

Diagnosis and Prognosis of B-Cell Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma (B-CLL/SLL) and Mantle Cell Lymphoma (MCL)

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ABSTRACT

Background: B-cell chronic lymphocytic leukemia/small lymphocytic lymphoma (B-CLL/SLL) and mantle cell lymphoma (MCL) show many overlapping morphologic and immunophenotyping features, however they have great difference in therapeutic regimens and prognosis.

The Aim of the Study: Is to determine the diagnostic and prognostic role of clinico-pathologic variables, CD23 and Cyclin D1 oncoproteins in B-SLL/CLL and MCL.

Patients and Methods: This study included 25 B-CLL/SLL cases and 25 MCL cases. All cases were carefully examined and stained using CD23 and Cyclin D1 immunostaining.

Results: There was significant difference between B-CLL/SLL and MCL regarding several items including pattern of growth, where interfollicular pattern was restricted to B-SLL/CLL while nodular and mantle zone pattern were confined to MCL; pseudo-follicles were only present in B-CLL/SLL. Transformed cells, plasmacytoid cells, peripheral blood lymphocytosis, significant longer survival and good prognosis were statistically more prominent in favor of B-CLL/SLL. On the other hand, cell cleavage, epithelioid histiocytes, plasma cells, naked nuclei, hyalinized venules, deposited hyaline material in background and reticular fibers in addition to higher mitotic index per 20 HPF were more significantly identified in favor of MCL. CD23 was expressed as membranous pattern in 16/25 (64%) of B-CLL/SLL cases and 1/25 (4%) of MCL cases. On the other hand, Cyclin D1 was expressed as nuclear staining in 18/25 (72%) of MCL cases and only 1/25 (4%) of B-CLL/SLL cases. Regarding B-CLL/SLL, age >60 years and mitosis $\geq 10/20$ HPF were independent prognostic factors of shorter survival by multivariate analysis. In MCL, Cyclin D1 overexpression and splenomegaly were independent prognostic factors of survival by multivariate analysis.

Conclusion: Cyclin D1 is not only implicated in tumor genesis of MCL, but also in progression and extension of the disease when expressed in high levels (50% cut off

value) and it seems to have prognostic impact in MCL. This can be used as a basis for future therapeutic strategies targeting cell cycle regulators. This study could support the concept that Cyclin D1 and CD23 immunostaining may be reliable diagnostic tools for discrimination between B-CLL/SLL and MCL.

Key Words: *B-cell chronic lymphocytic leukemia/Small lymphocytic lymphoma (B-CLL/SLL) - mantle cell lymphoma (MCL) - CD23 - Cyclin D1.*

INTRODUCTION

B-CLL/SLL and MCL are two types of peripheral small B-cell lymphomas that share many morphologic and immunophenotypic features (both are CD5+, CD19+, CD20+, CD10, and BCL-2+), making differentiation between them problematic and sometimes challenging. However, distinguishing the 2 types from each other is essential due to the great difference in therapeutic regimens and prognosis. MCL is a vexing and increasingly frequent problem for oncologists. The disease brings together the worst characteristics of high grade and low grade lymphomas, i.e. the course is not indolent and the disease is rarely curable [1]. Most MCL patients in a study by DiRaimondo et al. [2] were refractory to conventional therapy of B-CLL/SLL including fludarabine, however they showed a good response to fractionated cyclophosphamide-based therapy. On the other hand, B-CLL/SLL is usually low grade with a relatively longer overall survival and good response to therapy [3]. The availability of immunohistochemical markers considerably improved results of diagnosis in the last years. CD23 and cyclin D1 have been proved by some researchers

to be usually able to differentiate well between B-CLL/SLL and MCL [4-5]. The international prognostic index (IPI) is one available, validated tool for predicting prognosis based on clinical features (age, stage, level of lactate dehydrogenase, performance status, and number of extranodal sites [6]. However, the IPI is not an ultimate instrument because a considerable proportion of patients classified as "low" or "low-intermediate risk" eventually shows therapeutic failure [7]. Thus, we are still in need of more accurate factors to predict prognosis and survival. Our aim is to study the different histopathologic and clinical features of B-CLL/SLL and MCL to determine the most reliable ones for differentiation and prediction of prognosis, in addition to evaluation of the diagnostic role of cyclin D1 and CD23 protein expression in both tumors using immunohistochemical methods and investigation of their prognostic impact.

MATERIAL AND METHODS

This study comprised 50 lymph node biopsy specimens diagnosed as B-CLL/SLL (25 cases) and MCL (25 cases). Cases were selected after exclusion of CD19 negative cases to be sure of the B cell origin of the cases and CD5 negative to exclude other types of lymphoma. The cases were diagnosed during the period between 1997 and 2002 in the pathology department of the National Cancer Institute, Cairo University. Available information was collected including age, sex, extra-nodal sites of involvement, peripheral blood involvement (absolute lymphocytosis $>4000/\mu\text{l}$ with atypical morphology) [8], bone marrow involvement, serum LDH, B-symptoms [9]. Staging has been made for every case according to Ann-Arbor staging system [10] and evaluation of the performance status for patients according to Eastern Cooperative Oncology Group (ECOG) criteria [11]. The follow up period ranged from 24 to 84 months in which we calculated the time elapsed between excision biopsy and death or last contact with the patient. All cases were examined with special attention to various histological features mentioned below:

Architectural effacement whether partial or complete without any residual follicles [12].
Pattern of infiltration whether diffuse, vaguely nodular, mimicking primary follicles, or mantle zone (MZ) pattern in the form of wide mantles

around atrophic residual germinal centers (GCs) [13]. When a mixture of patterns was encountered, the predominant pattern is the one occupying $>50\%$ of the cross sectional area of all available sections [14]. # pseudofollicles which appear as pale rounded foci during scanning at x 40 magnification [15]. In high power (HP) view they comprise a mixture of prolymphocytes, paraimmunoblasts and small atypical lymphocytes [9]. They were counted at x100 magnification per low power field (LPF), and graded as: Negative if absent; present (+)=1-7/LPF; and abundant (++)= ≥ 7 /LPF (low power field). # naked GCs defined as bare GCs lacking normal lymphocyte cuff within the diffuse areas [16]. Naked GCs should not be taken as an evidence of MZ pattern [14]. # Size of nuclei and nuclear contour [17]. # Transformed cells namely prolymphocytes which are slightly bigger than small lymphocytes and have more open chromatin, a distinct nucleolus, and a greater amount of pale cytoplasm, and paraimmunoblasts which are even larger with vesicular chromatin and a prominent central nucleolus. They were graded semiquantitatively as absent (-), present (+)=1-20% of the neoplastic infiltrate, and abundant (++) $>20\%$ of the neoplastic infiltrate in each field. N.B: Care should be taken not to confuse paraimmunoblasts with immunoblasts which are slightly larger and having darker cytoplasm [15]. # epithelioid histiocytes either singly scattered or in collections [14]. They were graded semiquantitatively as absent (-), present (+)=1-15/HPFX400, and abundant (++) >15 /HPFX 400. # plasma cells and cells with plasmacytoid differentiation, monocytoid change, naked nuclei of follicular dendritic cells, centroblasts and immunoblasts [15]. # hyalinized walled, blunt ended venules [13]. They were graded as absent (-), present (+)=1-5/HPFX200, abundant (++) >5 /HPFX200. # deposited hyaline material in the background. # Microvessel density was estimated in order to evaluate degree of angiogenesis. We determine 3 hot spots by x100 magnification. Microvessels were counted within 3 hot spots at x200 magnification. The mean number is then calculated and graded as follows: Low <15 microvessels/HPF, Moderate= $15-30$ microvessels/HPF, High ≥ 30 microvessels/HPF [18]. # pseudorosettes [19], thick reticulin fibers, invasion of the LN capsule by the neoplastic infiltrate [9]. # Mitotic index/20 HPF was performed according to Yatabe et al. [13].

Determination of different cytologic variants of both B-CLL/SLL and MCL was done including: Mixed cell variant of B-CLL/SLL: Characterized by large aggregates of prolymphocytes extensively infiltrating and replacing 30-50% of the nodal architecture [20]; paraimmunoblastic variant of B-CLL/SLL: When there are sheets or large aggregates of paraimmunoblasts [15]; atypical cell variant of CLL/SLL: Formed entirely of slightly cleaved neoplastic lymphocytes and exhibiting wide pseudofollicles [15]; blastic variant of MCL: Applied when the neoplastic cells are larger than usual and have more finely dispersed nuclear chromatin, small nucleoli resembling lymphoblasts [21]; pleomorphic variant of MCL: Characterized by heterogeneous populations of larger tumor cells with irregular nuclei and small nucleoli [21]; monocytoid variant of MCL [22]; evaluation of presence or absence of transformed areas e.g. to diffuse large B cell lymphoma [23].

Immunohistochemistry: Four sections of 4µm thickness were prepared as follows: One test slide for staining by cyclin D1, one for staining by CD23, one for staining by CD19 as a B-cell marker when there were no plasma cells or plasmacytoid differentiation (38 cases), and the last one was negative control. CD5 was tested only if follicular lymphoma or marginal zone lymphoma was suspected especially in CD23 (-) (36 cases), cyclin D1 (-) cases. Primary antibodies were mouse monoclonal anti-human cyclin D1 (clone DCS-6) (0.2ml culture supernatant) (Dako, Copenhagen), monoclonal mouse anti-CD23 antibody (clone 1B12) (0.1ml supernatant) (Neo Markers, Westinghouse), mouse monoclonal anti-human CD19/B-Cell Ab-2 (clone 19CO2) (0.1ml culture supernatant) (Neo Markers, Westinghouse), and mouse monoclonal antihuman CD5 Ab-1 (clone 4C7) (7ml ready to use) (Neo Markers, Westinghouse). The detection kit is avidin-biotin complex peroxidase method (Neo Markers, Westinghouse). Antigen retrieval of CD23 and CD19 was performed in 10mmol/L citrate buffer, pH 6.0, while for Cyclin D1 and CD23, 0.1mmol/L EDTA, pH 8.0 was used. CD23 and CD19 primary antibodies were used at a dilution of 1:10 using PBS. Cyclin D1 antibody was used at a dilution of 1:25 using LSAB. CD5 antibody was ready to use without dilution. Primary antibodies were incubated with sections overnight. Di-Amino Benzidine (DAB) Chromagen was used as col-

oring solution. Mayer's haematoxyline was used as counterstain (Bio-Genex, San Ramon, USA).

Interpretation of Immunostaining: The evaluation of immunostaining for both Cyclin D1 and CD23 was scored for the percentage of immunopositive tumor cells. Cyclin D1 over expression was defined as positive in the nuclei of lymphoma cells with or without simultaneous weak staining of cytoplasm. Endothelial cells and histiocytes were used as internal positive control [24]. Negative (-)=<10% of cells positive, Regional (+)=10-50% of cells positive, Diffuse (++) >50% of cells positive [25]. According to Watson et al. [26] CD23 positivity was identified by significant labeling of neoplastic cells (in the form of membranous reaction) in any area of the section. If positivity was only restricted to the dendritic reticulum cell network, the case was considered negative. Negative (-)=No individual lymphoid cells positive, Regional (+) =≤50% of cells positive, Diffuse (++) >50% of cells positive. CD5 and CD19 positivity was identified by significant labeling of neoplastic cells (in the form of membranous reaction) in any area of the section.

Statistics: The data were collected and statistically analyzed using a personal computer with Statistical Package for the Social Sciences (SPSS), VERSION 11 software program. Value of <0.05 was considered statistically significant [27].

RESULTS

We excluded CD5 negative cases (3 cases out of the stained 36 cases by CD5, those were CD23 negative) and CD19 negative cases (2 CD19 negative cases out of 38 cases with no plasma cells or plasmacytoid differentiation) to exclude other histologic mimickers of our lymphoid malignancies. After exclusion of those cases the total number of studied cases was 50. The diagnosis of the cases was re-evaluated after marker results. There was statistically significant difference between B-CLL/SLL and MCL regarding age ($p=0.02$) as mean age was 60.08 ± 12.34 versus 53.80 ± 10.51 years for each type, respectively.

Histopathologic Features by Low Power Examination: There was a statistically significant difference between B-CLL/SLL and MCL

regarding predominant pattern of growth ($p=0.02$) with diffuse pattern being more common in B-CLL/SLL (96%) than MCL (68%), and restriction of nodular and mantle zone patterns to MCL and interfollicular pattern to B-CLL/SLL. Regarding pseudofollicles, they were only present in B-CLL/SLL cases ($p=0.000001$). There was no statistical difference between the two tumor types regarding either effacement or naked GCs ($p>0.005$).

Histopathologic Features of Studied Cases by High Power Examination: The neoplastic cells were purely non cleaved in 60% of B-CLL/SLL cases versus only 8% in MCL, while they were purely slightly cleaved in 56% of MCL cases versus 16% of B-CLL/SLL cases, thus recorded a highly statistically significant difference ($p=0.0003$) between them regarding cleavage. In B-CLL/SLL the characteristic transformed cells of prolymphocytic and paraimmunoblastic types were detected in 92% versus 36% in MCL ($p=0.000001$). There was a statistically significant difference between B-CLL/SLL and MCL regarding epithelioid histiocytes ($p=0.00001$), plasma cells ($p<0.05$), naked nuclei ($p=0.01$) and mitosis per 20HPF ($p=0.004$) in favor of MCL and regarding plasmacytoid cells ($p<0.05$) in favor of B-CLL/SLL. However, no statistically significant difference between the two tumor types regarding cell size, monocytoid cells, immunoblasts, centroblasts, eosinophils or neutrophils could be found.

Other Features: There was a highly statistically significant difference between MCL and B-CLL/SLL regarding hyalinized venules ($p=0.002$), deposited hyaline material in favor of MCL and reticular fibers being only restricted to MCL ($p=0.004$). No significant difference was detected regarding microvessel density, pseudorosette or capsular invasion.

There was a statistically significant difference between B-CLL/SLL (76%) and MCL (40%) regarding peripheral blood lymphocytosis. However, no significant difference could be detected between the two tumor types regarding lymphadenopathy, L.N mass, stage, serum LDH, extranodal involvement, bone marrow, spleen, liver, others and B symptoms ($p>0.005$).

Different Histological Types in B-CLL/SLL & MCL: Among the 25 B-CLL/SLL cases 3/25 (12%) were of mixed cell type, 1/25 (4%) was

atypical, and another case (4%) showed wide areas of transformation to diffuse large B cell lymphoma. The remaining 20 cases (80%) were of the conventional type. Regarding MCL, the blastoid variant has been encountered in 2/25 (8%), the pleomorphic variant in 1/25 (4%), and the monocytoid variant in 1/25 (4%). The remaining 21 cases (84%) were of the conventional type.

Immunohistochemistry: CD23 was positive in 16/25 cases (64%) of B-CLL/SLL. In 10/16 (62.5%) of positive cases >50% of neoplastic cells (diffuse pattern) stained with a membranous pattern of staining. The staining was moderate to strong in intensity in 14/16 (87.5%) of positive cases. Only 1/25 cases (4%) of MCL was positive for CD23 in which staining pattern was quite different. It was membranous but dot like, i.e. not continuous. CD23 also stained the follicular dendritic cells within the residual GCs in some cases of MCL exhibiting mantle zone pattern or with naked GCs, and the dispersed network of dendritic reticulum cells in 4/25 cases (16%). Therefore, CD23 immunoreactivity was highly statistically associated with B-CLL/SLL ($p=0.00004$). There was a statistically significant difference between CD23 positivity and abundance of transformed cells ($p=0.01$). The 3 mixed cell type cases were distributed as follows (one negative, one positive with regional distribution and one positive with diffuse distribution). The only atypical case and the case with wide areas of transformation to diffuse large B cell lymphoma exhibited diffuse CD23 positivity. No other histopathologic feature was statistically correlated with CD 23. There was a statistically significant difference between cases with CD23 positivity and advanced stage ($p=0.02$), and bone marrow involvement ($p=0.012$). The only CD23 positive MCL case was characterized histologically by complete effacement of architecture, mixed diffuse and nodular pattern of growth, small non cleaved neoplastic cells, no transformed cells, few scattered epithelioid histiocytes and hyalinized venules together with deposited hyaline material in the background, moderate degree of microvessel density, capsular invasion, and moderate mitotic index (14/20HPF). It showed cyclin D1 negativity. Clinically such case was a thirty-eight-year-old female with stage III at presentation, splenomegaly, peripheral blood absolute lymphocytosis, normal LDH level (177IU/L)

and good performance status. Interestingly, the survival time of this case was 45 months which is longer than that of most MCL cases included in our study.

Cyclin D1: Among the 25 MCL cases, 18 (72%) showed cyclin D1 positivity (10% cut off value). In such cases cyclin D1 reactivity was localized to the nucleus with or without simultaneous cytoplasmic staining. The staining extent and intensity were highly variable, from 25% to 90% of neoplastic cells, 10/18 (55.6%) of positive cases showed regional positivity and 8/18 (44.4%) of positive cases showed diffuse positivity. The intensity of 6/18 (33.3%) of positive cases was strong. Only one out of the 25 B-CLL/SLL cases demonstrated nuclear regional staining of cyclin D1 detected in 20% of the tumor cells and was weak in intensity. Therefore, Cyclin D1 nuclear immunoreactivity was statistically associated with MCL ($p=0.000004$). There was a statistically significant difference between cyclin D1 expression and immunoblasts ($p=0.01$), immunoblasts have been only detected in negative cases. The two cases of blastoid variant and one case of pleomorphic variant were stained positive for cyclin D1 with regional positivity, while the only case of monocytoid variant was negative. The cyclin D1 positive cases were characterized by higher mitotic rate with a mean of 18 ± 10.73 versus 8.14 ± 5.64 for the cyclin D1 negative cases ($p=0.032$). There was a statistically significant difference between cyclin D1 immunoreactivity and clinical stage ($p=0.05$), LDH level ($p=0.05$), increased number of extranodal involvement ($p=0.03$), and bone marrow involvement ($p=0.02$). The only Cyclin D1-positive B-CLL/SLL case was also CD23 positive and was

characterized histologically by complete effacement of architecture, diffuse pattern of growth, mixed small and medium sized cells with mixed non-cleaved and slightly cleaved nuclei, abundant transformed cells and epithelioid histiocytes, hyalinized venules, and moderate MI (14/20HPF). Clinically, such patient was a 51-year-old male, stage III at presentation, with hepatosplenomegaly, peripheral blood absolute lymphocytosis, high serum LDH level, B symptoms and very short survival time (3 months).

Survival Analysis: The longest survival time was observed in a case with B-CLL/SLL (67 months), and the shortest has been recorded by a case of MCL (1 month). MCL cases experienced a significantly ($p=0.01$) shorter survival time (mean 11.6 months) than B-CLL/SLLs (mean 34 months), Chart (1). In B-CLL/SLL, the CD23 (+) patients were characterized by significantly longer survival time than CD23 (-) group with a mean of 36.43 ± 22.38 months for the positive group versus 15.67 ± 10.05 months for the negative one, Chart (2). In MCL, cyclin D1 (-) patients were characterized by significantly longer survival time than cyclin D1 (+) patients with a mean and standard deviation of (21.3 ± 22.4) months for the negative group versus (6.9 ± 9.9) months for the positive one, Chart (3). Using multivariate analysis only mitosis and age >60 years proved to be independent prognostic factors of poor survival in B-CLL/SLL cases, Table (1). Moreover, multivariate analysis demonstrated that cyclin D1 positivity is the strongest predictor of short survival in MCL followed by splenomegaly regardless of other prognostic parameters, Table (2).

Table (1): Prognostic factors affecting overall survival of 25 B-CLL/SLL cases.

Variables	Un-favorable factor	Number of cases	Univariate analysis P	Multivariate Analysis (Cox Regression)	
				RR (95% Confidence Interval)	P
Cell Size	Small-medium	5	0.006	1.13 (0.16-7.74)	0.90
Mitosis/20HPF	$\geq 10/20$ HPF	4	0.02	5.56 (1.14-26.98)	0.03*
CD23	Negative	9	0.05	1.25 (0.23-6.65)	0.80
Age	>60ys	12	0.04	13.96 (1.59-122.57)	0.02*
Extranodal >1	Present	8	0.04	8.83 (0.55-140.11)	0.12

*Significant ($p\leq 0.05$).

Table (2): Prognostic factors affecting overall survival of 25 MCL cases.

MCL-variables	Un-favorable factor	No. of cases	Univariate analysis P	Multivariate Analysis	
				RR (95% Confidence Interval)	P
Epith histiocytes	Abundant	8	<0.001	1.0 (0.17-5.79)	1.0
Age	>60ys	8	0.02	1.0 (0.16-6.20)	1.0
Hepatomegaly	Present	9	0.04	1.0 (0.20-4.90)	1.0
Splenomegaly	Present	18	0.03	0.20 (0.04-0.98)	0.05*
LDH	> Normal	11	0.0007	1.0 (0.22-4.46)	1.0
Extranodal involvement	> 1	7	0.006	1.0 (0.03-29.06)	1.0
CylinD1	Positive	18	0.04	19.96 (1.72-153.57)	0.01**

* Significant ($p \leq 0.05$).
 **Highly significant.

SLL vs MCL

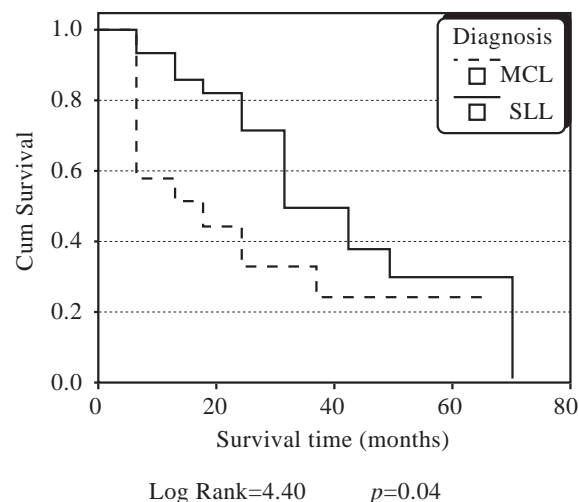


Chart (1): Kaplan-Meier survival curve of B-CLL/SLL and MCL groups ($p=0.04$).

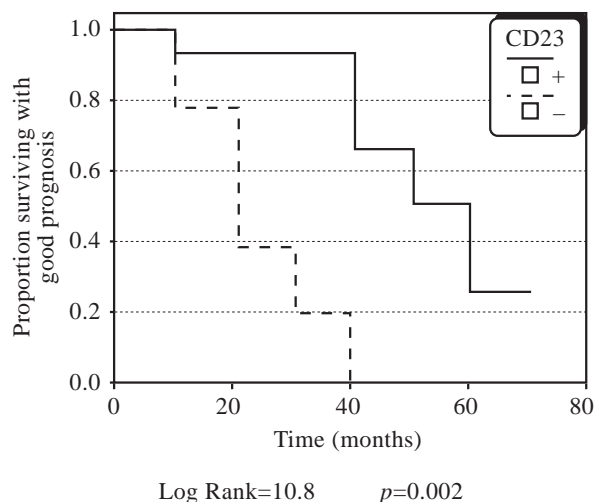


Chart (2): Kaplan-Meier survival curve of CD23 positive group in B-CLL/SLL.

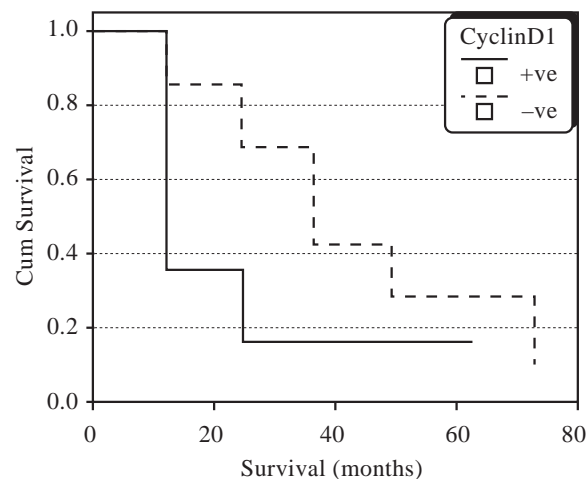


Chart (3): Kaplan-Meier survival curve of cyclin D1 in MCL cases.

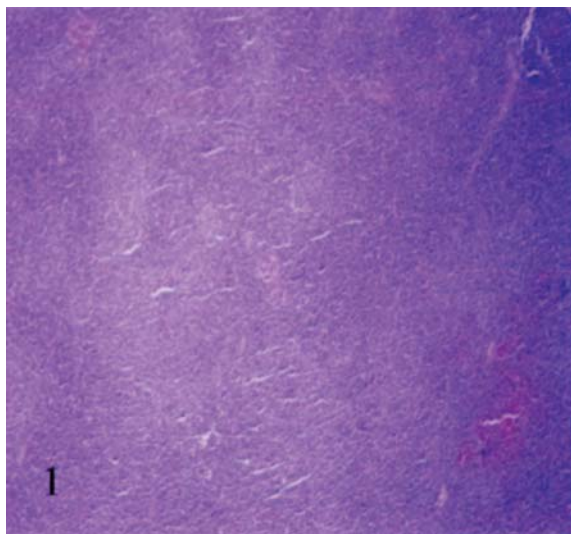


Fig. (1): A case of B-CLL/SLL with diffuse pattern of growth and scattered pseudofollicles (H & E x 52).

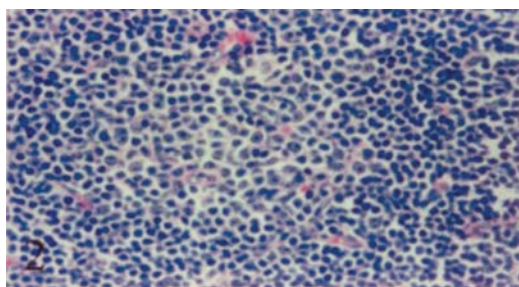


Fig. (2): High power view of the pseudo-follicle formed of a mixture of prolymphocytes and small neoplastic lymphocytes (H & E x 520).

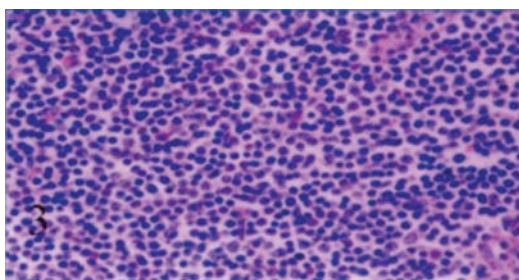


Fig. (3): A case of B-CLL/SLL formed of atypical small lymphocytes having rounded non cleaved nuclei and few scattered transformed cells (H & E x 260).

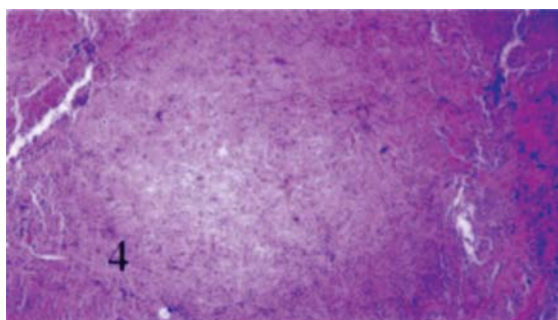


Fig. (4): A case of MCL with diffuse pattern (H & E x 130).

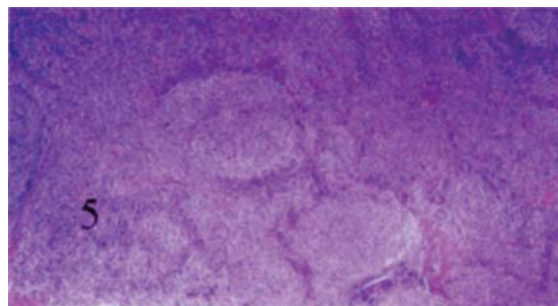


Fig. (5): MCL case with nodular pattern (H & E x 130).

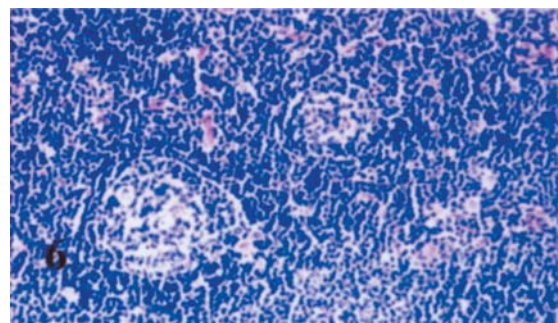


Fig. (6): MCL case with mantle zone pattern (H & E x 260).

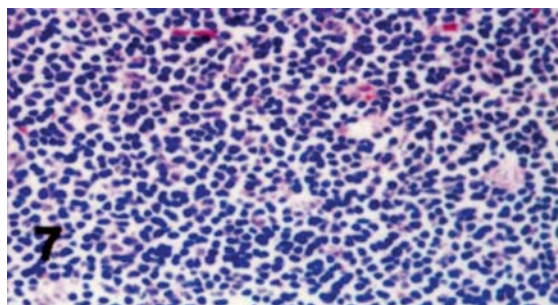


Fig. (7): MCL case showing monotonous population of cells with small slightly cleaved nuclei (H & E x 520).

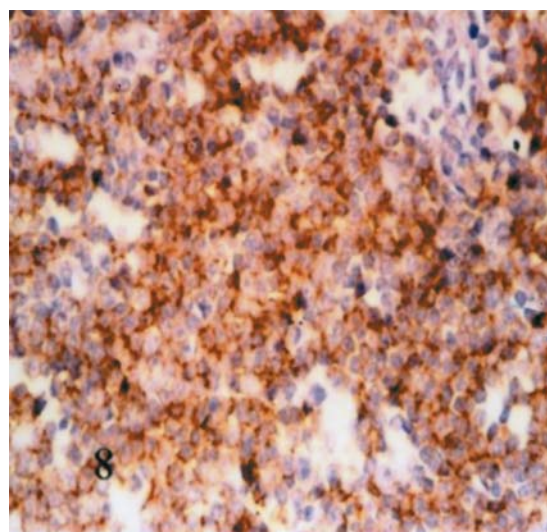


Fig. (8): A case of B-CLL/SLL stained for CD23 with membranous expression pattern. (Immunoperoxidase with Mayer's Haematoxylin x 520).

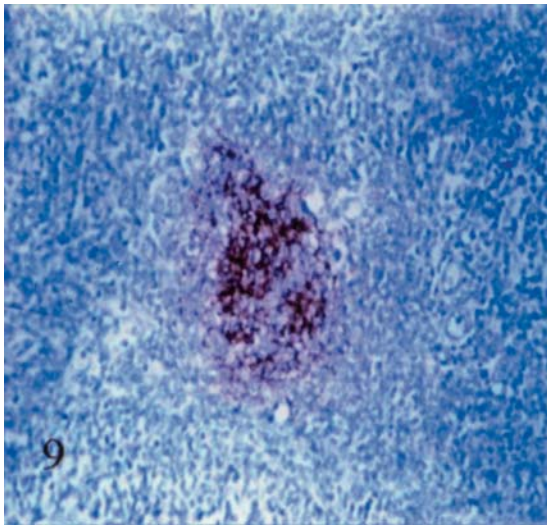


Fig. (9): A case of MCL with CD23 positivity confined to the dendritic reticulum cells within a residual GC. The surrounding tumor cells are negative. (Immunoperoxidase with Mayer's Haematoxylin x 260).



Fig. (10): MCL case showing positive nuclear cyclinD1 reactivity in almost all cells with moderate to strong intensity. (Immunoperoxidase with Mayer's Haematoxylin x 520).

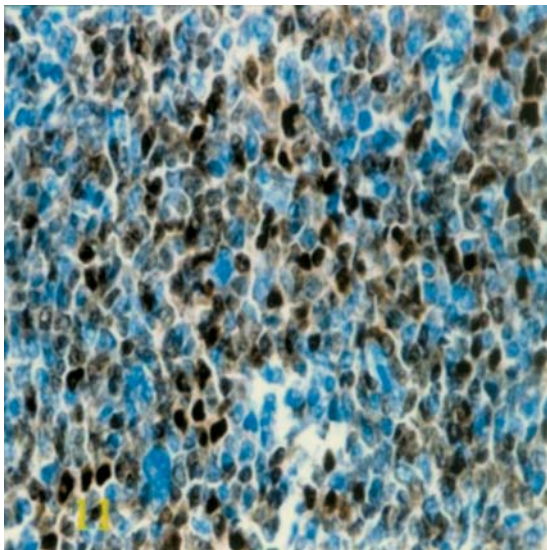


Fig. (11): Another case of MCL with regional (<50%) strong nuclear positivity for cyclinD1 (Immunoperoxidase with Mayer's Haematoxylin x 520).

DISCUSSION

By studying the histopathologic criteria, a diffuse pattern of growth was documented in 96% of B-CLL/SLL cases (24/25). This was in agreement with all researches made on B-CLL/SLL [15]. Here we describe a case of B-CLL/SLL with mixed pattern (interfollicular and diffuse) suggesting that interfollicular pattern represents an early phase of the disease, rather than a distinct type, that most probably

progresses later into diffuse pattern [12]. In MCL the patterns of growth were diffuse in 68%, nodular in 24%, and mantle zone in 8% of cases. This was different from those documented by Majlis et al. [28] in which the percentages of mantle zone and nodular patterns were reversed. This could be because most Egyptian patients usually present at a late stage making diffuse and nodular patterns constitute the majority of the cases (92%). This was supported by observations of Mokhtar and Khaled [9]. No mixture of mantle zone and diffuse patterns could be encountered supporting the hypothesis that these patterns represent stages of the disease starting by mantle zone then nodular and ending by being diffuse. Pseudofollicles have been detected in (72%) of B-CLL/SLL cases and absent from MCLs. This percentage was within the range documented by Dick, [23] and Gupta et al. [12] (60-87.5%). The presence of pseudofollicles was probably the most reliable histopathologic feature for discrimination between B-CLL/SLL and MCL ($p=0.000001$) as they were absent from all cases of MCL; this was in agreement with Kurtin, [29]. However, their absence in 28% of B-CLL/SLL made diagnosis difficult in such cases. In our study, there was statistically a significant difference between MCL and B-CLL/SLL as regards epithelioid histiocytes and hyalinized venules, where they could be detected in 100% and 60% of our included MCLs. This was in accordance with Arber et al. [30] who

documented them as clues for diagnosis of MCL. However, they could be also detected in a subset of B-CLL/SLL, 32% & 20%, respectively, in agreement with Dick [23]. Therefore, their presence is a good positive indicator of MCL but it does not exclude the possibility of B-CLL/SLL. Furthermore, we observed that the presence of deposited hyaline material in the background of the lymph node section, thick walled blood vessels and bands of sclerosis were significantly related to MCL. These findings were almost exclusively confined to cases with hyalinized venules. Pathogenesis of the two processes may be the same and needs further investigations. From the analysis of the clinical data of the studied cases, it has been found that the most frequent clinical finding in MCL cases after lymphadenopathy was splenomegaly (18/25 cases, 72%) with huge splenomegaly in 6/18 cases (33.3%). This was similar to the results of Letestu et al. [31] (45/60, 75%). Huge splenomegaly in our study was confined to cases with a mixed pattern of growth either nodular and diffuse or nodular and mantle zone; this was similar to observations published by Medeiros & Jaffe [32]. On the other hand, the most frequent clinical feature in B-CLL/SLL cases was peripheral blood lymphocytosis (19/25, 76%) consistent with data reviewed by Mokhtar & Khaled [9]. Regarding survival analysis, B-CLL/SLL cases experienced a significantly longer survival time (mean 34 months and median 30 months) than MCL cases (mean 11.6 months and median 6 months). This was in agreement with Döhner et al. [33]. The worst outcome was encountered in MCL patients rather than the B-CLL/SLL patients, despite similar clinical features, which may be due to better response of B-CLL/SLL cases to conventional therapy [3], in contrast to higher percentage of relapse and failure of response to usual therapeutic regimens in MCL [34]. Further confirmatory studies to explain the difference between the two neoplasms in biological and clinical behavior despite common cell origin are warranted.

The availability of immunohistochemical markers considerably improved results of diagnosis in the last years. CD23 and cyclin D1 have been proved by some researchers to be usually able to differentiate well between B-CLL/SLL and MCL [4-5]. Our results were concordant in finding that cyclin D1 positivity was

statistically related to MCL, while CD23 positivity was statistically related to B-CLL/SLL. Cyclin D1 overexpression is almost always confined to MCL rather than B-CLL/SLL because it usually results from t (11; 14) (q13; q32) which is the hallmark of MCL [35], while B-CLL/SLL is caused principally by defects that prevent cell turnover due to programmed cell death rather than by alterations in cell cycle regulation. In the vast majority of patients, B-CLL/SLL cells are predominantly represented by G0 quiescent cells that gradually accumulate in the patient's body, not because they are dividing more rapidly than normal, but because they are surviving too long [36]. Fournier et al. [37] reported that over expression of CD23 prevents hydrocortisone induced programmed cell death. Hence CD23 positivity is usually a feature of B-CLL/SLL. CD23 stained (16/25 cases, 64%) B-CLL/SLL cases with well defined membranous pattern. This percentage was more than that reported by Singh & Wright [5] (5/12 cases, 41.67%), and less than that reported by Kumar et al. [4] (93%). CD23 is an activation marker and many cytokines can modulate its expression on the cell surface. Therefore, it is possible that the CD23 negativity in some patients represents a shift in phenotype occurring with a change in the natural history of the disease with loss of this antigen at progression and consequent refractoriness to therapy [2]. CD23 mediates growth and differentiation signals in B cells [38]. This explains why CD23 positivity was directly related to abundance of transformed cells (prolymphocytes and paraimmunoblasts) in our studied B-CLL/SLL cases. This was also in agreement with Lampert et al. [39] who noticed high expression of CD23 in pseudofollicles of CLL in lymph nodes and spleen. Regarding clinical findings, high expression of CD23 was directly related in our study to bone marrow infiltration ($p=0.012$). This is not surprising when considering the fact that CD23 acts as an adhesion molecule which promotes adhesion of lymphocytes to high endothelial venules [40] and lost during transendothelial migration of neoplastic B-cells in B-CLL [24]. Our results declared that the CD23 positive group exhibited longer survival time than negative group. This could be explained by the fact that CD23 works as a ligand for CD21 [41] which is a molecule potentially involved both in T-cell dependent and T-cell independent responses. Thus, increased CD23 expression in lymphoma cells

leads to enhanced T-cell interaction resulting in improved autologous tumor response as reported by Lindroth et al. [7]. Our results are in agreement with Dadmarz and Cawley [42] who reported that CD23 expression seems to be prognostically favorable.

The incidence of cyclin D1 protein expression with intranuclear staining in MCLs was higher in our study (18/25 cases, 72%) than reported by Hashimoto et al. [43] (16/27 cases, 60%) who used monoclonal antibodies on frozen sections. On the other hand, our results were lower than those obtained by Singh and Wright [5] who reported that all cases of MCL showed diffuse nuclear staining with cyclin D1 polyclonal antibody on paraffin-embedded sections. This difference may be attributable to the type of fixative used, or antibodies (monoclonal vs. polyclonal). Other possible causes of negativity in 28% of our sampled MCL cases could be the low expression levels in these cases below the threshold for detection by immunohistochemistry, or absence of cyclin D1 overexpression in such cases which indicates that a cytogenetic abnormality other than Bcl-1 rearrangement might be implicated in the pathogenesis of these cases. Nonetheless, further investigations of this negative group may declare a subset of patients considered for different therapeutic approaches. Cyclin D1 positivity was the most independent prognostic factor of poor survival in MCL in our study. This was consistent with results by Hashimoto et al. [43] and Yatabe et al. [13]. Expression of cyclin D1 in a large series of MCL patients and its relation to bad prognosis and poor survival could be a base for future therapeutic regimens for MCL targeting cell cycle regulatory mechanisms and factors including cyclin D1 and cyclin dependant kinases (cdks). Actually Hahntow et al. [44] reported a new therapeutic regimen by a new class of cell cycle inhibitors entering clinical trials for CLL cases. These drugs exert their activity by inhibition of cdks and induce cell cycle arrest and apoptosis in cancer cells. This may be precisely valuable in MCL due to its strong relation to cyclin D1 [34]. From this study we can conclude that, Cyclin D1 is not only implicated in tumor genesis of MCL but also in progression and extension of the disease when expressed in high levels (50% cut off value). This can be used as a basis for future therapeutic strategies targeting cell cycle regulators. Therefore, it seems that

Cyclin D1 and CD23 immunostaining are reliable diagnostic tools for discrimination between B-CLL/SLL and MCL. Moreover, Cyclin D1 overexpression seems to have prognostic impact in MCL.

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