CEREBROSPINAL FLUID FLOW CYTOMETRY FOR DIAGNOSIS AND MONITORING OF NHL: A REGINA ELENA NATIONAL CANCER INSTITUTE EXPERIENCE

44

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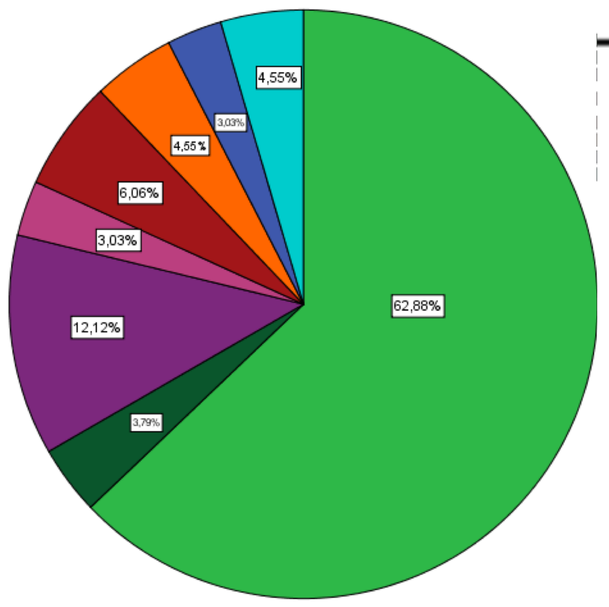
Background

Central nervous system (CNS) involvement in non-Hodgkin's lymphoma (NHL), significantly more frequent in aggressive subtype, has an incidence of 5-9% and represents a negative prognostic factor. Compared to cerebrospinal fluid (CSF) cytology, the gold standard for leptomeningeal infiltration diagnosis, flow cytometry (FC) has shown to be a more sensitive diagnostic tool for CNS infiltration. We report the role of FC in diagnosis and monitoring of CSF infiltration in a large cohort of NHL patients.



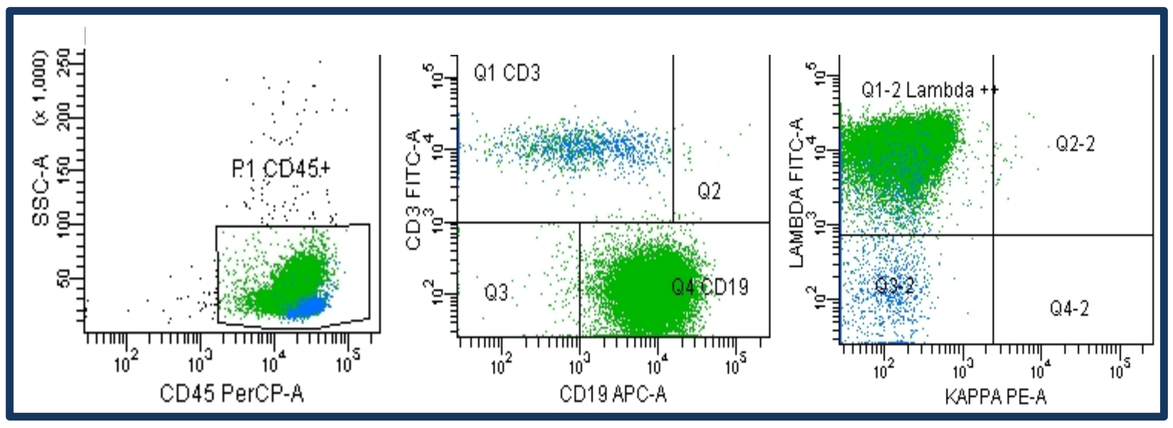
	Frequency	Percent
DLBCL	83	62,9
Burkitt Lymphoma	5	3,8
MCL	16	12,1
Anaplastic CD30+ Lymphoma	4	3,0
Periferal T-NHL	8	6,1
FL	6	4,5
Other	4	3,0
PCNSL	6	4,5
Total	132	100,0

Methods From January 2007 to June 2012, a total of **179** CSF samples, corresponding to 131 NHL patients, were characterized by FC and compared to cytology to evaluate the possible CNS involvement. Peripheral blood FC study was performed on 75 patients.

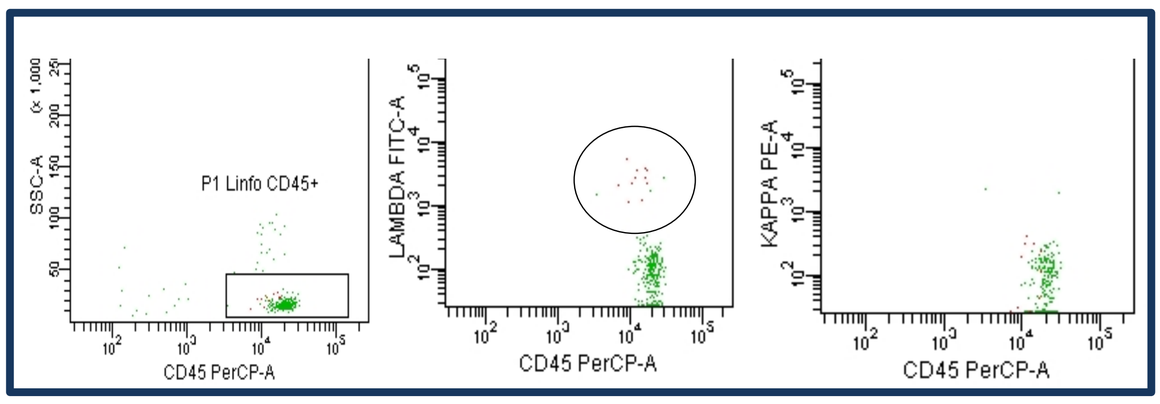
Results

According to the WHO classification, 83 were diffuse large B cell, 16 mantle cell, 8 peripheral T cell, 6 follicular, 5 Burkitt, 5 primary CNS, 4 anaplastic ALK+, 2 lymphoblatic, 1 NK and 1 dendritic cell NHL. A positive CSF cytology was documented in 3.8% of cases (5/131 patients), by contrast 6.9% of cases (9/131 patients) were positive by FC. None of the patients positive for cytology were negative by FC ($p < 0.0001$). In the 5 patients positive both by cytological and FC analysis, the percentage of pathological cells identified by FC was significantly higher compared to the 4 patients with negative cytology and positive FC (90% vs. 19.5%). A trend was observed suggesting that cytology is capable of CSF infiltration diagnosis only for high infiltration rates (Mann-Whitney U test $P=0.086$). Moreover, 48 samples were collected for minimum residual disease monitoring. In these patients, cytology was positive in 18.8% and FC positive 39.6% of cases (19/48 patients) ($P = 0.006$). Focusing on samples with low cell count (number of events lower than 50th and 25th percentile on immunophenotypic examination), cytological examination did not show positive cases (0/24; 0/12), by contrast FC identified 6 and 3 positive samples in the respective subgroups. The blood analysis showed the presence of circulating neoplastic cells in 8/75 patients with a correlation between blood and CSF involvement ($P = 0.014$). A T lymphocytes population was documented in all the CSF samples (100%), flanking the lymphoma cell in the positive cases.

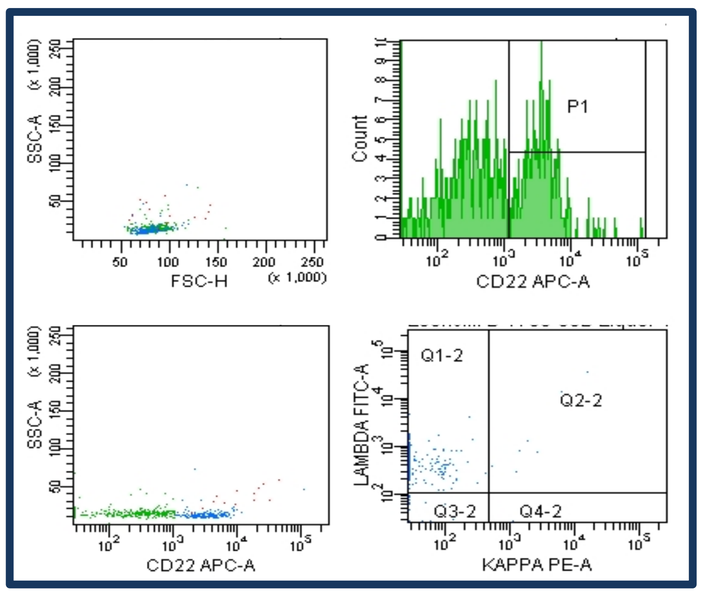
MCL flow-CSF at diagnosis



MCL flow-MRD



PCNSL
"occult" leptomeningeal disease



Conclusions

Compared to cytology, FC confirmed to be a more sensitive approach for CSF infiltration in NHL both in patients at diagnosis and for minimal residual disease assessment with a relevant diagnostic advantage in CSF samples with low cell count. A positive correlation was found between the presence of peripheral blood circulating lymphoma cells and CSF infiltration. As previously reported by our group in breast cancer neoplastic meningitis, a side population of reactive T lymphocytes was observed in all the case, documenting an active mechanism of T-lymphocytes migration to CSF.