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Flow cytometry of cell suspensions from lymph nodes: immunophenotype, DNA content and proliferative rate are strongly correlated with histopathology diagnosis

Sir,

A proposed REAL classification has reinforced the role of immunophenotyping in the diagnosis of lymphomas.[1] Immunophenotyping is performed with immunohistochemical staining, but the availability of flow cytometry (FCM) offers an alternative method.[2] Despite the loss of tissue architecture and potential lack of tumor representation, FCM has been reported to be effective, especially in B-cell NHL.[3] On the other hand, DNA content and cell cycle analyses have been studied mainly with regard to their clinical impact.[4] In this study lymph nodes were analyzed by FCM with a multiparameter approach which involved firstly, surface immunophenotyping and secondly, analysis of DNA content, S-Phase Fraction (SPF) and BCL2 expression, in order to evaluate their contribution to diagnosis.

Cell suspensions were prepared in an automated device, MediMachine (Dako, Denmark), and incubated with the following fluorescein or phycoerythrin labeled monoclonal antibodies (MnAbs): CD5, CD19, CD4, CD8, CD3, CD7, anti-κ, anti-λ, CD10 and CD23. Cells were immediately analyzed in a FACScalibur Cytometer (Becton Dickinson, San José, CA, USA). One aliquot was resuspended in 1 mL of cold methanol 70%, briefly stored at –20°, and then incubated with fluorescein labeled MnAb against BCL2 protein. Cells were treated with RNAse, and 20 µL of propidium iodide was added. DNA ploidy and cell cycle were analyzed using MODFIT software.

Results of surface B and T cell markers of 12 samples are given in Table 1.

In 4 samples a B-cell neoplasm was confirmed since CD19 positive cells accounted for more than 50% of the total population and the D value of κ/λ analyses was 0.83-0.97. In samples #1 and 11 a T-cell NHL was initially suspected, based on an abnormal asynchronous expression of CD3, CD5 and CD7. Results of the second step are as follows: among four cases of B-cell NHL, DNA content analysis disclosed aneuploid peaks only in case #4 (Figure 1A) and BLC2 expression was higher than 50% in 3 cases. Extremely helpful information was obtained in cases 1 and 11, since a high SPF was found. In six cases with immunophenotype, SPF and BCL2 unconclusive for diagnosis, when gating on the largest population, we could detect hyperploid peaks in two cases (Figure 1B). Anatomic

Table 1. Surface markers' expression, Bcl2 and S phase.

Sample	*CD3*	*CD7*	*CD5*	*CD4*	*CD8*	*CD10*	*CD19*	*CD23*	*k/l*	*Bcl2*	*S phase*	*Age*	*Diagnosis*
1	20	57	54	12	7	0	5	0	0.05	ND	9.68	28	TL-NHL
2	34	40	34	18	8	1	16	0	0.08	ND	2.91	14	NS-HD
3	55	60	61	46	18	1	30	0	0.19	8	2.7	35	LP-HD
4	11	13	11	7	6	0	73	7	0.97	48	1	71	CC-NHL
5	51	40	46	36	9	2	34	3	0.23	19	6.15	14	Reactive
6	44	40	36	29	8	3	55	10	0.86	67	1.23	64	CC-NHL
7	50	55	58	40	3	0	28	7	0.22	ND	0.35	30	Reactive
8	34	35	37	28	8	0	52	42	0.19	ND	0.51	29	Reactive
9	15	17	20	12	7	23	73	9	0.83	70	1.42	67	CC-NHL
10	75	80	85	67	7	0	14	9	0.41	4	1.76	71	NS-HD
11	45	20	25	18	8	0	22	13	0.28	23	12.82	45	ATC-NHL
12	6	5	6	5	3	40	92	15	0.97	87	0.2	62	CC-NHL

NHL: non-Hodgkin's lymphoma, HD: Hodgkin's disease; TL: T- lymphoblastic; NS: nodular sclerosis; LP: lymphocyte predominance; CC: centrocytic-centroblastic; ATC: anaplastic T-cell.

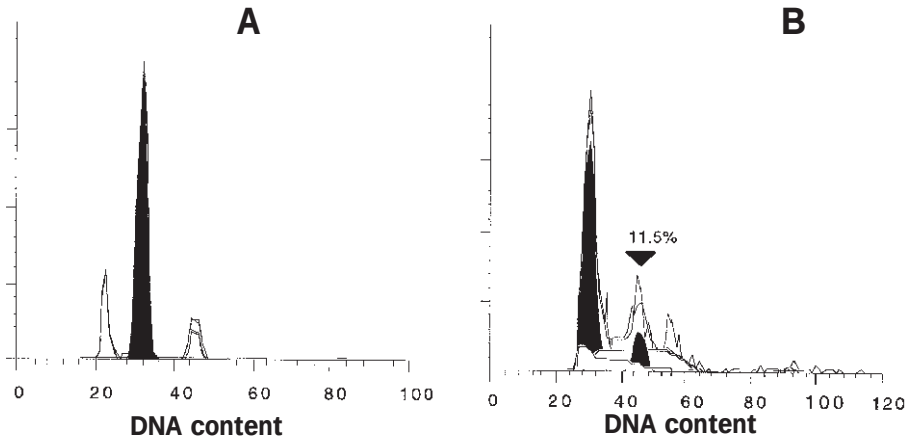

Figure 1. (1 A) DNA content showing hyper and hypoploid peaks in a case of B-cell NHL. (2B) Aneuploid peak is detected among the largest cells in a case of HD.

pathology results were in close agreement since all cases of B-cell NHLs were centroblastic-centrocytic NHLs, 2 cases of T-cell NHLs were T-cell lymphoblastic lymphoma and anaplastic T-cell lymphoma, and 2 cases of aneuploid peaks with normal populations were HD nodes. The remaining cases were reactive nodes, with the exception of a HD case, in which no aneuploid peaks were detected.

Only histopathology can provide diagnosis of lymphoid malignancies (i.e. a distinction between thymomas and T lymphoblastic lymphomas, ALK protein expression in anaplastic lymphomas and diffuse or follicular patterns of B-NHLs). However, B-cell NHLs were easily detected by FCM, confirming the feasibility of automated methods to prepare cell suspensions.[5] Likewise, the detection of a SPF of nearly 10% confirms the presence of a neoplasic process, although it has been previously reported that 6% is a satisfactory threshold to differentiate NHLs from reactive hyperplasias.[6,7] Almost 100% of HD are aneuploid, the detection being hampered by the scarce tumoral representation,[8] but a simple approach of gating[9] could detect two aneuploid peaks out of three cases of HD. In conclusion, we demonstrate the usefulness of FCM, especially using a multiparametric approach including DNA analyses, in acquiring diagnostic information rapidly from lymph nodes.

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