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Studying the Concept of Minimal Residual Disease Detection in Cases of CLL

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Abstract:

Despite improvements in the treatment of haematological malignancies over the last few decades, many patients relapse after an initial response to treatment. Such relapses are derived from the same clone detected at the time of diagnosis and is caused by Minimal Residual Disease (MRD). The present study focused on detecting MRD in CLL specimens using Material and Methods: Prospectively, 30 B-CLL (group A) patients admitted to hematology unit, Main University hospital of Alexandria was studied. Twenty healthy subjects (Group B) with matched age and sex was recruited as a control group in specimens for diseases other than CLL or haematological malignancies. After a written consent, each patient (before and after treatment) and controls underwent history taking and complete examination, CBC, bone marrow aspiration (when possible), Lymph node fine needle aspiration biopsy (when possible), and immunophenotyping. All specimens were subjected 4-color flow cytometry using a combination of CD5/19/20/79b to detect minimal residual disease (MRD) in peripheral blood or bone marrow samples. Results: Both groups did not differ as regard age or sex (p=0.92). CLL were found to be typical and atypical cases. The expression of CD79b showed a significantly higher MFI percentage in the atypical cases. CD20+/CD5+ is a significantly smaller population than CD20+/CD5- with a significantly higher MFI of CD20 in the CD5+ population. However the MFI of CD79b did not differ between the CD5+ and CD5- B cells. Conclusion: MRD was detected in all CLL patients regardless of the type of treatment received. The lowest threshold of detection was 0.15%. There is an atypical population of CLL which is characterized by higher MFI than typical cases. This finding can use as a phenotype detector for atypical CLL cases

Keywords

Keywords should be the keywords used in the article or related to the articles, Each keywords should be separated by comma or semi-colon, E.g. International Journal of Research, Edupedia Publications, ISOAR Journals, Book Publisher

1. Introduction

Chronic lymphocytic leukemia (chronic lymphoid leukemia, CLL) is a monoclonal disorder characterized by a progressive accumulation of functionally incompetent B-lymphocytes in peripheral blood, bone marrow and lymphoid tissues.

It is the most common form of leukemia found in adults. [1]The basic physiopathological defect in CLL is the resistance of neoplastic cells to programmed cell-death or apoptosis. This monoclonal population expresses CD19, CD5 and CD23with reduced expression of serum immunoglobulins (sIg) and CD79b.[2-4] Despite improvements in the treatment of haematological malignancies over the last few decades, many patients relapse after an initial response to treatment. Such relapses are derived from the same clone detected at the time of diagnosis and is caused by Minimal Residual Disease (MRD). Minimal residual disease (MRD) can be defined as the level of disease detectable by the most sensitive technique available. In the case of CLL, MRD-negative status has been arbitrarily defined as the presence of less than 1 CLL cell in 10 000 leucocytes (0.01%). [5] In addition, patients in whom it is possible to achieve MRD-negative status could be those with a biologically less aggressive disease and, hence, with a better prognosis. Also, increasing or prolonging treatment with the aim of reaching MRD-negative status may convey unnecessary risks such as myelotoxicity and infections, with increasing risk of developing secondary MDS/AML in 2- 4%. [6]

The characteristic phenotype of CLL cells facilitates the analysis of MRD by flow cytometry. The analysis of MRD by using two-colour flow cytometry CD5/CD19 or CD20/light chainwas the early approach and has a low sensitivity. Four-colour flow cytometry is now considered the standard technique to assess MRD and to detect cells rarely present in normal or reactive bone marrow. This techniqueis able to detect one tumour cell in 10^4–10^5 normal B cells. [7,8,9]

The present study at hand focus on detecting MRD in CLL specimens using a combination of four antibodies (CD5/19/20/79b) and a sequential gating strategy to effectively separate CLL cells from normal B cells.

Materials and Methods

The study was propsectively conducted on:

1) Thirty (30) patients of B-CLL cases admitted to hematology unit, internal medicine department main university hospital, Alexandria at presentation (if available) or during follow up.
2) Twenty (20) healthy subjects with matched age and sex will be recruited as a control group with 10 performing bone marrow aspirate for diseases other than CLL or haematological malignancies.

A written consent will be taken from all patients included in the study. Each patient and control will be subjected to the following:

1. History taking and complete physical examination.
2. CBC. [10]
3. Bone marrow aspiration, when possible [11]
4. Lymph node fine needle aspiration biopsy (when possible). [12]
5. Immunophenotyping.[13]
6. Four color flow cytometry using a combination of CD5/19/20/79b to detect minimal residual disease (MRD) in peripheral blood or bone marrow sample. [14]

The present study was reviewed and approved by the department's review and assessment and Faculty ethical committees

Statistical analysis of the data (15)

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0.(Armonk, NY: IBM Corp)(16) Qualitative data were described using number and percent. The

Kolmogorov-Smirnov test was used to verify the normality of distribution Quantitative data were described using range (minimum and maximum), mean, standard deviation and median. Significance of the obtained results was judged at the 5% level. The used tests were Chi-square test, Fisher's Exact or Monte Carlo correction, Student t-test, F-test (ANOVA) , Paired t-test , Mann Whitney test, Kruskal Wallis test, Wilcoxon signed ranks test.

Results

After comprehensive review of the results of the CLL cases we divided them into two subgroups:

- Group IA, which consists of the typical CLL cases (27 cases).
- Group IB, which consists of the atypical CLL cases (4 cases).

Also, the control cases were divided into two subgroups:

- Group IIA, which consists of the peripheral blood controls (10 cases).
- Group IIB, which consists of the bone marrow controls (10 cases).

Different comparisons were done to asses if a significant difference was found between any two subgroups mentioned above,

Discussion

Chronic lymphocytic leukaemia is the most common adult leukaemia in the world. The classic features of CLL include a clonal CD5 positive B lymphocyte population with a peripheral count of a minimum of 5000 cells/mcL, bone marrow lymphocytosis and varying degrees of cytopenias and hepatosplenomegaly. CLL is a clinically heterogeneous disease with an extremely variable clinical course and disease outcome. Nowadays there is growing recognition of the association of MRD status in CLL as a predictor of both progression free survival and overall survival.

The present work aimed at detecting MRD in CLL specimens using a combination of four antibodies (CD5/19/20/79b) and a sequential gating strategy to effectively separate CLL cells from normal B cells. In the present study; minimal residual disease was detected in all patients studied, with the lowest level detected was 0.15 %. Four cases of CLL with atypical morphology were identified. In the present study, the expression of CD79b was observed to vary from typical as compared to atypical CLL patients; with significantly higher MFI percentage in the atypical cases. Atypical CLL with its heterogeneous morphology is sometimes difficult to distinguish from non Hodgkin lymphoma and integration of morphologic and expanded immunophenotypic profile is critical in achieving accurate diagnosis (17) Cases of CLL with cytologically atypical cells were previously described and were reported to have a worse prognosis. (Frater 2001)(18) High 79b expression has been associated with atypical morphology, advanced clinical stage and short survival in CLL (Del Poeta 2008) (19).

In the normal bone marrow samples in the present study; a comparison between CD5+B cells and CD5- B cells as regards % and MFI was studied. The percentage of B cells in general (CD5+ and CD5-) is detected to be low in count. CD20+/CD5+ is a significantly smaller population than CD20+/CD5- with a significantly higher MFI of CD20 in the CD5+ population. However the MFI of CD79b did not vary between the CD5+ and CD5- B cells.

Conclusion:

1. MRD was detected in all CLL patients regardless of the type of treatment received.
2. The lowest threshold of detection was 0.15%

3. There is atypical population of CLL that is characterized by higher MFI than typical cases. This can be used as phenotype detector for atypical cases.

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