Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/mjafi

Original Article

Flowcytometric comparative analysis in acute leukemias between Indian and proposed minimal screening panel

Srishti Gupta [a], *Brig Tathagata Chatterjee* [b,*], *Col Sanjeevan Sharma* [c], *Brig Ajay Sharma* [d], *Col Prosenjit Ganguly* [e], *Col Jasjit Singh* [f], *Surg Capt Satyaranjan Das* [g]

[a] Resident (Pathology), Army Hospital (R&R), New Delhi 110010, India
[b] Commandant, 166 Military Hospital, C/o 56 APO, India
[c] Senior Adviser (Med & Hematology), Command Hospital (Southern Command), Pune 411040, India
[d] Consultant & Head (Hematology), Army Hospital (R&R), New Delhi 110010, India
[e] Senior Adviser (Pathology), Command Hospital (Eastern Command), Kolkata, India
[f] Senior Adviser (Med & Hematology), Army Hospital (R&R), New Delhi 110010, India
[g] Senior Adviser (Paediatrics & Hematology), INHS Asvini, Colaba, Mumbai, India

ARTICLE INFO

Article history:
Received 20 April 2015
Accepted 6 January 2016
Available online 29 March 2016

Keywords:
Acute leukemias
Flowcytometry
Indian Consensus screening panel
Proposed minimal screening panel
MPAL

ABSTRACT

Background: Acute myeloid leukemia and acute lymphoid leukemia differ substantially in response to therapy and course, and accurate differentiation of the two is fundamental to therapeutic decisions. Immunophenotyping is used for this purpose, and various guidelines have been proposed regarding a minimal screening antibody panel. Most of them have been found inefficient.

Methods: Eighty-two cases of consecutive acute leukemias reporting to this hospital over a period of two years were included in the study. Peripheral blood smear, bone marrow aspirate, and bone marrow biopsy were studied using morphology, cytochemical stains, and relevant immunohistochemical stains on selected biopsy specimens. Flowcytometry analysis was carried out using Indian consensus screening panel and our proposed minimal screening panel (PMSP) for comparison.

Result: Immunophenotyping using PMSP resulted in 95.12% accurate diagnosis versus Indian consensus minimal screening panel (ICMSP) with an accuracy of 92.68%. This result was statistically significant as per Chi Square tests.

Conclusion: PMSP can be used as a substitute for ICMSP, since it includes lineage-specific cytoplasmic antibodies, as well as lesser number of monoclonal antibodies, and enables us to diagnose mixed lineage leukemia. Fewer markers can be linked to a lower cost as well, which is relevant in a developing economy.

© 2016 Published by Elsevier B.V. on behalf of Director General, Armed Forces Medical Services.

* *Corresponding author*. Tel.: +91 8551910936.
E-mail address: ctathagat@hotmail.com (T. Chatterjee).

http://dx.doi.org/10.1016/j.mjafi.2016.01.002

0377-1237/© 2016 Published by Elsevier B.V. on behalf of Director General, Armed Forces Medical Services.

Introduction

The acute leukemias are a heterogeneous group of neoplasms arising from transformation of hematopoietic stem cells usually with a retained partial capacity of differentiation.[1] Acute leukemia is broadly divided into acute myeloid leukemia (AML) and acute lymphoid leukemia (ALL).[2] AML and ALL differ substantially in response to therapy and course, and accurate differentiation of the two is fundamental to therapeutic decisions.

We now know from available literature[3] that diagnostic evaluation of acute leukemia involves morphological, cytochemical, immunophenotypic, and cytogenetic evaluation. Using Romanovsky-stained smears alone, experienced morphologists are only 70–80% correct in separating ALL from AML. Helpful features are chromatin character, nucleoli, and cytoplasm. Because of morphologic overlap between leukemic and normal progenitors, immunophenotyping is generally more useful in classifying a recognizable leukemia. Immunophenotyping allows reproducible lineage assignment of some leukemia that would otherwise be difficult to classify, particularly in differentiating lymphoid from immature myeloid leukemia. In addition, intrapermeable cytoplasmic markers (myeloperoxidase, cCD22, cCD3, cCD79a) are more specific indicators of lineage commitment in acute leukemia than surface markers.[4,5] Hence, addition of immunophenotypic analyses including intrapermeable stains improves accuracy in delineation of ALL and AML to 95–98%.

Antibody panel selection is a critical step in specimen processing and plays a pivotal role in obtaining an accurate diagnosis. The ultimate test of any antibody screening panel for acute leukemia is its ability to accurately delineate ALL from AML. Surveys in the United States have revealed that laboratories on an average utilize 16–19 antibodies, a figure ranging from 5 to 45 antibodies.[6–8] Increasing the number of antibodies not only helps minimize the turnaround time, but it also adds to a higher cost. Thus, choosing the perfect minimal screening panel is not a trivial endeavor. There have been many publications around the selection of a panel of antibodies[9–12] but these panels have only been partially successful.[13]

A National meeting on Guidelines for immunophenotyping of hemato-lymphoid neoplasms by flowcytometry that was held on 14 March 2008 in Mumbai,[13] India, reached a consensus and recommended an Indian consensus minimal screening panel (ICMSP) comprising of ten antibodies CD10, CD19, CD7, CD5, CD13, CD33, CD117, CD34, HLA-DR, and CD45 followed by a secondary directed panel.

It is our opinion that this panel falls behind accurately in differentiating ALL from AML and mixed phenotype acute leukemia (MPAL), since it lacks intrapermeable antibodies (that are more lineage specific). This paper attempts to highlight these shortcomings and proposes an alternate minimal screening panel that addresses these issues.

Materials and methods

Patients and samples

Eighty-two consecutive newly diagnosed untreated acute leukemia cases were included in the study and analyzed at a single center. Peripheral blood and bone marrows were received with detailed clinical history. Hematological parameters namely hemoglobin (g/dl), total leukocyte count, differential leukocyte count, and platelet count were noted.

Methodology

We performed tests in the following order to diagnose cases of acute leukemia – (1) morphology alone (2) morphology and cytochemistry (3) immunophenotyping.

Final diagnosis was made with combined approach comprising of morphology, cytochemistry, immunophenotyping, and cytogenetics.

Morphology

Peripheral blood smears and bone marrow aspirates were stained with Leishman stain. Bone marrow biopsies were stained with hematoxylin and eosin as well as reticulin stain. Relevant immunohistochemical stains including anti-MPO and CD34 were performed in diagnostically difficult biopsy cases.

Cytochemical analysis

Air-dried fresh peripheral blood smear or bone marrow aspirate was stained with myeloperoxidase, α-naphthyl butyrate esterase, and naphthol AS-D chloroacetate esterase. Wet-fixed smears were stained with periodic acid Schiff stain. These cytochemical reagents were used according to manufacturer's (Merck Millipore) instructions.

Immunophenotyping

Flowcytometry (FCM) analysis was done on peripheral blood or bone marrow aspirate collected in EDTA (K2/K3) vacutainers in recommended concentration (0.34 M). Samples were processed within 24 h of collection. Samples with an inadequate cell count were excluded. Cell suspension was prepared by standard lyse wash technique.

For antibody staining, surface and cytoplasmic antigens were stained with commercially available (Beckman Coulter) flourochrome conjugated antibodies as per manufacturer's instructions. Flourochromes used were FITC, PE, ECD and PC-5.

First, surface staining was done for CD45 followed by cytoplasmic staining. Cytoplasmic antigen staining was done using intrapermeabilizing agents. Analysis was done using double laser 4-color Beckman Coulter FC500 FCM (Beckman Coulter, Miami, FL, USA). Controls were run before each test with IgG1, IgG2, and IgG3 labeled with FITC, PE, and ECD respectively.

The proposed minimal screening panel (PMSP) comprised of eight antibodies namely CD45, CD19, CD10, cCD3, cCD79a, cMPO, sCD3, and CD34. Contrary to the ICMSP, this panel leverages use of intrapermeable antibodies.

Comparative analysis was carried out by staining the same sample with both PMSP and ICMSP. The tubes used in both the panels are listed in Table 1. For a comprehensive analysis, additional antibodies were used in a few diagnostic cases, where either panel failed to subclassify the acute leukemia.

Gating strategy used was side scatter (SSC) versus CD45. Forward scatter versus SSC gating strategy was used, wherever blasts were CD45 negative. An antigen was considered

Download English Version:

https://daneshyari.com/en/article/3160848

Download Persian Version:

https://daneshyari.com/article/3160848

Daneshyari.com