

ORIGINAL ARTICLE

Utility of flow cytometric κ and λ light chain analysis of peripheral blood

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Summary

Purpose: Monoclonality in the peripheral blood can be shown by flow cytometric analysis of kappa (κ) and lambda (λ) light chain ratio of B lymphocytes. We aimed to show the utility of this method in patients with unknown causes of lymphadenopathy and/or splenomegaly.

Methods: This method was performed in 81 adult patients with undefined causes of lymphadenopathy and splenomegaly.

Results: 18 (22%) of these patients had clonality and all of them were diagnosed as B cell lymphoma later. None of the patients with benign causes had clonality in the peripheral blood. We could not find any relationship between presence

of clonality and type and stage of lymphoma and bone marrow involvement.

Conclusion: This method is easy to perform, cheap and non-invasive and yet it can give valuable information about the malignant nature of a suspected disease. If there is a sign of clonality in the peripheral blood, more invasive diagnostic procedures should be performed rather than watch and wait.

Key words: flow cytometry, kappa, lambda, light chain, lymphoma

Introduction

Non Hodgkin Lymphomas (NHL) arise from a clonal lymphoid cell. Mature B cell expresses either immunoglobulin light-chain κ or λ . CD19 is a pan B cell surface marker. The normal ratio of κ / λ expression on CD19 positive B cells is 2/1 [1-3]. If the lymphoma has clonal B cell origin, the lymphoid tissue shows an altered ratio of κ / λ . Abnormality in κ / λ ratio of peripheral B cells indicates the presence of lymphoma cells in the peripheral blood [4,5]. An altered κ and λ immunoglobulin light chain ratio was used as an indicator of monoclonality in other studies [6-8] and a number of authors reported that the altered of κ / λ ratio, dual expression of κ and λ light chains and lack of expression of κ and λ on CD19 positive B cells is

a sign of malignancy [9,10]. Immunophenotypic analysis of peripheral blood by using flow cytometry can be an useful tool to distinguish malignant B cell proliferation from benign causes.

Sobot et al. published a paper back in 1985, suggesting that clonality found by flow cytometry can be used as a tool for detection of minimal residual disease [1]. They also found a correlation between morphological bone marrow involvement and peripheral B cell clonality.

There are few studies in the literature about the utility of flow cytometric analysis of B cell light chains, but the positive and negative predictive value of the test remains unclear [8,9,11].

We aimed to show the usefulness of flow cyto-

metric analysis of light chain expression of CD19 positive B lymphocytes of the peripheral blood in patients with splenomegaly and lymphadenopathy of unknown causes.

Methods

Results of κ and λ analysis of CD19 positive B lymphocytes with flow cytometry performed at the Osmangazi University Hematology Laboratory between January 2010 and November 2014 were evaluated. The medical files were evaluated for κ and λ analysis and the final diagnosis of the patients. If the patients were diagnosed as B cell lymphoma, the stage of disease according to Ann Arbor staging system and the status of bone marrow involvement were recorded.

Statistics

Statistical tests were performed by using IBM SPSS for Windows 20.0 software package. P values < 0.05 were considered significant. The Shapiro-Wilk test was performed for testing normality and the x² test was used to compare categorical variables.

Flow cytometric procedure

Three-color flow cytometric immunophenotypic analysis was performed using fluorescein isothiocyanate (FITC), phycoerythrin (PE) and peridinin chlorophyll protein (PerCP) conjugated antibodies to κ, λ and CD19 respectively (Beckman Coulter, Fullerton, CA). Samples of EDTA anti-coagulated whole blood were obtained, white blood cells (WBC) and the concentration was adjusted < 20 million cells per milliliter. Two ml of the specimen were washed twice in phosphate-buffered saline wash buffer (Sigma-Aldrich, ST Louis, MO) and reconstituted to the original volume in the wash buffer. One hundred and fifty to 200 mcL of the cell suspension were added to 12x75 mm BD Falcon polystyrene staining tubes (BD Biosciences) containing 10 mcL of FITCκappa, PEλambda and PerCP19 antibodies. The samples were incubated for 15 min in the dark at room temperature. Then 2ml FACSlyse (BD Biosciences) were added and the specimens were incubated again in the dark at room temperature for 10 min. Specimens were centrifuged for 5 min at 1000g and the supernatant was discarded and then 100 mcL of wash buffer were added to each tube. 60 000 events per tube were acquired using a Becton Dickinson FACSCalibur flow cytometer and saved into an FCS 2.0 data file.

Interpretation of flow cytometry results

Patients with the following three flow cytometry patterns were reported as clonal:

1. Abnormal κ/λ ratio of CD19 positive B cells: CD19 and κ positive / CD19 and λ positive >3 and <0.5 were considered abnormal (Figure 1).

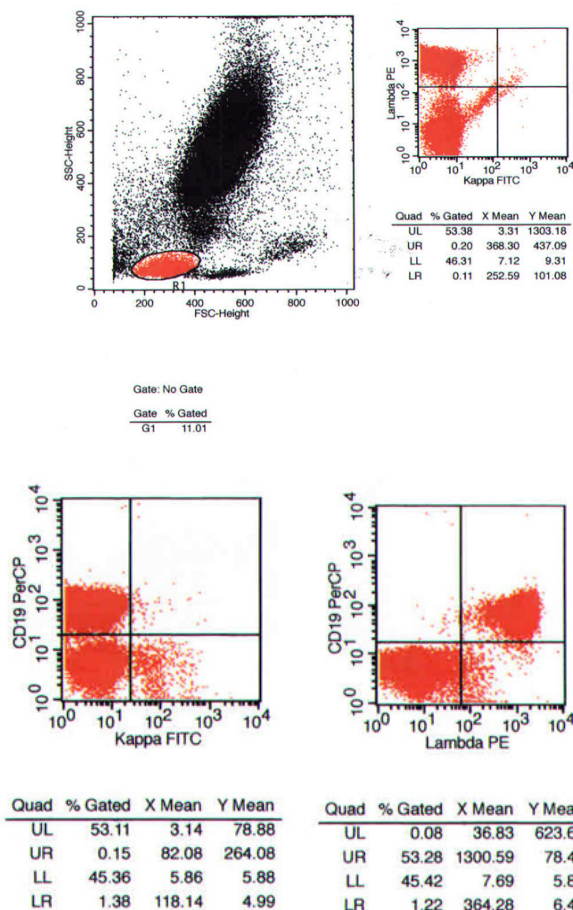


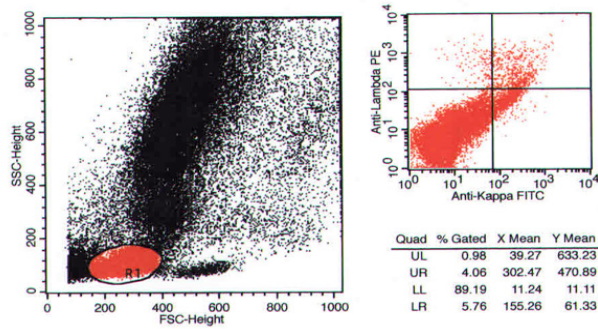
Figure 1. Lambda clonality of peripheral CD19 positive B cells.

- Dual positivity of κ and λ on CD19 positive B cells: if the sum of CD19 and κ positive and CD19 and λ positive B cells was > 10% of the total number of B cells, this was called as “dual positivity” (Figure 2).
- Lack of both κ and λ expression on CD19 positive B cells: if the light chain expression was present in < 5% of the total gated B lymphocytes, the condition was called “lack of expression” (Figure 3).

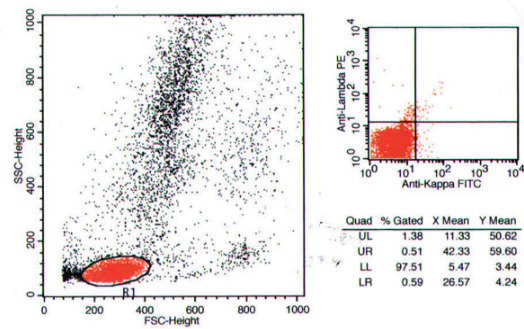
Results

Eighty one κ and λ analyses were done by flow cytometry between January 2010 and November 2014. Forty seven of these patients were male and 34 were female. Mean age was 57±15 years.

All patients had light chain analysis performed because they had either lymphadenopathy or splenomegaly or both of unknown causes. From the medical records of these patients, we identified three main indications for the test order: 1. There were no lymph nodes that could be easily removed; 2. The patient was unwilling to



Gate: No Gate
 Gate: % Gated
 G1 25.77



Gate: No Gate
 Gate: % Gated
 G1 60.71

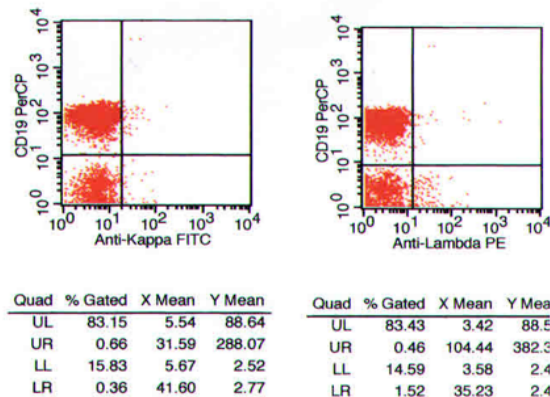
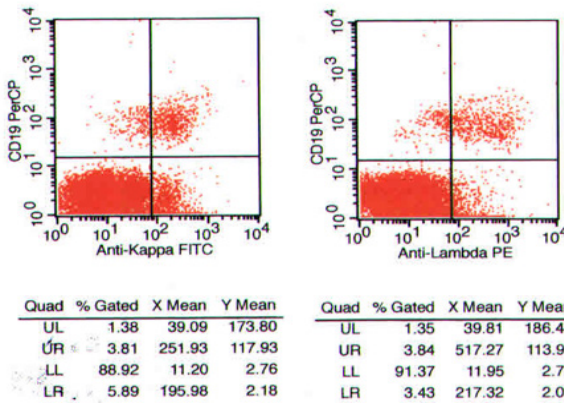


Figure 2. Dual clonality of peripheral CD 19 positive B cells.

Figure 3. Lack of light chain expression on peripheral CD 19 positive B cells.

give consent to invasive procedures; 3. Etiology for splenomegaly could not be identified and diagnostic splenectomy was planned. Three patients with isolated splenomegaly and B cell clonality in the peripheral blood were offered splenectomy and diagnosed as B cell non-Hodgkin's lymphoma (NHL) later.

Among these 81 patients, 18 (22.2%) had clonality of B cells in the peripheral blood. Nine of them had κ and 6 had λ clonality. One of the patients had clonal B cells which expressed both light chains (dual expression) and 2 had "lack of expression". None of the 41 patients who had be-

nign causes of lymphadenopathy or splenomegaly had clonality in the peripheral blood. Five patients had other malignant causes than B cell NHL: 2 classic Hodgkin's lymphoma and 3 T cell lymphoma. Neither of these 5 patients had B cell clonality by flow cytometry either (Table 1).

Thirty five patients were diagnosed as B cell NHL and 18 (51.4%) of them had clonality. In cases with clonality, clonal light chain type was the same in the peripheral blood and in the lymphoid tissue.

Among 18 patients with clonality, 11 (61.1%) had bone marrow involvement and 15 (83.3%) had

Table 1. Flow cytometry results

Diagnosis	Kappa clonality N(%)	Lambda clonality N(%)	Dual expression N(%)	Lack of expression N(%)	No clonality N(%)
B cell NHL *	9 (11)	6 (8)	1 (1)	2 (2)	17 (21)
HL or T cell NHL*					5 (6)
Benign causes *					41 (51)

*Number of patients, HL: Hodgkin's lymphoma, NHL: Non-Hodgkin's lymphoma

Ann Arbor stage III or IV disease. We could not find statistically significant relationship between the presence of clonality and the bone marrow involvement or the stage of the lymphoproliferative disease. Only one of the patients with bone marrow involvement had lymphocytosis in the peripheral blood (Figure 3).

Discussion

Results by simple flow cytometry showing circulating monoclonal B cells in NHL had been published long time ago [4,5,11]. Since then there are no reports mentioning the diagnostic importance of this test. κ and λ analysis by flow cytometry seems to be an "old fashioned" test, that is done and forgotten. We believe that it is not the case.

Our study was carried out in patients with unproven but suspected lymphoma in contrast to the other reports in the literature [4,5,11,12]. Johnson et al. considered κ / λ ratio range 4-0.5 and Sobol et al. used κ / λ ratio exceeding 3 or below 0.06 as a sign of clonal proliferation [1,8]. We considered the ratio as clonal if it was either over 3 or below 0.5. We defined dual expression according to Xu's excellent review about the subject [10]. We saw benign cases showing dual positivity of κ and λ in a small percentage of CD19 positive cells so we think dual positivity that is > 10% of the CD19 positive cells is critical. According to Kaleem et al., if the light chain expression is present in < 5% of the total gated B lymphocytes there is a "lack of expression" [9]. We found a few cases with lack of expression and dual positivity, but these entities should always be kept in mind for not to miss any clonal case.

If there was clonality by flow cytometric anal-

ysis of B cells, the positive predictive value of the test was 100%. It was also specific to B cell NHL. Hodgkin's lymphoma and T cell lymphomas did not show clonality, as expected.

We found clonality in 51.4% of B cell NHL cases. This may not be the true incidence of circulating B cell clonality in NHL since we did not perform the test in every consecutive lymphoma patient. Sobol et al. found 43% B cell clonality by flow cytometric method in their study [1].

There are some strong reports indicating that there is an association between clonality and disseminated disease and the presence of clonality can be used as a tool for monitoring B cell NHLs [1,8]. We were unable to show such a relationship between stage and type of lymphoma. Interestingly -although there was no lymphocytosis in the peripheral blood- there was clonality in flow cytometry and bone marrow involvement in 11 (13%) patients. This finding suggests that flow cytometric analysis of κ and λ light chains is more sensitive than conventional hematologic methods.

κ and λ analysis is useful for distinguishing malignant causes from benign ones. It is also useful to differentiate lymphomas arising from B cells from other cell lines. This relatively cheap to perform and non-invasive test can be done before PET scan or biopsies requiring open thoracotomy or laparoscopic investigation of the abdominal cavity. We also find κ and λ analysis very useful for patients with isolated splenomegaly. In these patients the only way to diagnose the patient is to perform splenectomy. If the patient has clonality in the peripheral blood it is much more easy to convince the patient and the surgeon about the procedure.

References

1. Sobol RE, Dillman RO, Collins H et al. Applications and limitations of peripheral blood lymphocyte immunoglobulin light chain analysis in the evaluation of non-Hodgkin's lymphoma. *Cancer* 1985;56:2005-2010.
2. Garrett JV, Scarffe JH, Newton RK. Abnormal peripheral blood lymphocytes and bone marrow infiltration in non-Hodgkin's lymphoma. *Br J Haematol* 1979;42:41-50.
3. Grey HM, Rabellino E, Pirofsky B. Immunoglobulins on the surface of lymphocytes. IV. Distribution in hypogammaglobulinemia, cellular immune deficiency, and chronic lymphatic leukemia. *J Clin Investig* 1971;50:2368-2375.
4. Ault KA. Detection of small numbers of monoclonal B lymphocytes in the blood of patients with lymphoma. *N Engl J Med* 1979;300:1401-1405.
5. Ligler FS, Smith RG, Kettman JR et al. Detection of tumor cells in the peripheral blood of nonleukemic patients with B-cell lymphoma: analysis of "clonal excess". *Blood* 1980;55:792-801.
6. Witzig TE, Banks PM, Stenson MJ et al. Rapid immunotyping of B-cell non-Hodgkin's lymphomas by flow cytometry. A comparison with the standard frozen-section method. *Am J Clin Pathol* 1990;94:280-286.
7. Wongchaowart NT, Rinehart DG, Hsi ED. The correlation dimension identifies B-cell immunoglobulin light chain restriction in peripheral blood flow cytometry data. *Am J Clin Pathol* 2006;125:600-607.

8. Johnson A, Cavallin-Stahl E, Akerman M. Flow cytometric light chain analysis of peripheral blood lymphocytes in patients with non-Hodgkin's lymphoma. *Br J Cancer* 1985;52:159-165.
9. Kaleem Z, Zehnbauer BA, White G et al. Lack of expression of surface immunoglobulin light chains in B-cell non-Hodgkin lymphomas. *Am J Clin Pathol* 2000;113:399-405.
10. Xu D. Dual surface immunoglobulin light-chain expression in B-cell lymphoproliferative disorders. *Arch Pathol Lab Med* 2006;130:853-856.
11. Smith BR, Weinberg DS, Robert NJ et al. Circulating monoclonal B lymphocytes in non-Hodgkin's lymphoma. *N Engl J Med* 1984;311:1476-1481.
12. Geary WA, Frierson HF, Innes DJ et al. Quantitative criteria for clonality in the diagnosis of B-cell non-Hodgkin's lymphoma by flow cytometry. *Mod Pathol* 1993;6:155-161.