Table 2: Summary of cytologic and histologic evaluation

FNA Diagnosis	# of cases (%)	Biopsy Follow up	CD4/CD8
Reactive	64 (75%)	5 (3R, 1HL, 1NHL)	4.1–29 (7.5)
Atypical	15 (18%)	8 (1R, 3HL, 4NHL)	4.6–14 (7.9)
HL	4 (5%)	3 (3HL)	5.3–13 (7.4)
NHL	2 (2%)	1 NHL	4–5.6 (4.8)

FNA: fine needle aspiration; R: reactive; HL: Hodgkin lymphoma; NHL: non-Hodgkin lymphoma. CD4/CD8 ratio range and mean.

All 85 aspirates were obtained on palpable lymph nodes by cytopathologists using 25 to 27 gauge needles. Aspirated material was immediately smeared on slides, air-dried, and stained with Diff-Quik and ultra-fast Papanicolaou stains. Additional material obtained for FCM was placed in RPMI solution. The cytologic diagnoses were grouped into four categories: reactive, atypical, HL, and NHL. Histologic follow-up was available in 17/85 (20%) of the cases.

Flow cytometric studies were performed using FACscan flow cytometer (Becton Dickinson, San Jose, CA). The specimens, suspended in RPMI solution, were centrifuged at 1500 rpm for 5 minutes, the supernatant was discarded, and red blood cells were lysed using Becton Dickinson FACS Lysing Solution. The cell pellets were then washed and resuspended in phosphate buffered saline. Aliquots of the cell suspension were then incubated with different combinations of monoclonal antibodies (three or four in each tube) including CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD19, CD20, CD23, HLA-DR, and Kappa/Lambda light chain (all antibodies from Becton Dickinson, San Jose, CA). An average of 10000 lymphocytes was collected in the lymphocyte gate using forward and side scatter.

Results

As shown in Table 1, the 85 lymph node aspirates were obtained from 85 patients ranging in age from 19 to 87 years. 29 patients were male and 56 were female. Eleven patients had a previous diagnosis of a lymphoproliferative disorder (1 HL, 6 mycosis fungoides, 2 NHL, 1 acute lymphoblastic leukemia, 1 Castleman disease). Lymph node sites were as follows: 14 inguinal, 43 cervical, 15 axillary, 2 epitrochlear, 5 submental, 2 subauricular, 2 intraparotid, 1 occipital, and 1 mediastinal. The lymph nodes ranged in diameter from 0.5 to 5 cm. CD4/CD8 T cell ratios ranged from 4 to 29. A CD4/CD8 T-cell ratio of 4 was chosen as the cut-off point because it represents twice the normal value (2), and a cut-off point of 3.9 was shown to represent the lower limit of CD4/CD8 ratio is cases of HL in a recently published report [13].

Overall, 64/85 (75%) lymph node aspirates were read as reactive on cytomorphology (Table 2). The cytologic findings in these cases consisted of a heterogeneous population of small and large lymphocytes with no cytologic atypicality mixed with tingible body macrophages with no definitive R-S cells or variants (Figure 1). 5 of the 64 reactive cases in which cytomorphology and FCM did not reveal evidence of a lymphoproliferative disorder had tissue follow-up because of persistent lymphadenopathy and high clinical suspicion. 3/5 cases (60%) confirmed the diagnosis of reactive lymphadenopathy.

The two remaining cases (40%) were positive for lymphoma. The first was diagnosed as nodular lymphocyte predominant Hodgkin lymphoma. Histologic evaluation revealed focal effacement of the nodal architecture by vague nodules of small lymphocytes admixed with occasional large atypical cells. Immunohistochemical stains performed on snap-frozen tissue showed that the small cells were mostly B-lymphocytes. The large atypical cells were positive for LCA, CD20 and EMA, and negative for CD15 and CD30. CD57 positive lymphocytes formed rosettes around the large cells. The second case was diagnosed as peripheral T cell lymphoma. The patient had a history of breast cancer treated by chemotherapy and radiation. The lymph node was enlarged due to an expansion of the interfollicular area by medium-sized atypical lymphocytes and increased vascularity with no significant increase in large cells. Immunohistochemistry confirmed that the atypical cells were CD3 and CD4 positive. CD8 positive T cells represented a minority of the cells. Flow cytometry failed to diagnose this case because a limited panel of antibodies against T-cell surface markers was used. The increased CD4/CD8 ratio (4.20) was thought to be a reactive change secondary to chemotherapy for breast cancer.

15 out of 85 cases were read as atypical. These cases consisted mostly of a heterogeneous population of small lymphocytes admixed with a population of intermediate or large cells, some showing angulated nuclei and prominent nucleoli with no definitive R-S cells or variants. 8/15 cases