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Cost-Effectiveness of Limited Screening Panel for Acute Lymphoblastic Leukemia Diagnosis in a Resource-Limited Setting

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ABSTRACT

Background. Flow cytometry is an invaluable tool in the diagnostic evaluation of acute leukemia and post therapy monitoring; however, majority of Filipino population cannot afford the cost. The use of a minimal screening panel which is both cost-effective and provides an accurate diagnosis of acute lymphoblastic leukemia is seen as an alternative.

Objectives. We aim to determine the cost-effectiveness and accuracy of using a minimal screening panel for the diagnosis of acute lymphoblastic leukemia (ALL).

Methodology. We selected a limited panel of 9 antibodies comprising of CD45/CD19/CD20/CD10/HLA-DR/CD34/cCD3/cCD79a/cTdt and retrospectively reviewed newly diagnosed cases of B-cell and T-cell ALL from September 2016 to December 2019 using this panel.

Results. Out of 719 bone marrow aspirates submitted for basic leukemia flow cytometric analysis we identified 268 ALL cases (239 B-ALL and 29 T-ALL).

In all cases, a diagnosis was established using the limited panel. Compared to the current cost of our comprehensive panel (₱ 9,903.60). This limited panel cost ₱ 3,062.29, that offers a 69.08% savings per test, which translated to a ₱1.2 million savings a year (for an average of 180 annual cases).

Conclusion. We underscore the utility of a limited panel for the diagnosis of ALL. Although this panel remains to be assessed with a larger validation cohort, its application in resource-limited developing countries is diagnostically useful and cost-effective.

Recommendation. The use of a limited panel of 9 antibodies is recommended as a screening panel for patients who are highly suspected of having ALL both clinically and initial bone marrow smear assessment.

Key words: limited screening panel, acute lymphoblastic leukemia, pediatric population, Filipino

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INTRODUCTION

The diagnosis of acute lymphoblastic leukemia (ALL) entails the integration of cell morphology, immunophenotype and genetics/cytogenetic studies.[1,2] Cellular morphology is the first step in the diagnosis of ALL, but given that there are no morphologic criteria to distinguish whether the blasts are of the B- or T-cell lineage, other ancillary tests were sought.

Flow cytometry is a crucial tool in the rapid diagnosis and accurate classification of leukemia.[3] It employs physical characterization including cell size, granularity and DNA content. These parameters are measured simultaneously as the suspension pass through a measuring device. Highly specific monoclonal antibodies are used to recognize surface, cytoplasmic and nuclear antigens present in leukocytes and these are labeled with the use of fluorochromes, the most widely used of which are FITC, phycoerythrin and allophycocyanin.[4-6]

Flow cytometric evaluation in addition to its diagnostic use can be utilized to assess relapse and or residual disease following therapy. The use of appropriate antibody panels

will aid in the identification of cell type, cell lineage, the stage of maturation and clonality.[4]

An extensive panel of antibodies is used in order to make a definitive diagnosis of acute leukemia. The antibody panel primarily include surface markers (i.e., CD45, CD34, CD117, HLA-DR, CD4, CD8, CD19, CD10, CD20, CD33, CD13, CD56, CD14, CD64, CD11c, CD41a, glycophorin A, anti-kappa and anti-lambda) and cytoplasmic markers (i.e., IgG2a, IgG1, cCD3, cCd79a, cMPO and cTdT).[7-10] However, the use of this panel is expensive and majority of Filipino population cannot afford the cost. In this setting, screening with the use of a limited number of antibody can help in reducing the financial burden of flow cytometry.[9,11-13]

METHODOLOGY

This study utilized B-cell and T-cell acute lymphoblastic leukemia diagnosed by flow cytometric evaluation coupled with cellular morphology. Bone marrow aspirate in heparinized tubes or peripheral blood in EDTA tubes were subjected to three-colored flow cytometry. For the diagnosis, a basic leukemia panel was used in all cases (Appendix A). The panel comprised of 23 antibodies including surface markers (CD45, CD4, CD8, CD34, CD117, HLA-DR, CD13, CD3, CD33, CD19, CD10, CD20, CD5, CD56, CD14, anti-kappa and anti-lambda) and cytoplasmic markers (IgG2a, IgG1, cCD3, cCD79a, cMPO and cTdT).

A limited panel of nine (9) antibodies (CD45, CD19, CD10, CD20, HLA-DR, CD34, cCD3, cCD79a and cTdT) were selected.[4,12,14] Using this panel, we retrospectively reviewed all newly-diagnosed pediatric (0-18 years old) cases of B-cell and T-cell acute lymphoblastic leukemia from September 2016 to December 2019.

The study is limited to patients from our institution and cases sent from other institutions for diagnosis were not included in the study population. Relapse and residual B-cell and T-cell lymphoblastic leukemia cases were also excluded from the study.

The computed minimum sample size for the study was 56. The sample size for the study was estimated using single population proportion formula with the following assumptions: 100% sensitivity, 100% specificity and 55% prevalence ALL based on the results of the study done by Artaiz et al.[15] The sample size was calculated using sample size estimation formula for diagnostic studies.

The data were tabulated and descriptive statistics were presented as frequencies and tables. The sensitivity, specificity and predictive values were calculated for the minimal panel compared with the basic leukemia panel.

RESULTS

A total of 719 bone marrow aspirate were submitted and subjected to a comprehensive flow cytometric analysis. Of this, 268 were ALL cases; 239 (89.2%) of which were B-lymphoblastic leukemia (B-ALL) and 29 (10.8%) were T-lymphoblastic leukemia (T-ALL). There were 59 cases by which ALL was the clinical consideration, however no abnormal blast population was noted on flow cytometry.

The commonly expressed B-cell antigens in B lymphoblastic leukemia were CD79a (97.4%), CD10 and CD19 (96.7%), cTDT (94.98%), HLA-DR (90.3%) and CD34 (85.4%). The other markers that yield positivity were: CD20 (44%), CD13 (34.3%), CD33 (17.57%) and CD45 (5%). A diagnosis of B-ALL was established with the use of the limited antibody panel in 100% of cases (239/239). This was based on the positivity of cCD79a and other B cell markers (CD10, CD19 and CD20) and immature markers namely CD34 and cTdt (Table 1).

All 29 T-ALL cases expressed cCD3 and CD5 (100%). Surface CD3 was expressed in 89.7 % of cases. Other markers that yield positivity were: cTdT (79.3%), CD8 (68.96%), CD4 (31%), CD34 (24.1 %), CD13 (17.2%) and CD33 (10.34%). Cytoplasmic CD79a was negative in all cases. A diagnosis of T ALL established with the use of the limited antibody panel in 100% of cases (29/29) (Table 2).

From this data, the sensitivity and specificity of the limited screening panel was at 100%. The positive and negative predictive values were both 100%.

The current cost of our basic leukemia panel is ₱ 9,903.60, compared to the limited panel which cost ₱ 3,062.29. This offers a 69.08% savings per test, which translates to a ₱ 1.2 million savings per year (for an average of the 180 annual cases) (Table 4) (Appendix B).

DISCUSSION

Immunophenotyping was used as a means of identifying and quantifying a single cell population which can be accomplished by staining the population of interest with two or more antibodies simultaneously.[6] There has always been a need of thorough and careful selection of marker

Table 1. Antigen expression of B-ALL cases

	B-Acute Lymphoblastic Leukemia (n= 239)						
	cTdT	CD34	cCD79a	CD10	CD19	CD20	HLA-DR
Positive	227 (94.98%)	204 (85.4%)	233 (97.5%)	231 (96.7%)	231 (96.7%)	106 (44.3%)	216 (90.3%)
Negative	12 (5.0%)	5 (14.8%)	6 (2.5%)	8 (3.3%)	8 (3.3%)	133 (55.6%)	23 (9.7%)

Table 2. Antigen expression of T-ALL cases

	T-Acute Lymphoblastic Leukemia (n= 29)									
	cTdT	CD34	cCD3	CD3	CD5	CD4	CD8	CD13	CD33	cCD79a
Positive	23 (79.3%)	7 (24.1%)	29 (100%)	25 (89.7%)	29 (100%)	9 (31%)	20 (68.96%)	5 (17.2%)	3 (10.3%)	NIL
Negative	7 (26.9%)	19 (73.1%)	NIL	1 (3.8%)	NIL	20 (68.96%)	9 (31.%)	24 (82.8%)	26 (89.7%)	29 (100%)

Table 3. 2x2 table for the computation of sensitivity, specificity and predictive values of the limited screening panel

Limited Panel Flow	Basic Leukemia Panel		Total
	Positive	Negative	
Positive	268	0	268
Negative	0	59	59
Total	268	59	327

Table 4. Cost of the basic leukemia panel compared with the limited screening panel

Costing	Basic Leukemia Panel	Limited Screening Panel
Cost Per Test	9,903.06	3,062.29
Annual Cost	1,782,550.80	551,212.20
Annual Savings		1,231,338.60

combinations based on their specificity in the identification of lineage, stage of maturation and aberrant phenotype expression. The use of appropriate monoclonal antibody clones and fluorochrome combinations must also be considered. The use of these markers in combination is more pertinent than that of individual markers, for they provide a unique immunophenotype enabling the identification of the cell population in question.[9,11,16,17]

The antibody panel for the limited flow cytometry were lineage-specific B-cell and T-cell markers which include cytoplasmic CD79a and cytoplasmic CD3, respectively. Cellular maturity was assessed by the presence or absence of the following markers: CD45, CD34, cytoplasmic TdT, CD10 and CD19.[1,8,9,18]

As opposed to the other antigens which are anchored on the cell membrane, TdT is found in the nucleus. Hence, TdT staining is performed intracellularly after rendering both the cell membrane and nuclear membrane permeable.[6,19]

Degree of lineage maturation of the population of interest was evaluated by CD45 and CD34, since these markers are considered the most efficient in defining immaturity. CD45 enabled one to differentiate hematopoietic cells by their pattern of intensity which can be correlated with both cell lineage and maturity. For this reason, CD45 was a major marker in the identification of blast population based on its dim expression and the exclusion of normal hematopoietic cells. Of the 268 diagnosed ALL cases, 5% of this expressed dim positivity to CD45. To further refine and confirm the gating of blasts, CD34 was used.[11,16,20] In this study, B-ALL and T-ALL expressed CD34 in 85.4% and 24.1% respectively.

Cytoplasmic CD79a expression appears early during B-cell commitment. This occurs after the expression of Tdt and prior to the acquisition of CD19. In conjunction with cytoplasmic CD79a, virtually all cases of B-ALL express CD19. CD19 is deemed a sensitive B-cell marker but has a low specificity prompting the need for cytoplasmic CD79a to improve lineage assignment.[11,16] HLA-DR is also a helpful marker in the detection of acute leukemia and may be the most sensitive marker for B-ALL.[13] 97.5% of all B-ALL cases expressed CD79a and the remaining 2.5% of cases did not, hence the use of another B-cell marker (e.g., CD19 or CD20) was warranted. According to Swerdlow et al., CD79a has been noted to be positive in a number of T-ALL cases, hence these individual markers are not specific. However, combined expression of these markers will strongly support the diagnosis of B-ALL.[1]

CD10 often expressed in B-ALL can represent a population of both immature cells and normal B cell development. This type of maker is needed in the comparison with and differentiation from normal B-cell development patterns.[11]

In order to differentiate B-ALL from T-ALL, the incorporation of cytoplasmic CD3 is warranted. Lineage specific cytoplasmic CD3 is constantly expressed at high levels in T-ALL, which made the gating of blasts easier.[1,16] The interpretation of cytoplasmic CD3 should be coupled with surface CD3 for the reason that most of the T-ALL cases express cCD3 with negative smCD3.[16] All 29 cases of T-ALL expressed cCD3 and none expressed CD79a.

In a study by Singh et al., a minimal panel of eight antibodies were proposed (CD45/CD34/CD19/MPO/cytoCD3/CD64/CD117/CD79a) and a diagnostic yield of 97.5% was achieved. Their study was based on a 200 population, by which only 5/200 required an additional set of antibodies to properly classify the leukemic process.[4] Our study had a sensitivity and specificity of 100%, which meant that all 268 ALL cases were duly diagnosed by the use of the proposed limited screening panel.

In 2018, the World Bank said that amidst the good economic performance of the country, poverty remains high and the pace of poverty reduction has been slow.[21,22] The additional expense of healthcare ancillary procedures adds to the financial burden of the average Filipino.[22] The use of the limited screening panel cuts the cost of flow cytometry by 69.08%, hence easing the financial burden.

CONCLUSION

We underscore the utility of a limited panel for the diagnosis of ALL. Although this panel remains to be assessed with a larger validation cohort, its application in resource-limited developing countries is diagnostically useful and cost-effective.

RECOMMENDATION

The use of a limited panel of 9 antibodies is recommended as a screening panel for patients who are highly suspected of having ALL both clinically and by initial bone marrow smear assessment. A study on limited screening panel that will extend to cases of acute myeloid leukemia is also proposed.

STATEMENT OF AUTHORSHIP

All authors fulfilled the ICMJE authorship criteria.

AUTHOR DISCLOSURE

The authors declared no conflict of interest.

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APPENDICES

Appendix A

Table 1. Basic leukemia panel composition

	Surface		
	FITC	**PE**	**PerCP**
1			CD45
2	CD4	CD8	CD45
3	CD34	CD117	CD45
4	HLA-DR	CD13	CD45
5	CD3	CD33	CD45
6	CD19	CD10	CD45
7	CD20	CD5	CD45
8	CD56	CD14	CD45
9	Anti-kappa	Anti-lambda	
	Cytoplasmic		
10	IgG2a	IgG1	CD45
11	cCD3	cCD79a	CD45
12	cMPO	cTdT	CD45

Appendix B

Table 2. Costing for basic leukemia panel

Tube #	Product Description	Test per Vial	SRP	Price per Tube
1	CD45 PerCP	200	32,602.00	163.01
2	CD4/CD8	100	56,010.64	280.05
2	CD45 PerCP	200	32,602.00	163.01
3	CD34 FITC	200	56,010.64	280.05
3	CD117 PE	100	35,265.96	352.66
3	CD45 PerCP	200	32,602.00	163.01
4	HLA-DR FITC	200	45,638.30	228.19
4	CD13 PE	200	62,234.04	311.17
4	CD45 PerCP	200	32,602.00	163.01
5	CD3 FITC	200	37,340.43	186.70
5	CD33 PE	200	20,317.00	101.59
5	CD45 PerCP	200	32,602.00	163.01
6	CD19 FITC	200	37,340.43	186.70
6	CD10 PE	200	47,712.77	238.56
6	CD45 PerCP	200	32,602.00	163.01
7	CD20 FITC	200	37,340.43	186.70
7	CD5 PE	200	47,712.77	238.56
7	CD45 PerCP	200	32,602.00	163.01
8	CD56 FITC	100	31,117.02	311.17
8	CD14 PE	200	33,191.49	165.96
8	CD45 PerCP	200	32,602.00	163.01
9	Anti-kappa/Anti-lambda	100	56,010.64	560.11
9	CD45 PerCP	200	32,602.00	163.01
10	Simultest IgG2a/IgG1	100	51,861.70	518.62
10	CD45 PerCP	200	32,602.00	163.01
11	cCD3 FITC	200	37,340.43	186.70
11	CD79a PE	100	40,265.96	402.66
11	CD45 PerCP	200	32,602.00	163.01
12	MPO FITC	100	33,191.49	331.91
12	TdT PE	100	37,340.43	373.40
12	CD45 PerCP	200	32,602.00	163.01
	FLUIDICS			2,505.48
			Total	**9,903.06**