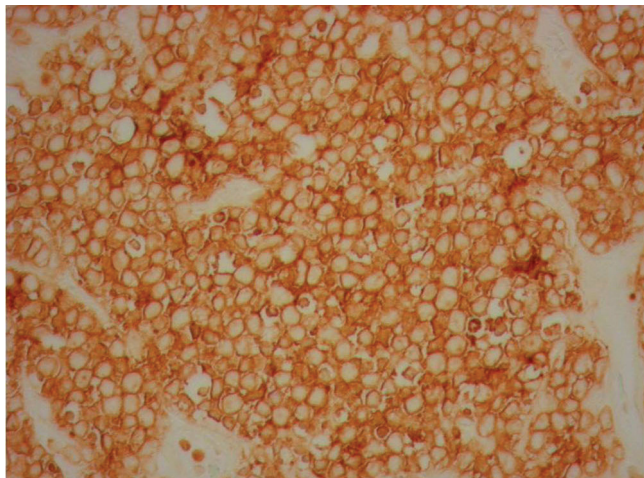


Fig. 1 - CD10 (x 400) – membranous immunoreactivity to anti-CD10 antibody in 100% of the tumor cells.

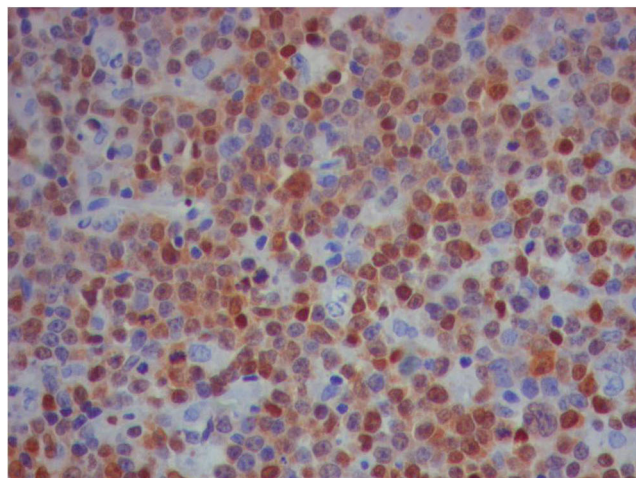


Fig. 3 - MUM-1 (x 400) – nuclear immunoreactivity to anti-MUM1 antibody in 90% of the neoplastic cells.

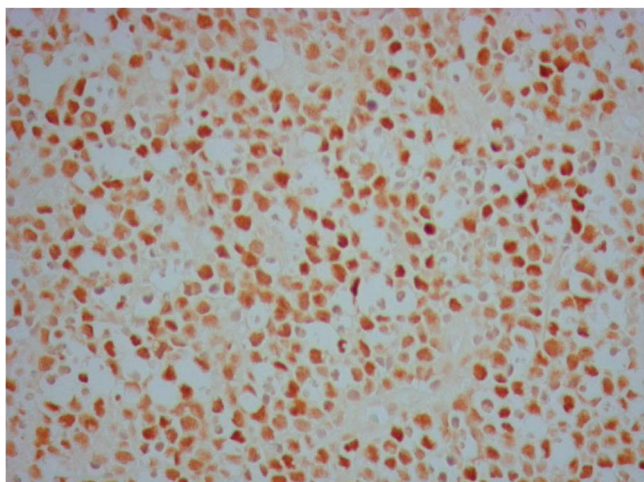


Fig. 2 - Bcl-6 (x 400) – nuclear immunoreactivity to anti-Bcl6 antibody in 70% of the tumor cells.

Discussion

The present study was designed to determine the cell origin profile according to Hans' classification in AIDS-related DLBCL. Higher prevalence of GC profile was observed (60%). This finding is in accordance with results of other series of HIV-infected patients.^{6,7} In a series of HIV-negative patients in the same institution, during a similar period, only 38% had the GC profile.¹¹ It has been postulated that antigenic B-cell stimulation in the context of relatively preserved immune function, typical of the HAART era, could disproportionately promote germinal center pathways of lymphomagenesis.⁷ Our results highlight the biological differences in the origin of the lymphoma in HIV-infected patients.

Previous studies have described high rates of co-expression of GC antigens and activation markers in HIV associated lymphomas.^{6,8} This might represent a final stage of intra-GC differentiation in ARL, suggesting a unique pathophysiology. Our results are in line with this observation and show CD10 and MUM-1 co-expression more prevalent than in HIV-negative series (12% x 1%).⁴ In other series of HIV-infected patients, this

Table 2 - Results in different series of GC profile following Hans' algorithm

DLBCL	CD10	BCL-6	MUM1	% positive Present study	% positive Chadburn's series	% positive Hans' series
GC	+	-	-	2	4	3
GC	-	+	-	5	2	14
GC	+	+	-	31	27	18
GC	+	-	+	12	2	1
GC	+	+	+	10	19	6
NGC	-	-	+	19	23	21
NGC	-	+	+	0	17	18
NGC	-	-	-	21	6	19

phenomenon was verified with a higher Bcl-6 and MUM-1 co-expression, not seen in our series.⁶ This low Bcl-6 and MUM-1 co-expression may be related to the difficulties in reproducing Bcl-6 results usually reported.^{6,12}

Regarding other main characteristics, we found ARL subtype distribution similar to previous studies, with 61% of DLBCL (74% if plasmablastic lymphoma included) and 16% of Burkitt's lymphoma. DLBCL accounts for 70-80% of systemic AIDS-related lymphomas in most reports, including the largest Brazilian one.¹³⁻¹⁵ In concordance with these series, our patients had a median age of 40 years, were male, presented with higher prevalence of extranodal disease at diagnosis and high or high-intermediate IPI.^{14,15}

In conclusion, the present study found AIDS-related DLBCL to be mostly of GC profile, different from the findings in HIV-negative series.

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Conflict of interest

All authors declare to have no conflict of interest.

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