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Original Article

The role of immunohistochemistry in the assessment of classical Hodgkin lymphoma microenvironment

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Abstract: Introduction: Classical Hodgkin lymphoma (CHL) has a unique cellular composition, containing a minority of neoplastic cells - Hodgkin and Reed-Sternberg (HRS) cells - in an inflammatory background. Investigations into this microenvironment have been given special importance in scientific hematopathology, playing an important role in elucidating its composition and its relationship to the prognosis of patients. Objective: To investigate microenvironment tumor markers in CHL, in order to analyze their interactions with clinical-morphological aspects of interest in onco-hematopathology. Methods: This retrospective study analyzed 184 patients with a pathologic diagnosis of CHL. Clinical data were reviewed from medical records. A morphological and immunophenotypic study with CD20, CD30, CD15, PAX-5, CD3, CD4, CD8, CD68, CD34, CD138 and PD-1 were performed. The data were tabulated and *p* value less than 0.05 was considered significant. Results: The time-to-cure was shorter in CD20+ patients, especially in those with more than 25% positivity (P=0.0183). The time-to-cure (P=0.0309) and the death (P=0.016) rates were shorter in PD-1 negative patients. Among patients with the presence of plasma cells in the microenvironment, those with lower numbers tend to be cured earlier (P=0.0374). Higher vascular density is associated with lower frequency of B symptoms (P=0.036) and presence of disease recurrence (P=0.004). Conclusions: The microenvironment is certainly the setting of increasingly robust studies and the findings of this work highlight non-neoplastic B lymphocytes, plasma cells, PD-1 lymphocytes, and vascular density, related to prognosis of CHL patients.

Keywords: Hodgkin disease, immunohistochemistry, tumor microenvironment, patient outcome assessment

Introduction

Cancer research in the last four decades has focused on the cancer cell, with the aim of understanding the oncogenes and tumor suppressor genes in which activation/upregulation or loss of function confer aberrant properties on normal cells, contributing to their malignant transformation. However, cancer cells do not manifest disease alone and research is moving more towards unveiling the roles of cells in the tumor microenvironment - self-sufficiency of tumor growth-stimulating signals, insensitivity of the tumor to factors that inhibit its proliferation, invasion of other tissues, and the ability to originate metastases, unlimited potential for multiplication, induction of angiogenesis and blocking of the natural mechanisms of cell death - in order to generate personalized therapy, in addition to changing the prognosis of many forms of cancer [1, 2].

The microenvironment of classical Hodgkin lymphoma (CHL) is composed of a wide variety of inflammatory and stromal cells. There is an important variability in the composition of this microenvironment, with few lymphocytes in the lymphocyte-depleted (LDCHL) subtype, a mixed cellular infiltrate in the mixed cellularity (MCCHL) subtype and an important fibrosis in the nodular sclerosis (NSCHL) subtype [3, 4]. Due to the infiltration of inflammatory cells, normal lymph node histology, separated into follicular B cells and areas with T cells, is lost. The infiltrate includes cells in order to eliminate Hodgkin and Reed-Sternberg (HRS) cells as well as cells that assist in the survival and proliferation of the tumor clone. In recent decades,

investigations about the microenvironment in CHL have played an important role in elucidating its composition and its relationship with the prognosis of patients. Currently, there is already evidence that HRS cells actively orchestrate the composition of the microenvironment in lymphoma. This association of HRS cells with the microenvironment and the difficulty in growing these cells in culture or in immunodeficient rats indicate that the interaction between neoplastic cells and the microenvironment plays a major role in the pathophysiology of the disease [5].

HRS cells secrete cytokines, such as IL-5, CCL5, CCL17, CCL20, CCL22 and CCL28, to attract inflammatory cells [6]. These lead to a microenvironment predominantly composed of T cells - T helper (Th) and regulatory T (Tregs) - as well as macrophages, eosinophils, neutrophils, mast cells, B cells, plasma cells and also fibroblasts and collagen fibers [6].

Many studies have focused on the impact of non-neoplastic cells on the pathophysiology of CHL, especially through the immunohistochemical expression of cells in the microenvironment in order to identify prognostic markers and therapeutic targets. A more precise understanding of the mechanisms of immune escape is needed in patients with CHL and this requires a more detailed characterization of the cell populations in the tumor microenvironment. This approach has important scientific and translational implications and may allow future molecular investigations in these patients with regard to this complex network of inflammatory cells, enabling the implementation of personalized therapies.

Materials and methods

Study population

We reviewed 184 tissue samples of patients diagnosed with CHL between 1998 and 2019 from three Brazilian healthcare centers - Botucatu School of Medicine, São Paulo State University (FMB UNESP), Luxemburgo Hospital e Sao Luiz/D'Or Hospital. The patients included in the study were selected from the pathology reports and had paraffin blocks available for reassessment, with conditions of use without compromising the sample. Nodular lymphocyte predominant Hodgkin lymphoma (NLPHL) and grey zone cases were excluded. The Botucatu School of Medicine, São Paulo State University (FMB UNESP) research ethics committee approved the study under number 2,931,940, also with the due consent of the other two institutions.

Morphological and immunohistochemical evaluation

The tissue samples stained with hematoxylin & eosin (H&E) were reevaluated by the study pathologists according to the morphological criteria defined by the WHO in 2017, in its publication *Classification of Tumors of Hematopoietic and Lymphoid Tissues*.

Immunohistochemical analysis was performed using the polymer detection system with staining with diaminobenzidine chromogen and counterstaining with hematoxylin. The markers used were: CD20 (L26, ready to use, Dako), CD30 (Ber-H2, ready to use, Dako), CD15 (Carb-3, ready to use, Dako), PAX-5 (SP34, ready to use, Ventana), CD3 (polyclonal, ready to use, Dako), CD4 (SP35, ready to use, Ventana), CD8 (SP57, ready to use, Ventana), CD68 (KP1, ready to use, Dako), CD34 (QBEnd/10, ready to use, Dako), CD138 (MI15/Sindecan, ready to use, Dako) and PD-1 (NAT-105/CD279, ready to use, Ventana). Epstein Barr virus infection was tested by immunohistochemistry - LMP-1/EBV (LMP1, ready to use, Dako) - and *in situ* chromogenic hybridization (CISH). The immunoexpression of CD30 and CD20 was evaluated in total sections of the original blocks. The other markers were evaluated in tissue microarray (TMA) blocks, made from representative sectors and selected from the total samples. The staining extent of CD20, CD3, CD4, CD8, CD138 and CD68 was graded in "plus" signs. Positivity up to 24.9% was defined as 1+, positivity between 25% and 49.9% was defined as 2+, positivity between 50% and 74.9% was defined as 3+, and positivity greater than 75% was defined as 4+. Blood vessels were evaluated by immunostaining for CD34 in endothelial cells, allowing the determination of vascular density, that is, blood vessel count in one high-power field (HPF).

Clinical review

Clinical aspects, including age at diagnosis, sex and ethnicity, B symptoms, extranodal involve-

ment, Ann Arbor staging, serum lactate dehydrogenase (LDH), treatment with doxorubicin, bleomycin, vinblastine and dacarbazine (ABVD regimen) and/or radiation therapy and/or bone marrow transplantation, bone marrow involvement or non-involvement, response to treatment (complete or progressive disease), recurrence and death were collected from patients' medical records. A poor outcome was considered for those patients with progressive disease, recurrence, or death due to the disease.

Statistical analysis

Analyses were performed using the statistical software SPSS for Windows, version 15.0 (SPSS Inc., Chicago, IL, USA), except for the survival curves that were performed in the statistical software R. The analysis was descriptive, followed by the Chi square test and Fisher's exact test to compare proportions. The Kaplan-Meier method was used to estimate patient survival and the log-rank test used to compare survival curves between groups. Significance was determined using Student's t-test and Mann-Whitney test, and statistical significance was set at p value <0.05.

Results

Clinical aspects

Out of 184 patients, 107 (58.2%) were men and 77 (41.8%) were women with a median age of 30.3 years (range: 13-79 years). The cervical location was the most common at diagnosis (n=88; 49.2%), followed by supraclavicular (n=30; 16.3%) and axillary (n=20; 11.2%) lymph nodes. Histologic subtypes were: 72.7% (n=133) nodular sclerosis, 15.3% (n=28) mixed cellularity, 1.6% (n=3) lymphocyte-rich, 1.6% (n=3) lymphocyte-depleted and 8.7% (n=16) unclassifiable. Sixty-seven percent of patients (n=76) had B symptoms, 14.3% (n=16) had bulky disease and 41.2% (n=47) had extranodal involvement (bone marrow, spleen, liver, spinal cord, lung, small intestine, bone, breast, abdominal wall or skin). Bone marrow infiltration was present in 19.9% (n=29) of the patients analyzed. Most patients had stage III and IV (n=61; 54.5%) at diagnosis. The first line of treatment was ABVD in 92.7% (n=102) and 57 (51.8%) patients also received radiotherapy and 8 patients received bone marrow transplantation (7.3%). A poor outcome (progressive or recurrent disease or death) was detected at the end of the segment in 33/101 (32.7%) of the cases. The clinical and histologic characteristics of all patients with CHL are shown in **Table 1**.

B lymphocytes

Out of 174 patients, 171 (98.4%) were positive for CD20. The disease-free survival rate was better in CD20+ patients in relation to negative ones, particularly in those with more than 25% of CD20 expression (P=0.183) (**Figure 1A**). This is not seen for overall survival (P=0.5281) (**Figure 1B**). Other than that, CD20+ patients tend to have lower frequency of extranodal involvement (59.09%, P=0.057) and higher frequency of lactate dehydrogenase (LDH) greater than 475.5 (50.96%, P=0.062).

Plasma cells

Out of 157 patients with available material, 70 (44.6%) were negative for CD138, 78 (49.7%) were 1+, and 9 (5.7%) were 2+ for this marker. Patients with a lower number of plasma cells (1+) tend to be cured before 2+ or negative patients (P=0.0374) (**Figure 2A**). This is not seen for overall survival (P=0.2224) (**Figure 2B**). The frequency of B symptoms was higher in CD138+ patients (65.7%, P=0.046).

CD4 and CD8 lymphocytes

The frequency of CD4 distribution was: 29 (20.3%) patients had 1+, 49 (34.3%) 2+, 46 (32.2%) 3+ and 19 (13.3%) 4+ of a total of 143 patients with available material. For CD8 the frequency was: 55 patients (36.2%) with 1+, 47 (30.9%) with 2+, 37 (24.3%) with 3+ and 13 (8.6%) with 4+ of a total of 152 patients. For these two markers, it was observed that up to 50 months, patients with a higher number of CD4-positive lymphocytes had better survival rates (P=0.015) (**Figure 3A**), while for CD8, patients with 4+ had a better survival rate than patients with 3+ (P=0.0385) (**Figure 3B**).

PD-1 lymphocytes

Out of 154 patients with available material, 143 (92.9%) were negative for PD-1 and 11 (7.1%) were positive, all of these displaying only 1+ (≤24.9%). The distribution of time-to-cure and death between these two groups was dif-

Table 1. Clinical and histologic characteristics of patients with classical Hodgkin lymphoma

	Total cases with available data
Sex	184
Female	77 (41.8%)
Male	107 (58.2%)
Age (years)	30.3 (13-79.4)
Location at diagnosis	179
Cervical	88 (49.2%)
Supraclavicular	30 (16.3%)
Axillary	20 (11.2%)
Inguinal	12 (6.7%)
Mediastinal	19 (10.3%)
Mediastinal (bulky)	8 (4.3%)
Retroperitoneal	1 (0.5%)
Spinal cord	1 (0.5%)
Bulky	112
No	96 (85.7%)
Yes	16 (14.3%)
Extranodal involvement[1]	114
No	67 (58.8%)
Yes	47 (41.2%)
Bone marrow involvement	146
No	117 (80.1%)
Yes	29 (19.9%)
B symptoms	113
No	37 (32.7%)
Yes	76 (67.3%)
Ann Arbour staging	112
I	10 (8.9%)
II	41 (36.6%)
III	22 (19.6%)
IV	39 (34.8%)
HIV	114
No	109 (95.6%)
Yes	5 (4.4%)
First line treatment	110
Without treatment	3 (2.7%)
ABVD	102 (92.7%)
Other than ABVD[2]	5 (2.7%)
Radiotherapy	110
No	53 (48.2%)
Yes	57 (51.8%)
Bone marrow transplantation	110
No	102 (92.7%)
Yes	8 (7.3%)
Treatment response	102
Complete	80 (78.4%)
Progressive disease	22 (21.6%)
Unfavourable outcome[3]	101
No	68 (67.3%)
Yes	33 (32.7%)
Histologic subtype	183
Nodular sclerosis	133 (72.7%)
Mixed cellularity	28 (15.3%)
Lymphocyte-rich	3 (1.6%)
Lymphocyte-depleted	3 (1.6%)
Unclassified	16 (8.7%)

[1]Bone marrow, spleen, liver, spinal cord, lung, small intestine, bone, breast, abdominal wall, and skin. [2]BEACOOP (bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine and prednisone), DHAP (dexamethasone, cytarabine and cisplatin), ICE (ifosfamide, carboplatin e etoposide) e GDP (gemcitabine, dexamethasone, and cisplatin). [3]Progressive disease, recurrence, and death. ABVD (doxorubicin, bleomycin, vinblastine and dacarbazine).

ferent (P=0.0309 and P=0.0016, respectively), showing better overall and disease-free survival in patients without immunoexpression for PD-1 (**Figure 4**).

Macrophages

In relation to CD68, of the total of 157 patients, 15 (9.6%) were negative for this marker, 85 (54.1%) had 1+, 39 (24.8%) exhibited 2+, 15 (9.6%) 3+ and 3 (1.9%) 4+. Patients with a higher CD68 expression (>50%) had a shorter survival rate (P=0.0288) (**Figure 5**).

Vascular density

In the CD34 evaluation, the median number of vessels was 8.0 vessels/HPF (1.0-43.0). Samples with values above 8.0 vessels/HPF are related to disease recurrence (P=0.090, Mann-Whitney test). Higher vascular density was also associated by the chi-square test/Fisher's exact test with a lower frequency of B symptoms (P=0.036) and the presence of recurrence (P=0.004) (**Table 2**; **Figure 6**).

Extension of immunoexpression and clinical aspects

The differences between the extension of immunoexpression of certain markers were statistically significant in relation to some clinical and morphological aspects: B lymphocytes and unfavorable outcome (P=0.045, Mann-Whitney test), CD138+ plasma cells and progressive disease (P=0.047, Mann-Whitney

test), CD138+ plasma cells and vascular ectasia (P=0.026, Mann-Whitney test), PD-1 positive cells and complete response to treatment (P=0.021, Mann-Whitney test), PD-1 positive cells and death (P=0.015, Mann-Whitney test). Other factors did not have significant associations. The specific microenvironment was not related to prognosis and treatment efficacy in different types of CHL.

EBV infection

The association between positive samples for LMP-1 in IHC with positive results for molecular evaluation by CISH was represented in 30 patients (93.8%), being statistically significant (P<0.0001). Comparing LMP-1 with EBER, having this as the gold standard, the sensitivity was 93.8%, the specificity was 91.3%, the positive predictive value was 20.3%, the negative predictive value was 98.3% and accuracy was 91.8% (**Table 3**).

Discussion

Studies have focused on the impact of non-neoplastic cells in the pathophysiology of classical Hodgkin's lymphoma (CHL), especially through the immunohistochemical expression of cells in the microenvironment in order to identify prognostic markers and therapeutic targets.

The present study showed that a lower positivity of CD20 cells in the microenvironment (1+) is related to a better disease-free survival rate. There are few studies through IHC or gene expression [7-12] on the role of non-neoplastic B cells in the pathogenesis of CHL and in response to therapy. B cells compete with HRS cells for survival signals such as CD40 ligand (CD40L) derived from T cells, which may partly explain the fact that greater amounts of B cells are associated with better overall survival in CHL [7]. Greaves et al. (2013) [7] and Tudor et al. (2013) [11] found a significant correlation between a high number of CD20+ cells and better survival, as did Jachimowicz et al. (2020) [12], who showed that low B-cell count is associated with a significant reduction in progression-free survival and overall survival.

In the present study, the presence of plasma cells was associated with B symptoms, and the finding of CD138+ plasma cells in an extension of up to 24.9%, was associated with a higher cure rate compared to the absence of plasma cells or to a higher number of them in the microenvironment (>25%). Plasma cells, even at low numbers, are able to synthesize high levels of cytokines and antibodies that can provide antitumor immunity through antibody-dependent cell cytotoxicity (ADCC) and phagocytosis, complement activation, and improved presentation of antigens by dendritic cells [13]. Plasma cells can also promote tumor growth by releasing immunosuppressive cytokines and producing ineffective antibodies in mediating the antitumor response [13].

RNA sequencing data from The Cancer Genome Atlas (TCGA) show the prognostic relevance of plasma cells in the tumor microenvironment in several malignant neoplasms. A high expression of B-cell and plasma cell genes is associated with increased overall survival in patients with melanoma, adenocarcinoma of the lung, pancreatic adenocarcinoma, and head and neck squamous cell carcinoma [13]. However, high levels of expression of these genes are associated with a poorer clinical outcome in patients with glioblastoma and clear cell renal cell carcinoma [13].

Regarding prognosis, studies are still controversial about the presence of plasma cells detected by CD138 which can contribute to a positive (in colorectal, esophageal, and gastric cancers and melanoma) or negative (breast cancer, ovarian cancer, and melanoma) prognosis. The absence of a prognostic effect has been reported in patients with esophageal cancer and non-small cell lung cancer [14]. High levels of plasma cells and B cells are also associated with increased biological aggressiveness in bladder cancer, increasing the probability of invasion. An increased chance of recurrence, in turn, is seen in patients with prostatic adenocarcinoma [13].

Gholiha et al. (2019) [15] and Visser (2019) [16] widely discussed the role of plasma cells in CHL. HRS cells produce IL-6, IL-21, and CCL28, which attract plasma cells to the tumor microenvironment. IL-6 is also associated with the presence of B symptoms and is the cytokine responsible for the differentiation and survival of plasma cells. Gholiha et al. (2019) [15] demonstrated that a larger infiltrate of plasma cells

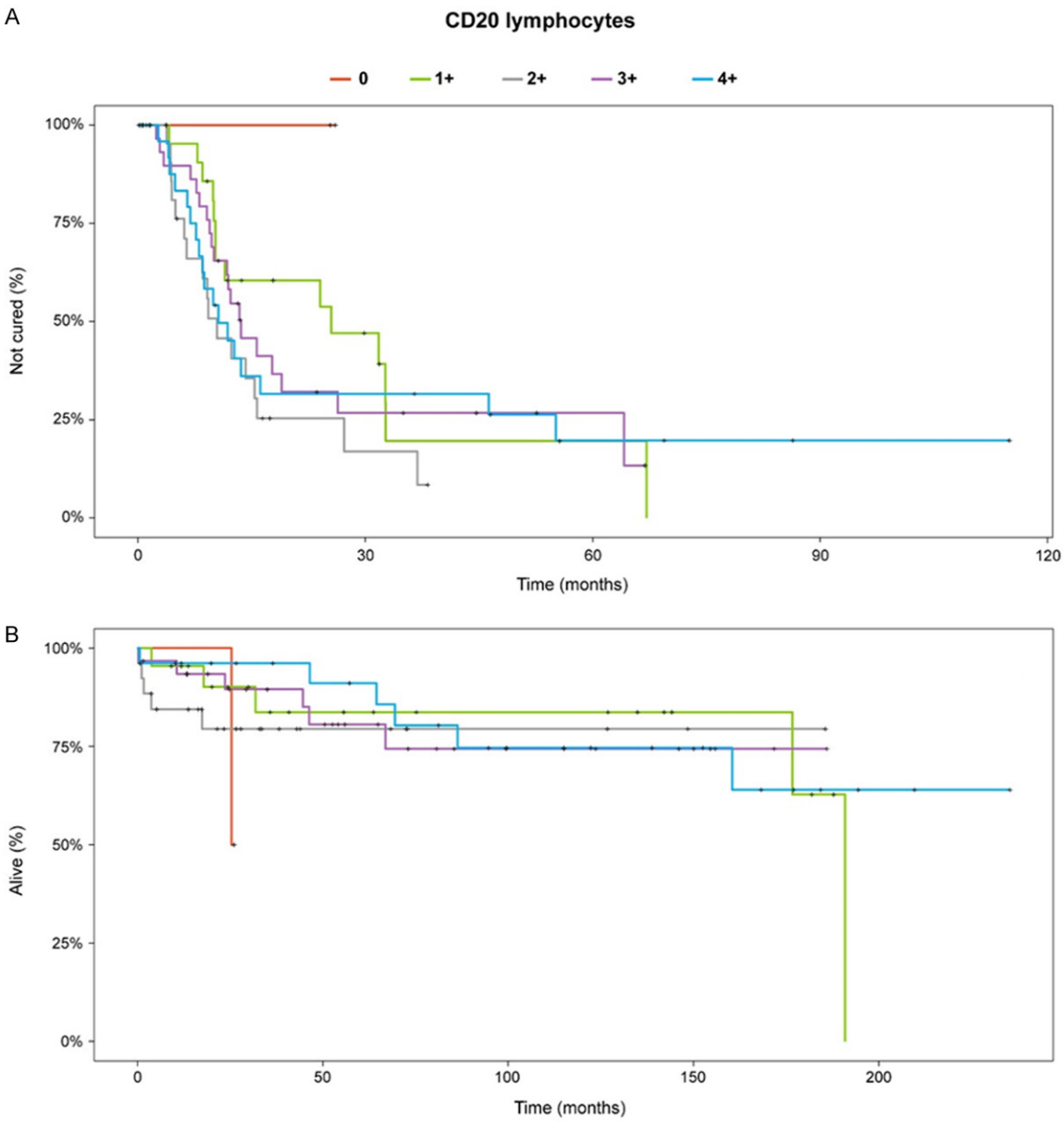

Figure 1. CD20 lymphocytes in the microenvironment. A. Kaplan-Meier estimator of time to cure by variable CD20 on lymphocytes in the microenvironment (P=0.0183). The disease-free survival rate was better in CD20+ patients in relation to negative ones, particularly in those with more than 25% of CD20 expression (above 2+). B. Kaplan-Meier estimator of time to death by variable CD20 on lymphocytes in the microenvironment (P=0.5281). There is no difference in the overall survival between the different groups (P=0.5281).

is associated with B symptoms, advanced staging, greater number of eosinophils and lower survival. Tudor et al. (2013) [11] did not show any impact of plasma cells on patient survival.

The extent of CD4 and CD8 labeling also influenced patient survival. The present study showed that patients with higher numbers of CD4 lymphocytes had better survival rates. For CD8, a discrepancy was observed between patients with 3+ and 4+, which can be explained by sampling, by interference from censored cases or by the probable critical value of these markers so that the “plus sign” system is not effective for evaluation. CD4 positive T cells play a central role in the CHL microenvironment and are typically in close contact with HRS cells (a phenomenon known as a lymphocytic rosette). This morphologic manifestation can

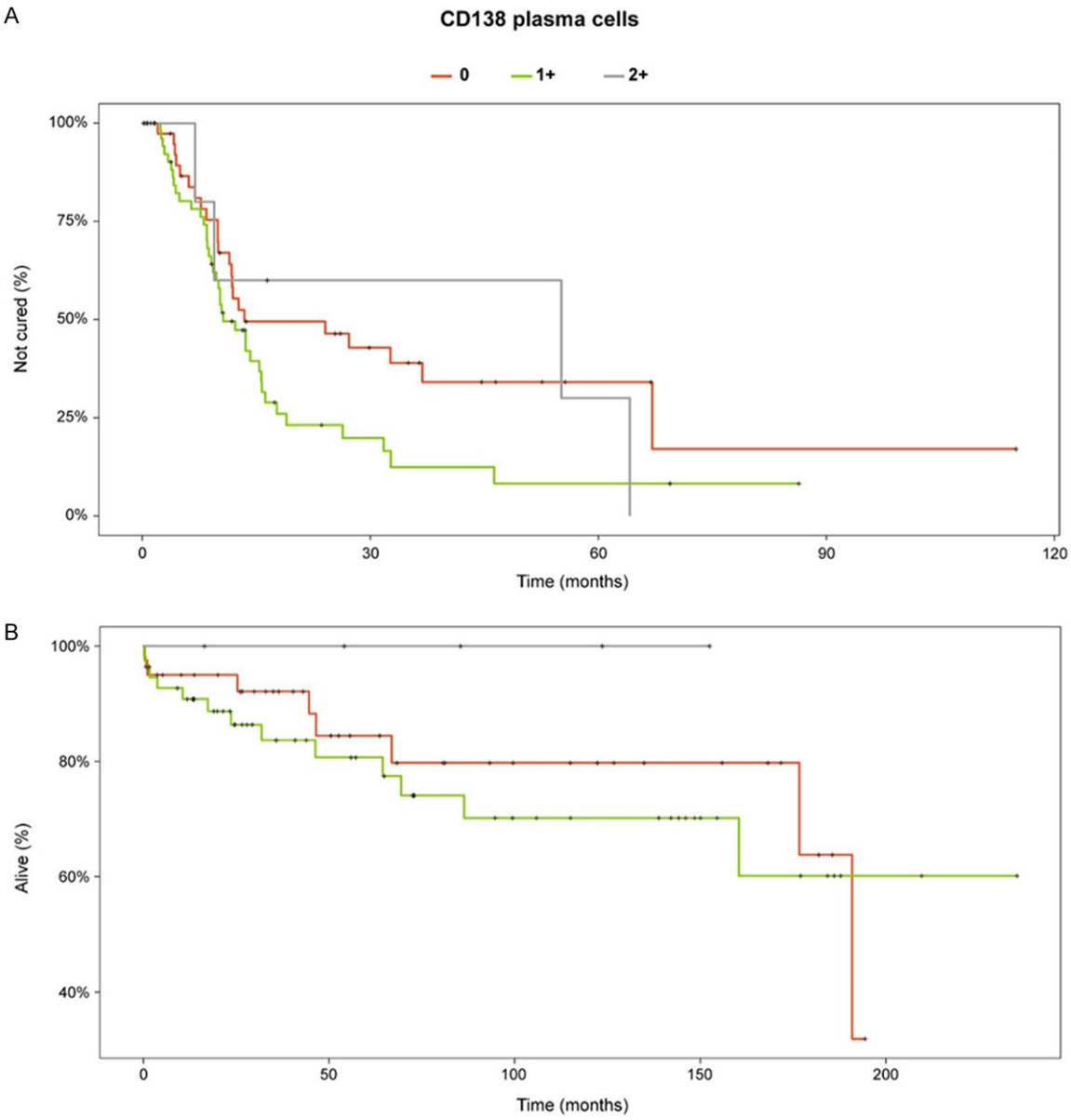

Figure 2. CD138 plasma cells in the microenvironment. A. Kaplan-Meier estimator of healing time by variable CD138 (P=0.0374). The disease-free survival rate was better in patients with a lower number of plasma cells (1+) than in 2+ or negative patients (P=0.0374). B. Kaplan-Meier estimator of time to death by variable CD138 (P=0.2224). There is no difference in the overall survival between the different groups (P=0.2224).

function as a source of survival through mainly Th2 cells, but it also forms a physical barrier against cytotoxic T cells. CD4 positive T cells are attracted to HRS cells that produce chemokines and cytokines that also recruit eosinophils, mast cells, macrophages, and neutrophils. Unlike their role in solid tumors, CD4+ T cells can play a major role in the PD-1 mediated antitumor response in CHL by HLA class II-dependent mechanism [17, 18]. In addition, T cells, in particular Tregs, have the function of rescuing HRS cells from attack by cytotoxic T lymphocytes and NK cells. This may be particularly relevant in cases of CHL in which HRS cells are infected with EBV and express viral antigens that can shape the microenvironment by attracting more regulatory T cells and saving HRS cells from the need for other signs of survival and suppression of cytotoxic T lymphocytes [19-21].

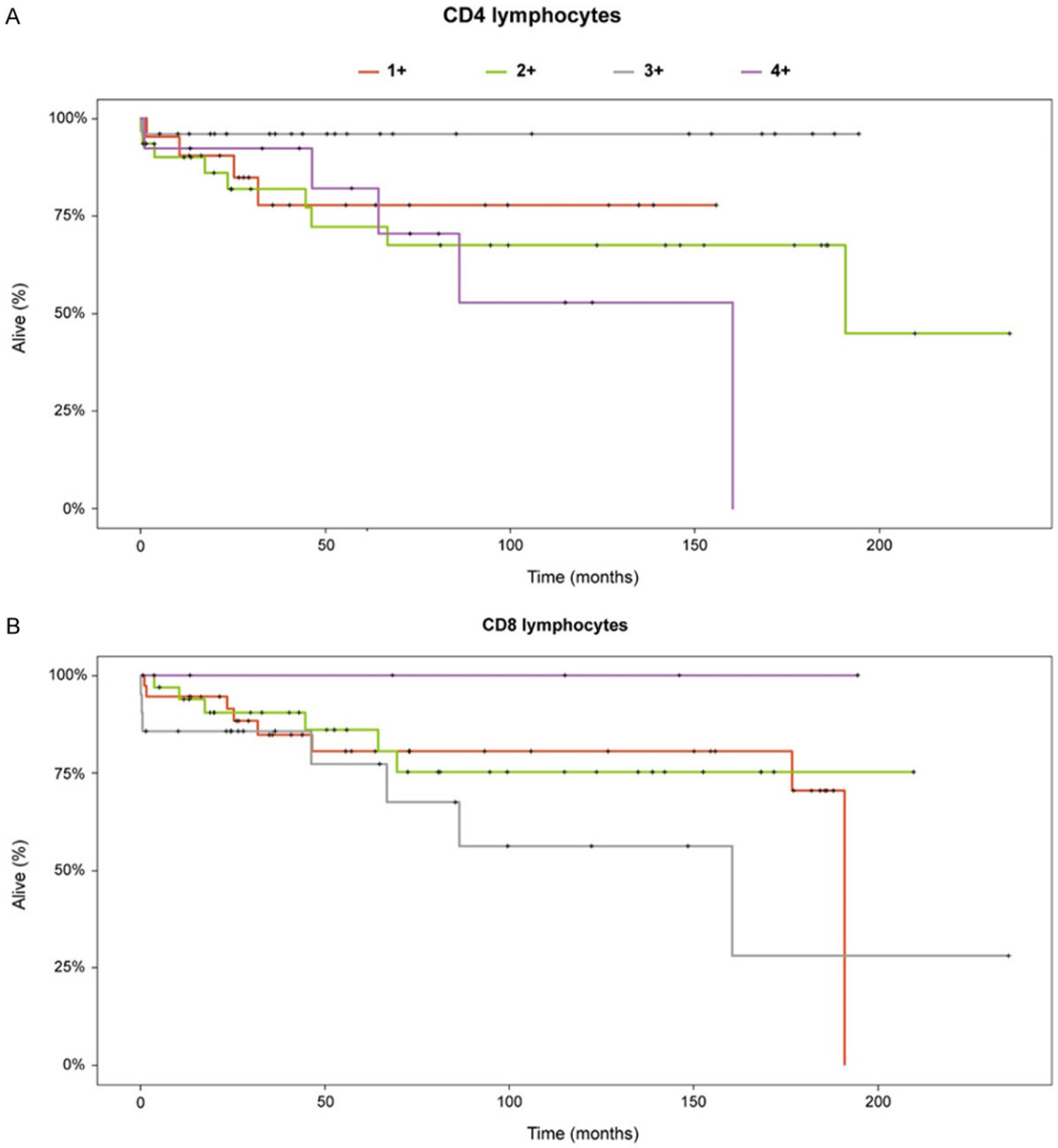

Figure 3. CD4 and CD8 lymphocytes in the microenvironment. A. Kaplan-Meier estimator of time to death for the variable CD4 in the microenvironment (P=0.015). Up to 50 months, patients with a higher number of CD4-positive (4+) lymphocytes had better survival rates (P=0.015). B. Kaplan-Meier estimator of time to death by variable CD8 in the microenvironment (P=0.0385). Up to 50 months, patients with a higher number of CD8-positive (4+) lymphocytes had a better survival rates (P=0.0385).

The rate of T helper cells (CD4+) and effector T cells (CD8+) may be correlated with the prognosis of patients with CHL. High levels of CD8+ and low levels of CD4+ are associated with a favorable outcome in one study [22]. Alvaro-Naranjo et al. (2005) [23] demonstrated that low CD8+ had worse outcome in patients at an early stage. In this study, a high number of cytotoxic T cells, demonstrated through the expression of TIA1 (cytotoxic granule-associated RNA binding protein) and granzyme B, also correlated with lower survival. This negative prognosis of increased numbers of cytotoxic T lymphocytes has also been confirmed by two other studies [24, 25].

The presence of PD-1 positive lymphocytes in the present study is associated with a lower

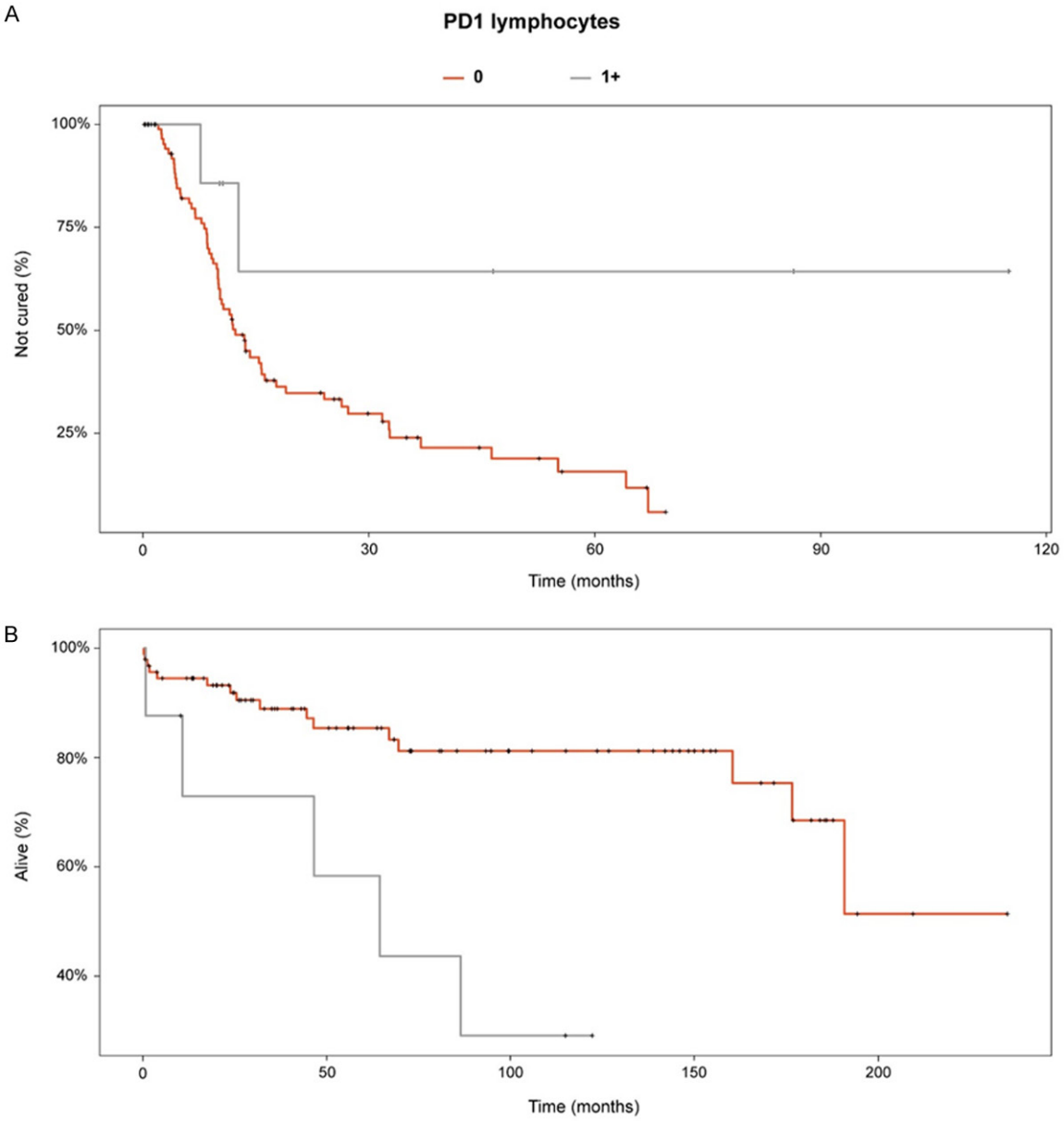

Figure 4. A. Kaplan-Meier estimator of time to cure by variable PD-1 (P=0.0309). The disease-free survival rate was better in patients without immunoexpression for PD-1 (P=0.0309). B. Kaplan-Meier estimator of time to death by variable PD-1 (P=0.0016). The overall survival rate was better in patients without immunoexpression for PD-1 (P=0.0016).

cure rate and a lower survival rate. The expression of PD-L1 is observed in most HRS cells in approximately almost all cases of CHL [26]. An immune avoidance mechanism involves signaling between PD-L1 expressed at high levels in HRS cells and its receptor (PD-1) expressed in CD4+ and CD8+ T lymphocytes, suppressing the effector function of T cells and consequently, disabling the antitumoral function of these cells. HRS cells also induce the expression of PD-L1 in macrophages, further promoting the immunosuppressive environment [27]. Hollander et al. (2017) [28] showed that high proportions of both PD-1 and PD-L1 by IHC in microenvironment leukocytes are associated with lower disease-free survival and overall survival. Muenst et al. (2009) [29] also showed that a greater number of PD-1-positive lymphocytes is a negative prognostic marker for overall survival in patients with CHL.

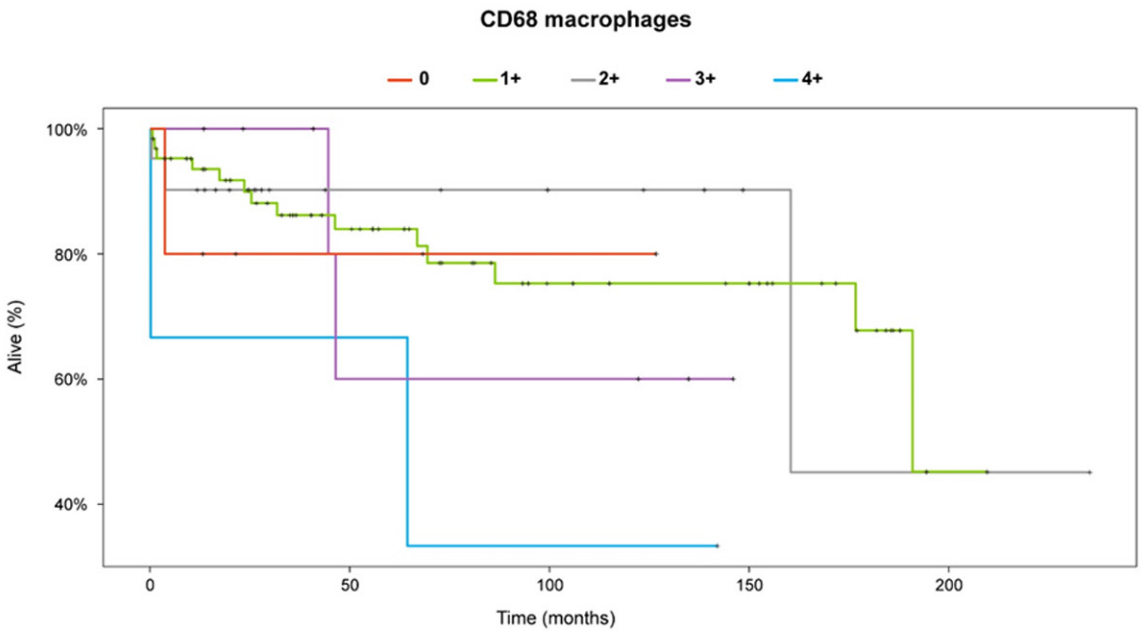

Figure 5. Kaplan-Meier estimate of time to death by variable CD68 (P=0.0288). The overall survival rate was lower in patients with a higher CD68 expression (>50%) (P=0.0288).

Table 2. Clinical characteristics in relation to vascular density[1]

	Vascular density		*p*-value[2]
	<8 vessels/HPF	>8 vessels/HPF	
B symptoms	42 (63.6%)	24 (36.4%)	0.036
Recurrence	1 (11.1%)	8 (88.9%)	0.004
Bulky disease	11 (68.75%)	5 (31.25%)	0.41
Extranodal involvement	21 (53.85%)	18 (46.15%)	0.837
Bone marrow involvement	12 (57.1%)	9 (42.9%)	1.000
Ann Arbor III e IV	29 (54.7%)	24 (45.3%)	0.839
Unfavorable outcome[3]	15 (50.0%)	15 (50.0%)	0.369

[1]Vascular density median. [2]P<0.05. [3]Progressive disease, recurrence, and death.

The increased number of CD68+ and/or CD163+ macrophages is well established in the literature as a predictor of adverse outcome in patients [30, 31], and the present study corroborates these findings, demonstrating that patients with higher CD68 expression (>50%) had worse survival rates.

In the study presented here, it was observed that higher vascular density (>8 vessels/HPF) is related to a lower frequency of B symptoms and the presence of recurrence. Angiogenesis is the hallmark of tumor growth and progression in solid and hematological neoplasms and can provide prognostic information on CHL. Korkolopoulou et al. (2005) [32] investigated angiogenesis in patients with CHL using CD34 immunoexpression and observed that overall survival was adversely affected by vascular density and that it decreased with staging. Glimelius et al. (2005) [33], through immunoexpression by CD31, demonstrated the same results, showing that patients with a higher vessel count exhibited a worse disease-free survival rate.

In conclusion

- Patients with CD20 positivity in the lymphocytes in the inflammatory background are cured faster than the negative ones, particularly those with more than 25% positive cells (P=0.0183).

- PD-1 negative patients have a higher cure rate and longer survival than PD-1 positive patients (P=0.0309 and P=0.0016, respectively).

- Patients with less than 25% of CD138-positive plasma cells in the microenvironment tend to be cured before patients with more than 25% positivity or negative patients for this marker (P=0.0374).

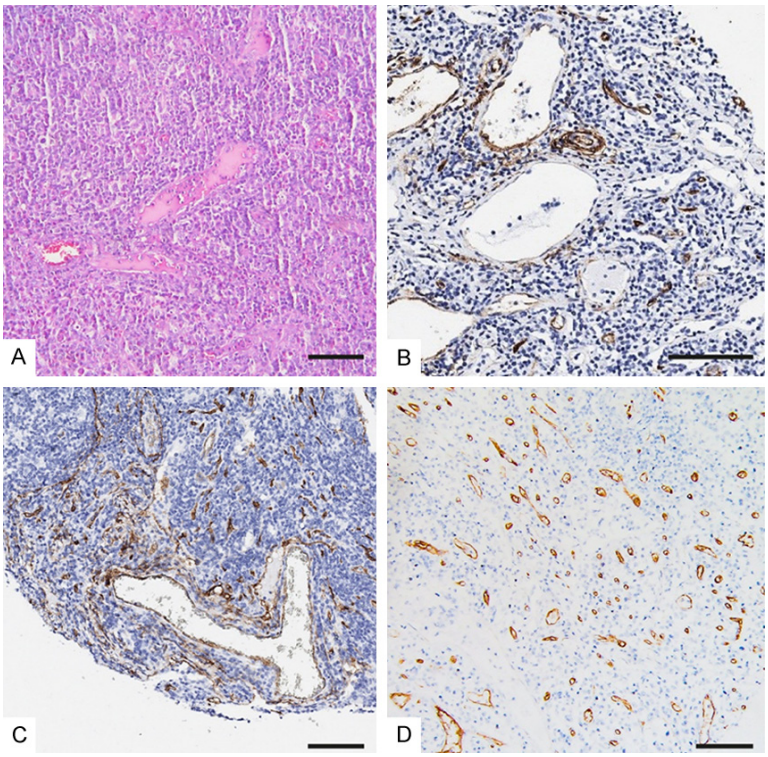

Figure 6. Ectasia and vascular density. A. Vascular ectasia on morphology (H&E). Dilated blood vessels and congestion are observed in the center of the image. B. Vascular ectasia by CD34. Dilated blood vessels are observed throughout the image. C. Ectasia and vascular density by CD34. There is a dilated blood vessel and an increased number of blood vessels observed by CD34 immunostaining. D. Vascular density by CD34. There is an increased number of blood vessels observed by CD34 immunostaining. Scale bar =100 μm. Original Magnification =200x. H&E: hematoxylin and eosin.

Table 3. Diagnostic power of LMP1 in detecting EBV infection, using EBER (CISH) as the gold standard

Measure	
Sensitivity (%)	93.8%
Specificity (%)	91.3%
Positive predictive value (%)	20.3%
Negative predictive value (%)	98.3%
Accuracy (%)	91.8%
Kappa index (%)*	0.77%
p value†	<0.001

*Kappa agreement coefficient (κ): <0.00 (no agreement), 0.00-0.20 (poor agreement), 0.21-0.40 (mild agreement), 0.41-0.60 (moderate agreement), 0.61-0.80 (substantial agreement) and 0.80-1.00 (almost perfect agreement). †The significance level was 5% (*p* value <0.05).

- Vascular density is related to a lower frequency of B symptoms (P=0.036) and the presence of recurrence (P=0.004).
- Patients with a higher number of CD4-positive lymphocytes had better survival (P= 0.015).
- Patients with a higher CD68 expression (>50%) had shorter survival (P=0.0288).

The present study, unlike previous studies, worked with "plus" sign methodology for the quantification of cells in the microenvironment, which is a method of greater reproducibility in the healthcare practice. However, a greater understanding of cell interactions can be performed with multiplex immunohistochemistry and computational cell counting in order to improve the results obtained.

Larger and prospective cohorts are necessary for clarification and a more precise understanding of the interaction between the tumor and the immunologic and clinical response of patients with CHL. Background inflammatory cells are known to be an important component in the tumor microenvironment, and there are important scientific and translational implications to these findings, which may influence the development of therapeutic approaches in the future.

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Disclosure of conflict of interest

None.

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