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Ultrasound-guided fine needle aspiration cytology combined with flow cytometric immunophenotyping for rapid characterization of deep-seated non-Hodgkin's lymphoma recurrence

We performed US-guided fine needle aspiration cytology combined with flow cytometry in 55 patients with deep lesions and a history of non-Hodgkin's lymphoma (NHL). Forty-seven patients were found to be affected by B-NHL, 5 by T-NHL, 3 by metastatic carcinoma. Immunocytochemistry, morphology and flow cytometry identified 58%, 77% and 100% of the 52 patients with NHL recurrence, respectively.

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We tested the role of flow cytometry compared with morphology, immunocytochemistry and cytogenetics in the assessment of deep lesions in 55 patients (median age 60 years) evaluated for possible NHL recurrence. At time of revaluation, after informed consent US-guided fine needle aspiration cytology (FNAC) of the target lesion was performed using an EUB 525 Hitachi instrument (Tokyo, Japan) with 3.5- or 7.5-MHz probe, puncture adaptor and 0.71 mm (22G) diameter modified Chiba needle 150 or 200 mm in length. Smears stained with Diff-Quick, Papanicolau and Giemsa were analyzed by two expert pathologists. The remaining cytological material was placed i) in 5 mL of isotonic saline solution and processed for flow cytometry; ii) in 15 mL of RPMI-1640 cell culture, and processed for immunocytochemistry and for cytogenetics. Flow cytometry was performed using Becton Dickinson equipment (Palo Alto, CA, USA) and a panel of fluorescein or phycoerythrin-conjugated monoclonal antibodies (MoAbs). After erythrocyte lysis, forward/side light scatter and CD45/side light scatter were used as primary gating methodologies. Normal cut off values for each lymphoid cell population were set according to standard procedures;[1] B-cell monoclonality definition required κ:λ or λ:κ ratio equal to or greater than 10:1, T-cell monoclonality was diagnosed on the basis of abnormal T-cell antigen expression.[2] Immunocytochemical and cytogenetic studies were performed according to standard procedure.[3,4] Three to 5 samples were obtained from each patient; all FNAC procedures were well tolerated. As for morphologic assessment, both pathologists agreed in diagnosing NHL in 40 patients, adenocarcinoma in 2, dysgerminoma in one and reactive lymphoid hyperplasia in 12. Immunocytochemistry, performed in 52 patients, demonstrat-

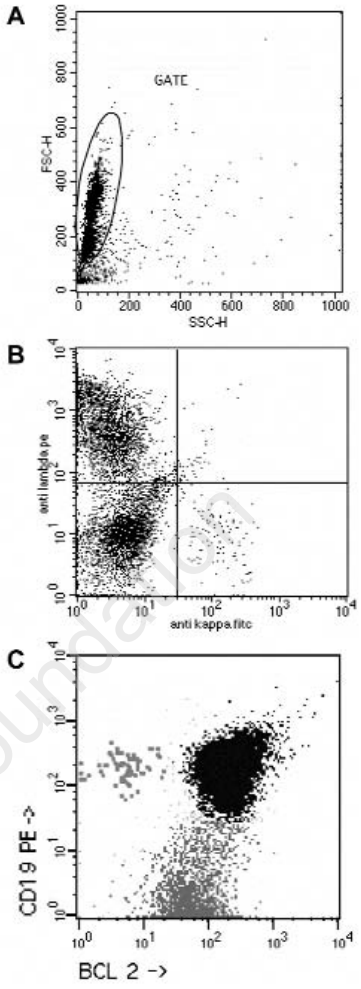

Figure 1. Flow cytometry scattergrams of a representative case of follicular center cell lymphoma (grade I). (A) Gate including low side scatter (small) cells. (B) B-cells with l restriction. (C) Neoplastic follicular B-cells with high expression of both CD19 and bcl-2 (upper-right), normal T-cells with intermediate bcl-2 expression (1 log less intensity) (down), and residual normal follicular B-cells, bcl-2 negative (upper-left).

Table 1. Results of a multidisciplinary approach for identifying NHL recurrence by US-guided fine needle aspiration.

Initial diagnosis	*N. of patients*	*Lesion site at revaluation*	*Flow cytometry I/T*	*Morphology I/T*	*Immunochemistry I/T*	*Cytogenetic I/T*	*Final diagnosis*
DLBCL	13	para-aortic (6), iliac (3), spleen (2), kidney (1), thyroid (1)	13/13	13/13	13/13	3*/7	aggressive NHL (13)
FCL/I	11	para-aortic (8) iliac (2), spleen (1)	10/11	6/11	5/10	1°/3	aggressive NHL (5) indolent NHL (5) metastatic carcinoma (1)
FCL/III	3	para-aortic (3)	3/3	3/3	3/3	0/2	aggressive NHL (3)
SLL	10	para-aortic (4), iliac (3), liver (2), spleen (1)	10/10	7/10	5/10	0/0	aggressive NHL (3) indolent NHL (7)
MZL	8	iliac (4), para-aortic (1), spleen (1), ileum (2)	6/8	8/8	3/6	0/3	indolent NHL (6) metastatic carcinoma (2)
MCL	5	para-aortic (2) iliac (2), spleen (1)	5/5	1/5	1/5	5#/5	aggressive NHL (5)
PTCL	3	para-aortic (2) kidney (1)	3/3	3/3	0/3	0/0	aggressive NHL (3)
MF	2	soft tissue (2)	2/2	2/2	0/2	0/0	aggressive NHL (1) indolent NHL (1)

*DLBCL: diffuse large B-cell lymphoma; FCL/I or III: follicle center cell lymphoma/grade I or III; SLL: small lymphocytic lymphoma; MZL: marginal zone lymphoma; MCL: mantle cell lymphoma; PTCL: peripheral T-cell lymphoma; MF: mycosis fungoides. *Trisomy 12, inv 1 + trisomy 8 or 3, del (6q), in one patient each; °hyperdiploidy; #t(11;14); I/T: identified as malignancy/tested.*

ed B-cell antigens with λ or κ light-chain restriction in 30 patients, CD20 antigen with no immunoglobulin staining in 14 and non-diagnostic findings in 8. At flow cytometry, mononuclear cell concentration of analyzed suspensions ranged between 4×10^4 to 6×10^6 cells (median: 7×10^5). Flow cytometry identified 47 patients with monoclonal B-cell expansion and 5 with a T-cell lymphoproliferative disorder, clearly discriminating between large and small lymphoma cells. Monoclonal B-cells ranged from 64 to 98% (mean: 84.5) in the lymphoid gate; this was reflected in the κ/λ or λ/κ ratio observed (κ= mean 73:1, range 29 - 96:1; λ= mean 87.6:1, range 77 - 97:1). Twelve patients had a typical pattern of follicular center cell lymphoma (FCL) with bcl-2 overexpression in 4 of 6 tested cases (Figure 1), 10 of diffuse large B-cell lymphoma, 7 of small lymphocytic lymphoma (SLL), 6 of marginal zone lymphoma, 5 of mantle cell lymphoma (MCL), 3 of peripheral T-cell lymphoma and one of mycosis fungoides; the remaining 8 patients presented with B- or T-large, not otherwise specified lymphoma cells. Flow cytometric and morphologic results were available the same day of the procedure. Cytogenetics, performed in 20 cases, showed normal karyotype in 2 patients, clonal markers in 9 (Table 1) and non-diagnostic results in 9. Overall, 52 patients were found to be affected by B- or T-cell NHL, whereas 3 had diagnosis of metastatic carcinoma, with cytometric absence of lymphoid markers (Table 1). Flow cytometry and immunocytochemistry agreed in diagnosing the presence of a clonal disease in only 30 cases; in 22 cases immunocytochemistry failed to detect a B- or T- monoclonality clearly documented at FACS analysis. Flow cytometry and morphology agreed in detecting NHL in 40/52 cases, and were discrepant in 12/52. Within 30 days from the FNAC, the latter 12 patients underwent laparoscopic biopsy at the same site of FNAC or excisional biopsy of a superficial lymphadenopathy. These 12 patients had small mature B-cell lymphomas (5 cases FCL- I, 4 MCL and 3 SLL); there was full agreement between histology and flow cytometry in identifying them. When stratifying the relapsed NHLs as aggressive (33 cases) or indolent (19 cases), taking into account cyto-morphological and flow cytometric results, as reported by Young *et al.*,[5] we found that 9 patients had relapsed with a disease more aggressive than that at first presentation (Table 1). In our hands, the diagnostic accuracy in the setting of NHL was significantly higher by flow cytometry than by cyto-morphology and immunocytochemistry (100% versus 77% and 58%; p=.003, by χ^2 testing). Surgical biopsies were performed as part of this study only in patients with equivocal cytologic results, and in all cases histology confirmed the flow cytometric subtyping of the malignancy. Flow cytometry rapidly provided an accurate definition of the size of neoplastic cells, a clear-cut distinction between T- and B-cell lymphomas, a better determination of κ/λ monoclonality, and an excellent discrimination between subtypes of small B-cell NHL. Although cytogenetics was an ancillary tool in this study, it sometimes helped in NHL classification (e.g. MCL). In conclusion, our data suggest that US-guided FNAC combined with flow cytometry is a rapid, reliable and safe mini-invasive procedure for identifying and sub-classifying NHL recurrence.[5-8]

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Low-grade non Hodgkin's lymphomas in the elderly: impact of a low-dose fludarabine-based combination regimen (mini-FLEC)

Regimens combining fludarabine with cyclophosphamide and mitoxantrone or doxorubicin have shown to be an effective therapy for elderly patients with advanced-stage low-grade non-Hodgkin's lymphomas (LG-NHL) although complicated by frequent treatment-related neutropenia and infections. We evaluated the efficacy and toxicity of a low-dose fludarabine-based combination regimen (mini-FLEC) in 20 elderly patients with advanced LG-NHL.

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Despite the wide range of treatment options, how to treat and when to treat elderly LG-NHL patients are still open questions; the effectiveness of the treatment and the quality of life should both be taken into consideration.[1-3] The efficacy of several fludarabine-based regimens (more often in association with mitoxantrone and/or cyclophosphamide) in LG-NHL patients has been widely demonstrated,[4-6] although the relevant incidence of therapy-related toxicity, including severe neutropenia and documented infections, still adversely affects the clinical outcome especially in elderly patients.[7]

We have previously shown the effectiveness and low toxicity of a low-dose fludarabine-based regimen (FLEC) including epirubicin and cyclophosphamide, in 30 patients with LG-NHL.[8] In that study we recorded a satisfactory overall response rate of 79% with 43% complete remissions (CR) and 36% partial remissions (PR). Therapy-related toxicity was mild regardless of age and consisted mainly of transient neutropenia and fever of undetermined origin while only 2/30 patients experienced a documented infection. Thus, we decided to extend the study to elderly patients with advanced stage LG-NHL by further reducing the doses of epirubicin and cyclophosphamide. Our intent was to offer an effective and fairly well tolerated treatment to a population of patients often showing a less responsive disease.[9]

Between September 1996 and March 2000, 20 consecutive elderly patients with *de novo*, relapsed or refractory LG-NHL received 5 monthly cycles of the mini-FLEC regimen (epirubicin 30 mg/m^2 i.v. on day one, fludarabine 15 mg/m^2/day (max 25 mg) i.v. from day 1-4 and cyclophosphamide 200 mg/m^2/day i.v. from day 1-4) after having given informed consent, according to institutional guide-lines. Prednisone was administered at a dose of 40 mg/m^2/day i.v. from day 1-4 only in the first cycle. No infection prophylaxis was given. All 20 patients enrolled were evaluable for response as they received at least 3 cycles of mini-FLEC and their pertinent data are listed in detail in Table 1. It should be noted that we also included in the study 8 patients with mantle cell lymphoma, formerly defined as a LG-NHL, which often behaves clinically as an aggressive lymphoma.

Six CR (30%) and 11 PR (55%) with an overall response rate of 85% were recorded. Only 3 patients did not respond. As expected, better results were achieved in the untreated group of patients in whom the response rate was 100% (4 CR and 6 PR) (Table 1). The median time to achieve CR was after 4 courses (range, 3 to 5) and the median duration of CR was 40 months (range, 6-61). Three of 6 patients who achieved CR relapsed after 6, 12 and 30 months, while the remaining 3 are still in CR after 50, 51 and 61 months. Eight of 11 patients, who had a PR, developed progressive disease after 3-15 months. Ten patients died of disease progression: all 3 patients who had no response, 6 of the partial responders whose disease progressed and 1 patient who obtained a CR but then relapsed and died after 6 months. One patient died of causes not related to the lymphoma, after 56 months of stable PR. The histologic subtype did not influence the response rate, although all 6 patients with follicular center (grade I) lymphoma subtype achieved a response (4 CR and 2 PR). Appreciable results were also recorded among the 8 patients with mantle cell lymphoma (2 CR and 5 PR) confirming the efficacy of a fludarabine-based regimen in this subtype of lymphoma with an unfavorable prognosis.[10] The overall survival and progression-free survival at 4 years were 48% and 45% with a median duration of 40 months (range 4-61) and 33 months (range 6-58), respectively. Overall and progression-free survival curves are shown in Figure 1.

The mini-FLEC regimen was very well tolerated and was given in an outpatient setting to most of the patients. No differences in therapy-related adverse effects were recorded between treated and untreated patients. All 82 cycles (mean 4, range 2-5) were evaluated for toxicity. Hematologic toxicity consisted mainly of transient grade III-IV neutropenia documented in 10/20 (50%) patients and in 20/82 (25%) cycles. Nevertheless, no dose reduction was applied and only 3/82 cycles were postponed by one week. Three of 20 patients received granulocyte colony-stimulating factor. One patient developed grade II thrombocytopenia. Extra-hematologic toxicity consisted of grade I nausea and vomiting observed in 7/82 cycles. A short-lasting fever of unknown origin (mean 3 days) was observed in 7/20 patients and in 10/82 cycles, most