Lymphogram: a rapid flow cytometry method for screening patients with lymphocytosis

Lymphogram is a reagent that permits simultaneous enumeration of T-, B- and NK-lymphocytes and subpopulations in a single flow-cytometric test. Such a reagent is thus potentially attractive as a first-line test in the investigation of lymphocytoses as it could obviate the need for expensive and labor-intensive conventional multi-reagent panels. However, to our knowledge there have been no previous reports directly comparing the Lymphogram with a standard immunophenotyping. We have, therefore, conducted such a study.

Thirty-three samples from patients with known T-cell lymphocytoses with the diagnoses of large granular leukemia (3 cases), NK-leukemia (2), T-cell prolymphocytic leukemia (4), T-cell non-Hodgkin's lymphoma (2), T-cell acute lymphoblastic leukemia (4), and T-cell lymphocytosis (18) were included. Samples were analyzed using both a conventional flow cytometry panel and the Lymphogram reagent (Cytognos, Salamanca, Spain), which contains CD8-FITC, CD19-FITC, CD56-PE, CD3-PE and CD4-PEcy5. Conventional flow-cytometry was performed using the following antibodies (MoAb): CD5-FITC/CD19-PE, CD4-FITC/CD8-PE, CD3-FITC/CD16-PE and CD57-FITC/CD-56PE (Caltag). Lymphogram staining was performed according to the product insert. Stained cells were acquired using a FACScan flow-cytometer running CELLQUEST software (Becton-Dickinson). The Lymphogram analysis was performed using the PAINT-A-GATE program in a sequential manner. In the first step, $CD19^+$, $CD56^+$ and $CD3^+$ populations were identified (Figure 1a). In the second step, the T-cell subsets were assessed (Figures 1b-e). The results of the Lymphogram were compared with those obtained in the standard way (Table 1). The Lymphogram correctly identified the lineage in 32/33 cases of lymphocytosis and there were no cases of misclassification. The results obtained by the two methods showed no major discordance as 107/154 of the results

Table 1. The comparison of the results obtained with conventional immunophenotyping (C) and with the Lymphogram reagent (L).

Pat. no.#	Diagnosis	CD3C	CD3L	CD19C	CD19L	CD56C	CD56L	CD4C	CD4L	CD8C	CD8L	Ratio CD4/CD8C	Ratio CD4/CD8L
1	LGL leukemia	79	77.2	9	11	nd	11.5	24	25.7	46	50.5	0.5	0.5
2	LGL leukemia	77	52.9	nd	3.1	21	15.6	19	15.2	56	55.5	0.3	0.3
3	LGL leukemia	81	77.6	1	0.3	7	9	7	11.6	72	70	0.1	0.2
4	NK leukemia	6.9	8.3	1.5	1.9	90.2	90.5	4.7	4.9	72.4	63	0.1	0.1
5	NK leukemia	19	14.8	3	8.4	4	2	9	8.1	19	8.2	0.5	1.0
6	Sézary syndrome	86	80	7	5.8	1	0.7	70	72.5	16	16.5	4.4	4.4
7	T-ALL	nd	3	0	0.4	50	44	3	2.2	2	1.1	1.5	2.0
8	T-ALL	7	5	1	0.5	0	1.4	2	3.4	4	2.9	0.5	1.2
9	T-ALL	40.5	37.9	8.3	11.8	3.4	1.6	64	70	61.6	56.3	1.0	1.2
10	T-ALL	72	80.6	11	3	5	4.2	58	54.2	55	54.4	1.1	1.0
11	T-lymphocytosis	86	77.7	9	2.2	2	7.6	37	37.2	46	55	0.8	0.7
12	T-lymphocytosis	78	83.9	12	7.7	4	3.1	17	21	54	65	0.3	0.3
13	T-lymphocytosis	98	97.4	0	0	2	0.8	18	16	80	82	0.2	0.2
14	T-lymphocytosis	nd	94.6	0.3	1.5	nd	0.4	12.7	13.4	82.8	82.7	0.2	0.2
15	T-lymphocytosis	54	60	9	11.8	nd	8	12	14.5	64	63	0.2	0.2
16	T-lymphocytosis	82	81.7	10	nd	2	4	9	14.2	76	77	0.1	0.2
17	T-lymphocytosis	80	84.2	13	10.8	11	3	48	57.3	31	37	1.5	1.5
18	T-lymphocytosis	80	84	1.2	4.2	5.7	6.4	82	72.5	9.5	10	8.6	7.3
19	T-lymphocytosis	77	77.1	5	3.4	9.2	10	64.3	70	12.4	15	5.2	4.7
20	T-lymphocytosis	69.3	73.5	12.6	9.5	nd	8.4	55	66	16.4	20	3.4	3.3
21	T-lymphocytosis	87	89	7.9	1.9	nd	3	77	79.7	13	7.2	5.9	11.1
22	T-lymphocytosis	98	98	0	0	2	1	69	77	20	21	3.5	3.7
23	T-lymphocytosis	80	75.1	16.4	23.2	2	2	69	76.3	16	19.2	4.3	4.0
24	T-lymphocytosis	79.7	84.9	13.7	11.6	5.4	2	43.8	58.2	33.7	36	1.3	1.6
25	T-lymphocytosis	88	94	2.3	1.9	5.2	3.7	22	9.4	4	2.4	5.5	3.9
26	T-lymphocytosis	49.1	47.8	5.4	7.1	nd	41.7	34.2	40.4	13.2	26	2.6	1.6
27	T-lymphocytosis	86.1	89.8	5.4	5.4	nd	4	21.8	22.1	29.9	39	0.7	0.6
28	T-lymphocytosis	60	56.7	3.3	4	14	7.9	49.3	33.9	21.6	18.7	2.3	1.8
29	T-NHL-Skin	78	80	1	5.5	14	8	4	9.1	75	79	0.1	0.1
30	T-PLL	0	1.3	2	0.3	1	0.2	94	80	0	1	na	80.0
31	T-PLL	1	0.3	0	0.4	1	0.1	94	98	0	0.1	na	980.0
32	T-PLL	90.2	84.9	0.3	1.7	0.2	1.84	96.3	78.9	0.4	2.63	240.8	30.0
33	T-PLL	81	89.7	9	3.8	2	3	76	83	8	10	9.5	8.3

Mean difference	4.2	2.5	2.4	5.8	3.6
Median difference	3.7	1.7	1.5	4.6	2.2
No cases with difference <5%	21 (68%)	25 (81%)	20 (77%)	17 (51%)	24 (73%)
No cases with difference <10%	29 (93%)	31 (100%)	26 (100%)	27 (82%)	30 (91%)
ND	2	2	7	0	0
Overall mean difference			2.7		
Overall median difference			2.3		

Legend: T-LGL: T-cell large granular leukemia; T-ALL: T-cell acute lymphoblastic leukemia; NHL: non-Hodgkin's lymphoma; T-PLL: T-cell prolymphocytic leukemia; ND: not done.

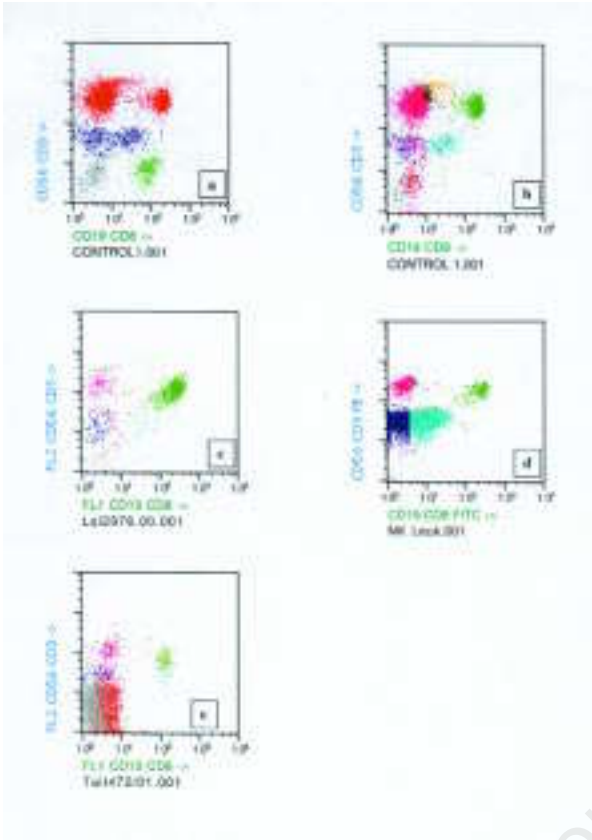

Figure 1. a) first step of the Lymphogram analysis (normal pattern); b) second step of the Lymphogram analysis (normal pattern); c) pattern characteristic for LGL; d) pattern characteristic for NK-leukemia and e) pattern of T-ALL. Grey, unclassified events; red, $CD3^+CD4^-CD8^-$; pink, $CD3^+/CD4^+$; green, $CD3^+/CD8^+$, yellow, $CD3^+/CD8^+$dim; blue, $CD56^+/CD8$; cyan, $CD56^+/CD8^+$; black, $CD3^+CD4^+CD8^+$ subpopulations.

for the 5 monoclonal antibodies only differed by less than 5%, and 143/154 by less than 10%. A small number of discordant results were noted, including the CD4 percentage in 6 patients and the CD3 in 3. The Lymphogram failed to classify one case with a $CD56^-$ NK cell disorder.

Lymphogram allows the simultaneous analysis of the major circulating lymphocyte sub-populations, T, B, and NK, with a single test vial and provides a rapid identification and enumeration of these lymphocyte populations that could be useful when a patient's material is limited. The test can be performed on a standard flow-cytometer fitted with one laser as, although there are 5 MoAb included in the reagent, only 3 fluorochromes are used and the distinction relies on staining intensity.[2,3]

We have undertaken a side-by-side comparison of this rapid method with the standard immunophenotypic analysis. A previous study by Bellido *et al.*[1] applied the Lymphogram to 108 samples for immunophenotyping B-cell and T-cell lymphocytoses. By additional testing with anti-κ and anti-λ in cases in which the $CD19^+$ cells exceeded the normal range, the authors were able to determine clonality of B-cells. However, this study[1] did not include a comparison between the standard flow-cytometry and the Lymphogram.

The present study showed no major discrepancies between the conventional flow-cytometry and the Lymphogram, which should encourage the use of the Lymphogram as it is time-saving. A small number of samples with lack of agreement between the Lymphogram and conventional flow-cytometry can be explained by differences in gating (physical vs immunologic gate) and analysis (lack of negative control in the Lymphogram and therefore subjectivity of the analysis process with the PAINT-THE-GATE software). A higher percentage of $CD4^+$ cells in the conventional flow cytometry analysis can be explained by the inclusion of monocytes (which are $CD4^+$) into the total (cases #18, 28, 30, 32).

We have shown here that the Lymphogram is as effective as multi-reagent panels in the initial classification of most cases of lymphocytoses, but that additional studies with an extended panel of conventional MoAb will be required in some patients. In particular, care must be taken when NK malignancy is suspected, as only cases expressing the CD56 marker on their surface can be recognized. Of the 2 cases of NK leukemia analyzed, one case presented with cells co-expressing $CD56^+CD8^+$ and thus the diagnosis of NK leukemia would have been suggested by the Lymphogram. In the other case the cells were $CD3^-$, $CD4^-$, $CD8^-$ and $CD56^-$ and the malignancy would not have been detected as an NK-leukemia when tested with the Lymphogram alone. Upon testing with conventional cytometry, a population of $CD16^+$ NK cells was detected. However, the excess of unclassified Lymphogram events in this patient would have indicated further studies.

In conclusion, the Lymphogram appears to be a simple, cost-effective and reliable tool for initial screening of lymphocytoses.

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