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Lymphoma Diagnosis: Lessons Learned from the Comparison of Histology and Cytology Associated with Flow Cytometry

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Abstract

For lymphoma diagnosis, the Flow Cytometry (FCM) and cytology associated with FCM (C-FCM) performed on Fine Needle Aspiration (FNA) or cell suspension/imprints from fresh tissue display a good concordance (from 85% to 90%) with the diagnosis made using histological data. Herein is reported a retrospective series of discordant cases, five of them are discussed in details, and some recommendations are proposed for the interpretation of C-FCM data. Firstly, this review highlights the importance of analyzing simultaneously the cytological and FCM data. In particular, the cytological data are crucial to interpret FCM data and/or to complete Ab panels when the strategy of the laboratory is to systematically perform a first screening, which does not always allow detecting lymphoma cells. Secondly, this report underlines that cytology and FCM analysis should be followed by a confrontation/discussion with a pathologist. Finally, C-FCM appears to be a rapid and particularly important technique to guide the choice of the following diagnosis tools (IHC and genetic).

Keywords: Cytology; Flow cytometry; Lymphoma; Histology

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Introduction

In accordance with the current WHO classification [1], an integrated morphologic, immunophenotypic, and molecular approach is crucial for lymphoma diagnosis. In this context, the Flow Cytometry (FCM) and cytology associated to FCM (C-FCM) performed on Fine Needle Aspiration (FNA) or cell suspension/imprints from fresh tissue display a good concordance in terms of diagnosis with histology (from 85% to 90%) [2]. Herein, the focus is made on discordant cases encountered in a single hematologic department, they are presented as short cases, and some recommendations for the C-FCM interpretation are proposed. A series of 324 Lymph Node (LN) samples (from January to December, 2019, in the Hematology unit of the Lyon-Sud Hospital Group/ Hospices Civils de Lyon, which is one of the largest French recruitment center) evaluated by touch LN imprints cytology and FCM with the corresponding histopathological analysis were retrospectively studied by an experimented cytologist and FCM interpreters [3]. The C-FCM was in agreement with histology in 295 (91.05%) cases. The distribution of the different types of lymphomas analyzed herein differed than the one reported in a recent series [4] (Table 1), which could be explained as only a fraction of all LN biopsies received by the pathology unit were secondarily addressed to Hematology unit and as the present study included samples only for one year. In 29 (8.95%) cases, the first diagnostic hypothesis made by cytology was discordant with the histologic diagnosis. These discordant cases were Hodgkin lymphoma (HL, all different subtypes, n=6), diffuse large B-cell lymphoma (DLCBL, n=5), angioimmunoblastic T-cell lymphoma (AITL, n=5), anaplastic large cell lymphoma (ALCL ALK+/ALK-, n=3), follicular lymphoma (FL, n=2), in situ mantle cell lymphoma (*in situ* MCL, n=1), marginal zone lymphoma (MZL, n=1), MALT lymphoma (n=1), T-cell/ Histiocyte-Rich Large B-Cell Lymphoma (THRBLCL, n=1), LNH B, NOS (n=1), benign lesions (n=1), sarcoma (n=1), and Schwannoma (n=1). Among these cases, 28/29 (96.55%) were samples sent for newly/suspected lymphoma; in contrast, 228/295 (77.20%) concordant cases were samples sent for newly/suspected lymphoma. The analysis was made by non-experimented interpreters in 25/29 discordant cases (86.20%). As in a previous study [4], the discordant cases were classified as

Table 1: Distribution of cases.

Main entities	Count	Proportion among all samples (lymphoma, reactive lesions, and non-hematopoietic neoplasm) n=324	Proportion among lymphomas n=201	
Mature Lymphoid neoplasm	155	47.80%	77.10%	
Mature B-cell lymphomas	131	40.40%	65.20%	
DLCBL	30			22.90%
BL	1			0.70%
FL (grade 1,2, or 3A)	53			40.40%
MCL	13			9.90%
MALT lymphomas	2			1.40%
NMZL/SMZL	8			3.90%
LL/CLL	19			14.50%
B-cell lymphoma, NOS	4			2.80%
THRLBCL	1			0.7 00%
Mature T-cell lymphomas	24	7.40%	11.90%	
AITL	16			66.70%
ALK+-ALCL	6			25.00%
PTCL, NOS	2			8.30%
Precursor lymphoid neoplasm	2	0.60%	0.90%	
HLs	44	13.60%	21.90%	
Reactive lymphoid lesions	97	29.90%		
Non-hematopoietic neoplasm	26	8.00%		

DLBCL: Diffuse Large B-Cell Lymphoma; BL: Burkitt Lymphoma; FL: Follicular Lymphoma; MCL: Mantle Cell Lymphoma; NMZL: Nodal Marginal Zone Lymphoma; SMZL: Splenic Marginal Zone Lymphoma; LL/CLL: Lymphocytic Lymphoma/Chronic Lymphoid Leukemia; THRLBL: T-cell/Histiocyte-Rich Large B-Cell Lymphoma; AITL: Angio-Immunoblastic T-cell Lymphoma; ALCL: Anaplastic Large Cell Lymphoma; PTCL, NOS: Peripheral T-Cell Lymphoma, NOS; HL: Hodgkin Lymphoma

Table 2: Discordances between C-FCM and histology.

Category A = misclassification of lymphoma subtype	6
low grade B-NHL instead of DLBCL	2
DLBCL instead of low grade B-NHL	0
misclassification NHL subtype	4
misclassification NHL instead of HL	0
Category B = malignant to benign lesions or vice versa	21
lymphoma or other neoplasm instead of benign lesions	1
benign lesions instead of lymphoma or other neoplasm	18
suspect lesions to overt lymphoma	2
Category C = lymphomas to other malignancies or vice versa	2
lymphomas instead of other malignancies	1
other malignancies instead of lymphomas	1

HL: Hodgkin Lymphoma; DLBCL: Diffuse Large B-Cell Lymphoma; NHL: Non-Hodgkin Lymphoma

major or minor changes according to the guidelines of the European society of medical oncology (Table 2) [5,6]. The cytology of LN imprints was reviewed by an experimented hematologist/cytologist for all cases and FCM data were re-interpreted a posteriori by a FCM expert for the 25 available cases (Table 3). The most frequent discordances were misclassification of malignant to benign lesions or *vice versa* (21/29, 75.86%), which mainly included False Negative (FN) diagnosis (18/21, 85.71%): Benign lesions instead of HL (n=5), DLCBL (n=2), AITL (n=3), ALCL (n=3), THRLBCL (n=1), LF with partial involvement (n=1), in situ MCL (n=1), MZL (n=1), and Schwannoma (n=1). In all cases but one case of LH, one AITL, one ALCL, one LF with partial involvements, and one *in situ* MCL, the expert cytological analysis could correct the diagnosis. After a second FCM analysis, the correct diagnosis could be proposed for 3 AITL cases, 1 ALCL case, and 1 MZL case and suggested in all LH cases. In 1 case, a False Positive (FP) diagnosis (1/21, 4.76%) was made by cytology (DLCBL instead of benign lesion). In the latter case, the analysis by a cytologist expert could correct the diagnosis, but the review of FCM data could not be performed. For 2 cases, cytological analysis was difficult and did not lead to a conclusion of lymphoma. The histologic diagnosis corresponded to one AITL and one FL, that were retained after a second cytologic and FCM analysis in both cases. Misclassification of lymphoma subtype was observed for 6 cases: Low grade B-NHL instead of DLCBL (n=2), LH instead of DLCBL (n=1), DLCBL instead AITL (n=1), THRLBCL instead LH (n=1), and FL instead of LNH-B, NOS (n=1). In 2 cases of grade misclassification, the second cytology and FCM analysis corrected the error; the second cytology analysis could correct the diagnosis in all 4 remaining cases but the FCM data was contributive in the diagnosis correction only one case (THRLBCL *vs.* LH).

Misclassification between lymphoma and non-hematopoietic was observed in 2 cases: LNH B instead of sarcoma (that could be corrected by a experimented review of cytology and re-interpretation of FCM data since most cells were CD45 negative) and non-hematopoietic neoplasm instead of MALT lymphoma (that could be corrected by review of imprints but FCM was not contributive as the cellular suspension was not concentrated enough).

After this review of cases by experimented cytologic/immunophenotype interpreters, the diagnostic concordance with histology was to 98.20% (318/324, only 6 discordant cases; Table 3).

Discordant Cases

Case 1: classic Hodgkin Lymphoma (cHL)

A 49-year-old female was admitted for general weakness, B symptoms, and fever. She presented multiple lymphadenopathies (axillar, cervical, and mediastinal). Her blood count was the following: Leukocytosis at 18.6 G/L associated with anemia (hemoglobin: 108 g/L, VGM: 77 fL). Lactate Dehydrogenase (LDH) and β-2 microglobulin levels were normal.

Imprints from a fragment of axillar LN biopsy were performed, as well as cell suspension for FCM analysis. Imprints were hypocellular including fibrotic fragments, few eosinophils, and small lymphocytes. This case was first diagnosed as a reactive lymphadenopathy.

However, the careful cytologic analysis and examination of all imprints allowed to identify Hodgkin or Reed-Sternberg (HRS) cells, frequently reduced to their nucleus (Figure 1, black arrow), consistent with the histologic diagnosis. In routine, the screening Antibodies (Abs) panel used was designed to analyze the different subset of lymphoid population, and did not allow the identification of HRS-cells. In this case however, the distribution of lymphoid subsets was quite different to what is usually observed in reactive conditions (low count of CD19 B-cells [17%] and quite large count of T-cells [75%], especially CD4 T-cells representing 85% of T-cells) and might orientate the diagnosis to cHL (see Case 1 - Educational message) [7,8].

Table 3: Discordant cases.

Histologic diagnosis (n=29)	First cytology diagnosis	Corrected diagnosis by experimented interpreters	
		Cytology (n=29)	FCM (n=25)
HL (n=6)	Benign lesions (n=4) THRLBCL (n=1)	5 (83.30%)	6* (6/6, 100%)
DLCBL (n=5)	Benign lesions (n=2) MZL (n=1)#, FL (n=1)#, HL (n=1)	5 (100%)	2# (2/4, 50.00%)
AITL (n=5)	Benign lesions (n=3) DLCBL (n=1), LNH B, NOS (n=1)	4 (80.00%)	3 (3/4, 75.00%)
ALCL (n=3)	Benign lesions (n=3)	2 (66.66%)	1 (1/2, 50.00%)
THRLBCL (n=1)	Benign lesions	1 (100%)	1 (100%)
LF (n=1)	LNH B, NOS	1 (100%)	1 (100%)
LF partial involvement (n=1)	Benign lesions	0	0
in situ MCL (n=1)	Benign lesions	0	0
MZL (n=1)	Benign lesions	1 (100%)	1 (100%)
MALT (n=1)	Non hematopoietic neoplasm	1 (100%)	0
LNH B, NOS (n=1)	FL	1 (100%)	0
Benign lesion (n=1)	DLCBL	1 (100%)	na
Sarcoma (n=1)	LNH B	1 (100%)	1 (100%)
Schwannoma (n=1)	Benign lesions	0	0
		23/29 (79.30%)	16/25 (64.00%)
Final discordance after review		6/324 (1.80%)	

HL: Hodgkin Lymphoma; THRLBL: T-cell/Histiocyte-Rich Large B-cell Lymphoma; DLBCL: Diffuse Large B-Cell Lymphoma; AITL: Angio-Immunoblastic T-cell Lymphoma; MZL: Marginal Zone Lymphoma; FL: Follicular Lymphoma; ALCL: Anaplastic Large Cell Lymphoma; MCL: Mantle Cell Lymphoma; na: not available
*CMF analysis was suggestive of HL but did not allow identifying tumor cells
#partial infiltration

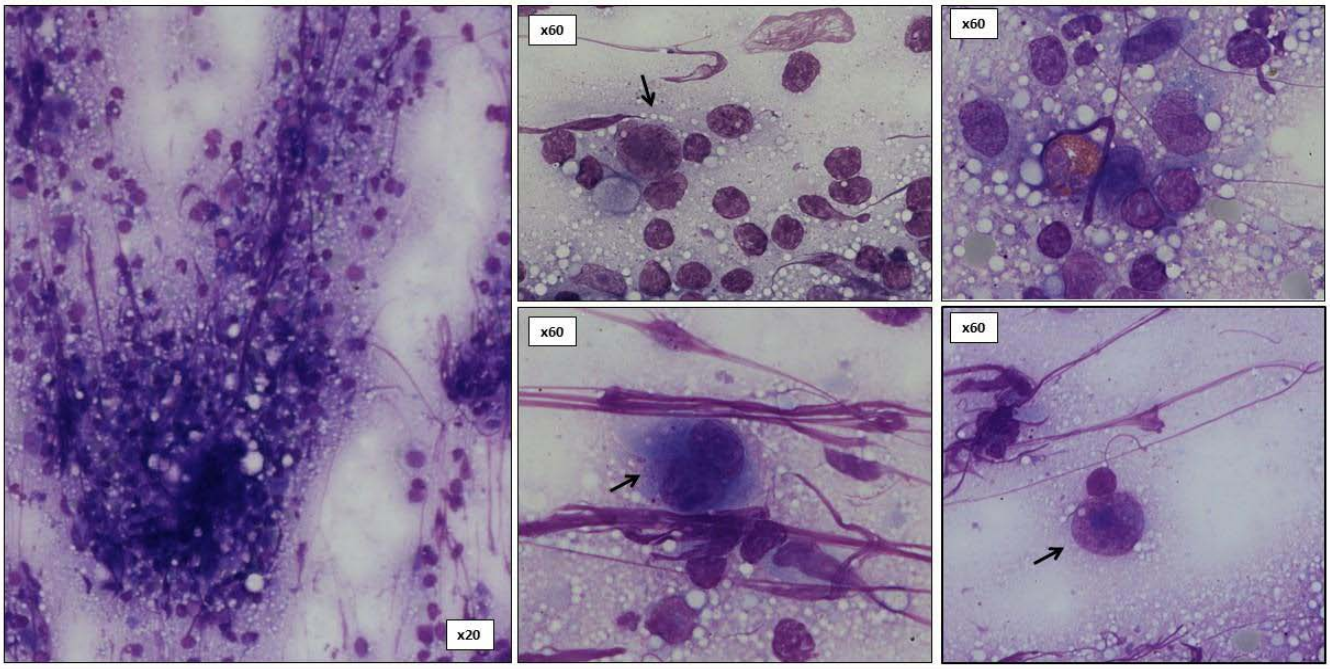

Figure 1: Classic Reed-Sternberg cells are large, have abundant slightly basophilic cytoplasm, and have at least two nuclear lobes or nuclei (black arrow). The nuclei are large and often with rounded contours, with a prominent, often irregular, pale chromatin, and usually one prominent nucleolus. Hodgkin cells have the same aspect but are mononuclear (black arrow) (Magnification x20 and x60, May-Grünwald Giemsa (MGG) staining).

Educational message: All (the maximum amount and at least 3) imprints should be analyzed to search HRS cells especially if the first imprints are suggestive of HL because of the inflammatory background (eosinophils, plasma cells, numerous histiocytes with epithelioid features, isolated or in small granuloma/aggregates) in an appropriate clinical context. Few reports in the literature have described the identification of HRS cells by FCM analysis, which requires a specific combination of Abs [9]. However, a low count of B-cells associated with a preponderance of CD4 T-cells in the absence of other immunophenotypic alterations on lymphocyte population is highly suggestive of cHL [7,8]. Although some authors do not recommend sending a cHL-suspicious sample for immunophenotyping by FCM, in these situations the characterization of the background cell population will add helpful information. Obviously, the FCM results

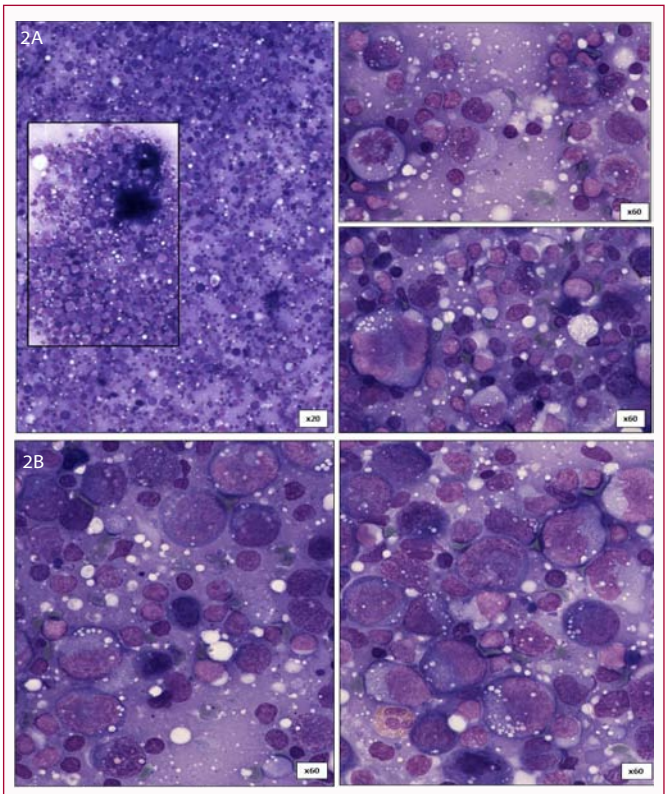

Figure 2: Lymphoid-rich tissue with effaced Lymph Node (LN) architecture and scattered very large-sized cells with irregular nuclear contours (convoluted or multilobed) and abundant basophilic cytoplasm. Sometimes, they could mimic Hodgkin or Reed-Sternberg (HRS) cells. Few mitoses suggest the aggressiveness of the lymphoma. (Magnification x20 and x60, May-Grünwald Giemsa (MGG) staining).

should be interpreted in perspective with the cytological parameters because an abnormal repartition of the lymphoid subset can also be observed in T-cell lymphoma (see Case 4). In Case 1, complementary immuno-cytochemistry with anti-CD30 and anti-CD15 Abs into imprints can be particularly informative.

Case 2: Anaplastic large cell lymphoma (ALK+ALCL)

A 56-year old female treated for Bartonella infection 2 years earlier was admitted for severe abdominal and back pain associated with B symptoms and recurrence of multiple lymphadenopathies. LDH (650 UI/L) and the C-reactive protein (CRP, 62.1 mg/L) levels were elevated, blood cell count was normal.

Imprints from a fragment of cervical LN biopsy were performed, as well as cell suspension for FCM analysis. In the imprints, normal LN architecture was effaced and displayed numerous large-sized cells dispersed into an inflammatory background (small lymphoid cells, eosinophils, histiocytes; Figure 2). This case was first interpreted as a reactive lymphadenopathy with immunoblastic hyperplasia. In addition, the FCM analysis was not contributive as only the compartment of small lymphocytes was analyzed and displayed normal distribution of the different lymphoid subsets: CD19 B-cells (27.1%), CD3 T-cells (61.9%) with CD4 T-cells (18.5%) and CD8 T-cell (48.8%).

Expert cytological analysis combined with adjusted FCM analysis allowed to correct the diagnosis (ALK+ ALCL). Indeed, the second FCM analysis focused on large cells using a combination of pan-T Abs and allowed to identify few atypical CD4+ T-cells that lost surface CD3 and CD7 expression. The tumor CD4 T-cells strongly expressed CD30.

Educational message: Cytology was clearly atypical: Disappearance of normal LN architecture and presence of numerous atypical large and medium-sized cells with highly irregular (convoluted or multilobed) and hypochromatic nuclei and basophilic cytoplasm different from reactive centroblasts and/or immunoblasts (Figure 2). Against the diagnosis of reactive follicular hyperplasia, they were not a lot of follicles with Germinal Center (GC) including a predominance of large cells (centroblasts) along with scattered tangible-body macrophages. Finally, this lymphoma diagnosis can overlap with - and are sometimes difficult to distinguish from - cHL diagnosis. Both display an inflammatory background that can be rich in epithelioid histiocytic cells and eosinophils. The interpretation of FCM data combined with cytological data is crucial so as: (1) not to give FN negative result due to an analysis focusing only on the small lymphocytes compartment, and to properly specify the size settings for FCM gating; (2) to orientate the choice of the suitable Abs panel for FCM, including pan-T Abs and anti-CD30. In a cHL context, the neoplastic cells in ALCL usually express T-cell markers.

Case 3: DLCBL, NOS

A 39-year-old pancreas-transplanted type 1 diabetic male presented severe abdominal pain. PET-scan revealed a mesenteric mass associated with multiple adenopathies. LDH level was elevated (335 UI/L). The blood cell count and CRP level were normal.

Imprints from a fragment of the mesenteric mass biopsy were performed, as well as cell suspension for FCM analysis. Imprints displayed a more or less heterogeneous and polymorphic large-sized cell population on a necrotic background. Besides, there were small lymphocytes; some had cleaved nuclei, and few macrophages with apoptotic bodies (Figure 3). The FCM analysis detected lambda monotypic CD19+ B-cells (75%) associated with CD3 T-cells (17%) among which CD4 T-cells (11%) and CD8 T-cells (46%; Figure 3). Considering the identification of centrocytes (small lymphocytes with cleaved nuclei), this case was first interpreted as a Follicular Lymphoma (FL).

The diagnosis was corrected into DLCBL upon second cytological and FCM analyzes, confirming that monotypic B-cells were larger than the reactive T-cells (Figure 3).

Educational message: Few centrocytes (small to medium-sized cells with cleaved nuclei) were identified, but these one are normally found in GC and therefore their present could not argue alone in favor of a FL diagnosis. Against the diagnosis of FL, the centrocytes represented a minority of the lymphoid cells, the main population being composed of large-sized cells. However, the diagnosis of FL (grade 3) or DLCBL by FL transformation could still be discussed. The necrosis background argued in favor of an aggressive lymphoma. The FCM analysis contributed to retain the diagnosis of large B-cells lymphoma by highlighting monotypic light-chain B-cells and a majority of large-sized cells identified on cell-size dot plot (Forward Scatter [FSC]/Side Scatter [SSC]).

Case 4: Angioimmunoblastic T-cell lymphoma (AITL)

A 54-year-old male was admitted for erythematosus rash with pruritus and eosinophilia (1.3 G/L) after treatment by carbocisteine and solupred for influenza infection, which initially orientated the diagnosis towards a DRESS syndrome. However, no clinical improvement was observed. The patient then presented fever,

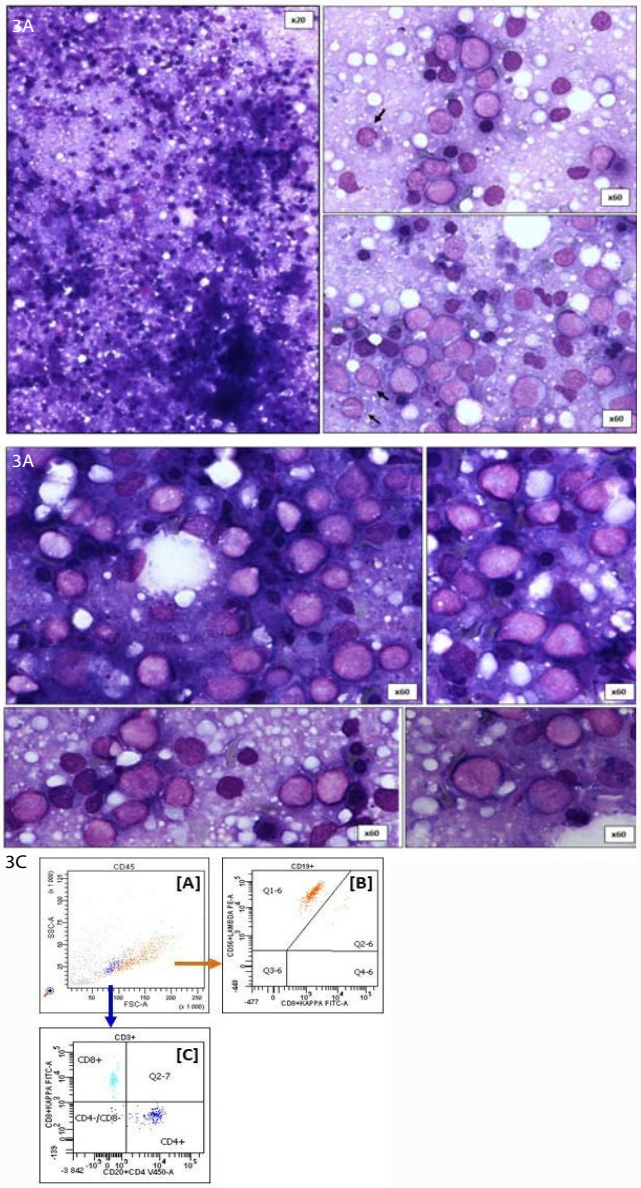

Figure 3: Lymph Node (LN) with necrosis background and a mixture of small- and large-sized lymphoid cells. Large cells display variable nuclear features, including round or irregular nuclei and a variable number of nucleoli. Some rare small lymphocytes are identified as centrocytes (arrow; magnification x20 and x60, May-Grünwald Giemsa (MGG) staining). Dot-plot of multi-parametric Flow Cytometry (FCM) obtained from cell suspension: large CD19+ B-cell lymphoma (orange) and residual T-cells (blue)(Forward Scatter/Side Scatter [FSC/SSC] dot-plot [A]), lambda monotypic B-cells (dot-plot obtained with gating settings for CD19+ [B] and repartition of CD4+ and CD8+ T-cells (dot-plot obtained with gating settings for CD3+ [C]).

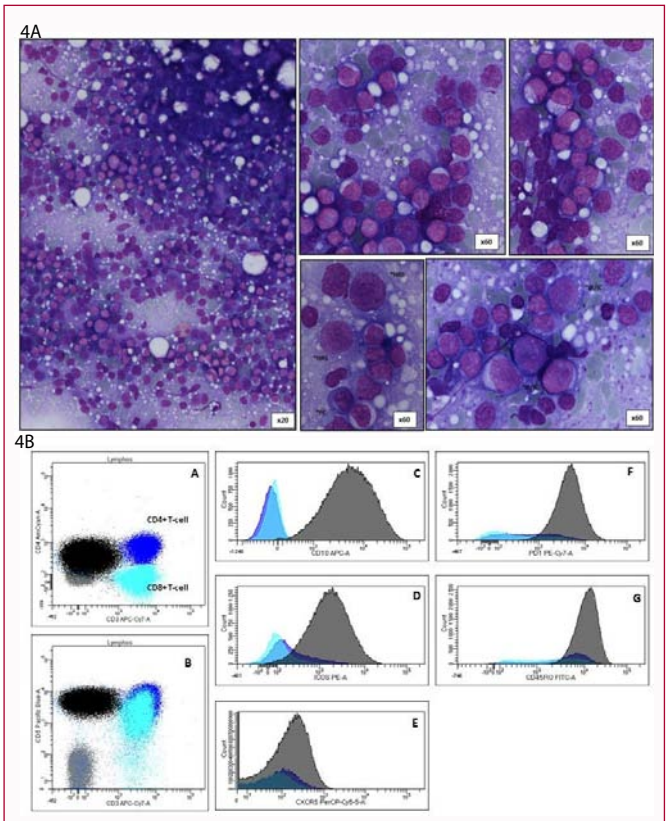

Figure 4: Partial effacement of Lymph Node (LN) architecture with inflammatory background (eosinophils, mast cells, histiocytes, and Plasma Cells [*PC]) and polymorphous lymphoid cells including predominant small-to-medium-sized lymphocytes that show minimal atypical features (pale cytoplasm, irregular nucleus) and large-sized cells mimicking Hodgkin or Reed-Sternberg (HRS)-cells (*HRS) and/or Immunoblasts/Centroblasts (*IB/CB). In the interfollicular zone, an expanded FDC meshwork could be observed. Dot-plot and histograms of multi-parametric Flow Cytometry (FCM) obtained from cell suspension (all gating settings for lymphocytes): residual CD4+ (blue) and CD8+ (turquoise blue) T-cells, atypical CD4+ T-cells that lost CD3 expression (black, A), residual T-cells, and atypical CD4+ T-cells expressing CD5 (B), atypical CD4+ T-cells strongly expressing CD10 (black, C) and ICOS (black, D) and dimly expressing CXCR5 (black, E). The PD-1 (black, F) and CD45RO (black G) were positive.

enlarged LN at multiple sites, and hepatosplenomegaly. LDH (582 UI/L) and CRP (26 mg/L) levels were elevated. A polyclonal hypergammaglobulinemia was detected.

Imprints from a fragment of cervical LN biopsy were performed, as well as cell suspension for FCM analysis. Imprints displayed a partially effaced LN architecture by a polymorphous lymphoid population: Small lymphoid cells displaying minimal cytological atypical features (abundant and pale cytoplasm) mixed with large lymphoid cells (immunoblasts), eosinophils, mast cells, plasma cells, and histiocytes. In the interfollicular zone, an expanded Follicular Dendritic Cell (FDCs) meshwork was detected (Figure 4). This case was first interpreted as a reactive lymphadenopathy.

The expert cytological analysis interpreted with FCM data (suitable Abs combination) allowed to correct the diagnosis. Indeed the FCM analysis detected CD4 T-cells with an atypical profile (loss of surface CD3 expression and strong CD10 expression). In addition, these CD4 T-cells displayed the immunological profile of THF (ICOS+/CXCR5+) cells, arguing in favor of a diagnosis of AITL (Figure 4). Note that in this case the cytological analysis of peripheral blood smear associated with FCM has enabled the identification of AITL involvement.

Educational message: The AITL diagnosis may be difficult as this morphology can be encountered in a variety of reactive and neoplastic conditions such as auto-immune diseases, MZL, and more rarely cHL. As for main other lymphomas, the definitive diagnosis requires complementary immunological, genetic, and molecular data. For this difficult diagnosis, the analysis of peripheral blood and/or bone marrow smears may provide useful information for the interpretation of LN imprints. In peripheral blood, lymphoid compartment usually contains small-sized atypical lymphocytes (minimal cytologic atypical features = clear-to-pale cytoplasm,

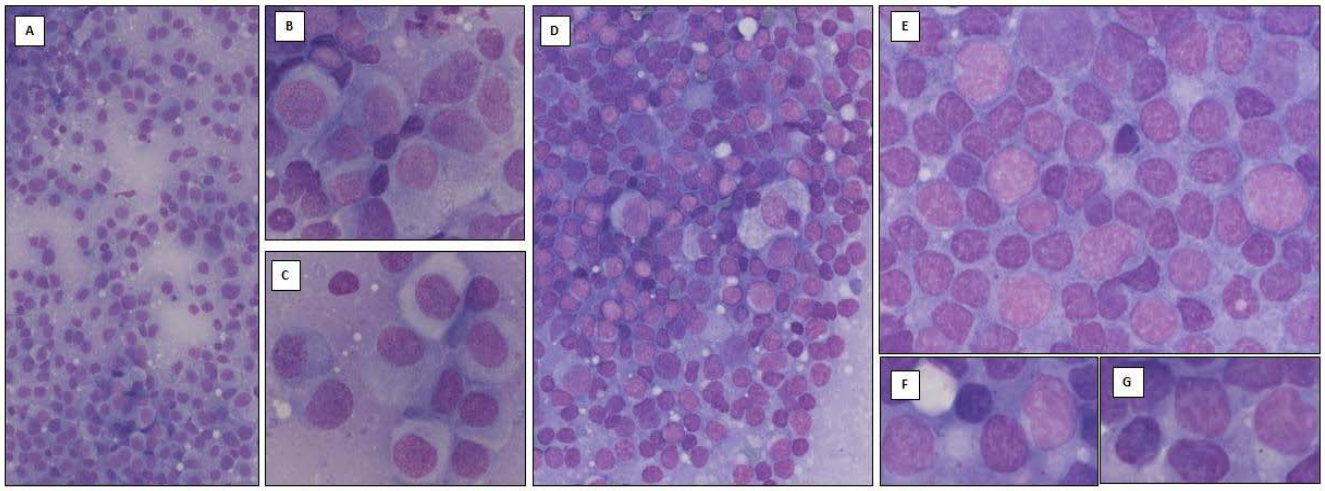

Figure 5: Partial effacement of Lymph Node (LN) architecture with metastatic Paget disease (large-sized cells with abundant and pale cytoplasm (magnification x20 [A], x60 [B,C], May-Grünwald Giemsa [MGG] staining), associated in some area with atypical lymphoid cells (small-to-medium-sized cells, irregular nucleus, and dispersed chromatin (magnification x40 [D], x100 [E-G], May-Grünwald Giemsa [MGG] staining).

irregular nucleus as observed in LN imprints) mixed with eosinophilia and large-sized lymphoid cells and/or sometimes plasma cells [10]. The heterogeneity of the lymphoid population contrasts with the homogeneity of a small B-cells lymphoma dissemination. Moreover the heterogeneity of lymphoid contingent can argue in favor of reactive lymphocytosis, but a lymphopenia is usually observed in AITL, conversely to the lymphocytosis observed in mononucleosis syndrome and/or other reactive conditions. In numerous cases, the neoplastic CD4 T-cells present no atypical profile such as loss of pan-T antigens. Therefore the choice of Ab panel should be guided by cytology. The inclusion of CD10 in our routine panel since 2005 [10,11], and more recently TFH markers (CXCR5, ICOS, PD-1) has consistently reduced false-negative results. When a sole screening Ab panel is used, the interpretation should be carefully interpreted with cytology and clinical data to allow the orientation of second Ab panel. However, AITL is sometimes particularly difficult to diagnose and ancillary molecular tests are frequently performed: IGH or TCR gene rearrangement analysis, as well as Next-Generation Sequencing (NGS). The mutations of IDH2, TET2, and DNMT3A are frequently observed in AITL [1]. Among these, IDH2 R172 mutations appear to be specific for AITL, whereas the others can be detected in other PTCLs, in particular those with a TFH-cell like immunophenotype.

Case 5: Histology corrected by C-FCM = MCL associated with extramammary Paget disease

An 85-year-old male, followed for an extramammary Paget disease, was admitted because of enlarged LN at multiple sites. Blood cell count was normal.

Imprints from a fragment of inguinal LN biopsy were performed, as well as cell suspension for FCM analysis. Imprints displayed a partially effaced LN architecture by large non-hematopoietic cells with abundant and pale cytoplasm. Considering clinical data, they corresponded to LN metastatic sites of the Paget disease (Figure 5). In the reduced area of the fragment, lymphoid tissue persisted and its meticulous analysis allowed to identify a subset of atypical lymphoid cells [small-to-medium sized-cells, irregular nucleus, and dispersed chromatin (Figure 5), suggesting an involvement of small B-cell lymphoma. This hypothesis was confirmed by FCM analysis that allowed characterizing the lymphoma entities as MCL. Indeed, FCM detected lambda monotypic B-cells expressing CD5 and CD38 and not CD200. Interestingly, C-FCM allowed correcting the histological diagnosis that had not initially identified MCL. Then, additional IHC including in particular Cyclin D1 expression confirmed the diagnosis of MCL associated with metastatic sites of Paget disease.

Educational message: This case illustrates the importance of analyzing cytological data concomitantly with FCM data. If cytological data were strongly suggestive of an involvement of lymphoma, in this context of metastatic sites of Paget disease and reduced area of analysis of lymphoid tissue, the detection of monotypic B-cells by FCM allowed confirming and characterizing the origin of lymphoma cells. Importantly, this case underlines the complementarity and usefulness of C-FCM for pathologists. Firstly, the cytology may sometimes detect subtle atypical features easier than histology. Secondly, FCM may be more sensitive since it identifies antigen co-expression more easily than Immunohistochemistry (IHC). Indeed, the co-expression may be difficult to evaluate by immunohistochemistry and, in this case, it did not initially identified CD20+ B-cells that co-expressed CD5. The detection Cyclin D1+ B-cells by IHC confirmed the MCL diagnosis. This case underlines the importance of the discussion/interaction between pathologists and cytology/immunophenotype interpreters.

Discussion

The immunological, cytogenetic, and molecular tools, in particular the recent NGS, have led to a more precise classification of lymphoma as illustrated by the recent WHO classification that comprises numerous subtype entities [1]. The characterization of specific entities, indispensable to the optimal clinical management of patients, leads to a more complex diagnostic strategy, and the need to prioritize the different diagnostic tools. Obviously, C-FCM cannot replace histology, but it has become an ancillary technique useful for pathologists. It provides rapid results (24 h to 48 h) and first orientation of neoplastic cells lineage (T or B-derived lymphoma). The detection of monotypic B-cell light-chain expression is more sensitive using FCM than using IHC. Moreover, thanks to the multiple available Ab combinations, FCM provides complementary information in difficult diagnosis, such as T-cell lymphoma (that requires frequently an extensive panel of Abs not always available

for IHC), difficult histologic diagnosis, or composite lymphomas. Therefore, the first screening by C-FCM may orientate the histology diagnosis, especially the choice of Abs for IHC and molecular tools. This is achieved by discussion between pathologists/cytologists and flow-cytometer interpreters.

However, many neoplastic LN or masses can display heterogeneous infiltration patterns and the fraction of tissue analyzed by C-FCM can be a sample that is devoid of malignant cells. Therefore, the cytological control of cell suspension and/or LN imprints is crucial for an optimal interpretation of FCM data. Besides, adverse storage conditions or manipulations due to the difficulty encountered in obtaining enough cells from some types of tissue could also explain FN results (e.g. skin). As reported in the present review and in the literature [12], some entities of lymphoma are particularly difficult to analyze by FCM, such as DLBCL, either because the gating settings was not performed on the neoplastic cells, or because the tissue contains important necrosis and/or fibrosis. On the other hand, in the latest WHO classification, the definitive diagnosis of some entities, such as the high-grade B-cell lymphoma with MYC and BCL2 and/or BCL6 rearrangements, requires the integration of specific IHC markers (not available for FCM), and genetic and complete molecular data.

Furthermore, FCM could detect antigen expression that may be useful as prognostic or therapeutic markers, especially with the emergence of the new treatment by CAR-T cells and human bi- and/or tri-specific Abs. In addition, FCM can be easily standardized [13-15], and allows the quantification of antigen expression [16]. Finally, FCM data interpretation of hematological neoplasia is a high-complexity assay that requires analysts to be specifically trained. Importantly, FCM data interpreters should be experienced to recognize the immunological profiles of lymphoid cells that derive from normal counterpart and those that orientate the diagnosis to lymphoma. In the same way, the contribution of cytology in lymphoma diagnosis requires to be performed by an expert cytologist. Herein, the discordant cases were due to non-experimented cytologists rather than experimented ones. The great experience of the interpreters is crucial to be able to discuss the significance of the cytology and FCM findings. As showed herein, the diagnosis was improved if the cytological and FCM data were simultaneously analyzed by an experimented cytological/immunological interpreter or at least by two different interpreters.

Conclusion

Firstly, this review highlights the importance of analyzing simultaneously the cytological and FCM data. In particular, the cytological data are crucial to interpret FCM data and/or to complete the Ab panel when the strategy of the laboratory is to systematically perform a first screening, which does not always allow detecting lymphoma cells. Secondly, this report underlines that cytology and FCM analysis should be performed preferentially by an experimented interpreter (or at least 2 different interpreters) and ideally followed by a confrontation/discussion with a pathologist. Finally, C-FCM appears to be a rapid and particularly important technic to guide the choice of the following diagnosis tools (IHC and genetic).

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