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Statistical Analysis of ZAP-70 And CD38 Expression in Chronic B-cell Leukemia Patients

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Chronic lymphocytic leukemia (CLL) cells with unmutated immunoglobulin heavy chain gene (U-IGHV) differs from those with mutated IGHV (M-IGHV) in the expression levels of a relatively small subset of genes. It is observed heterogeneous clinical behavior of patients with chronic lymphocytic leukemia according to gene expression profile: a) indolent disease and a lack of disease-related complications for a long period; b) progressive and/or symptomatic disease which requires therapy relatively soon after the diagnosis. Most clinically relevant markers, which can be used as a surrogate marker for expression of U-IGHV are zeta-chain associated protein of 70 kDa (ZAP-70) and CD38. The aim of our study is to evaluate whether expression profiles of these markers differ in different countries. Based on our results we concluded that ZAP-70 and CD38 have differential expression.

Key words: CLL, U-IGHV, M-IGHV, ZAP-70.

Introduction

The clinical behavior of patients with chronic lymphocytic leukemia (CLL) is heterogeneous [1]. Some patients have indolent disease and lack of disease-related complications for many years; others develop progressive and/or symptomatic disease requiring therapy within in a relatively short time after diagnosis. Early treatment of the CLL could put patients at risk for therapy-related complications that might compromise their quality of life and/or survival [4].

Traditional staging systems could not distinguish an aggressive disease course from the good prognosis in patients.

In our research we are focusing on the new and most clinically relevant markers: zeta-associated protein 70 (ZAP-70) and CD38 expression. Gene expression analyses found out that CLL cells with unmutated immunoglobulin heavy chain gene (U-IGHV) are different from CLL cells with mutated IGHV (M-IGHV) in the expression levels of a relatively small subset of genes, one of which encodes the zeta-chain associated protein of 70 kDa [4]. The same finding was revealed for U-IGHV leukemia-cell which are known to express CD38. In this respect, ZAP-70 and CD38 expression analyses

can provide complementary prognostic information identifying three patient subgroups with good, intermediate and poor prognosis [3].

Comparing two markers, ZAP-70 has been emerged as the most promising surrogate marker for the IGVH mutation status. However, the combination of ZAP-70 and CD38 increases the prognostic power than usage each separately.

Most patients with CLL that express U-IGHV and/or CD38, but lack expression of ZAP-70, will not require therapy by current criteria for many years after diagnosis [1, 3].

Patients who have CLL cells with M-IGHV, apparently lack leukemia-cell expression of ZAP-70. Such patients have a relatively indolent clinical course and might not have the same risk-benefit ratio with early therapy as patients with CLL cells that express ZAP-70, who on average require therapy within 3 years after the diagnosis [4].

Prognostic predictions in B-cell chronic lymphocytic leukemia (B-CLL) at early clinical stage are based on biological disease parameters, such as ZAP-70 and CD38 protein levels, genomic aberrations as well as IGVH mutation status. The levels of ZAP-70 and CD38 stays sustainable over the time in the majority of patients [2].

The use of these prognostic markers could not obviate clinical monitoring for other features associated with disease progression, such as lymphocyte doubling time, progressive lymphadenopathy, measurement of beta-2-microglobulin levels, or development of disease-related symptoms or disease-associated cytopenia [4, 7].

Average treatment-free survival time in patients whose leukemic cells were ZAP-70(+)/CD38(+) was 30 months compared to 130 months in patients with a ZAP-70(-)/CD38(-) status. In patients with discordant ZAP-70/CD38 results, the average treatment-free survival time was 43 months [6, 7].

The aim of our study was to estimate the difference of expression profiles of these markers in different countries.

Materials and Methods

To evaluate expression rate of ZAP-70 and CD38 markers we applied flow cytometry and immunocytochemistry in 320 B-CLL patients.The median age of the patients while diagnosis was 56 (42-96years). We performed flow cytometry and Immunocytochemistry to detect expression of ZAP-70 and CD38. CLL cells also were analyzed for CD19, CD20, CD23, and CD38, using monoclonal antibodies (mAbs) conjugated to allophycocyanin (APC), peridinin-chlorophyll-protein (PerCp), fluorescein isothiocyanate (FITC), or phycoerythrin (PE) (Becton Dickinson, Pharmingen, Dako). ZAP-70 expression is thought to be positive if the percentage of CLL cells expressing ZAP-70 is greater than 20% and the optimal threshold for CD38 (+) equals 35% or more of the CLL cells.

We performed comparison of expression profiles of two prognostic markers in different countries: Germany, USA, Egypt, India and Georgia [1, 4, 5, 6, 8, 9].

Results and Discussion

We revealed, that the percentage of both ZAP-70/CD38 negative expression levels were close in all three countries: Germany, Egypt and Georgia: 47,6%, 48,0% and 49,5 % respectively. Both ZAP-70/CD38 positive expression levels appeared highest in India (56,0%) compared to other countries as Germany (23,4%), USA, Mississippi (35,3%), Georgia (35,3%) and Egypt (48,0%). This fact also was mentioned by Indian researchers, however they did not give explanation [5]. Discordant expression of these two markers showed lowest levels in Georgia (17,2%) compared to Germany (29,0%)

and Egypt (42%). In our opinion it was caused by technical aspects of estimating marker expression. According to some authors FCI (Flow Cytometry) technique is sensitive, more objective and quantitative in comparison with microscopic detection of manual absorbance-based enzyme immunohistochemistry products [10]. We started to use flow cytometry later then immunocytochemistry, after that we revealed, flow cytometry was more sensitive and precise comparing to immunocytochemistry.

Immunocytochemistry expression of CD38 (total expression level is negative) and ZAP-70 (total expression in CLL cells is positive) is shown on figures 1 and 2, respectively (**Figs. 1, 2**).

Results from flow cytometry were shown on figure 3 and 4. It was demonstrated CD 38 positive cell percentage on CD19(+)CLL cells (**Fig. 3**) and ZAP-70 expression level estimated on CD20(+)CLL cells (**Fig. 4**)

As it was shown above data for these marker expressions differ between countries.

With the respect of value of prognostic markers we included ZAP-70(+)/CD19(+)/CD5(+) and percentage of CD38(+)/CD19(+)/CD5(+) B-CLL cells into a routine diagnostic B-CLL panel to predict outcome.

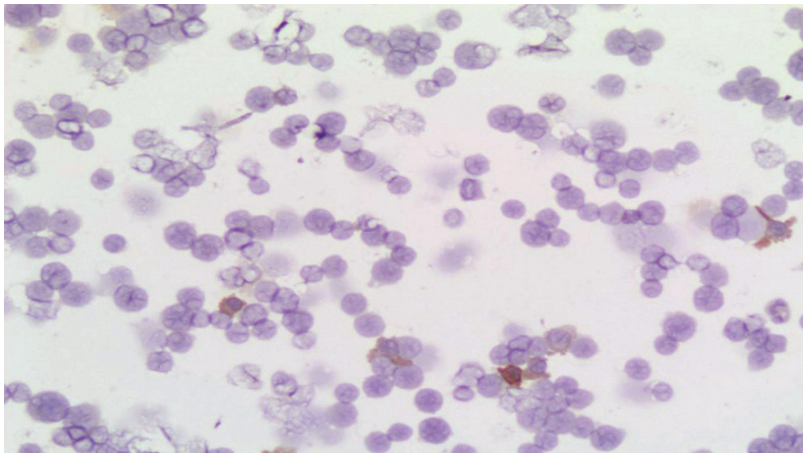

Fig. 1. Expression of CD38 by immunocytochemistry

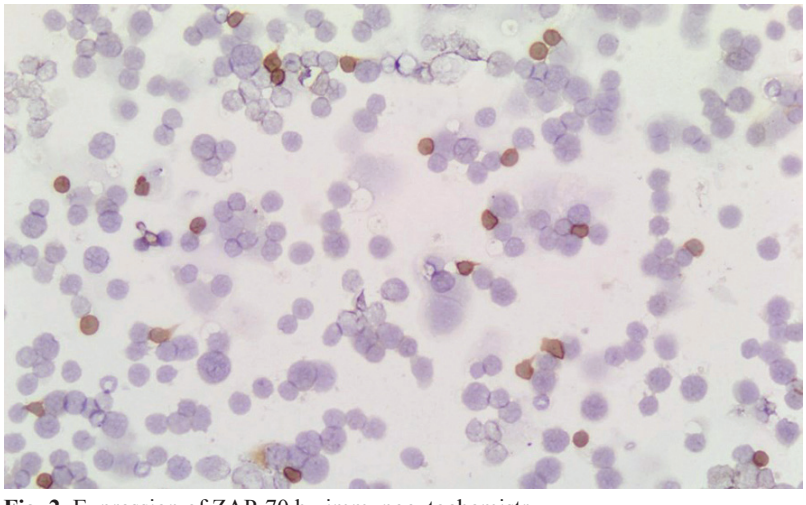

Fig. 2. Expression of ZAP-70 by immunocytochemistry

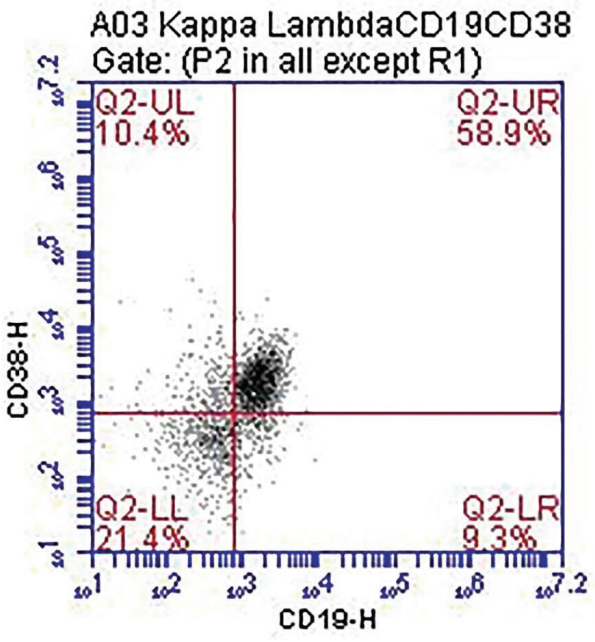

Fig. 3. Flow Cytometry image of CD19/CD38 expression

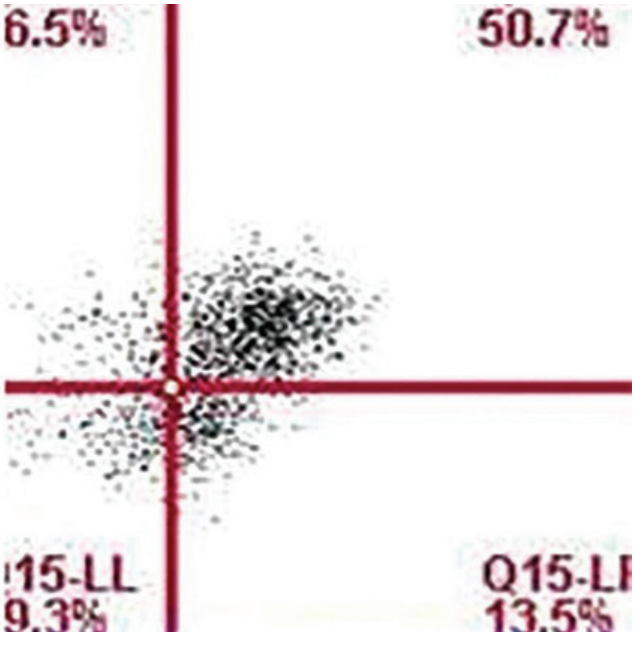

Fig. 4. Flow Cytometry image of CD20/ZAP-70 expression

Clinical studies are in progress to evaluate the potential benefit of early therapy in newly diagnosed patients who have adverse prognostic markers. Such studies might determine whether patients at high risk for early disease progression benefit from therapy administered soon after diagnosis. Also we continue working to reveal the reasons for differences of disease outcome [7].

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