UTILITY OF IMMUNOLOGICAL MARKER CD14 IN IDENTIFYING MONOCYTIC COMPONENT IN ACUTE LEUKAEMIA

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ABSTRACT

Objective: To assess the usefulness of immunological marker CD 14 in identifying malignant cells of monocytic lineage

Material and methods: Eight thin smears each from peripheral blood and bone marrow of seventeen acute leukaemia patients were obtained. Romanosky dye (Leishman) and cytochemcial stains (Peroidase, Periodic acid Schiff, non specific esterase) were applied. Two bone marrow smears were then treated with immunological marker CD 14.

Results: CD14 reacted with malignant monocytic lineage cells in 16/17 (94.11%) acute leukaemia cases and that no reactivity was observed in other cells of myeloid as well as lymphoid lineage. Addition of immunological marker CD14 to the battery of morphocytochemical diagnostic tools has increased diagnostic efficacy from 82.35% to 94.11%, highlighting the usefulness of CD14 immunological marker.

Conclusion: CD14 surface marker is a useful tool for identifying monocytic component in neoplastic cells of acute leukaemia patients.

Key words: Immunological marker CD14, monocytic component, acute leukaemia.

INTRODUCTION

The identification of different leukocytes and their respective stages of maturation has mostly been based upon standard morphological and enzymatic criteria.[1,2] However the use of these routine laboratory methods have not always been sufficient to characterize unequivocally leukaemic cells, classifying 80 to 85% cases only.[3] Remaining 15 to 20% patients are a diagnostic dilemma.

In order to receive proper treatment, they need to be properly diagnosed and classified. This gap in knowledge is filled by the use of immunological markers. In the current study the utility of one such immunological marker CD 14 is being evaluated following application on smears of bone marrow aspirate of seventeen suspected patients of acute monocytic leukaemia and acute myelomonocytic leukaemia.

MATERIAL AND METHODS

Seventeen patients of acute leukaemia clinically and morphocytochemically suspected of involving monocytic component at Armed Forces Institute of Pathology (AFIP) Rawalpindi and Pathology department LRH Peshawar were evaluated. This study was performed at AFIP- Rawalpindi and all the cases were analyzed by the three authors first individually and then collectively before making final opinion.

History, physical findings were recorded and the following specimen were collected from each patient.

1 3ml peripheral blood in EDTA container.

2 Bone marrow aspirate spread as thin films on eight glass slides.

3 Romanosky stain (Leishman).

4 Cytochemical stains (Peroxidase, PAS, Non-specific esterase).

5 Immunological marker CD14.

Peripheral blood and bone marrow thin push smears were prepared on glass slides and allowed to dry up for 20 minutes. These smears were then wrapped in aluminum foil and stored at temperature less than -20 °C. Smears of all seventeen patients were collected in similar fashion ever a period of one year and were analyzed collectively later on with immunological marker CD 14. Complete blood picture including platelet court, total leukocyte count, haemoglobin estimation were performed at the time of patient's visit on sysmex hematology analyzer and reports filed.

Bone marrow aspirate was obtained from posterior iliac spine in adults and from medial side of tibial tuberosity in children under 2 years of age. Smears of bone marrow aspirate were prepared and stained with Leishman, PAS, Peroxidase and non-specific esterase stains with and without sodium fluoride using standard method.[2] All patients were having more then 30% blast cells in the bone marrow.

Immunological marker CD14 commercially available stain which reacts with monocytic component was applied using peroxidase anti-peroxidase (PAP) method[3] modified by signet laboratories. Inc. in their universal immunoperoxidase staining kit (murine). At least three hundred nucleated cells were assessed in two different smears and percentage calculated for determining positively with immunological marker CD 14.

RESULTS

There is no consensus on the cut-off point for considering a specimen to be positive with a marker but commonly used criteria is: positivity in greater than 20% of leukaemic cells in acute leukaemia and positivity greater than 30% of leukaemic cells in chronic lymphoproliferative disorders.[6]

The morphological and cytochemical features of blast cell populations in the peripheral blood and bone marrow smears were suggestive of mixed myeloid and monocytic component in 6/17 (35.29%) cases and solely monocytic in 8/17 (47.05) cases. As a whole morphology and enzymatic study were able to classify 14/17 (82.35%) acute leukaemia cases as predominantly involving monyctic cell lineage.

Acute leukaemia 8/17 (47.05%) cases which were reported morphocytochemically

to constitute mainly of monocytic malignant cells, all reacted strongly with CD 14 immunological marker in >90% blast cells population. 6/17 (35.29%) cases of acute leukaemia defined as comprising of mixed myeloid and monocytic component on morphology and cytochemistry also showed strong reactivity with CD 14 but in only 40% blast cell population. A similar pattern of 40% blast cells positivity was observed in another 2/17 (11.77%) cases of acute leukaemia in whom morphology and cytochemistry were not clearly decisive.

As a whole in 16/17 (94.11%) acute leukaemia cases, monocytic component was determined where as the blast cells of 1/17 (5.88%) patients did not react with CD14 monoclonal antibody.

DISCUSSION

Acute myeloid leukaemia is the most common type of acute leukaemia in adults. Despite considerable improvements in the rate of remission after chemotherapy most patients ultimately die with relapse leukaemia.[7] The "Non-Specific Esterase" which can be demonstrated cytochemically is a reliable marker of the human monocytes and histiocytes. This "non specific esterase" is in reality a group of enzymes and not a single protein.[8] These enzymes have different electromobilities and substrate specificities and are sensitive to heat and organic solvents. Therefore the practical exploitation of non-specific esterases has largely been restricted to fresh tissues including smears, imprints and frozen tissue sections. In paraffin embedded tissues where enzyme surface markers are not applicable, muramidase has been used as marker for monocytes and histiocytes. Muramidase is not specific for monocytes or histiocytes but is also present in granulocytes. Alpha antitrypsin is also used as marker for monocytes but not designated as specific.[9]

Problems inherent to the enzymic study of the non specific esterase may be alleviated by immunochemical study of monocytes. Our current immunochemical study reveal that "CD14" a specific antibody reacts exclusively with normal monocytes and with neoplastic cells of monocytic leukaemia and it does not react with neoplastic cells of granulocytic leukaemia.

Immunohistochemical stains have recently been used for the positive identification of different cell types in mixed populations and for the diagnosis of undifferentiated acute leukaemia and lymphoma.[5,10,11,12,13,14,15] Panel of monoclonal antibodies are also applied for studying the correlation of immunophenotying with morphological classification or morphocytochemical classification and/or cytogenetic classification.[3]

According to French, American and British (FAB) haematologists, diagnosis and classification on the basis of morphology alone has a reproducibility upto 65%, whereas supplementation by cytochemistry improves the reproducibility upto 85%. A similar pattern was observed in the current study, where morphology and cytochemistry could diagnose and classify 14/17 (82.35%) patients.

Addition of the immunological marker CD14 to the morphocytochemical tools in this study has enhanced the identification and reproducibility to 94.11%. This is in accordance with immunological marker studies performed in other parts of the world.[15,17,18] where the diagnostic and classification accuracy has increased from 95% to 97%.

Blast cells of one out of seventeen (5.88%) acute leukaemia patient did not react with CD14 monoclonal antibody. This feature is also in accordance with other studies performed in other parts of the world, where it has been observed that blast cells of upto

20% patients do not react with CD14. In cases of acute leukaemia where morphology of neoplastic cells is suggestive of monocytic leukaemia but first line cytochemistry and CD 14 analyses are equivocal addition of another specific marker "CD11c" is recommended in order to define the nature of neoplastic cells.[19,20]

CONCLUSION

1. Addition of CD14 monoclonal antibody to the morphocytochemical tools has greatly enhanced the monocytic identification capacity from 82.35% to 94.11%.
2. Blast cells of one out of seventeen (5.88%) patient did not react with CD14. this suggest that another immunological marker may be required to confirm the lineage of these neoplastic cells.

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