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DOCTORAL THESIS

B-cell lymphocytic lymphoma — clinicobiological, immunohistochemical, and biomolecular correlations

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Introduction

B-cell small lymphocytic lymphoma (B-SLL) is the tissue (predominantly nodal) form of B-cell chronic lymphocytic leukemia (B-CLL), a neoplasm of mature but immunologically inert B lymphocytes. The two entities are indolent malignancies and are regarded as the same disease with different manifestations, according to the latest World Health Organization (WHO) classification of hematopoietic and lymphoid tumors and the International Workshop on CLL (iwCLL) group [1,2]. The primary sites of involvement are the peripheral blood, spleen, lymph nodes, and bone marrow. The malignant B lymphocytes in B-CLL and B-SLL have identical pathologic and immunophenotypic features. The term B-CLL is used when the disease presents as primary involvement of the peripheral blood (accumulation of monoclonal B lymphocytes >5 × 10^9/L), whereas B-SLL is used when the lymphoproliferative process is confined to lymphoid tissue, mainly lymph nodes.

CURRENT STATE OF KNOWLEDGE

1. Etiology and epidemiology of B-cell small lymphocytic lymphoma/chronic lymphocytic leukemia

1.1. Etiology of SLL/CLL

The exact etiology of SLL/CLL is unknown. Genetic factors appear to play a greater role in the development of this condition than environmental factors. A limited number of risk factors relate to occupational exposures such as chemicals, radiation, and tobacco. An increased risk of developing SLL/CLL has been reported among rubber industry workers and individuals exposed to benzene. However, these associations have not been conclusively demonstrated.

1.2. Global and national incidence and prevalence

CLL/SLL is the most common chronic lymphoproliferative disorder of adults in Western countries, accounting for approximately 25–35% of leukemia cases in the United States (US) [3]. In Europe, the incidence is 4.2 per 100,000 per year and rises to >30 per 100,000 per year in individuals aged >80 years [4]. In approximately 10% of cases, the disease presents as primary nodal involvement similar to non-Hodgkin lymphoma; this presentation accounts for 5% of all non-Hodgkin lymphoma cases.

The disease is diagnosed in people aged 50–70 years, with a median age at diagnosis of 70 years. Although incidence increases with age, it can also be diagnosed in young adults (30–39 years) [5,6].

2. Pathophysiology of SLL/CLL

SLL/CLL arises from mature CD5-positive B lymphocytes but exhibits intrinsic heterogeneity reflecting differences in ontogeny, epigenetic programming, and antigen exposure. The B lymphocyte is functionally incompetent, arrested along the differentiation pathway. Based on gene-expression and genomic methylation analyses, three epigenetic subgroups have been delineated—naive-like (n-CLL), intermediate (i-CLL), and memory-like (m-CLL)—which correspond to the somatic hypermutation status of the immunoglobulin

heavy-chain variable region (IGHV)—unmutated IGHV (U-IGHV) and mutated IGHV (M-IGHV)—and to clinical evolution. These subgroups support the existence of distinct transformation trajectories (naive vs memory), which may explain why some clones are predominantly anergic whereas others are proliferative and chemotactic [7–9].

3. Diagnosis of B-cell small lymphocytic lymphoma/chronic lymphocytic leukemia

CLL/SLL typically has an insidious onset. Most patients are asymptomatic in the early stages. The diagnosis is often incidental, following a routine complete blood count (CBC) showing an increased absolute lymphocyte count, or after the discovery of painless lymphadenopathy.

Ancillary investigations are performed both to establish the diagnosis and to assess prognosis, since patient management depends on disease stage, the presence of adverse factors, and the criteria for active disease.

In CLL, clinical suspicion arises when the CBC shows leukocytosis with absolute lymphocytosis. The absolute lymphocyte count should be >5,000/μL (5 × 10^9/L). On the peripheral blood smear, lymphocytes are small and apparently mature, with scant basophilic cytoplasm, a dense nucleus with condensed chromatin, and no visible nucleoli; characteristic Gumprecht “smudge” cells (fragile malignant lymphocytes) may be seen.

In patients with SLL, the lymphocyte count may be normal or only slightly elevated; by definition, SLL patients have an absolute lymphocyte count <5,000/μL (5 × 10^9/L) at diagnosis [1]. Diagnosis requires histopathologic confirmation from a lymph node biopsy.

Staging and risk stratification for CLL use the Rai and Binet systems, respectively, whereas patients with SLL are staged using the Lugano-modified Ann Arbor system.

4. Prognostic and predictive markers in B-cell small lymphocytic lymphoma/chronic lymphocytic leukemia

Prognostic and predictive markers are measurable variables associated with disease course and treatment response. Some markers may serve both roles. Prognostic markers are associated with clinical outcomes (e.g., progression-free survival, overall survival) regardless of treatment type. They indicate disease activity, extent, and growth rate. The main markers used in clinical practice are:

- clinical staging
- TP53 mutations and del(17p)
- mutational status of the immunoglobulin heavy-chain variable region (IGHV)
- lymphocyte doubling time
- Beta-2 microglobulin
- del(11q), del(13q), trisomy 12

The clinical utility of other prognostic markers (CD38, ZAP-70, NOTCH1, BIRC3, SF3B1 mutations) remains unclear.

Predictive markers provide information about response to specific therapies and thus guide treatment selection.

5. Treatment and response to treatment in B-cell small lymphocytic lymphoma/chronic lymphocytic leukemia

A hallmark of CLL/SLL is that not all patients require treatment at diagnosis. Asymptomatic patients diagnosed at limited disease stages—Rai 0–II, Binet A/B, or Ann Arbor I/II—are eligible for a “watch-and-wait” approach, given the generally indolent course and the lack of survival advantage with early therapy in clinical trials, whether using chemotherapy or targeted agents [2].

Treatment is indicated for patients with active disease, according to the International Workshop on CLL (iwCLL) guidelines [2]. Active disease is defined by: progression of marrow failure evidenced by worsening anemia or thrombocytopenia; progression of lymphadenopathy or splenomegaly; progressive lymphocytosis with an increase >50% over two months or a lymphocyte-doubling time <6 months; autoimmune complications inadequately responsive to

corticosteroids; functionally significant or symptomatic extranodal involvement; and the presence of disease-related B symptoms (≥10% involuntary weight loss in the last 6 months, ECOG ≥2, non-infectious fever, night sweats in the absence of infection). Hemoglobin <11 g/dL or platelets <100 × 10^9/L usually indicate treatment initiation. The absolute lymphocyte count alone is not an indication for treatment in the absence of symptoms and marrow involvement [2]; however, a leukocyte count >200,000/μL may warrant therapy due to the increased risk of hyperviscosity syndrome (e.g., transient ischemic attacks).

For patients eligible to start therapy, several factors influence regimen selection: age, performance status and comorbidities, presence of TP53 mutations or del(17p), IGHV mutation status, patient preference, and therapeutic goals.

For patients with limited, asymptomatic disease, the standard is observation with clinical and laboratory follow-up. This strategy is supported by randomized clinical trials and meta-analyses showing no clinical benefit mandating treatment, as well as a double-blind, placebo-controlled randomized trial of ibrutinib versus observation that showed increased toxicities and similar overall survival with ibrutinib [10–13].

In patients with localized SLL confined to a single nodal area and who are asymptomatic, surveillance is likewise recommended rather than systemic therapy or radiotherapy (RT). Evidence for RT is based on small retrospective series [14].

All patients eligible for treatment should be encouraged to enroll in clinical trials. When this is not feasible, therapy is chosen according to national and international guidelines (NCCN, ESMO) [15,16]. Given the availability of multiple agents with distinct mechanisms and toxicity profiles, the choice is influenced both by disease-related factors and by patient profile (comorbidities, concomitant medications, patient preference, access, and anticipated adherence). From a genetic-risk perspective, evaluations required before any line of therapy include TP53 status and del(17p), and IGHV mutation status if not already known [17,18].

First-line treatment strategies include: continuous BTK inhibitor (BTKi) therapy—ibrutinib, acalabrutinib, or zanubrutinib—until progression; fixed-duration BCL-2 inhibitor therapy—venetoclax combined with the anti-CD20 monoclonal antibody obinutuzumab for 12 cycles; lead-in ibrutinib (3 cycles) followed by the ibrutinib–venetoclax combination for a total of 12 cycles [16].

Chemoimmunotherapy (CIT) of limited duration—such as fludarabine–cyclophosphamide–rituximab (FCR)—may be considered only in patients with a favorable genetic profile defined by mutated IGHV, no TP53 abnormalities, and additionally a non-complex karyotype (fewer than five chromosomal aberrations), and when targeted therapies

are not reimbursed [16,19]. Progression-free survival (PFS) is generally inferior with CIT compared with targeted therapy, though this has not translated into an overall survival (OS) disadvantage in most studies [20,21].

Although chemotherapy remains an option for fit, younger patients with favorable genetics, targeted therapies should be the preferred first choice, given superior PFS and OS results. When choosing between fixed-duration therapy—ibrutinib–venetoclax or venetoclax–obinutuzumab—and continuous BTKi therapy, fixed-duration regimens are preferred due to lower toxicity and the possibility of retreatment at relapse, limiting clonal selection and reducing adverse events, particularly in patients with favorable genetic risk, where an additional PFS benefit has been observed [22–24].

Patients with TP53 abnormalities (mutation/deletion) should initiate BTKi therapy, as reported PFS data suggest longer disease control with continuous regimens.

In relapsed/refractory disease, chemotherapy should not be administered because it is associated with low PFS and OS rates [25,26]. After relapse following CIT or late relapse after fixed-duration venetoclax-based regimens, two therapeutic options are available: fixed-duration venetoclax–rituximab or continuous BTKi.

Despite CLL and SLL being considered biologically similar and managed similarly in guidelines, there are limited real-world data specific to SLL because it accounts for only 10–15% of CLL/SLL cases. Moreover, these patients were excluded from most CLL trials but, conversely, were included in trials for indolent non-Hodgkin lymphomas (e.g., follicular lymphoma), leading to uncertainties regarding optimal SLL management.

PERSONAL CONTRIBUTION

1. Research methodology

1.1 Aim and objectives of the research

We chose this research topic because, although B-cell small lymphocytic lymphoma (B-SLL) is considered the same disease as chronic lymphocytic leukemia (B-CLL), there are few data and studies focused on the characteristics and management of "pure" lymph node SLL.

Moreover, although national and international guidelines recommend similar treatment irrespective of presentation as B-SLL or B-CLL [150–153], real-world studies indicate that most B-SLL patients receive treatments and are managed according to protocols for indolent non-Hodgkin lymphomas (iNHL) [154–156]. This stems in part from the exclusion of B-SLL patients from CLL clinical trials, while they were, conversely, enrolled in iNHL trials. Thus, between 1999 and 2020, of 56 clinical trials cited in the NCCN treatment guidelines, B-SLL patients were included in only 38% of CLL-targeted studies but were enrolled in 16 non-CLL trials for various iNHL subtypes [157]. Excluding B-SLL from B-CLL trials leads to underassessment and potentially suboptimal management, as outcomes from leukemic presentations are extrapolated to nodal presentations, where disease course, treatment response, and risk profile may differ. It is also well known that B-CLL patients with deletion of the short arm of chromosome 17 (del(17p)) have a poor prognosis, significantly shorter progression-free and overall survival, and resistance to chemotherapy [158]; however, this evaluation is not performed routinely in B-SLL.

In this thesis, we will assess the presence of del(17p), the immunohistochemical expression of p53, and the relationships between these markers and clinical/biological prognostic parameters; we will evaluate their impact on therapeutic decision-making and treatment response, and the feasibility of enrolling patients in clinical trials to improve survival and quality of life.

Research aim

To validate, through clinico-morphologic analysis complemented by immunohistochemical and molecular studies, diagnostic and prognostic markers in nodal B-cell small lymphocytic lymphoma, with the goal of improving risk stratification and personalizing clinical management.

Research objectives

1. Primary objective

Describe and correlate the clinico-biological and morphological characteristics of SLL patients with their IHC and molecular profiles (p53 IHC, del(17p)), and relate these to clinical outcomes.

2. Secondary objectives

2.1 Evaluate the role of p53 IHC (pattern and intensity of expression) and integrate it into a practical prognostic algorithm.

2.2 Determine the prognostic and therapeutic implications of the IHC/molecular profile (p53 IHC and del(17p)), separately and in combination, on time-to-first-treatment (TTFT) and progression-free survival (PFS).

2.3 Identify and validate independent factors associated with TTFT and PFS and formulate recommendations for personalized management, based on these analyses.

1.2 Study design and patient selection

This was a prospective observational study of 46 patients diagnosed with B-cell small lymphocytic lymphoma (B-SLL) in the Department of Pathology of the Constanța County Clinical Hospital and treated in the Hematology Unit (Internal Medicine II) and Internal Medicine I. The study period was January 1, 2017–December 31, 2022. Patient enrollment ended on December 31, 2022, and follow-up continued until December 31, 2024.

Fluorescence in situ hybridization (FISH) and immunohistochemical analyses were performed at the Research and Development Center for Morphological and Genetic Studies in Malignant Pathology (CEDMOG), Ovidius University of Constanța.

The study was conducted with patients' written informed consent and with approval from the Ethics Committee.

Inclusion criteria

- Age >18 years
- Patients diagnosed with B-SLL based on histopathology and immunohistochemistry and treated within the Hematology Unit
- Adequate biological material: FFPE blocks
- Minimum complete clinical file: demographics, staging, laboratory tests, treatments, follow-up
- Absolute count of clonal lymphocytes in peripheral blood <5,000/μL, or <10,000/μL in the absence of demonstrated clonality

Exclusion criteria

- Age <18 years
- Patients diagnosed with other types of B-cell lymphomas
- Refusal to sign informed consent

1.3 Data collection

Data were collected prospectively from the first visit onward throughout each patient's clinical course, with creation of a study database.

The clinical examination included assessment of the patient's general condition in order to calculate the Eastern Cooperative Oncology Group (ECOG) performance status; evaluation of palpable peripheral lymphadenopathy (location and size); assessment for hepatomegaly and splenomegaly; and documentation of disease-related B symptoms.

Laboratory and biochemical investigations included:

- Complete blood count parameters (hemoglobin, leukocytes with differential, platelets)
- Liver function (transaminases)
- Viral hepatitis markers (HBV, HCV) and HIV serology
- Renal function (creatinine, uric acid, urea)
- Serum LDH
- Inflammatory markers — ESR
- Serum beta-2 microglobulin
- Coombs test, bilirubin, reticulocytes
- Serum iron, ferritin, vitamin B12, folic acid

Because patients with SLL present with nodal disease, imaging consisted of contrast-enhanced CT of the neck, chest, abdomen, and pelvis to identify all nodal and extranodal sites and to quantify lymphadenopathy. Given the low avidity of SLL, PET/CT was not performed.

Each patient was staged according to the Ann Arbor/Lugano classification.

Therapeutic decisions included: watch-and-wait; chemotherapy; monoclonal antibodies as monotherapy or combined with chemotherapy; Bruton tyrosine kinase inhibitors (BTKi); radiotherapy; and surgery.

Patients were monitored with periodic visits per protocol, and treatment response was assessed as complete response, partial response, stable disease, or progressive disease. Follow-up was carried out from the time of diagnosis until the end of the study period, according to protocol.

1.4 Histopathological and immunohistochemical examination

p53 expression was evaluated on FFPE tissues because this is a rapid testing method that can help identify high-risk patients (TP53 abnormalities). We used the p53 antibody, clone DO-07, isotype IgG2b, kappa, with nuclear positivity (p53 **Zeta** antibody, which reacts with both mutant and wild-type p53). We applied a pattern-based approach: **wild-type** = variable nuclear staining in 1–50% of tumor cells; **overexpression** = strong, diffuse nuclear staining in >50% of tumor cells; **null** = complete absence of signal in tumor cells with a positive internal control. Classification followed recommendations used in routine diagnostic practice for mature B-cell lymphomas; “overexpression” and “null” were considered abnormal (“mutant-like”) patterns.

1.5 Determination of TP53 gene status by FISH

Deletion of 17p13.1 was assessed by fluorescence in situ hybridization (FISH) using a locus-specific probe for TP53 deletion (Figure 3) (CytoCell for P53 gene deletions — TP53 — LPS 037, Oxford Gene Technology IP Limited, UK) on paraffin-embedded tissue sections. Samples were analyzed with a fluorescence microscope.

1.6 Statistical analysis and data interpretation

Experimental data were processed on a personal computer using Microsoft Excel and the MedCalc Software statistical package (personal license). The procedures applied were: descriptive statistics (to characterize categorical variables and discrete/continuous numerical variables defined in the database); charts/graphs; parametric statistical tests (Student's t-test for comparing the means of two independent samples); nonparametric statistical tests (the χ^2 test of association—link—between two categorical variables, with calculation, in certain situations, of the odds ratio, OR, and relative risk, RR). For continuous variables, the Shapiro–Wilk test was used to verify the normality assumption (given the small sample sizes, $n < 50$). The significance level was set at $\alpha = 0.05$ for all tests.

1.4 Results obtained

At diagnosis, the mean age was 65 years (range: 36–77 years). The study cohort included 16 women (34.78%) and 30 men (65.2%). A lower frequency of B-SLL diagnosis was noted in females compared with males.

Regarding performance status (ECOG), the cohort comprised 21 patients (63%) with ECOG 0–1 and 17 patients (37%) with ECOG ≥2.

By Ann Arbor stage at diagnosis, there were 2 patients in stage I (4.3%), 16 in stage II (34.8%), 23 in stage III (50%), and 5 in stage IV (10.9%). Overall, 18 patients (39.10%) were diagnosed with limited disease (stages I/II) and 28 (60.87%) with advanced disease (stages III/IV), indicating that most patients presented with advanced-stage disease.

At diagnosis, 12 patients (26.1%) had B symptoms (involuntary weight loss in the last 6 months, profuse night sweats, or non-infectious fever), while 34 (73.9%) did not. The presence of B symptoms at diagnosis correlated with disease stage: among advanced stages, 8 patients in stage III and 4 patients in stage IV had B symptoms, whereas no patient with limited-stage disease had B symptoms ($\chi^2 = 14.79$, $df = 3$, $p = 0.002$).

At diagnosis, 20 patients (43.47%) had a spleen of normal size, while 26 (56.52%) had splenomegaly of varying degrees. Splenomegaly was present in 25 patients (89.28%) within the advanced-disease subgroup, compared with only one patient in the limited-disease subgroup, supporting an association between splenomegaly and disease stage ($\chi^2 = 30.5$, $df = 1$, $p < 0.0001$; statistically significant).

Hepatomegaly at diagnosis was observed in 22 patients (47.82%), while 24 (52.17%) had no hepatomegaly.

According to the International Prognostic Index (IPI), the cohort included 9 patients (19.6%) at low risk (IPI 0–1), 12 (26.1%) at low-intermediate risk (IPI 2), 20 (43.5%) at high-intermediate risk (IPI 3), and 5 (10.9%) at high risk (IPI 4).

Most patients with limited-stage disease at diagnosis had an ECOG performance status of 0 or 1 (38.89% and 55.56%, respectively), and only a small number had ECOG ≥2 (5.56%). In contrast, in advanced stages the majority had ECOG ≥2 (57.14%); ECOG 0 and ECOG 1 were seen in 10.71% and 32.14% of patients, respectively. There was a statistically significant association between ECOG and disease stage ($p < 0.0013$; Fisher's exact $p = 0.0004$).

In the study cohort, 16 patients (34.78%) had lymphadenopathy <5 cm at diagnosis and 30 patients (65.21%) had lymphadenopathy >5 cm. In limited-stage disease, 77.78% (14) had lymphadenopathy <5 cm and only 22.22% (4) had lymphadenopathy >5 cm; in stages III/IV, most patients had lymphadenopathy >5 cm (26 patients, 92.86%). This shows a statistically significant difference ($\chi^2 = 23.57$, $df = 1$, $p < 0.0001$; Fisher's exact $p = 0.000001$).

With respect to comorbidities, 9 patients (19.6%) had a score of 0–1, 22 patients (47.8%) had a score of 2–3, and 15 patients (32.6%) had a score ≥4. In limited stages, most patients had a comorbidity score ≤3, whereas patients with advanced stages more often had a score ≥4.

In the study cohort, 28 patients (60.86%) had anemia at diagnosis, of whom 21 (75%) had hemoglobin <10 g/dL. The minimum recorded hemoglobin was 5 g/dL and the maximum 16.2 g/dL. The mean value was 10.74 g/dL with a standard deviation of 2.54 (Shapiro–Wilk $W = 0.98$).

A higher proportion of patients in advanced stages III/IV had hemoglobin <10 g/dL (60.7%) compared with stages I/II, where 83.3% had hemoglobin >10 g/dL. Thus, hemoglobin is a negative prognostic factor for diagnosis and disease course, with statistical significance $p = 0.0001 < \alpha = 0.05$, $r = 0.64$, 95% CI −0.78 to 0.42. There is a statistically significant and fairly strong negative association between advanced disease stage and the presence of anemia. Among patients presenting with anemia at diagnosis (60.86%), 15 had disease-related anemia—8 (28.6%) due to autoimmune hemolytic anemia (AIHA), 7 (25%) secondary to hypersplenism—and 13 (46.4%) had anemia from other causes.

Regarding distribution by disease stage and β2-microglobulin at diagnosis, the plotted data show that patients in stages I/II (94.44%) had β2-microglobulin <3.5 mg/L, whereas those in stages III/IV had values >3.5 mg/L (78.57%). Thus, β2-microglobulin is a negative

prognostic factor, with statistical significance ($\chi^2 = 22.85$, df = 1, $p < 0.0001$). β2-microglobulin significantly discriminated between Ann Arbor I/II and III/IV patients, with an area under the curve (AUC) of 0.876 (95% CI, 0.78–0.95; $p < 0.001$). The optimal threshold by Youden's criterion was 3.5 mg/L, corresponding to 85% sensitivity and 80% specificity.

In this cohort, most patients had elevated LDH at diagnosis (58.7%). By disease stage, most early-stage patients had normal LDH (77.78%), whereas most advanced-stage patients had elevated LDH (82.14%) ($\chi^2 = 15.87$, df = 1, $p < 0.0001$; Fisher's exact $p < 0.0001$). Among patients with lymphadenopathy <5 cm at diagnosis, 12 (75%) had normal serum LDH and 4 (25%) had elevated values. Among those with lymphadenopathy >5 cm, 7 (23.33%) had normal LDH and 23 (76.66%) had elevated LDH ($\chi^2 = 11.24$, df = 1, $p = 0.0008$; Fisher's exact $p = 0.001$).

By lymph node size at diagnosis, most patients with lymphadenopathy <5 cm had normal serum β2-microglobulin—13 patients (81.25%)—and only 3 (18.75%) had elevated values. Among patients with lymphadenopathy >5 cm, 20 (66.66%) had elevated and 10 (33.33%) had normal β2-microglobulin ($\chi^2 = 9.3$, df = 1, $p = 0.002$; Fisher's exact $p = 0.004$). β2-microglobulin significantly discriminated patients with lymphadenopathy >5 cm from those with <5 cm, with an AUC of 0.807 (95% CI 0.664–0.909; $p < 0.001$). The optimal threshold by Youden's criterion was >3.2 mg/L, corresponding to 80% sensitivity and 81.25% specificity.

Regarding the initial therapeutic approach, 12 patients (26.1%) did not require treatment ("watch and wait"), while 34 (73.91%) initiated therapy: 20 (43.5%) received monoclonal antibody (mAb)–based chemoimmunotherapy (CIT), 9 (19.6%) received chemotherapy, and 5 (10.9%) received Bruton tyrosine kinase inhibitors (BTKi). Of the 34 who started treatment, 15 (44.1%) achieved a complete response (CR) and 19 (55.9%) were non-responders. Response varied by treatment type: in the chemotherapy group, 2 achieved CR vs 7 without CR; among those receiving mAb + chemotherapy, 8 achieved CR vs 12 without CR; and all 5 patients treated with targeted therapy (BTKi) achieved CR. There was a significant association between treatment type and achieving CR ($\chi^2 = 8.22$, df = 2, $p = 0.01$).

The clinical cases included (n = 46) were exclusively nodal SLL confirmed on lymph node biopsy, defined by diffuse effacement of architecture with mandatory proliferation centers (pseudofollicles) on hematoxylin–eosin. No biopsy was classified as nodal monoclonal B-cell lymphocytosis (CLL/SLL-like proliferation/in situ), which is defined by limited tissue infiltrates with preserved architecture and without proliferation centers. p53 IHC staining was assessed on representative fields, avoiding necrosis and artifacts. p53 overexpression was defined as strong nuclear signal (2–3+) in ≥40% of tumor cells, not restricted to proliferation

centers. The null pattern was defined as absence of staining in tumor cells in the presence of an adequate internal control. Cases with mosaic, weak/moderate positivity below this threshold were considered p53 wild-type and were not classified as overexpression.

According to p53 IHC expression pattern, 26 patients (56.5%) showed wild-type (normal) expression, 11 (23.9%) showed a null pattern, and 9 (19.6%) showed overexpression. Treatment response differed by p53 IHC pattern: among patients with aberrant expression (null/overexpression), 3 achieved CR and 17 did not; among those with wild-type expression, 12 achieved CR and 2 did not ($\chi^2 = 16.21$, df = 1, p = 0.0001).

In the study cohort, testing for del(17p) showed its presence in 14 patients (30.43%); 32 (69.56%) had no del(17p). Among patients who initiated treatment (73.91%), response differed by del(17p) status: in the del(17p)+ group, only 3 (20%) achieved CR vs 12 (80%) without CR; among del(17p)− patients, 12 (63.15%) achieved CR and 7 (36.84%) did not ($\chi^2 = 6.147$, df = 1, p = 0.013). All patients with del(17p) had an aberrant p53 IHC pattern. Among patients without del(17p) by FISH, 26 had wild-type p53 expression and 5 had aberrant expression ($\chi^2 = 28.306$, df = 1, p < 0.0001).

In multivariable Cox analysis (N = 46; 34 events), both aberrant p53 IHC and del(17p) were independently associated with a shorter time-to-first-treatment (TTFT) ($\Delta -2LL = 34.09$; df = 2; p < 0.0001). After mutual adjustment, aberrant p53 IHC had HR 3.23 (95% CI 1.10–9.49; p = 0.033) and del(17p) had HR 5.75 (95% CI 1.70–19.40; p = 0.0049). Model C-index = 0.742 (95% CI 0.695–0.788).

Both variables are independent predictors of earlier treatment initiation. del(17p) remains a very strong adverse signal, while aberrant p53 IHC adds incremental risk—likely capturing cases with TP53 mutations without deletion. These findings are consistent with the univariate analyses, which showed very short medians in del(17p)+ and/or p53-aberrant subgroups.

1.5 Conclusions

In B-cell small lymphocytic lymphoma, clinical presentation and course depend on tumor burden. Large lymph nodes (≥5 cm) and splenomegaly clustered in advanced disease and were associated with an earlier need for treatment and a shorter PFS. Ann Arbor/Lugano staging remains relevant: advanced stages had a clearly inferior course compared with limited stages, supporting systematic use of staging and comprehensive imaging at diagnosis and again when monitoring treatment response.

B2-microglobulin is a serum marker with clinical value for stratifying lymphoma patients, efficiently discriminating between early and advanced stages.

Although elevated serum LDH is not, by itself, an indication to initiate therapy, it signals biologically active disease and may raise suspicion for transformation or a high tumor burden.

TP53 abnormalities drive adverse prognosis: both del(17p) and aberrant p53 IHC (null/overexpression) identified patients with much shorter TTFT and PFS. In practice, any TP53 signal should be treated as high risk. All patients with del(17p) had aberrant p53 IHC, and a subset without deletion also showed an aberrant pattern—suggesting TP53 mutations without deletion. Where access to NGS is limited, combining 17p FISH with p53 IHC is a useful triage tool to avoid chemotherapy in high-risk patients.

The higher use of chemotherapy alone or combined with monoclonal antibodies reflects that SLL is often managed as indolent NHL, even though current standards favor chemotherapy-free regimens for most patients.

1.6 Originality of the thesis

This thesis stands out in that it:

- Presents the first prospective study in Romania dedicated to SLL, integrating systematic clinical, paraclinical, and imaging assessment (complete staging per current practice) within a consistent cohort.
- Provides a distinctive translational component: in the context of limited access to NGS, it implements and standardizes pattern-based p53 IHC as a surrogate marker of TP53 alterations (mutations), evaluated in parallel with FISH for del(17p) on FFPE tissue. This combination enables early identification of high-risk patients, prevents inappropriate initiation of chemotherapy in cases suspected of TP53 abnormalities, and guides the selection of targeted therapies.
- Proposes a practical diagnostic workflow—p53 IHC plus TP53 FISH— that is easy to implement in resource-limited centers, and standardizes the initial evaluation by consistently including clinical parameters and tumor-burden metrics.
- Through this integrated, practice-ready approach, the thesis makes an original contribution to SLL patient care in Romania and offers a replicable model at both institutional and network levels.

List of Publications

1. Andreea-Georgiana STOICA, Miruna CRISTIAN, Mariana ASCHIE et al, 17p13.1 Deletion in Small B-cell Lymphocytic Lymphomas: A Prognostic Factor and Diagnostic Approach, Documenta Haematologica - Revista Romana de Hematologie | 2025, Vol. 3, Nr. 1, https://doi.org/10.59854/dhrrh.2025.3.1.11

2. Stoica AG, Tica I, Ciocodei SL, Mitroi AF, et al. Primary Pulmonary Small B-Cell Non-Hodgkin Lymphoma – Case Presentation. ARS Medica Tomitana. 2020;26(2):80–84. doi:10.2478/arsm-2020-0016, https://doi.org/10.2478/arsm-2020-0016

3. MARIANA ASCHIE, ANDREEA GEORGIANA STOICA et al, Synchronous Association of Two Types of Indolent Lymphomas, REV.CHIM.(Bucharest) 69 No. 12 2018, 3653-3655, https://doi.org/10.37358/RC.18.12.6812

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3. Rebecca L Siegel , Tyler B Kratzer et al, Cancer statistics, 2025, CA Cancer J Clin 2025 Jan-Feb;75(1):10-45. doi: 10.3322/caac.21871.

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