

8-14-2009

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Atypical Morphology and Genetic Aberrations in Small Lymphocytic Lymphoma

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Title: Atypical Morphology and Genetic Aberrations in Small Lymphocytic Lymphoma (SLL)

Background: Common recurring genetic abnormalities with prognostic relevance are detected by fluorescence in situ hybridization (FISH) in 80% of SLL cases. Given the heterogeneity in outcome, we evaluated SLL lymph nodes for morphologic clues that predict genetic profiles. Thus, the pathologist could devise a directed approach to performing these ancillary studies.

Design: We identified 41 cases of SLL. H&E sections were evaluated for four morphologic features: expanded proliferation centers (EPC) comprising >35% of surface area, >10 large cells per 40X hpf outside of proliferation centers (LC), marked nuclear contour irregularities in tumor small cells (NCIS), and nuclear contour irregularities in large cells (NCIL). FISH for del13q14, trisomy12, del11q22, and del17p were performed on paraffin sections and interpreted blindly. Statistical analysis was performed using exact normal scores test and the Jonckheere-Terpstra procedure.

Result: FISH was determined in 100% of cases. 27/41 cases (66%) had FISH abnormalities with the following frequencies: del 13q14 (56%), del 11q (26%), trisomy 12 (22%), and del 17p (4%). There was an association between NCI in small cells and the presence of a worse FISH abnormality ($p = 0.0006$), as well as an association between NCI in large cells and a worse FISH abnormality ($p=0.001$). EPC ($p = 0.45$) and LC ($p = 0.44$) weren't associated with FISH.

Conclusion: NCIs in SLL may signal underlying adverse FISH abnormalities (del 11q) and worse prognosis. Given that SLL morphology does not substantially predict FISH, performance of these specialized tests should be directed by clinical parameters.

Introduction:

Chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) is a neoplasm of clonal mature B cells involving peripheral blood, tissue, and/or bone marrow.¹ The diagnosis is suggested by morphology and confirmed with immunophenotypic studies. The diagnosis is initially suspected when the complete blood cell count demonstrates an absolute lymphocytosis, and when increased numbers of mature lymphoid cells are seen on the peripheral blood smear. Immunophenotyping most often reveals a characteristic CD19+, CD20 (dim +), sIg restricted (dim +), CD5+, CD23+ and FMC-7 (-) profile. Patients are most commonly asymptomatic, with CLL/SLL being discovered as a lymphocytosis during routine blood work. If a patient does display symptoms, they would include night sweats, weight loss, lymphadenopathy, hepatomegaly and/or splenomegaly.²

Classically and still today, prognosis is largely based on clinical staging systems³ developed in the 1970's and presented in Table one. One of the staging systems, devised by Rai et al.,⁴ predicts survival time based on signs and symptoms. This has proven useful for some, but not for all patients. These systems predict patients with overall short/poor survival based on the presence of marrow failure (e.g. anemia, diffuse infiltration pattern). Unfortunately, in a portion of cases, these systems cannot predict, for example, that an early stage patient will experience an aggressive disease course.

Given that not all patients' disease course can be reliably predicted, investigators utilize additional markers that aid in prognostication. For example, elevated soluble CD23⁵ and lymphocyte doubling time⁶ are associated with more aggressive disease. Even more recently, CD38⁷, ZAP-70⁸, immunoglobulin heavy chain variable gene region mutational status (IgVH)⁹ and genetic aberrations, as detected by fluorescence in situ hybridization (FISH), have been described and evaluated for their use as prognostic markers. Each of these markers has been shown to have independent prognostic significance.

The use of FISH to detect recurring genetic aberrations within the B-CLL cells has proven to have significant prognostic capability. Dohner et al., in a hallmark article, revealed that FISH can identify a genetic abnormality in ~80% of CLL/SLL cases and that outcome can be reliably correlated to these aberrations.¹⁰ These findings are summarized in Table two. When deletion 13q14 is detected as the sole abnormality, it portends a significantly better prognosis compared to a deletion of p53 or ATM. If deletion 13q14 is present along with another aberration, the prognosis associated with the latter aberration prevails. ATM deletions tend to be associated with progressive and marked lymphadenopathy with a relatively poor prognosis.¹¹ A p53 deletion has the worst overall prognosis and highest likelihood for failure of treatment¹². Both ATM and p53 encode for tumor suppressor genes.

Although there is a wide variety of tools and parameters available to predict prognosis in CLL/SLL patients, it has not been studied whether or not the tissue morphology of CLL/SLL provides some predictive clues as to the presence of underlying favorable or unfavorable FISH abnormalities. Tissue biopsy (of an enlarged lymph node worrisome for a neoplastic process) is often the first step in evaluating lymphadenopathy in a patient. Given that the tissue is readily available to initially make the diagnosis, we hypothesized that morphologic abnormalities, aside from those typically expected in SLL, may be predictive of a FISH abnormality. This eliminates the need in some cases for FISH studies. Then, we investigated morphologic abnormalities in SLL and correlated them with underlying FISH aberrations.

Materials and Methods:

Case selection and inclusion criteria: All of the tissue-based SLL cases were retrieved from the archival pathology files at UNM for a total of 41 cases. In order for a case to meet criteria for this study, the diagnosis of SLL must have been previously established by a hematopathologist. Cases described as having atypical morphology or having any component of transformation were excluded from the study. This study was approved by the Human Research and Review Committee (HRRC).

Morphologic review: The morphologic features of each case were reviewed on full (H&E) sections in a blinded manner assessing for specific atypical features as described below. Each case was examined independently by two hematopathologists (KR was one) and one medical student (AB). Reproducibility was assessed by comparing the results between examiners (primarily the hematopathologists) and revisiting discordant results at the microscope until resolution was met. Discordant results were 10% overall.

The morphologic features assessed were: 1) expanded proliferation centers (EPC) comprising >35% of total surface area, 2) >10 large cells per 40X hpf outside of proliferation centers (LC), 3) marked nuclear contour irregularities (NCI) in tumor small cells, and 4) NCI in large cells. These features can be seen in figures 1-4. The presence of each morphologic feature was recorded individually. When looking for EPC and LC, the results were recorded as present or absent and then assigned a score of 1 or 0, respectively. Nuclear contour irregularities for both small and large cells were divided into three categories: none, mild irregularities, and marked irregularities. These were assigned a score of 0, 1, or 2, respectively.

FISH: FISH was used to detect genetic aberrations by noting the absence of specific DNA sequences (deletion), and one trisomy, known to be related to CLL/SLL using paraffin-embedded tissue and an automated imaging analysis system (MetaSystems™). Tissue microarrays (TMA) composed of 1.5mm

cores of fixed, paraffin-embedded tissue were prepared from all cases.¹³ FISH probes for 13q14, centromere 12, 11q22 (ATM) and 17p (p53) were hybridized to the TMA's. Analysis for del 13q14, del 11q, del 17p and trisomy 12 were performed on each case using MetaSystems™.

The data acquired from FISH was grouped and scored based on the genetic aberration found. Each case was assigned a score from 0 to 3 based on the FISH result. The higher the FISH score the worse the prognosis. A FISH score of 3 indicates del 11q (ATM) or del 17p (p53) is present. A FISH score of 2 represents trisomy 12. A score of 1 means no genetic aberrations were found. Finally, a score of 0 denotes del 13q14, the genetic aberration with the best prognostic outlook. If del 13q14 was found with another aberration, the score for the other aberration was assigned.

Data Analysis: Morphology scores, as described above, were tested for association with FISH scores. The association of the FISH scores with the EPC and LC morphology scores was carried out using the exact normal scores test. The association between large and small cell NCI and FISH scores was determined using the Jonckheere-Terpstra procedure. This allowed for inclusion of the information contained in the natural ordering of the categories.

Statistical calculations were made with Statgraphics Centurion XV version 15.2.06 (StatPoint, Inc. ,Herndon, VA), with data management carried out using Microsoft Excel 2007 (Microsoft Corporation, Redmond, WA) and StatXact-4 for Windows (Cytel Software Corporation, Cambridge, MA).

Two tailed tests and a Type I error rate of 0.05 were employed throughout.

Results:

Patients: Of the 41 cases, 44% were from female patients and 56% were from male patients. The age of the patients, at time of biopsy, ranged from 34 to 92, with a mean of 65 years of age. Seventeen patients were deceased.

Morphologic Review: 100% (41) of cases were examined looking for the atypical morphologic features listed above. Of the 41 cases reviewed, 83% (34/41) of cases had at least one specified atypical morphological feature with 50% (17/34) of cases displaying only one morphologic feature.

Fluorescence in situ Hybridization (FISH): FISH was successful in 100% of the cases (41). 27/41 (66%) cases were found to have FISH abnormalities. Out of the 27 cases with an abnormality, 56% had del 13q14, with 48% having del 13q14 as the sole abnormality. 26% of cases were found to have del 11q, 22% had Trisomy 12 and 4% had del 17p. 14/41 (34%) cases did not display any of the four abnormalities tested.

Morphology versus FISH: Table three demonstrates that when nuclear contour irregularities exist in large cells, there is a higher likelihood of a greater FISH score, with a $p = 0.001$ using the Jonckheere-Terpstra test. The greater FISH scores represent genetic aberrations with worse prognosis. As seen in the table, there are more cases with nuclear contour irregularities in large cells with greater FISH scores than with lower scores.

Table four compares the frequencies of NCI in small cells to FISH scores. The association between NCI in small cells and FISH scores was found to be statistically significant, with a $p = 0.0006$ using the Jonckheere-Terpstra test. There is a greater chance of a worse FISH score when nuclear contour irregularities in small cells are found under the microscope.

Comparison of EPC with the genetic aberrations using FISH did not yield a statistically significant association, with a $p = 0.45$. Looking at associations between LC and genetic aberrations also did not prove statistically significant; $p = 0.44$.

Discussion:

The prognosis of patients with small lymphocytic lymphoma/chronic lymphocytic leukemia still relies predominantly on the Rai or Binet systems. However, given that these systems do not always accurately predict which patients are likely to progress more quickly, a number of surrogate markers have been identified which aid in further prognostication (e.g. soluble CD23, lymphocyte doubling time, CD38, ZAP-70 and FISH genetic abnormalities). The finding that specific genetic aberrations correlate with survival time has allowed for better risk stratification of patients and better prediction of which patients may have a more aggressive disease course while others have a slow disease course when both start at the same stage of disease. Utilization of such ancillary tests is costly and need not be performed in every patient. Therefore, we hypothesized that the histology of cases of SLL may provide some clues to the underlying FISH genetic status, thus potentially allowing for only a subset of cases to have FISH testing performed.

In our study, we found that the presence of nuclear contour irregularities in either small or large tumor cells suggests an underlying genetic aberration that predicts a worse prognosis (e.g., del11q and del17p) ($p < 0.05$). We did not find any statistically significant associations of expanded proliferation centers or increased large B-cells outside of proliferation centers and FISH abnormalities. We identified seven cases with del 11q and one case with del 17p, which although similar frequencies to those seen in the Dohner study, are too few in number to make a strong prediction despite a statistically significant result. Thus, the SLL morphologic abnormalities examined in this study are largely not predictive of the underlying FISH genetic status. Therefore, in certain clinical scenarios (e.g. young age of the patient at diagnosis), performing ancillary tests with prognostic significance such as FISH should be considered as clinically warranted.

Limitations to this study are found primarily in its sample size. A larger sample size would be useful in order to potentially have more cases with del 17p and thus have enough cases to divide del 11q

and del 17p into two groups. Having more cases could lead to stronger associations with the morphologic features. As with some pathologic studies, reproducibility can be a limitation.

Small lymphocytic lymphoma has a number of prognostic tests available in order to accurately inform patients of their average survival time. Nuclear contour irregularities and their association with a worse genetic aberration may be informational, but should not replace fluorescence in situ hybridization as a predictor of del 11q or del 17p.

Acknowledgments:

Thanks to Dr. Tandberg for his assistance with statistical analysis and data presentation. I would also like to thank my research mentor, Dr. Reichard, for all of her help and support.

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Tables:

Table Error! Main Document Only.: Rai System of Clinical Staging of CLL

Stage	Symptoms	Survival time (months)
0	Lymphocytosis (Defined as bone marrow lymphocytosis* as well as blood lymphocytosis**)	>150
I	Lymphocytosis and enlarged lymph nodes	101
II	Lymphocytosis with enlarged spleen and/or liver	71
III	Lymphocytosis and anemia (hemoglobin <11 g/100ml or hematocrit <33%)	19
IV	Lymphocytosis and thrombocytopenia (platelet count <100,000/cu mm)	19

*Bone marrow lymphocytosis is defined as 40% or more lymphocytes observed in the marrow.

**Blood lymphocytosis is defined as absolute lymphocytes greater than 15,000/cu mm

Table 2: Genetic Aberration Frequency in CLL/SLL (adapted from Dohner et al, ref. 9)

	Deletion 13q14	None*	Trisomy 12	Deletion 11q22	Deletion 17p
Frequency of occurrence	55%	18%	16%	18%	7%
Target gene	Unknown	NA	Unknown	ATM	P53
Median survival	133 months	111 months	114 months	79 months	32 months

*No aberration found using the probes listed

Table 3: Frequencies for NCI in large cells by FISH score

	FISH = 0	FISH = 1	FISH = 2	FISH = 3	Total
NCIL = 0	2 (66.67%)	1 (33.33%)	0 (0%)	0 (0%)	3 (7.32%)
NCIL = 1	11 (45.83%)	7 (29.17%)	3 (12.50%)	3 (12.50%)	24 (58.54%)
NCIL = 2	0 (0.00%)	6 (42.86%)	3 (21.43%)	5 (35.71%)	14 (34.15%)
Total	13 (31.71%)	14 (34.15%)	6 (14.63%)	8 (19.51%)	41 (100.00%)

Table 4: Frequencies for NCI in small cells by FISH score

	FISH = 0	FISH = 1	FISH = 2	FISH = 3	Total
NCIS = 0	3 (100%)	0 (0%)	0 (0%)	0 (0%)	3 (7.32%)
NCIS = 1	9 (32.14%)	12 (42.86%)	4 (14.29%)	3 (10.71%)	28 (68.29%)
NCIS = 2	1 (10.0%)	2 (20.0%)	2 (20.0%)	5 (50.0%)	10 (24.39%)
Total	13 (31.71%)	14 (34.15%)	6 (14.63%)	8 (19.51%)	41 (100.00%)

Figures:

Figure 1: Expanded proliferation center

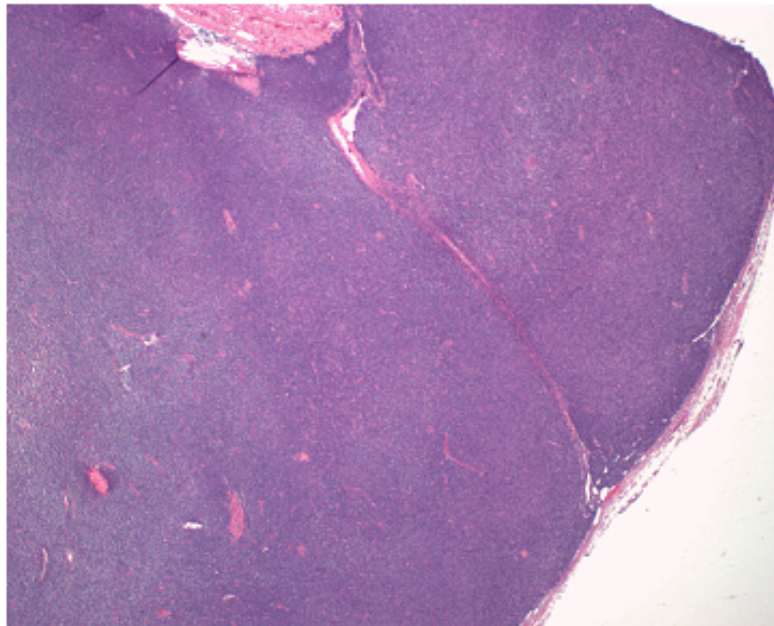
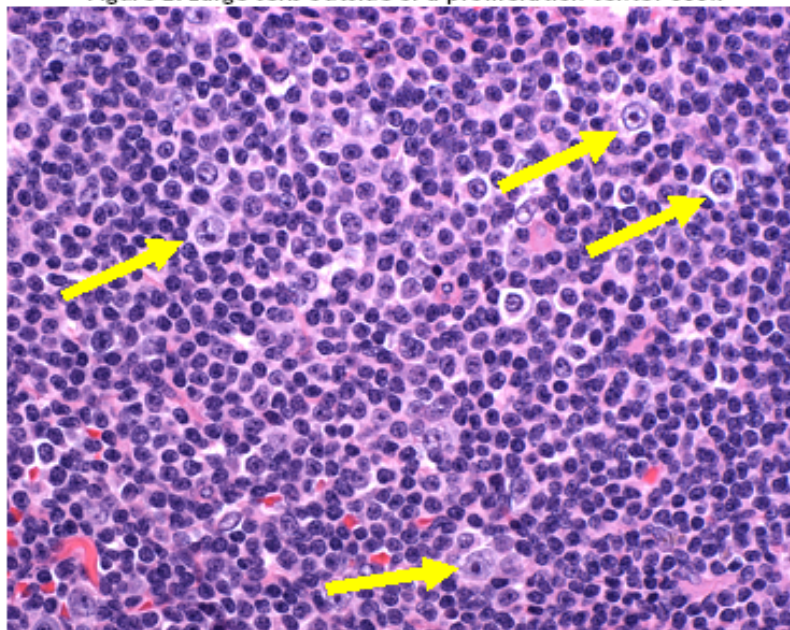
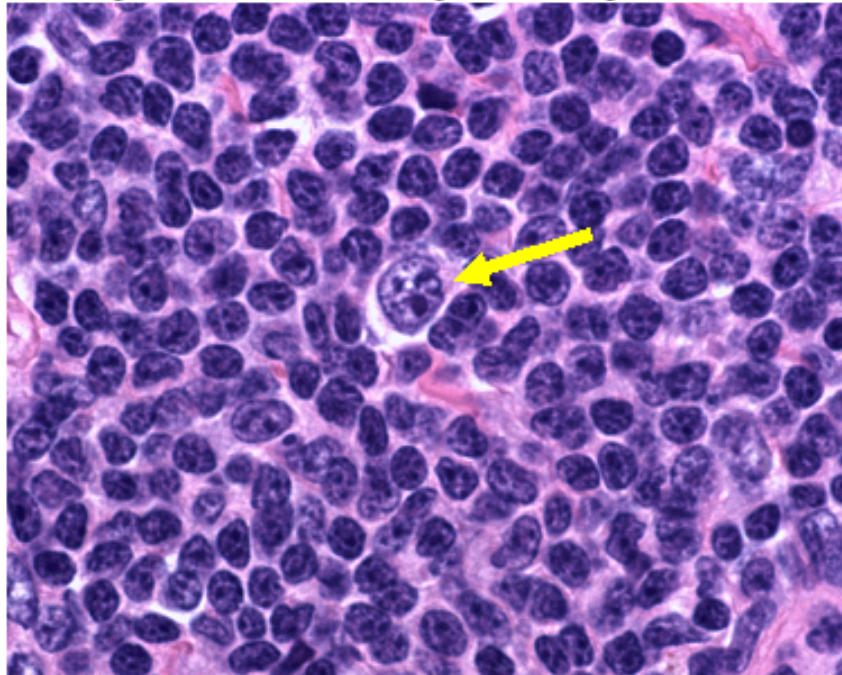


Figure 2: Large cells outside of a proliferation center 600x



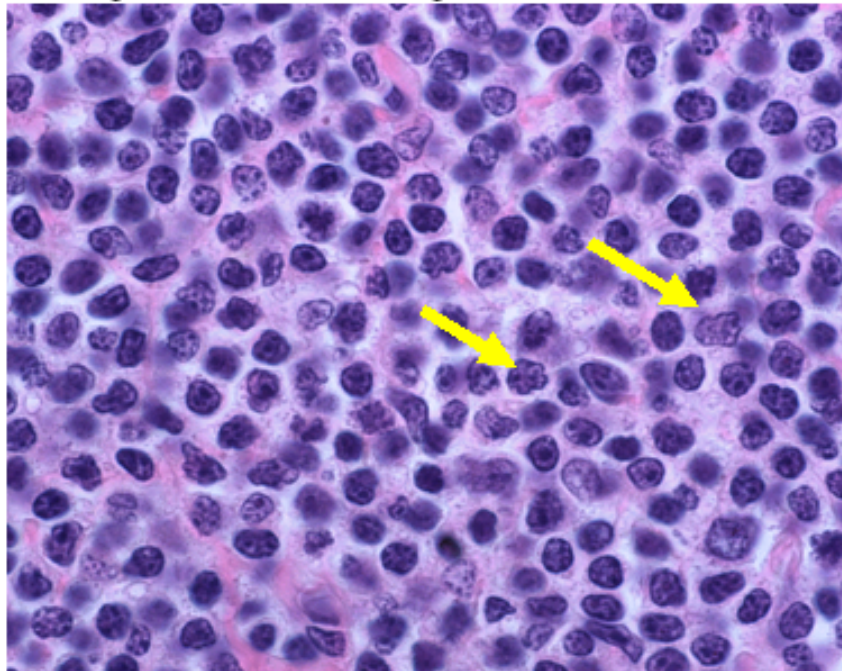
Yellow arrows show examples of large cells

Figure 3: Nuclear contour irregularities in large cells 1000x



Yellow arrow shows example of nuclear contour irregularity in a large cell

Figure 4: Nuclear contour irregularities in small cells 1000x



Yellow arrows shows examples of nuclear contour irregularity in a small cell