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Evaluation of the pathogenetic role of cellular subsets of the Follicular Lymphoma Microenvironment

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Esame finale anno 2022

To my Father

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CHAPTER 1 - INTRODUCTION

1.1 Follicular Lymphoma: clinical and epidemiological features

Follicular Lymphoma (FL) accounts for about 20% of all lymphomas, with the highest incidence rates reported in the USA and western Europe. It affects predominantly adults, usually in the sixth decade of life and with a male-to-female ratio of 1:1.7. A well known risk factor is the agricultural exposure to pesticides and herbicides (1). FL predominantly involves the lymph nodes, but also involves the spleen, bone marrow, peripheral blood, and less commonly Waldeyer ring. Pure extranodal presentations are un common, in these cases the most commonly affected sites include the gastrointestinal tract, soft tissue, breast, and ocular adnexa. Most patients have widespread disease at diagnosis, with peripheral and visceral lymphadenopathy and splenomegaly. The bone marrow is involved in 40—70% of cases. Despite the extension of the disease, patients are usually asymptomatic: B symptoms such as fever and weight loss are quite uncommon. The disease has always been considered virtually incurable because of its waxing and waning clinical course in which long remission periods alternate with frequent relapses. In about one third of case we assist to progression to large B-cell lymphoma (LBCL) with a significant worsening of the prognosis.

1.2 Morphology and Phenotype

FL is a non Hodgkin lymphoma typically composed of the two types of B cells normally found in germinal centres called centrocytes and centroblasts. The centrocytes (CC) are small to medium-sized cells with angulated, elongated, twisted, or cleaved nuclei; inconspicuous nucleoli; and scant pale cytoplasm; while centroblasts (CB) are large cells with usually round or oval nuclei, vesicular chromatin, 1—3 peripheral nucleoli, and a rim of cytoplasm (Figure1). Most cases of FL have a predominantly follicular growth pattern, with closely packed follicles that efface the nodal architecture. Neoplastic follicles have attenuated or absent mantle zones a typical back to back arrangement, with CC E CB randomly distributed. This feature is very helpful in the differentiation from reactive

germinal centers, where the subsets of centroblasts and centrocytes show a typical polarization. **(Figure1).**

The number of centroblasts varies from case to case and is the basis of grading: the evaluation takes in to account the absolute number of CB in ate least 10 neoplastic follicles at 40X HPF. Being the Grade 1characterized by 0-5 CB/HPF; grade 2 by 5-10 CB/HPF; grade 3 has more than 15 CB/HPF and is further subdivided in 3A: with >15 CB and CC and 3B bult up by CB only.

The tumor cells are usually positive for surface immunoglobulin (IgM with or without IgD, IgG, or rarely IgA). They express B-cell-associated antigens as CD19, CD20, CD22, CD79a and are usually positive for BCL2, BCL6, and CD10. Some cases, that are most commonly grade 3B, lack CD10 but retain BCL6 expression. Proliferation index ki 67 frequently correlates with FL grade being under 20% in grade 1-2 and over 20% in grade 3.

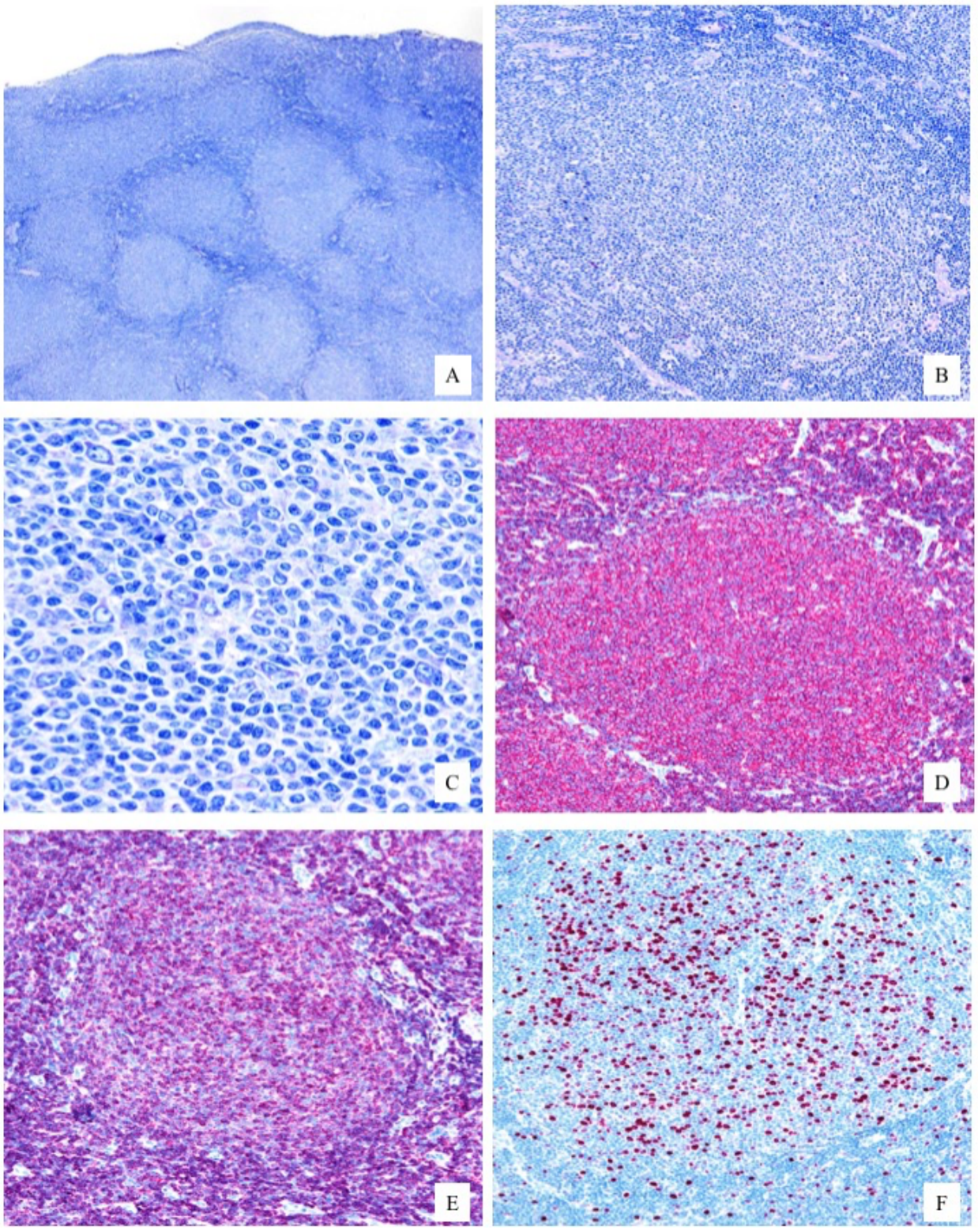

Figure 1: A "back to back follicles"; **B** unpolarized pathologic germinal center; **C** Centrocytes and Centoblasts within the germinal center **D** diffuse and intense CD20 expression in the neoplastic cells in inter and inrafollicular areas. **E** Bcl2 expression in the pathologic germinal center. **F** Ki 57 index in the germinal center.

1.3 Pathogenesis:

The hallmark genetic lesion of FL is the translocation t (14; 18) (q32; q21): it is caused by an error during the physiological rearrangement of the immunoglobulin (IGH) genes in the VDJ region and brings the BCL2 gene under the control of the IGH promoter, thus resulting in constitutive activation of its transcription and in FL cells protection from apoptosis. In physiological condition these cells, that are undergoing the process of antigen affinity maturation, should not be immortalized in case of genetic rearrangement process fails.

The expression of the BCL2 protein in the CG is by far the most distinctive element of FL. BCL2 is expressed by the neoplastic population in variable proportions: in 85-90% of FL grade 1-2, and in 50% of the FL grade 3; this aberrant expression together with other morphological clues allows to make the differential diagnosis with a reactive follicular hyperplasia (1).

Not all LFs are BCL2 +: in some cases this negativity is only apparent, being due to a mutation in the BCL2 gene that modifies the epitopes recognized by the most frequently used immunohistochemical antibody (Clone 124) resulting in negative staining; however, the use of antibodies directed towards other epitopes of BCL2 protein (Clones SP66, Clones E-17), allows to identify most of the true positive cases.

Nonetheless a fraction of FL cases are actually BCL2- (2,3). In these cases, the genetic lesions underlying the development of the disease are translocations and / or amplifications of the BCL6 gene, or mutations that lead to the overexpression of BCL6 and p53, resulting in a typical late germinal center phenotype (4).

Interestingly many FLs in extranodal sites tend to be of higher grade (grade 3) and may lack BCL2 protein and the BCL2 translocation (1)

The BCL2 negative FL are however diagnosed and treated as the BCL2 positive ones, as a difference in terms of impact on the prognosis has not yet been found (5).

Along with these alterations, 90% of FLs also show other genetic lesions such as loss of 1p, 6q, 10q and 17p, and "gains" in chromosomes 1, 6p, 7, 8, 12q, x, and 18q.

Deletion or mutation at the 1p36 locus that contains the TNFRSF14 gene is also possible, the impact of which on prognosis is yet to be confirmed. The FL is also subject to important epigenetic modifications, which appear early in the natural history of the disease, the most common being the “gain of function” of the H3K27 methyltransferase and of the EZH2 gene; mutations are also observed in the genes that regulate the "remodeling" chromatin as CREBP and MLL2 .

The number of changes increases in proportion to the degree of the lymphoma and is associated with the events of progression and transformation.

1.4 Prognostic Factors

Due to the extreme variability in outcome, many efforts were made in order to predict prognosis, need for therapy and risk of evolution in FL patients.

The clinical score called FLIPI and its evolution FLIPI 2 are widely used in clinical practice to stratify patients in risk classes (low, inrtermediate and high). They turned out to be robust and easy-to-use, but yet, taking into account only clinical parameters (age, hb levels, size of mass, stage…) without considering biological features of the disease by itself.

In fact, marked heterogeneity in outcome remains within each risk group and further insights in the biology of FL are needed to enable individualized therapies.

Pastore and colleagues recently identified a panel of 7 genes (EZH2, ARID1A, MEF2B, EP300, FOXO1, CREBBP, and CARD11), mutated in FL neoplastic cells, which seemed to have an impact on prognosis, however a genetic evaluation of each patient is not suitable for the current clinical practice and this molecular scoring remained the only attempt (6).

At present, the most effective unfavorable prognostic indicator is progression of disease (POD) within 24 months (POD<24) from first line treatment (7). Despite its clinical efficacy, this parameter requires two years' evaluation and the administration of a first unsuccessful line of therapy.

1.5 Immune microenvironment

Recently, the genome wide approach underscored the pivotal role of FL microenvironment in the evolution of this disease; differences in the biology of the host immune/inflammatory microenvironment (ME) have been suggested as cause of heterogeneity in clinical course and outcome of these patients.

FL ME is composed by macrophages, follicular T cells, non neoplastic B-cells and follicular dendritic cells. As happens in non neoplastic germinal centers, where these reactive cells are essential for the development and maintenance of B-cell response, it is well demonstrated that pure FL cell lines cannot be cultured without a favorable ME and even *in vitro* short term growth requires survival signals from feeder or cytokines.

In 2004, Dave et al. described the ME impact on FL biology. By gene expression profiling (GEP) they identified two different immune response (IR) signatures, involving T-cells and macrophages which independently predicted FL outcome.

A primary signature (Immune-response 1) included a complex mixture of T-cell and macrophage markers specifically associated with long overall survival rates, whereas a second signature (Immune-response 2) that included genes coding for markers specifically restricted to tumor-associated macrophages (TAM) and dendritic cells, associated with shorter survival rates. (8).

T lymphocytes

Our work focused on the number and distribution of CD8+ cytotoxic T lymphocytes and on PD1+ T "follicular t helper" (Tfh) T lymphocytes, taking into account the emerging data about their impact on antitumor immunity.

CD8+ cytotoxic lymphocytes are key elements in the immune response against neoplastic cells. Their activation with the binding of both the antigen and the major histocompatibility complex, triggers a series of cellular processes that results in the induction of apoptosis in the target cell via the TCR signalling pathway. Both in physiological and in pathological conditions, this activity can be inhibited by the initiation of signal cascades mediated by Tfh lymphocytes, with the well described phenomenon of the exhaustion of their function (9).

Tf h lymphocytes in physiological conditions carry out their function inside the CGs, particularly they are involved in the development and increase of antibody affinity of B cells. When the IGH rearrangement of GC cells turn out to be successful, Tfh lymphocytes , prevent their apoptosis through the activation of specific pathways, involving IL-4 and CD40.

In pathological conditions Tfh lympocytes appear to be able to promote FL cells immune escape and growth through a cyclic cross talk involving CCL17, CCL22, and IL4 pathway with recruitment of Treg lymphocytes, with the reduction of cell proliferation and the production of pro-inflammatory cytokines and a consequent attenuation of the process of immune response (10).

Both in physiological and pathological conditions, Tfh elements show different intensity of PD1 molecule expression in intra- and interfollicular sites and this difference can be considered an epiphenomenon of their functional maturative process, which starts in the paracortical of the lymph node, involves the passage in the interfollicular areas, and ends with the presence of "mature" elements at the level of the germinal center. In the first two phases the expression of PD1 remains weak/moderate, while at the level of the GC is typically intense, indicating the presence of a complete activation status of signalling and a effective modulation of the immune response (11, 12).

Macrophages

Macrophages are versatile cells characterized by an extraordinary functional plasticity: they can be immune-stimulatory or immune-suppressive, pro- or anti inflammation and in tumoral setting they can favor or restrain disease development.

This heterogeneity has been oversimplified by the concept of functional polarization which identifies two types of macrophages, M1 and M2, with distinct and opposite functions.

M1, or classically activated macrophages, are activated by Th-1 cytokines like INF□ and bacterial products; they secrete immunostimulatory cytokines that fuel the adaptive immune response and may acquire cytotoxic activity against transformed cells. M2, or alternatively activated macrophages, develop in a Th-2 cytokine-rich ME such as IL-4, IL-13; they have high scavenging activity and produce several growth factors that activate the process of tissue repair and suppress adaptive immune responses.

M2 macrophages may suppress cytotoxic T cell activity and attract T-reg lymphocytes, thus facilitating tumor growth and immune escape. In 2010 Steidl and colleagues recognized a gene expression profiling signature of TAMs being associated with poor prognosis and correlated an increased number of CD68+ macrophages with a shorter PFS and a higher risk of relapse after autologous hematopoietic stem-cell transplantation (13). FL cells recruits macrophages and switches their pro-inflammatory M1 phenotype to an immunesuppressive “M2” one, to escape the immune surveillance. In this setting, mechanisms of interaction between Macrophages and neoplastic clone still need to be clarified and could represent a possible therapeutic target (14). CD163 was proposed as the marker of M2-like phenotype and CD163+ TAMs, and it indeed resulted as a strong predictor of adverse outcomes in other ematologic diseases (14).

CHAPTER 2 – AIM OF THE STUDY

-Assess the presence of T lymphocytes as constituents of the follicular microenvironment, specifically identifying follicular T helper elements (Tfh) by characterization with PD1 and CD8+ cytotoxic T lymphocytes responsible for antitumor immune surveillance.

-Assess the presence and number of macrophage elements in particular TAM with M2 polarization

-Assess any difference in expression of molecules in BCL2+ and BCL2- LFs.

- Identify possible correlations with clinical data in order to define immunostaining patterns with potential prognostic significance, to be included in routine diagnostic practice in order to facilitate the risk framing and clinical management of patients with FL.

CHAPTER 3 – MATERIAL AND METHODS

3.1 Patient Selection

For this study, 46 cases of grade I to IIIA nodal LFs, including LF BCL2+ and LF BCL2-, were selected from the archives of the Hemolymphopathology Unit of Sant'Orsola Hospital in Bologna, Italy, frome saplse collected from 2012 to 2016. All specimens were formalin fixed and paraffin embedded. Each case has follow-up of at least 5 years and is provided with data regarding symptomatology at presentation, stage at diagnosis, FLIPI score, therapy, and any information about events of recurrence or transformation.

All 46 cases were re-evaluated in light of the diagnostic criteria outlined by the WHO 2017 classification, and the diagnosis of FL was reconfirmed by two experienced hematopathologists.

3.2 Immunostaining

Immunostaining for biomarkers were performed at the Biosciences Laboratory of Istituto Scientifico Romagnolo (IRCCS) IRCCS (Meldola, Italy) using the Ventana Benchmark ULTRA staining system (Ventana Medical Systems, Tucson, AZ, USA) .

CD68 (PG-M1) (Dako), CD163 (MRQ-26) (Ventana Medical Systems, Tucson, AZ, USA), anti-CD8(SP57) (Ventana Medical Systems, Tucson, AZ, USA), PD-1 (clone NAT1, CNIO) antibodies were used.

For the single staining with CD68-PGM1 and PD, tissue sections were incubated for 32 minutes with antibodies diluted in antibody diluent (Ventana Medical Systems) 1:100, 1:4 respectively.

Double immunostaining was performed with CD163 and CD8 ready to use antibodies.

The Optiview DAB Detection Kit (Ventana Medical Systems) and the ultraView Universal Alkaline Phosphatase Red Detection Kit (Ventana Medical Systems) were used for single and double immunostaining. All the tissue sections were counterstained for 16 minutes with Hematoxylin II (Ventana Medical System) after each immunostaining.

3.3 Slides evaluation

The content and distribution of positive elements for each staining was evaluated under an optical microscope (Olympus BX51). For single staining the absolute number of lymphoid elements positive for the molecule of interest was evaluated for each case in a minimum of 10 fields at high magnification (HPF,400x), both considering separately the intra- and interfollicular areas. Intrafollicular areas were defined as the complex consisting of germinal center and mantle while interfollicular areas as the zones interposed between neoplastic follicles. The average value of expression of the molecules on the 10 HPF for each case, expressed as a continuous variable, was used for subsequent correlations with clinical and anamnestic data. In double-staining the ratios between the two evaluated molecules were evaluated for each case in a minimum of 10 fields at high magnification (HPF,400x), both considering separately the intra- and interfollicular areas.

3.4 Statistical analysis

Statistical analysis was performed by parametric tests for mean expression values of the different molecules (Student's t-test; Anova test) and nonparametric indicators of linear correlation (Pearson's correlation) between expression values and clinical data, significance was considered with a p-value less than 0.05.

Histopathologically, LF grade, KI 67 growth fraction, and positivity for BCL2 were considered.

All temporal data were expressed in months: Overall Survival (OS) was measured from the date of diagnosis until the date of death from any cause or the last clinical follow-up, Progression Free Survival was considered from the date of confirmation of complete remission by clinical and instrumental evaluation until the date of the last follow up or death from any cause. Disease free survival "DFS", defined as the time elapsed between the date of initiation of first-line therapy until the date of initiation of a second therapy for relapse, and the POD 24 "progression of disease" at 24 months

from diagnosis, which assesses the presence or absence of disease relapse within 24 months from the first treatment, were also evaluated. The latter is now one of the most widely used indexes in the management of the LF patient following first-line therapy.

CHAPTER 4 - RESULTS

4.1 Patient Characteristics

46 cases of patients (24 M and 22 F) with LF were evaluated, with a mean age of 58 years, just 3 of them symptomatic at diagnosis, with disease stage ranging from I to IV, and a mean follow-up of 60 months. Samples include all grades from I to IIIA, 30 FL were BCL2+ and 16 BCL2-. 22 patients out of 46 experienced relapse of the disease, both within 24 months (12/22) and after (10/22), in two patients the disease was always present, while the remaining 22 had complete remission. Most of patients have an intermediate risk FLIPI score (24/46), 18 were at low risk and only 4 at high risk.

Patients Features	
Mean age (years)	58 (34-80)
Sex	
F	22
M	24
Stage	
I	10
II	13
III	7
IV	16
Follow-up (months)	60
FLIPI Score	
High	4
Intermediate	24
Low	18
Relapsed (total) POD24	22 12/22

4.2 PD1 evaluation in intrafollicular areas

Intrafollicular PD1 expression was assessed by considering the mean value of positive elements in 10 HPF for each case.

There were no statistically significant differences in intrafollicular PD1 expression between BCL2+ FL and BCL2- FL.

A positive correlation was observed between age at diagnosis and the number of total PD1+ lymphocytes (Pearson's correlation, p value 0.0398) .

Moreover, the absolute PD1 number was significantly higher in patients in whom POD was found within 24 months (Student-test, p value 0.002). (Figure 2)

No other statistically significant correlations were observed.

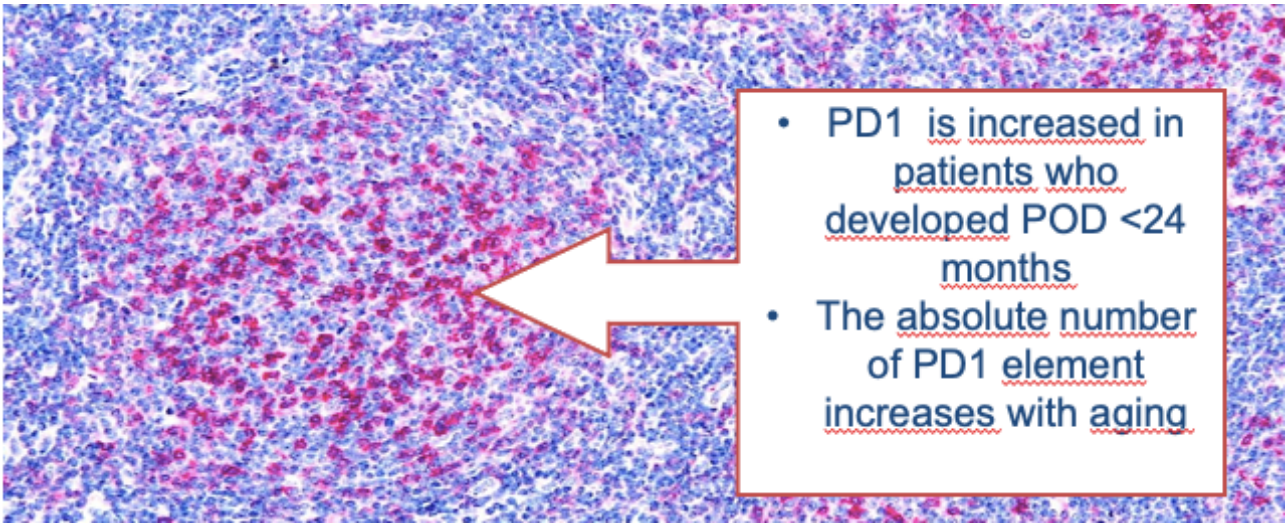

Figure 2 PD1 expression in intrafollicular areas and its significance

4.3 PD1 evaluation in interfollicular areas

Interfollicular PD1 expression was assessed by considering the mean value of positive elements in 10 high power fields for each case.

Interfollicular PD1 expression was assessed by considering the mean value of positive elements in 10 high power fields for each case

There were no statistically significant differences in interfollicular PD1 expression between BCL2+ FL and BCL2- FL.

The value of total interfollicular PD1 expression was significantly different among the four stages of disease (Anova test p value 0.005), being higher in stages I and II than in stages III and IV.

It was also observed that the value of interfollicular PD1 expression was significantly lower in patients who experienced POD24 (Student-test, p value 0.005). (Figure3)

No other statistically significant correlations were observed.

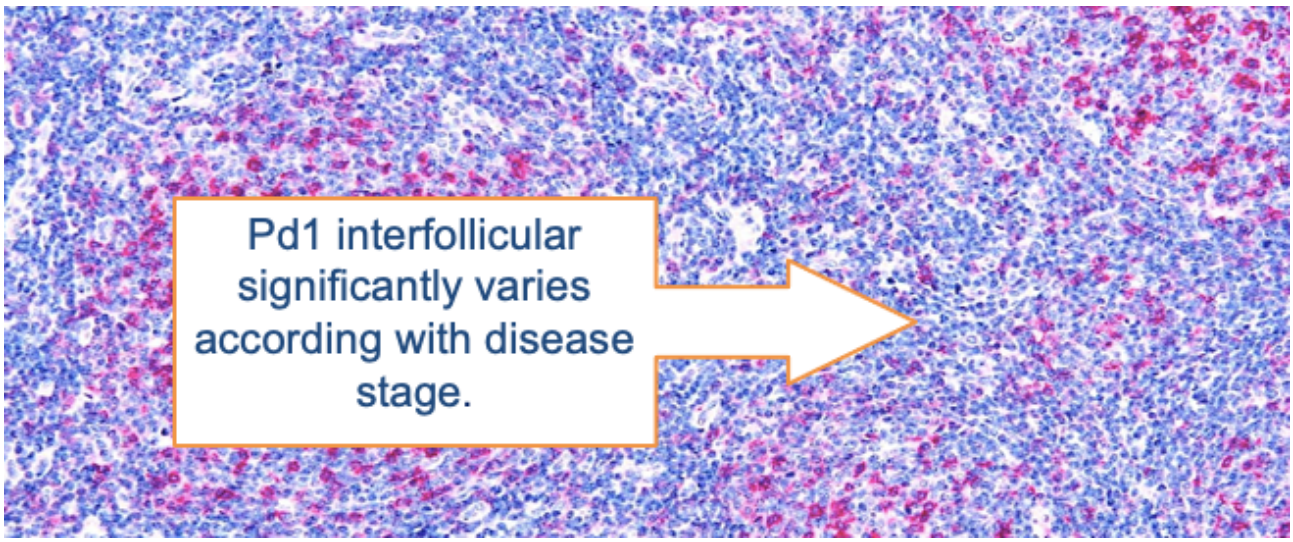

Figure 3 PD1 expression in interfollicular areas and its significance

4.4 CD8 evaluation in intrafollicular areas

Intrafollicular CD8 expression was almost null in all cases.

4.5 CD8 evaluation in interfollicular areas

Interfollicular CD8 expression was assessed by considering the mean value of positive elements in 10 high power fields for each case. In BCL2+ FL, the amount of CD8+ in interfollicular areas

positively correlated with OS (Pearson's correlation, p value 0.04), in all stages and all risk classes. (Figure 4)

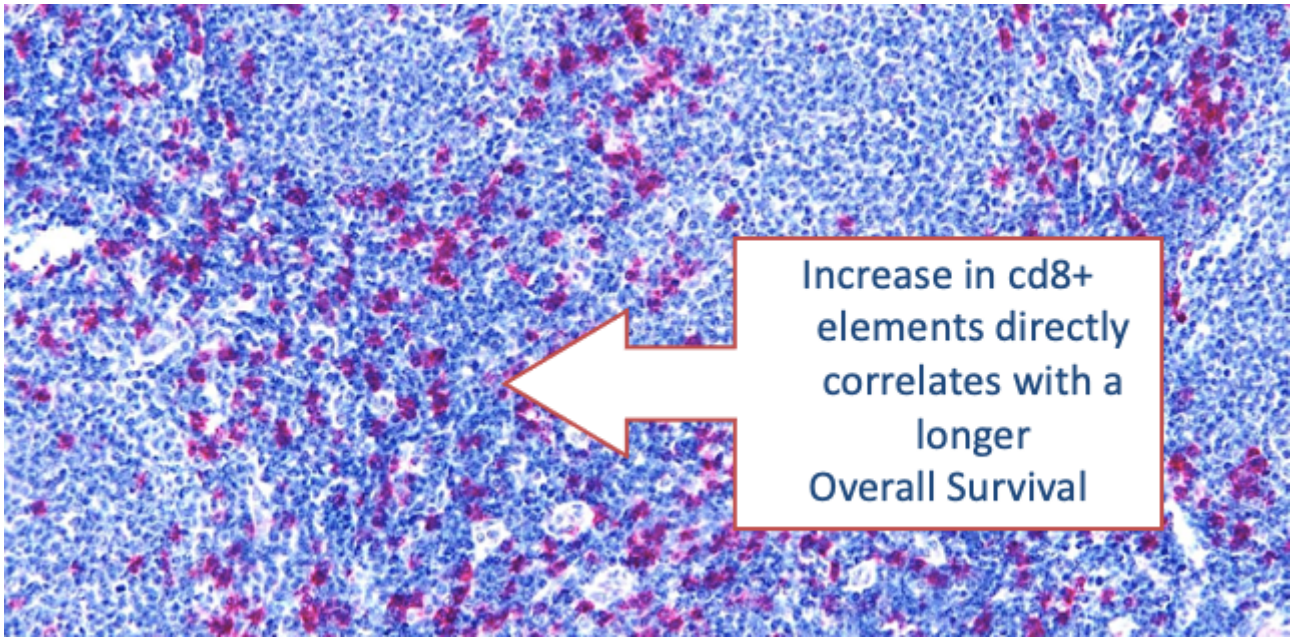

Figure 4 CD8 expression in interfollicular areas and its significance

4.6 CD68PGM1 evaluation in intrafollicular areas

Intrafollicular CD68PGM1 expression was assessed by considering the mean value of positive elements in 10 HPFs for each case.

BCL2- FL showed an higher absolute number of CD68PGM1 positive elements than BCL2+ cases.

We found a positive correlation between intrafollicular CD68PGM1 macrophages and months of disease free survival DFS (p=0.04). (Figure 5).

4.7 CD68PGM1 evaluation in interfollicular areas

Interfollicular CD68PGM1 expression was assessed by considering the mean value of positive elements in 10 high power fields for each case.

No statistically significant correlations were observed considering the marker by itself, an interesting data came from CD163/CD68PGM1 ratio, see below.

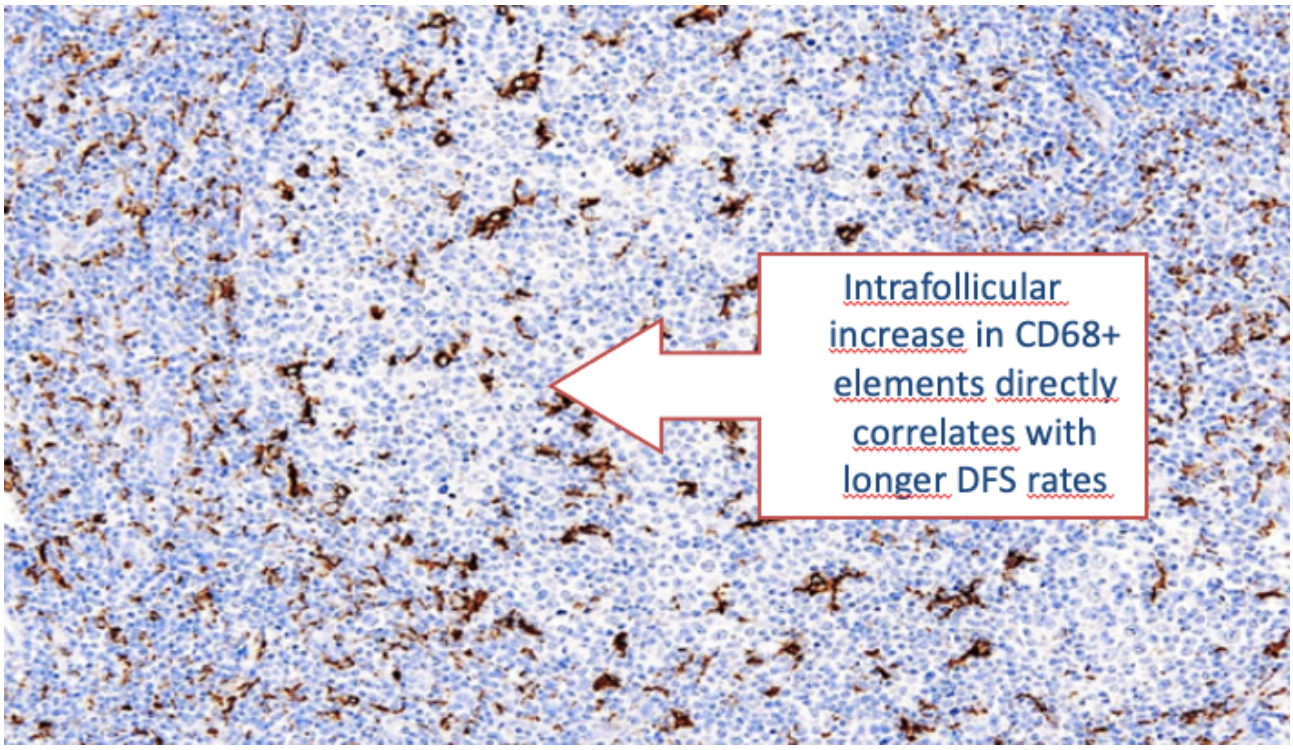

Figure 5 CD68PGM1 expression in intrafollicular areas and its significance

4.8 CD163 evaluation in inter and intrafollicular areas

Interfollicular and intrafollicular CD163 expression were assessed by considering the mean value of positive elements in 10 HPF for each case.

No statistically significant correlations were observed considering the marker by itself, interesting data came from its evaluation related both to CD68PGM1 and CD8.

4.9 Combined evaluations: markers ratios

Patients with a *high inter-follicular* CD163/CD8 ratio were enriched in POD24 events ($p = 0.01$) (Figure 6)

Moreover, patients who experienced POD24 had an higher overall CD163/CD68 ratio ($p = 0.01$).

As expected, we didn't found significant correlations involving intra-follicular CD163/CD8 and PD1/CD8 ratios due to the substantial absence CD8+ elements.

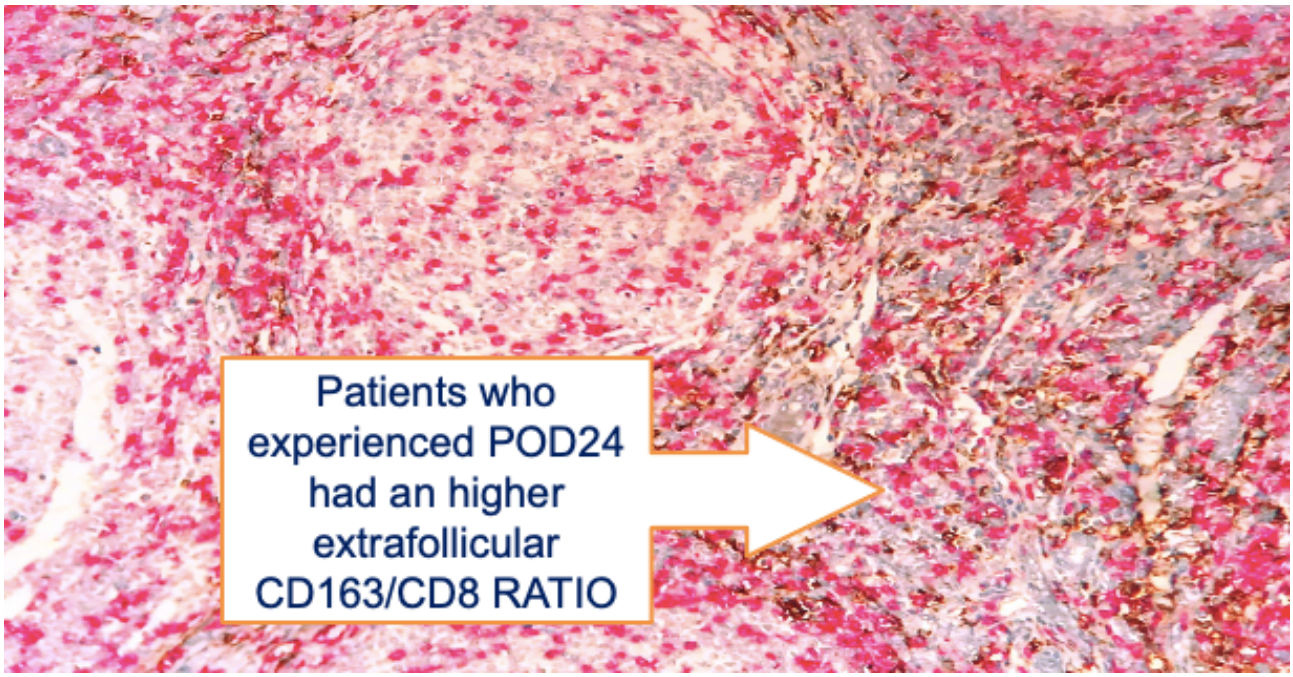

Figure 6 CD163/CD8 expression in interfollicular areas and its significance

CHAPTER 5 - DISCUSSION

The high frequency of lymphoproliferative diseases, the characteristic “wax and wane” clinical course made up of alternating complete remissions and relapses, and the high outcome variability have made FL a “virtually incurable” disease where an adequate stratification of patients is highly necessary but still lacking.

Even though hematologist rely on trelatively robust clinical indexes as FLIPI and FLIPI 2 scores, which can attribute a specific risk rate to affected individuals, there is still extreme heterogeneity in the clinical course within each risk class.

Although another useful indicator in the therapeutic management of patients with follicular lymphoma is the occurrence of early relapses, particulary within 24 months from diagnosis (7), further efforts are needed to identify diagnostic tools useful to define the most appropriate therapeutic strategy for each patient.

The relevance of the microenvironment in the development and progression of many lymphoid and nonlymphoid neoplasms (including FL) is well known, and although through assessments of gene expression profiles it has already become evident how the composition of the microenvironment impacts prognosis (8), such investigations are not available on a large scale for routine practice.

Starting specifically from this type of information about cell populations, that constitute an active part in tumor development, the first studies of immunohistochemical description of the composition of the microenvironment and its correlation with clinical data have begun (12-16).

The aim of our study is to identify a surrogate of the abovementioned molecular investigations, which can be inserted in the routine diagnostic practice and can be of immediate feedback for the clinician and , consequently, of great utility in the setting of the therapeutic strategy for each patient.

Our work focused on the distribution and expression characteristics of "follicular t helper" T, cytotoxic T lymphocytes and macrophages as elements of the ME of FL, to understand whether the immunohistochemical evaluation of the expression of specific markers can be included in the routine diagnostic pathway with potential relevance in defining the prognosis and therapy of FL patients.

PD1 amount and distribution significance

Although many studies with different techniques have been published, the impact of PD1 positive elements on FL prognosis is still widely debated (17).

Our data suggest that the location of PD1 positive elements may be mor significant that the mere quantity of positive cells. In fact, the presence of PD1 positive elements in intrafollicular areas relates with unfavorable prognosis, being higher in patients who develop a progression of disease within 24 months. At the same time, interfollicular PD1+ lymphocyte number inversely correlate with the stage of disease. This finding is in agreement with what previously known about the ability of FL cell to recruit Tfh lymphocytes to enable mechanisms of immune escaping (10): if they are present within the GC in their full activated status they contribute to FL development and progression with an higher risk of early relapse. On the other hand, if the recruitment by the neoplastic cells does not occur, the PD1 positive elements remain accumulated in the interfollicular areas without probably being able to perform their function of support to the pathological cells. This hypothesis could explain why the number of these elements in the interfollicular areas inversely correlates with the stage of FL.

CD8 amount and distribution significance

From the previous paper from the Lunenburg Lymphoma Biomarker Consortium (16) we knew that the presence of an higher number of CD8+ lymphocytes positively correlated with a longer OS only

in the advanced stages (III and IV) of the disease. In our work emerged the same statistically significant positive correlation, for all stages (I to IV) and all risk classes (Low to High FLIPI score) of BCL2+ FL. Interestingly, in the above mentioned work it is not specified if the result related to CD8+ elements had been obtained from a mixed population of both BCL2+ and BCL2- FL or from BCL2+ ones only.

In our evaluation the correlation is present only for the BCl2+ ones. This finding could suggest how a different pathogenesis of the two subtypes, although not having an apparent impact on prognosis, could still affect differently the interactions of neoplastic cells with the microenvironment.

CD68PGM1 amount and distribution significance

As happens for PD1, the impact of CD68PGM1+ macropaheges on prognosis is still widely debated. The main difference concerns the studies before and after the introduction of rituximab (monoclonal antibody against CD20) in the standard treatment of FL.

In most cases it is noted that the presence of macrophages in a therapeutic regimen without monoclonal antibody has an unfavorable impact and that this impact is reversed by treatment with anti-CD20 antibody. However, also in the post rituximab era different studies lead to variable results (18-20). We found a positive correlation between the amount of intrafollicular CD68PGM1+ macrophages and months of disease free survival and no correlation with CD68PGM1+ outside the follicles or the total amount. Our evaluation was made on the samples collected at the diagnosis and all of our patient received a first line treatment that included rituximab. We can assume that the intra-follicular macrophage component is therefore the one that has an impact on the patient's prognosis but it is beyond the scope of this study to define whether the presence of rituximab in the therapeutic regimen counteracts a possible negative effect of the CD68PGM1+ subset within the germinal center or whether it performs a synergistic action with it in the elimination of pathological elements.

Combined evaluations: markers ratios significance

As reported in the results chapter , no statistically significant correlations were observed considering the CD163 marker by itself but interesting data came from its evaluation related both to CD68PGM1 and CD8. From the literature we knew that the presence of CD163+ macrophages (indicating the M2 polarized macrophages) is associated with less favorable clinicopathological features of neoplasms than the presence of CD68+ macrophages (21-22). We found out that patients who experienced POD24 had an higher overall CD163/CD68 ratio, meaning that the higher the number of M2-polarized macrophages the worse the prognosis, intuitively because of their ability to suppress self anti tumoral immune response. Moreover, also the interfollicular CD163/CD8 ratio was positively associated with an increased risk of developing POD24. We can reasonably presume that the effects coming from an higher number of CD163+/M2 polarized macrophages could be counterbalanced by an high number of CD8+ active cytotoxic T cells. This result being in line with the previous discussed one about the protective effects coming from an higher number of CD8+ elements in interfollicular areas.

CHAPTER 6 – CONCLUSION AND PERSPECTIVES

The aim of our work was to evaluate some cellular subpopulations that constitute the microenvironment of follicular lymphoma and to assess their prognostic impact on the disease.

Specifically, our evaluation was carried out by means of immunohistochemical stainings in order to build a prognostic assessment tool based on simple and cost effective investigations that can be performed in a reproducible manner directly on the diagnostic sample taken from the patient.

We obtained interesting data for all the cell subsets we evaluated, consequently further steps are needed. We aim to enlarge our evaluation cohort and create separate training and test sets of cases to validate our finding with the final purpose of building a practical and reproducible immunohistochemical score which can directly provide the clinicians precious prognostic information alongside the diagnosis of the disease.

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