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Original Article

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

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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.

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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 literature³ 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) fluoro-chrome conjugated antibodies as per manufacturer's instructions. Fluorochromes 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

Table 1 – Tubes used in proposed minimal screening panel and Indian consensus minimal screening panel.

Tube Number	Antibody–fluorochrome combination
Proposed minimal screening panel	
Tube 1	cMPO–FITC cCD3–ECD cCD79a–PE CD45–PC5
Tube 2	CD19–FITC CD 10–PE CD 45–ECD CD 34–PC5
Tube 3 (isotypic control)	IgG1–FITC IgG1–PE IgG1–ECD CD45–PC5
Tube 4	sCD3–PC5 CD 45–ECD
Indian consensus minimal screening panel	
Tube 1 (isotypic control)	IgG1–FITC IgG1–PE IgG1–ECD CD45–PC5
Tube 2	CD 19–FITC CD 10–PE CD 45–ECD CD34–PC5
Tube 3	CD 33–PE CD 45–ECD HLA DR–PC5
Tube 4	CD 13–PE CD 45–ECD
Tube 5	CD 7–FITC CD 117–PE CD 45–ECD CD 5–PC5

positive, if >20% of blasts gated showed positivity for specific monoclonal antibody (based on WHO 2008 criteria 2).

Immunophenotyping results for both panels were compared against the final diagnosis to study each panel's accuracy.

Cytogenetic evaluation

Peripheral blood or bone marrow aspirate was outsourced for cytogenetic evaluation, as the facility was not available at our center.

Statistical analysis

SPSS software version 11.5 was used to analyze the data, and chi square test was used to compare the results of the two screening panels. A *p* value of <0.05 was considered significant.

Results

Patients' age varied between 2 years to 65 years (median age was 31 years). Fifty-two patients were males and 30 patients were females.

1. On the basis of morphology alone, 77/82 cases could be classified into AML (*n* = 50) and ALL (*n* = 27). The remainder (*n* = 5) of the cases could not be classified. 25/50 AML cases were further attempted to be subclassified according to Revised FAB classification into – AML M0 (*n* = 0), AML M1 (*n* = 0), AML M2 (*n* = 3), AML M3 (*n* = 8), AML M4 (*n* = 5), AML M5 (*n* = 4), AML M6 (*n* = 5), and AML M7 (*n* = 0). Rest of the AML cases (25/50) could not be subclassified.
2. Using combined morphology and cytochemistry, 80/82 cases of acute leukemia were classified into AML (*n* = 43), ALL (*n* = 36), and MPAL (*n* = 1). The remainder (*n* = 2) of the cases could not be classified. 40/43 AML cases were further subclassified according to revised FAB classification into M0 (*n* = 0), M1 (*n* = 6), M2 (*n* = 6), M3 (*n* = 8), M4 (*n* = 10), M5 (*n* = 4), M6 (*n* = 6), and M7 (*n* = 0).
3. FCM immunophenotyping: Using both ICMSF and PMSP results were seen as shown in Fig. 1. Using ICMSF, 10/45 AML cases were further sub classified into AML without differentiation (*n* = 2) and acute promyelocytic leukemia (*n* = 8). Using PMSP, 8/41 AML cases were further subclassified as acute promyelocytic leukemia.

Frequency of expression of various markers used in both the screening panels was studied for each lineage (myeloid, B and T-lymphoid and MPAL) and is shown in Fig. 2.

4. Combined morphology, cytochemistry, immunophenotyping, and cytogenetics data: Based on morphology, cytochemistry, immunophenotyping, and cytogenetics, 34 (41.46%) patients had ALL, 45 patients (54.88%) had AML, and 3 patients (3.66%) had MPAL. AML and ALL were classified as per WHO classification as shown in Table 2.

Retrospective analysis

We found that using morphology alone, 62/82 (75.60%) cases were correctly diagnosed classifying them into AML (*n* = 39) and ALL (*n* = 23).

Combined morphology and cytochemistry tests correctly diagnosed 77/82 (93.90%) cases successfully classifying them into AML (*n* = 42), ALL (*n* = 34), and MPAL (*n* = 1). Two cases of MPAL were incorrectly diagnosed as AML and ALL each. One case of AML M0 was incorrectly diagnosed as ALL by this technique.

ICMSF correctly diagnosed 76/82 (92.68%). Successful classifications are denoted in Fig. 3. Note that in a few diagnoses, AML, B-ALL, and T-ALL detection was a false positive. Moreover, all MPAL cases went under the radar and were never caught under ICMSF.

PMSP correctly diagnosed 78/82 (95.12%) cases. Successful classifications are denoted in Fig. 4. Note that none of the cases diagnosed were false positives. In addition, all MPAL cases were caught using PMSP.

Percentage accuracy of each technique listed above is plotted as a line diagram (refer Fig. 5).

Statistical comparative analysis

ICMSF had higher sensitivity only for AML cases. For AML and MPAL, PMSP had significantly higher sensitivity and specificity (refer Table 3).

Fig. 1 – Results from the flowcytometric analysis using Indian consensus minimal screening panel and proposed minimal screening panel in acute leukemia cases. Note that since some of the cases were not accurately diagnosed, this figure serves purely to represent test results.

Fig. 2 – Frequency of surface and cytoplasmic markers in acute leukemia.

Table 2 – Tabulation of acute leukemia cases using morphology, cytochemistry, immunophenotypic and cytogenetic features.

Acute leukemia (as per WHO classification)	Cytogenetics/subclassification	Occurrence (out of 82 cases)	Frequency (in %)
AML (NOS)	AML with minimal differentiation	2	2.40
	AML without maturation	5	6.10
	AML with maturation	7	8.54
	Acute myelomonocytic leukemia	7	8.54
	Acute monoblastic leukemia	4	4.88
	Acute erythroleukemia	6	7.32
	Acute panmyelosis with myelofibrosis	1	1.22
	Total	32	39.02
AML with recurrent translocations	t(8;21)(q22;22)	2	2.40
	t(15;17)(q22;q12)	8	9.76
	Inv16 (p13.1q22)	2	2.40
	MLL gene rearrangement	1	1.22
	Total	13	15.85
B-ALL	B-ALL (NOS)	13	15.85
	Recurrent translocation [t(9;22), MLL gene rearrangement]	12	14.63
	Burkitt Leukemia	1	1.22
	Total	26	31.70
T-ALL	–	8	9.76
MPAL		3	3.66

PMSP had higher positive predictive value compared to ICMSP. In fact, the positive predictive value for PMSP was measured at 100% in all three types of leukemias (refer Table 3).

ICMSP had higher negative predictive value compared to PMSP for AML cases. For ALL and MPAL cases, however, this

was not the case, and PMSP again displayed a 100% negative predictive value (refer Table 3).

For AML cases, ICMSP had 97.56% accuracy while PMSP had 95.12% accuracy. For ALL and MPAL cases, PMSP had 100% accuracy bettering ICMSP's accuracy of 93.90% and 96.34% respectively (refer Table 3).

Fig. 3 – Accuracy of classification using Indian consensus minimal screening panel (ICMSP) when compared against actual diagnosis. Note that in a few cases, AML, B-ALL and T-ALL diagnosis was sometimes a false positive. Moreover, all MPAL cases went under the radar and were never caught under ICMSP.

Fig. 4 – Accuracy of classification using proposed minimal screening panel (PMSP) when compared against actual diagnosis. Note that none of the cases diagnosed were false positives. In addition, all MPAL cases were caught using PMSP.

Fig. 5 – Accuracy of different diagnostic techniques used in this study plotted as a line diagram for comparison. Techniques used were (a) morphology alone (b) morphology and cytochemistry (c) Indian consensus minimal screening panel (d) proposed minimal screening panel.

These findings indicate that PMSP is a better panel for ALL and MPAL. Overall, statistically, PMSP had higher specificity, sensitivity, predictive values (positive and negative), and accuracy than ICMSP.

This difference is significant as per the likelihood-ratio chi-square tests ($G^2 = 184.952$, $df = 25$, $p \leq 0.05$). See [Tables 4 and 5](#) for more details.

Discussion

FCM enables a rapid and accurate diagnosis in hematolymphoid malignancies hence is considered the gold standard for diagnosing acute leukemias. However, there is a lack of uniform guidelines regarding the minimal number of

Table 3 – Comparison of various statistical indices between the Indian consensus and proposed minimal screening panel.

	ICMSP	PMSP
	Sensitivity	
AML	97.78%	91.11%
ALL	94.12%	100.00%
MPAL	0.00%	100.00%
	Specificity	
AML	97.30%	100.00%
ALL	93.75%	100.00%
MPAL	100.00%	100.00%
	Positive predictive value	
AML	97.78%	100.00%
ALL	91.43%	100.00%
MPAL	NA	100.00%
	Negative predictive value	
AML	97.30%	90.24%
ALL	95.74%	100.00%
MPAL	96.34%	100.00%
	Accuracy	
AML	97.56%	95.12%
ALL	93.90%	100.00%
MPAL	96.34%	100.00%

antibodies to be used in a screening panel without compromising the accuracy of diagnosis.

Cytoplasmic markers in acute leukemia

cMPO, cCD79a, and cCD3 were used in the study of the minimal screening panel as they have been reported to be lineage specific by numerous studies.^{14–16} Intracellular cytoplasmic antigen detection was most useful in establishing correct lineage affiliation in cases lacking expression of B, T, or myeloid surface membrane antigens.

cCD3

cCD3 expression was seen in 100% T-ALL cases which obviously makes it a good marker and showed no aberrant expression.

Table 5 – Chi-square test results.

	Value	df	Asymp. Sig. (2 sided)
Pearson Chi-square	250.247 ^a	25	.000
Likelihood ratio	184.952	25	.000 ^b
Linear-by-linear association	40.577	1	.000
No. of valid cases	82		

Note:

^a 32 cells (88.9%) have expected count <5. The minimum expected count is 0.07.

^b Likelihood ratio Chi-square was observed since Pearson's chi-square assumption was violated.

cCD79a

cCD79a expression was seen in 96.15% B-ALL cases with an aberrant expression in 6.67% AML cases (even though the intensity was found weaker in comparison to ALL blasts). Arber et al.¹⁷ have reported high incidence of aberrant expression of cCD79a in AML cases too.

Despite some aberrant expression, cCD79a is a good choice since it helps mark B-cell lineage ahead of other B-cell markers. Recent evidence suggests that CD19 surface antigen expression is not the first differentiation event in human B-cell ontogeny and rather CD10 is an upstream marker.^{18–20} This has been demonstrated by the fact that CD19+ B-cells can be grown from a progenitor pool with TdT+ CD34+ CD10+, CD19– cells. The same investigators have also found that these progenitors that lack CD19 already express cCD79a in addition to CD10, which implies that cCD79a precedes CD19 antigen expression.²¹ This finding was seen in one of our patients with an ambiguous immunophenotype {CD10+, CD79a+ CD7+ CD34+, cCD3–, cMPO–, CD19–} but in whom cCD79a heralded B-cell lineage commitment.

cMPO

Recent reports have demonstrated better sensitivity using flowcytometric methods, than with immunoenzymatic or cytochemical methods, to detect MPO in the cytoplasm of CD34+ hematopoietic progenitors.^{5,22–24} In our study, we found cMPO expression in 91.11% of the cases without any aberrance. However, cMPO was found negative in two AML M0 cases, one case of AML M6 and one case of acute

Table 4 – Indian consensus and proposed minimal screening panel cross tabulation (chi square test).

	Proposed minimal screening panel						Total
	APML	AML	MPAL	T-ALL	B-ALL	U*	
Indian Consensus Minimal Screening Panel	AML M0					2	2
	APML	8					8
	AML		32	1		2	35
	T-ALL			8	1		9
	B-ALL				24		26
	U*		1		1		2
Total	8	33	3	8	26	4	82

Note: U* – unclassified.

panmyelosis with myelofibrosis (APMF). Let us look at these cases in detail:

- AML M0 cases are usually cMPO negative and absence of other lineage-specific markers further helped to confirm the diagnosis. Secondary directed panel was employed using CD13 and CD33 in such cases and they were found to be positive thus ruling out acute undifferentiated leukemia.
- In our study, we had 6 cases of AML M6 out of which one case of AML M6b was negative for cMPO (but found to be positive for CD117 used in ICMSP). However, this case had already been classified using combined morphology and cytochemistry negating the need for FCM analysis.
- Lastly, PMSP could not diagnose a case of APMF due to absence of myeloid associated antigens. However, blasts in APMF are cMPO negative²⁵ and use of additional extensive secondary panel employing antibodies against myeloid (CD13, CD33, CD117), monocytic (CD11c, CD14, CD4), megakaryocytic (CD41), and erythroid (Glycophorin A) markers based on detailed clinical history and bone marrow biopsy morphology helped clinch the diagnosis.

Apart from these exceptions, cMPO was found to be positive in all other subtypes of AMLs (41 cases) including AML M5 (all 4 cases). Thus, we can confidently say that cMPO was found to be a very competitive and most frequently expressed myeloid marker. This is in line with a previous report that demonstrated cMPO in virtually all cases of AML.²⁶

Overall, in our study, all three markers were found to be lineage-specific antigens due to maximum positivity in a particular lineage with least aberrant expression. The result was in concordance with a study conducted by Thalhammer Scherrer et al.¹⁵

Our results also confirm previous findings that cCD3^{4,27–29}, cCD79a,^{20,30–32} and cMPO^{33,34} are the earliest identifiable, specific B, T, and myeloid markers. Also, they are expressed in virtually all cases of B and T-ALL and subtypes of AML respectively.

The practical significance of using three intracellular cytoplasmic stains was two-fold. Firstly, they increased diagnostic precision for all three lineages. Secondly, when used in combination with other markers (CD34, CD10, and CD19), they were able to identify MPAL.³⁵ cCD3 expression was useful in confirming the T-cell lineage. Intracellular cCD79a and cCD3 detection was useful in providing evidence of bi/triphenotypic leukemias and aberrant lymphoid marker positive AML. Our data, though based on a small number of acute leukemia patients, indicates that flowcytometric detection of cytoplasmic antigens helps to classify acute leukemias more accurately. Hence, their use should be encouraged in a primary minimal screening panel.

Comparison of other markers used in the two panels

Using PMSP, acute leukemias could be correctly delineated into AML versus ALL in a majority of cases. All B-ALL cases and T-ALL cases could be classified. However, 4 cases (two cases of AML M0, one case of AML M6, and a single case of very rare APMF) could not be classified due to the absence of cMPO expression. On the contrary, ICMSP could diagnose and

classify these cases as AML due to the expression of myeloid associated antigens (CD33 and CD13).

However, it has been emphasized by Thalhammer Scherrer et al.¹⁵ in their study that combined morphology and cytochemistry is able to classify most cases of AML and immunophenotypic analysis using minimal screening panel is required mostly in differentiating AML M0 from ALL. With this background, in some cases where cMPO is frequently negative, these diagnoses can sometimes be derived from preliminary tests already.

As a side note, we found cMPO (part of PMSP) had greater expression than CD33 (part of ICMSP) in myeloid lineage. cMPO was expressed in 41/45 AML cases whereas CD33 was expressed in 39/45 AML cases and CD13 was expressed in 35/45 AML cases. Interestingly, Thalhammer Scherrer et al.¹⁵ found CD33 to be the most frequently associated myeloid antigen followed by cMPO and CD13. Thus, our finding differs from the study done by them in this regard.

On FCM, PMSP successfully classified all three MPAL cases (based on WHO 2008 classification for MPAL²⁵). These three cases were all misdiagnosed by ICMSP that categorized two cases as B-ALL and one case as AML due to lack of cytoplasmic antibodies in the ICMSP.

Using ICMSP, 24/26 B-ALL cases were correctly delineated. Two B-ALL cases were misdiagnosed. One case was over-diagnosed as T-ALL. This case lacked CD19 antigen expression and pan T marker CD7 was found to be positive. This case was diagnosed correctly as B-ALL using PMSP due to presence of lineage-specific antigen cCD79a and lack of cCD3, which is expressed in all T-ALL cases. The second B-ALL case showed moderate positivity for both CD13 and CD33 in addition to CD19 and CD10. This case could not be classified concretely into myeloid or lymphoid by ICMSP due to lack of lineage-specific antigen cCD79a.

ICMSP has a couple of key markers that are known for aberrant expression. CD13 and CD33 (part of ICMSP) showed aberrant expression in 5/26 (19.23%) and 2/26 (7.69%) B-ALL cases respectively. Studies have also shown that CD13 and CD33 positivity is commonly seen in 6–16% and 3–10% of ALL cases respectively^{36–38} and they are frequently associated with t(9;22).^{39,40} In our study, we found Philadelphia chromosome t(9;22) positivity in 10/26 B-ALL cases out of which 7/26 expressed aberrant myeloid antigens.

Similarly, aberrant expression of lymphoid antigens CD7 (ICMSP), CD3 (PMSP), CD19 (ICMSP and PMSP), and CD10 (ICMSP and PMSP) was seen in AML cases. CD7 was seen in 5/45 (11.11%) cases of AML, CD10 in 2/45 (4.44%) AML cases, CD19 in 2/45 (4.44%) AML cases, and sCD3 in 3/45 (6.66%) AML cases. According to available literature, CD7 expression is seen in 20% of AML, CD19 in 5% of AML, CD10 in 3% of AML, and sCD3 in 1% of AML.^{41–43} This was in close concordance with our study results.

CD117 (part of ICMSP) is a specific marker for AML arising de novo or in association with myelodysplastic syndromes. Overall, we found CD117 expression in 46.67% of AML cases. Others have reported CD117 expression by flowcytometric analysis in 23–91% of AML cases.^{44–46} In a study by Hans et al.,⁴⁷ CD117 was found to be highly specific marker for differentiating acute myeloid from acute lymphoid leukemia. In fact, the specificity for the myeloid lineage was found to be greater than

that of CD13 and/or CD33. Despite the high level of specificity of CD117 for myeloid lineage, they found no cases in which CD117 positivity resulted in reassignment of lineage from lymphoid to myeloid.

In our study, CD117 expression was not seen in any acute lymphoblastic leukemia case. Moreover, CD117 positive AML cases were all cMPO positive too, except in a single AML M6 case that was already evident from combined morphology and cytochemistry analysis. Hence, CD117 offered no significant advantage in a screening panel.

CD34 antigen (used in both panels) is expressed on hematopoietic progenitor cells of all lineages as well as the most pluripotent stem cells. CD34 antigen expression is highest on the most primitive stem cells and is gradually lost as lineage committed progenitors differentiate. In our study, CD34 was found positive in 62.19% of acute leukemias. 65.38% of B-ALL and 50% of T-ALL expressed CD34 positivity. 67.56% of non-APML cases, 25% of APML, and 100% of MPAL cases expressed CD34. Both the cases of APML with CD34 positivity were hypogranular variants of APML that are known to frequently express CD34. However, CD34 expression was heterogeneous in AML as it was found in 100% of AML M0, 33.34% of M1, 66.67% of M2, 25% of M3, 50% of M4, 100% of M5 cases, and 66.67% of M6 cases.

In a study by Basso et al.,⁴⁸ correlation of CD34 with various subtypes was published in which CD34 expression was seen in 90–100% of FAB subtype M0, 70–90% of M1, 20–40% of M2, 0–5% of M3, 20–60% of M4, 50–80% of M5a, 10–30% of M5b, 20–40% of M6, and 40–60% of M7 cases.

CD34 was found to be less sensitive in detection of hematopoietic progenitor cells as HLA-DR was found to be positive in 68.29% of acute leukemias, 69.23% of B-ALL, 37.5% of T-ALL, 81.08% of non-APML cases, and 100% of MPAL cases. HLA DR expression was not seen in any APML case. This result was in concordance with a study done by Kaleem et al.⁴⁹

CD45 (leukocyte common antigen) was used to identify leucocytes on CD45 versus SSC plot. It expressed in 90.24% of acute leukemias with 95.55%, 100%, and 80.76% positivity in AML, T-ALL, and B-ALL cases. CD45 was used in both the screening panels.

sCD3 used in PMSP was seen positive in 37.5% of both T-ALL cases and APML cases. sCD3 positivity in APML cases in our study could be due to contamination of T lymphocytes in the gated population or nonspecific staining. In retrospect, since all T-ALL cases were positive for cCD3, inclusion of sCD3 did not justify its presence in the PMSP. Hence we believe that this marker can be omitted in future studies.

CD7 and CD5 (used in ICMSP) did not offer any specific advantage on cCD3 used in PMSP, as all T-ALL cases were positive for cCD3. CD7 showed expression in 100% of T-ALL with aberrant expression in 13.33% of AML cases and 8.33% cases of B-ALL. However, CD5 showed no aberrant expression and was found expressed in 87.5% of T-ALL cases.

Comparison of results from the two panels

PMSP was more sensitive than ICMSP in cases of ALL and MPAL. Though it was less sensitive in cases of AML, it had 100% positive predictive value across all three lineages (ALL, AML, and MPAL), which was much higher than ICMSP's positive

predictive values. This is hugely in favor of PMSP, since treatment for AML and ALL is different and delay in correct classification can lead to increase in morbidity.

PMSP also had higher specificity than ICMSP in all three types of acute leukemias. In fact, the specificity of PMSP was 100% in all cases. This is especially significant in developing countries, where cost of treatment is high, and specificity is preferred over sensitivity.

Thus, from the scope of our study, we can deduce that PMSP may substitute ICMSP providing better results.

It is interesting to note that PMSP measured at 100% for various statistical indices (at least 2/3 occurrences per index). The authors advise that readers be cautiously optimistic and understand that this figure may or may not extrapolate over larger sample sizes (82 cases studied in this research). For example, if we had received a case with a single population of blasts that met criteria for B or T-ALL that also showed unequivocal evidence of monoblastic differentiation, PMSP may not have picked it up.

It must also be noted that preliminary tests (combined morphology and cytochemistry) alone diagnosed more cases correctly (93.9%) than the ICMSP (92.68%) that used FCM and larger number of antibodies. This may be attributed to the expertise of the staff in the tertiary care center and may not always be the case elsewhere. Nonetheless, the PMSP diagnosed the highest number of cases correctly (95.12%).

Conclusion

Combined morphology and cytochemistry classifies most of the acute leukemia cases into myeloid versus lymphoid. However, FCM is needed to further subclassify the acute leukemia and identify blasts in difficult cases.

PMSP can substitute ICMSP as it classifies most of the acute leukemia cases into myeloid, lymphoid, and MPAL. PMSP takes advantage over ICMSP in cases of MPAL, which according to the latest WHO criteria (2008)³⁵ requires cytochemical stains. Addition of cytoplasmic markers should thus be used in screening panels even though their use may require higher expertise.

PMSP uses lesser number of antibodies and is accurate due to use of lineage-specific antibodies. ICMSP uses antibodies, which are not lineage specific and that are known to show aberrant expression.

Authors encourage the reader to build upon their findings in this paper. For example, the authors suspect that if sCD3 is removed from the PMSP to create a 7-marker alternate minimal screening panel, the same results will be observed. Further, authors recommend that sCD3 be replaced in the PMSP by a more useful marker such as a monocytic marker or CD33/CD13/CD117 that will help identify more AML cases.

Comprehensive panel is required for finding out the subtypes of AML as well as certain rare types of acute leukemia. A minimal screening panel only serves the purpose of delineation and must be treated as a primary panel. It is recommended to follow such a panel by a secondary directed panel to further subclassify acute leukemia as well as to determine its prognosis.

Conflicts of interest

The authors have none to declare.

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