important clinical and laboratory differences. HCLv is the rarest of them with an incidence of less than 1 case:1.000.000 inhabitants/year. Unlike HCL, HCLv generally presents with elevated blood counts, there is no monocytopenia and the abnormal cells present an identifiable nucleolus. HCLv cells do not express CD25 or present *BRAF* mutation mutations, findings that are almost universally present in HCL.

Aims: The aim of this study was to describe the laboratory findings of all HCLv cases diagnosed at Grupo Fleury from 2015 to 2018.

Methods: A retrospective descriptive study of the laboratory findings of all HCLv cases diagnosed at Grupo Fleury from 2015 to 2018. Complete blood counts were obtained with a Sysmex XE 5000 counter and submitted to morphology review. Blood or bone marrow immunophenotypic prophiles were obtained using an 8-color FACS Canto II cytometer for acquisition and Inifinicyt for analysis. Antibodies against CD3, CD4, CD5, CD8, CD10, CD11c, CD19, CD20, CD22, CD23, CD25, CD38, CD45, CD56, CD79b, CD103, CD123, CD200, KAPPA, LAMBDA, FMC7, IgM, IgD, IgG and IgA were used.

Results: 7 cases were diagnosed during this period (0.04% of all of suspected oncological cases submitted immunophenotyping). Median age was 86 years old (range: 58–89), and 3 were males. Median CBC values were [median (range)]: hemoglobin = 10.1 g/dL (8.8–12.7); lymphocytes = 2.4 x10^9/L (1.4–16.5); monocytes = 0.53 x10^9/L (0.2–3.1); platelets = 91 x10^9/L (76–97, and an outlier of 1.106 x10^9/L). No cases presented monocytopenia Flow cytometry (5 of peripheral blood and 2 of bone marrow aspirate), demonstrated universal positivity for CD19, CD20, FMC-7, CD103, and CD11c (partial expression in one case). No case demonstrated CD5, CD10, CD25, CD38 or CD123. Of note, CD200 expression was negative or mild in all cases. Five cases were IgM+/IgD+, one IgM+, and the other was IgG+. Two demonstrated kappa expression and 5 were lambda light chain restricted. Karyotype was normal in all of the 3 cases analyzed.

Summary/Conclusion: Flow cytometry, along with morphology, was the main sources of diagnosis, as no specific genetic alteration is known. The presence of CD103 and CD11c in the absence of CD123 and CD25 suggest HCLv, excluding classical HCL. CD200, classically strongly expressed in classical HCL was negative or only weakly expressed in HCLv and may help on diagnosis.

PB2064 USE OF RNASCOPE TECHNOLOGY TO DETERMINE STAT-3 EXPRESSION IN HUMAN DIFFUSE LARGE B-CELL LYMPHOMA

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Background: Diffuse large B-cell lymphoma (DLBCL) is the most common and one of the most heterogeneous lymphomas. Therefore, it is critical to further stratify cases of DLBCL into biologically similar and clinically meaningful subgroups, which will not only guide prognostic assessment and facilitate therapeutic decisions, but also stimulate further research to understand the pathogenesis and develop potential novel treatments.

Signal transducer and activator of transcription 3 (STAT3) is a transcription factor that exerts important biological functions related to cell proliferation, differentiation, survival, angiogenesis and immune response.

Aims: In this study, we used RNAscope technology in order to evaluate STAT3 RNA expression in human DLBCL in selected groups of activated B-cell–like DLBCL (ABC-DLBCL) patients and germinal center B-cell–like DLBCL (GBC-DLBCL) patients.

Methods: This retrospective study reviewed data from patients diagnosed with DLBCL collected from the archive of the Section of Pathology of the University of Bari, Hospital Policlinico, Bari, Italy, between 2009 and 2013. All patients had pathologically confirmed DLBCL. Tumors were divided into two histological subgroups: one that includes ABC patients and another that includes GCB patients.

Results: The results have shown that ABC DLBCL tissue samples contained more STAT3-positive cells as compared with GCB tissue samples. Moreover, the immunofluorescence analysis showed that tumor vessels in ABC samples appeared lined by endothelial cells expressing both FVIII and STAT3 signals, while in GCB samples, only few vessels expressed both FVIII and STAT3. These data confirm other reports showing that STAT3 is highly expressed and activated in ABC-DLBCL samples and the tumor vessels appeared lined by endothelial cells expressing both FVIII and STAT3.

Summary/Conclusion: An improved understanding of tumor biology and the role of the tumor microenvironment has led to advances in the diagnosis, classification, prognostics, as well as novel treatments of patients with hematologic malignancies. Ongoing dynamic and correlation studies of tumor biology and the contribution of the tumor microenvironment should be promoted in the context of novel drug development in order to identify optimal therapies for various lymphomas, and improve the curability of these diseases.

PB2065 DIAGNOSTIC VALUE OF THE NEW SYSMEX XN 2000 AUTOANALIZER IN THE STUDY OF LYMPHOCYTOSIS.

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Background: The new Sysmex analyzers of the XN series (Sysmex Corporation, Kobe, Japan) incorporate new leucocytes channels (WNR, WPC or WDF) based on fluorescence flow cytometry. Fluorescence flow cytometry, through these channels, enables separation of different leukocyte populations and generates numerous cellular parameters. The FSC (forward light scatter) intensity is proportional to the cell size, the SSC (side light scatter) to cellular complexity (granularity) and the SFL (lateral fluorescent light) to DNA/RNA content of the cells (nucleus). Several flags (QFLAGS) alert of blasts, abnormal (ABN) lymphocytes (neoplastic) and atypical (ATYP) lymphocytes (reactive) have been developed based on the cells scatter light behavior. High fluorescence lymphocytes (HPLC) can also be quantified. Moreover, Syxmex XN analyzers incorporate non-routine parameters, such as those of the lymphocyte populations: three related to the midpoint LY-X (the lateral scattered light intensity), LY-Y (the fluorescent light intensity), LY-Z (the FSC intensity); and three with the distribution area LY-WX (the lateral scattered light distribution width) LY-WY (the fluorescent light distribution width) and LY-WZ (the FSC distribution width). In a previous study, we observed that the lymphocyte parameters and the alarms of Sysmex-XN (age, number of lymphocytes, Q-FLAG, LY-Z, and LY-WZ) are useful tools to guide the diagnosis of lymphocytosis with an overall diagnostic value 87% [1].

Aims: To determine the diagnostic value of these parameters obtained in a previous study [1]in a different population of lymphocytosis.

Methods: 217 new cases with lymphocytosis > 4.0x10^9 / l were recruited. A peripheral blood immunophenotype (IF) was performed to distinguish polyclonal (n = 144) and monoclonal (n = 73) lymphocytosis. This study also allowed the separation of 33 cases of chronic lymphatic leukemia (LLC), 18 cases of LLC zap70 + and 22 marginal lymphomas. All the samples were analyzed with the XN-2000 for WDF and WPC channels. The diagnostic value of the statistically significant variables obtained from a previous study [1], including Age> 60 years; Lymphocytes> 7 x 10E9 / L and> 12 x 10E9 / L; QFLAG ATYP + ABN> 205 and> 345; LY-Z> 64,> 65 and> 67; LY-WZ> 590 were applied in this new group of samples.

Results: When we consider age (60years) as a cut-off point, in the group < 60 years we only observed 3 cases with monoclonal lymphocytosis, which was considered unrepresentative.

In the group >60 years we observed 71 polyclonal and 70 monoclonal lymphocytosis (table). XN-based parameters showed a global classification value of 83%. Regarding monoclonal lymphocytosis, 54 of the 70 patients (77%) were correctly classified. The most predictive variables of clonal lymphocytosis were QFLAG ABN + ATYP (PPV 83%) and absolute lymphocyte count > 7 x 10^9/ L (PPV 72%).

>60 years(n = 161)						
	QFLAG (ABN+ATYP)		LY-Z(ch)	LY-WZ(ch)	Lymphocytosis (x10e9/l)	
	≥205	≥345	≥65	≥590	≥7	≥12
Polyclonal (n = 71)	6/71	2/71	0/71	2/71	7/71	0/71
Monoclonal (n = 70)	30/70	11/70	6/70	6/70	18/70	20/70

Table. Laboratory diagnosis of lymphocytosis based on XN parameters.

Summary/Conclusion: The results were similar to our previous study and confirmed the utility of these parameters in an initial study of lymphocytosis. The positivity of these variables had a high predictive value of monoclonal lymphocytosis. Therefore, it could be used as a routine practice in laboratories.