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A Flow Cytometric Scoring System in the Diagnosis of Mantle Cell Lymphoma

ORIGINAL INVESTIGATION

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ABSTRACT

Objective: Our aim was to evaluate the contribution of flow cytometry (FC) analysis in patients with a histopathological diagnosis of mantle cell lymphoma (MCL) and to develop a reliable scoring system.

Materials and Methods: We assessed the results of FC analysis in peripheral blood or bone marrow samples of 36 patients diagnosed with MCL by histopathological evaluation. Multivariate analysis identified 5 variables associated with MCL: CD23 negativity, CD5/19 common positivity, CD20/22 common bright positivity, FMC7 positivity, and clonal sIg bright positivity. A 5-point scoring system was devised using these parameters.

Results: Twenty-five patients (69.4%) scored 5 points, 8 (22.2%) scored 4 points, and 3 (8.4%) scored 3 points. The diagnosis of MCL was confirmed by FC immunophenotyping in 91.6% of the patients.

Conclusion: Our results suggest that a quintet diagnostic scoring system, which is considered to be reliable in the diagnosis of MCL, could make a substantial contribution to diagnosis and should be more widely introduced in practice.

Keywords: Flow cytometry, mantle cell lymphoma, scoring system

INTRODUCTION

Mantle cell lymphoma (MCL) is classified in the B-cell lymphoma group; the genetic marker of the disease t(11;14) translocation leads to the overexpression of cyclin D1, which plays an important role in the pathogenesis of MCL. The incidence rate is approximately 0.2 to 0.3 individuals per 100,000 person-years. The median age at diagnosis is approximately 60 years. Patients usually present with lymphadenopathy, commonly with the involvement of the bone marrow, spleen, and gastrointestinal tract. Patients with MCL have a much worse prognosis than those with other lymphoproliferative disorders (LPDs). Because flow cytometry (FC) has started to be used for the identification of acute and chronic leukemia, it has become an increasingly used immunophenotyping technique in the assessment of chronic LPDs. However, a misleading condition can occasionally be observed in the differential diagnosis between MCL and other LPDs. In this context, Matutes et al. (1) proposed a scoring system using immunophenotyping for the differential diagnosis of LPDs in 1994. The authors focused on differentiating MCL from LPDs, particularly from chronic lymphocytic leukemia (CLL). Classical CLL is distinguished from MCL on the immunophenotype as it usually expresses CD23, has weak cell surface expression of immunoglobulin (sIg), and lacks the expression of FMC7 and CD22. These markers were subsequently revised (with the replacement of CD22 by CD79b) by Moreau et al. (2). MCL most commonly has the phenotypes CD5+, CD23−, FMC7+, CD79b+, and sIg strong positive (3). However, who could not achieve full points in the scoring systems were considered as having atypical LPD (4). For instance, the prevalence of CD23+ MCL has been reported as 15–55% (5-7), while there are a considerable number of CD23− CLL patients (8). As confusion in immunophenotypic scoring remained, Medd et al. (9) proposed a scoring system that included 4 markers, platelet count, CD20 and CD38 positivity, and CD23 negativity, in 2011. Subsequently, Juncà et al. (10) reported a new quartet scoring system that included CD11c negativity instead of platelet count. Herein, our aim was to investigate the contribution of FC with the most reliable markers for the diagnosis of patients with histopathologically proven MCL and to select a quintet scoring system.

MATERIALS and METHODS

Patient specimens

We evaluated the results of FC analysis in the peripheral blood and bone marrow samples of 36 patients who presented to the Hematology Unit of Erciyes University, School of Medicine and who were diagnosed as having MCL by histopathological evaluations between January 2011 and January 2013. FC was performed at the time of initial diagnosis before treatment. The diagnosis of MCL was made by evaluating biopsy materials obtained from

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the lymph node, bone marrow, and other tissues (lung, muscle, stomach, intestines, etc.). Written informed consent was obtained from the patients. In all patients, the diagnosis was confirmed by demonstrating positive cyclin D1 expression in immunohistochemical analysis in formalin-fixed, paraffin-embedded tissue. A fluorescent in situ hybridization (FISH) test (LSI IGH/CCND1 XT Dual color, Dual fusion Translocation Probe, Abbott/Vysis, UK) was performed to detect t(11;14) (q13;q32) in all patients' peripheral blood at the time of diagnosis.

Flow cytometry analysis

Flow cytometry analysis was performed on cell suspensions obtained from the peripheral blood or bone marrow specimens. A combination of 4- and 6-color immunophenotype analyses were performed using a FACSCanto II flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA). Peripheral blood and bone marrow specimens were incubated for 15 min after staining with a monoclonal antibody. Then, the samples were incubated for 10 min in 10% ammonium chloride in the dark. The combinations of fluorescein isothiocyanate (FITC), phycoerythrin (PE), peridinin chlorophyll protein, PECY-7, allophycocyanin, and APCCY-7 fluorochrome were used for the antibodies. The target antigens and monoclonal antibody clones included HLA DR, CD45, CD10 (HI10a), CD5 (L17F12), CD19 (SJ25C1), CD20 (L27), CD22 (S-HCL-1), CD23 (EBVCS-5), FMC7, kappa (TB28-2), and lambda (1-155-2). All antibodies were purchased from BD Biosciences. After centrifugation of a cell suspension, the supernatant was removed and the specimen was washed with washing solution twice. Pellets were re-suspended in 0.5 mL of specimen, and upon the acquisition of 25,000 lymphocytes per tube, all collected events were analyzed using the BD Diva software. Cell populations of interest were gated using either CD45/side-scatter or CD19/side-scatter dot plots. A daily calibration was performed using BDFACS 7-color setup beads (BD Biosciences). Isotypes (IgG1-FITC and IgG2-PE) were used as the negative controls.

The diagnostic scoring criteria were defined as follows: CD 5/19 common positivity, CD 23 negativity, CD 20/22 common bright positivity, FMC7 positivity, and clonal sIg bright positivity. The fluorescence intensity was measured using a logarithmic scale with signal intensity ranging from 10^0 to 10^4. A weak expression was defined when majority of the population was in the 1st log percentile above the control, whereas a strong expression was when majority of the population was in the 2nd log percentile or higher above the control. For the results of the analysis, 10^0–10^1 was defined as weak positivity, 10^1–10^2 as moderate positivity, and $>10^2$ as bright positivity.

Statistical analysis

To test data normality, Shapiro–Wilk's test was used, and the histogram and q-q plots were examined. Chi-square analysis was used to compare differences between the categorical variables, while an independent sample t test and Mann–Whitney U tests were used for continuous variables. The Spearman rank test was used for correlation analysis. The values were expressed as frequencies, percentages, mean and standard deviation or median, and the 25th–75th percentiles. Statistical analyses were performed using IBM SPSS Statistics 21.0 (SPSS Inc.; New York, USA) software. $P<0.05$ was considered statistically significant.

RESULTS

There were 36 patients diagnosed as MCL histopathologically. Cyclin D1 was positive in all patients. Of the patients, 20 were men (55.6%) and 16 were women (44.4%), with a mean age of 61.5±10.5 years (range: 32–85 years). Table 1 lists the laboratory data of the patients. In majority of the patients, diagnosis was made not only by bone marrow biopsies (19 patients) but also by biopsies of the lymph nodes (10 patients) or other tissue types, such as lung, gastrocnemius muscle, skin, bowel, stomach, and nasopharynx (7 patients). FISH t (11;14) testing was positive in 24 patients (66.6%).

Flow cytometry analysis was performed in 17 peripheral blood (47.2%) and 19 bone marrow (52.8%) samples. In the FC analysis, 25 patients (69.4%) met all criteria with 5 points. Figure presents the results of the FC analysis in a patient who achieved 5 points (Figure 1). In addition, 8 patients (22.2%) scored 4 points, while 3 patients (8.3%) scored 3 points. Of the 8 patients who scored 4 points, there was no CD5/19 common positivity in 4, while negative FMC7 and positive CD23 were detected in 2 patients. Of 3 patients who scored 3 points, there was weak CD23 positivity but no CD5/19 common positivity in 1 patient. In another patient, there was weak CD23 positivity but no CD20/22 bright positivity. In addition, FMC7 was negative and there was no clonal sIg bright positivity in the remaining patient who scored 3 points (Table 2).

The diagnosis of MCL had been confirmed in 25 patients who scored 5 points in diagnostic scoring system. Eight patients were considered as having atypical MCL, and 3 who scored 3 points were interpreted as having unclassified lymphoma according to FC analysis. Thus, the diagnosis of MCL was confirmed in 33 patients (91.6%) who scored 4 or 5 points by FC scoring system. When data of the remaining 3 patients were evaluated, it was found that the diagnosis of MCL was made by the histopathological evaluation of biopsy materials obtained from bone marrow, nasopharynx and gastrocnemicus muscle. The statistical analysis in these 3 patients was similar to those who scored 4 or 5 points, with the lower FMC7 being the exception. In the analysis of peripheral blood samples, it was seen that of 17 patients, 13 (76.5%) scored 5 points, while 3 (17.6%) scored 4 points and 1 (5.9%) scored 3 points. In the analysis

Table 1. Laboratory data of patients

Variables	Median (range)	Reference ranges
Hb (g/dL)	12.0 (10-14)	12-18
WBC (10^9/L)	16.0 (5.6-45.0)	4.8-10.8
Plt (10^9/L)	245 (168-380)	130-400
BUN (mg/dL)	21 (14-45)	7.9-21
Cre (mg/dL)	0.9 (0.64-1.88)	0.84-1.44
AST (u/L)	33 (24-57)	0-35
ALT (u/L)	40 (27-65)	0-45
LDH (u/L)	275 (148-396)	140-280

Hb: hemoglobin; WBC: white blood cells; Plt: platelet count; BUN: blood urea nitrogen; Cre: creatinine; AST: aspartate transaminase; ALT: alanine transaminase; LDH: lactic dehydrogenase

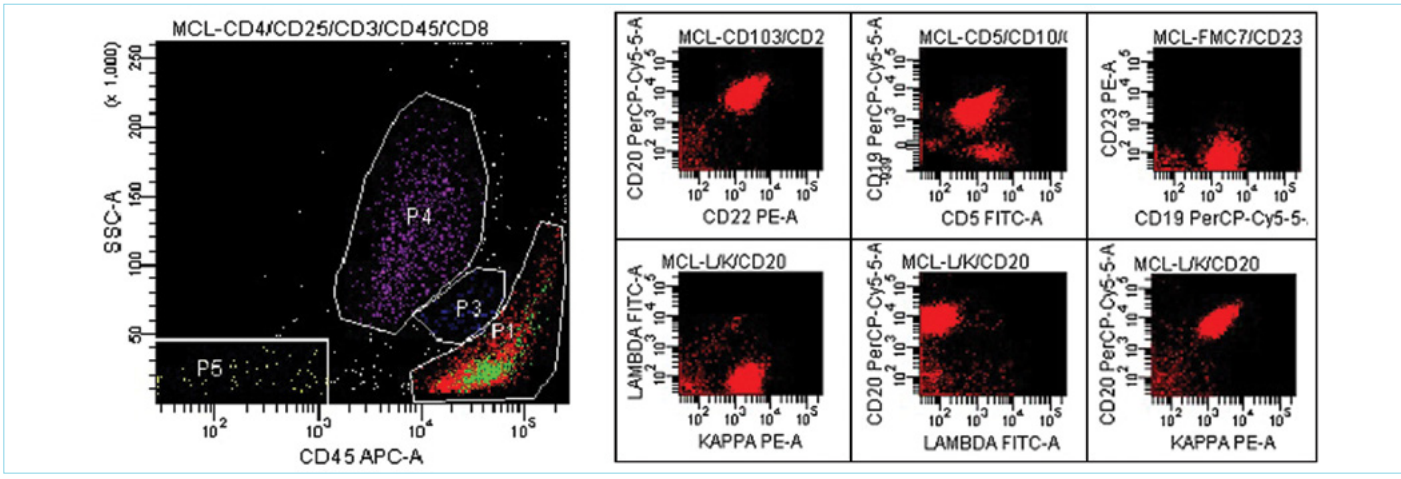

Figure 1. Flow cytometric features of a patient with MCL
MCL: mantle cell lymphoma

Table 2. Results of flow cytometric analysis

	Diagnostic score		
	5	**4**	**3**
CD5/19 common positive patients	25	4	2
CD23 negative patients	25	6	2
CD20/22 common bright positive patients	25	8	2
FMC7 positive patients	25	6	1
Clonal sIg bright positive patients	25	8	2
Diagnoses	**MCL**	**Atypical MCL**	**Unclassified Lymphoma***

MCL: mantle cell lymphoma

*Of 3 patients who scored 3 points, there was weak CD23 positivity but no CD5/19 common positivity in 1. In another patient, there was weak CD23 positivity but no CD20/22 bright positivity. In addition, FMC7 was negative, and there was no clonal sIg bright positivity in the remaining patient who scored 3 points.

of bone marrow samples, it was seen that of 19 patients, 12 (63.2%) scored 5 points, while 5 (26.3%) scored 4 points and 2 (10.5%) scored 3 points. No significant finding was obtained by the results of analysis performed in either bone marrow or peripheral blood.

In the statistical analysis, no significant differences were detected between groups regarding age, gender, CD5, CD19, CD20, CD22, CD23, and Kappa and Lambda values. Also, it was found that FMC7 values were significantly lower in the group including patients who scored 3 points when compared to those who scored 4 or 5 points (p=0.027; Fisher's exact test). However, it was found that age, platelet count and Hb values in the group including patients who scored 3 points were similar to those who scored higher points. Using the Mann–Whitney U test and Fisher exact test, no significant difference other than FMC7 values were detected between the groups. In conclusion, the diagnosis was confirmed by FC in 91.6% of the patients who were diagnosed as histopathologically proven MCL.

DISCUSSION

In the present study, we aimed to select a quintet scoring system using FC with the most reliable markers in patients with a histopathological diagnosis of MCL. Most studies in the literature generally address quartet scoring systems, but there is a contradiction regarding reliable markers (9, 10). However, it has been reported that CD23 negativity and CD20 strong expression are the most reliable markers (11). In our study, we did not only assess CD20 positivity, but also the common and strong expression of CD20/22. In the diagnosis of MCL, CD20/22 common bright positivity is an important marker in addition to CD23 negativity. This finding allows discrimination of MCL from CLL in some part, since CD20/22 common positivity can be observed in CLL, but it leads to a weak florescent intensity.

In fact, there are numerous markers that could be used in the immunophenotypic diagnosis of MCL. In a study by Junca et al. (10), CD38 positivity and CD11c negativity were particularly emphasized and these markers may have a major role to play in the diagnosis of MCL. However, as cost is an important issue in our healthcare system, we have to use the smallest number of markers in FC analysis as possible. Thus, we selected a scoring system by identifying 5 markers considered to be the most reliable ones in the diagnosis of MCL. We think that this scoring system should enable accurate diagnosis with feasible costs. The antibodies used in the study allowed us to discriminate MCL from both CLL and other LPDs.

The availability of immunohistochemical markers has considerably improved the results of diagnosis in the last few years (12, 13). Due to the presence of variable phenotypes of MCL, there is no consensus on the selection of specific markers. For example, there are CD5+/CD10– MCL as well as CD5+/CD10+ MCL patients. Moreover, there may be CD5–/CD10– MCL patients. Similarly, the FMC7 marker, which is considered to be important in the diagnosis of MCL, may be negative in some MCL patients as seen in the unclassified group that scored 3 points in our study (14). In our study, CD5 negativity was detected in 5 patients (13.9%). In the literature, there are CD5– MCL patients (10–30%), which is consistent with our findings (15-18).

The t(11;14) translocation by FISH, which is accepted as the gold standard in the diagnosis of MCL, was found to be positive in 66.6% of our patients (19-22). Medd et al. (9) addressed the differential diagnosis of FISH-negative MCL patients in addition to MCL patients confirmed by FISH positivity. The authors emphasized that other LPDs, such as atypical CLL or splenic lymphoma, should be considered in the differential diagnosis. As our study included patients with MCL confirmed by histopathological evaluation, we think that FISH negative patients did not affect the results. In fact, the finding of FISH negativity in 33.3% of the patients is consistent with that in the literature.

CONCLUSION

The confirmation of the diagnosis of MCL in 91.6% of the patients by a quintet scoring system is the key success point of our study. In MCL, diagnosis and in particular, differential diagnosis are extremely important as it has a much worse prognosis than other LPDs. In this context, the contribution of an FC scoring system, which is a convenient and practical method, becomes increasingly important in the diagnosis of hematological malignancies and should not be neglected. By developing an international scoring system, MCL can be diagnosed, and its management can be initiated as soon as possible. In conclusion, we think that diagnostic scoring systems for MCL should be standardized and generalized for daily practice.

Ethics Committee Approval: Ethics committee approval was received for this study.

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Author Contributions: Conceived and designed the experiments: MK, MYK, SS, AU. Performed the experiments: MK, DC, BE. Analyzed the data: SK, LK. Wrote the paper: MK, MC. All authors have read and approved the final manuscript.

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