Bone Marrow Involvement by Nasal NK Cell Lymphoma at Diagnosis Is Uncommon

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Abstract

To look for subtle evidence of marrow involvement in nasal NK cell lymphoma at diagnosis, we retrospectively studied trephine biopsy specimens from 25 consecutive patients by 2 sensitive techniques: CD56 immunohistochemistry and Epstein-Barr virus–encoded RNA in situ hybridization (EBER ISH). Only 2 patients had marrow involvement by NK cell lymphoma at diagnosis. In 3 additional patients, marrow involvement developed during or after systemic recurrence. All 5 positive cases were revealed by EBER ISH, but only 3 cases showed CD56 immunoreactivity. Among the 5 cases, only 2 were recognized by morphologic assessment. All 5 patients died, often within a short period, compared with a mortality of 50% for patients without demonstrable marrow involvement. Marrow involvement is distinctly uncommon in nasal NK cell lymphoma at diagnosis, and EBER ISH is the most sensitive technique for the demonstration of occult NK cell lymphoma. Despite the low frequency of marrow involvement in nasal NK cell lymphoma, EBER ISH is worthwhile to identify the minor subgroup of patients with a high likelihood of early death due to disease and when autologous bone marrow or peripheral blood stem cell transplantation is contemplated.

NK cell lymphomas are a group of recently characterized hematolymphoid malignant neoplasms comprising at least 3 overlapping categories: nasal NK cell lymphoma, nasal-type (extranasal) NK cell lymphoma, and aggressive NK cell lymphoma/leukemia.1,2 Despite differences in topographic predilection, these categories share many similarities in morphologic features, immunophenotype (CD2+, surface CD3–, cytoplasmic CD3+, CD56+), and genotype (germline T-cell receptor genes, strong association with Epstein-Barr virus [EBV]).3 Nevertheless, the majority of patients with nasal NK cell lymphomas have localized disease at diagnosis (stage I or II) while only approximately one fifth of patients with nasal-type NK cell lymphoma have stage I disease.1,2 Marrow involvement is distinctly uncommon in the former (0%-2%), but more common in the latter (15%-25%).1,2,4 It is, however, well known that in malignant lymphoma, marrow biopsy specimens interpreted on morphologic grounds as “negative for lymphoma” may harbor occult lymphoma cells demonstrable only by more sensitive immunologic or molecular techniques.5-8

Since the outcome of patients with nasal NK cell lymphoma is poor,2-4 with a high proportion of patients eventually developing additional sites of involvement, we question whether the disease might be more disseminated at diagnosis than that revealed by conventional staging (including morphologic evaluation of marrow). Since the bone marrow is a frequent site of dissemination in malignant lymphoma, analysis of marrow involvement provides a means to address this issue. We therefore used CD56 immunohistochemical and EBV-encoded RNA (EBER) in situ hybridization (ISH) techniques to determine the frequency of marrow involvement by nasal NK cell lymphoma at diagnosis. These markers were chosen because they are expressed commonly in nasal NK cell lymphoma but almost never in normal hematopoietic cells.9,10
Materials and Methods

Case Selection
During the period April 1996 to March 2000, 25 consecutive patients were diagnosed as having nasal NK cell lymphoma on nasal or nasopharyngeal biopsy at the Queen Elizabeth Hospital, Hong Kong, People’s Republic of China. Their clinical and laboratory records were reviewed. All patients had undergone marrow biopsy for lymphoma staging at the time of diagnosis, and some underwent follow-up marrow biopsies (12 samples from 9 patients during postchemotherapeutic assessment or at relapse). All the 37 trephine biopsy specimens were retrieved for review and further study.

Immunohistochemical Staining for CD56
Sections cut from the formalin-fixed paraffin-embedded trephine biopsy specimens were immunostained with a CD56 monoclonal antibody (123C3, Monosan, Uden, the Netherlands). Antigen retrieval was achieved by heating the hydrated slides in EDTA buffer at pH 8 in a pressure cooker for 2.5 minutes. The staining was performed in an automated immunostainer (Ventana ES, Ventana, Tucson, AZ) using the labeled streptavidin-biotin peroxidase detection system (Dakopatts, Glostrup, Denmark). An appropriate positive control was mounted on every slide to ascertain validity of the stain.

ISH for EBV
Paraffin-embedded sections of the trephine biopsy specimens were studied for the presence of EBV by nonisotopic ISH technique using fluorescein-conjugated peptide nucleic acid probes (code no. Y5200, Dakopatts) that were complementary to EBER1 and EBER2 encoded by EBV. Positive labeling was observed in the nuclei of cells latently infected with EBV. There was no problem in detecting EBER in the trephine biopsy specimens because the samples were decalcified in EDTA instead of acid. The detection step was performed using the Fab fragment of rabbit antifluorescein and the streptavidin-biotin peroxidase system, together with an amplification step in the Ventana ES automated immunostainer. An appropriate positive control (including a lymphoepithelioma-like carcinoma and an NK cell lymphoma) was mounted on every slide to ascertain validity of the test.

Results
Clinical Features and Treatment Outcome
The series included 20 men and 5 women, with ages ranging from 20 to 81 years (mean, 52.5 years). Most had stage I disease (18/25, 72%) on routine staging; 4 patients had stage II disease, and 3 patients had stage III or IV disease at diagnosis. The blood cell counts were normal at diagnosis in 17 patients but showed mild anemia or leukocytosis in 7 patients. One patient had pancytopenia at diagnosis, and he also had disseminated disease with lymphadenopathy, hepatosplenomegaly, and marrow involvement. The hemoglobin level was 10.1 to 16.2 g/dL (101-162 g/L; mean, 13 g/dL [130 g/L]); the leukocyte count was 2,200 to 14,100/µL (2.2-14.1 × 10^9/L; mean, 7,100/µL [7.1 × 10^9/L]); and the platelet count was 64 to 430 × 10^9/µL (63-430 × 10^9/L; mean, 234 × 10^9/µL [234 × 10^9/L]).

Sixteen patients were treated with local radiotherapy and combination chemotherapy, and 4 of them also received autologous peripheral stem cell transplantation. Nine patients were treated with radiotherapy (4 cases) or combination chemotherapy (5 cases) alone. The chemotherapy regimens included CEOP (cyclophosphamide, epirubicin, vincristine, and prednisolone) and ProMACE-CytaBOM (prednisone, methotrexate, doxorubicin, and cyclophosphamide plus doxorubicin, etoposide, cytarabine, bleomycin, and vincristine). One patient developed local recurrence in the nasopharynx at 3 months, and 1 had cervical lymph node metastases at 4 months. Two had central nervous system involvement at 4 and 7 months, respectively, and 2 others had systemic relapse (1 with cutaneous recurrence at 2 years followed by hepatic involvement after 4 months, and 1 with ovarian and peritoneal involvement at 15 months followed by marrow involvement at 22 months). One patient developed isolated marrow relapse at 3.5 months after initial diagnosis.

Fifteen patients (60%) died of disease at 2 weeks to 31 months (mean, 8.3 months; median, 6 months), and 9 were in complete remission at a median follow-up of 15 months (range, 2-36 months; mean, 13.8 months). One patient was alive with disease at 9 months.

Morphologic Assessment and Special Studies
Among the trephine biopsy specimens obtained from the 25 patients for initial diagnosis, only 2 (8%) showed evidence of involvement on special studies. In 1 patient, although the marrow blood preparation showed presence of 20% bland-looking large granular lymphocytes, the trephine biopsy specimen did not show obvious light microscopic evidence of lymphomatous infiltrate. Nevertheless, isolated lymphoma cells could be highlighted by CD56 immunohistochemical and EBER ISH staining. This also was the only patient with pancytopenia and disseminated disease at diagnosis. In the other patient, rare lymphoma cells were demonstrable by EBER ISH alone. The survival of these 2 patients was short: 2 weeks and 4 months, respectively, after diagnosis.

Among patients with negative biopsy findings at diagnosis, 3 patients exhibited marrow involvement on follow-up...
assessment. In 2 patients, an interstitial to nodular lymphomatous infiltrate (CD56+, EBV+) could be identified on morphologic examination. One was the patient with isolated marrow relapse, while the other was the patient with marrow involvement following ovarian and peritoneal relapse. In the third case, the patient developed central nervous system relapse, and concomitant marrow relapse was demonstrated by EBER ISH (with isolated positive cells) but not by CD56 immunostaining. All 3 patients had been treated with local radiotherapy and combination chemotherapy before the development of marrow involvement, and they died of disease at 3.5 months, 8 months, and 22 months, respectively, all within 1 or 2 weeks after the detection of marrow involvement.

Discussion

The frequency of marrow involvement in non-Hodgkin lymphomas at diagnosis is highly variable, ranging from as low as 3% in mediastinal large B-cell lymphoma to as high as 73% in B-cell small lymphocytic lymphoma/chronic lymphocytic leukemia.14 It is generally higher in the small cell lymphomas and follicular lymphomas. The reported frequencies typically are based on morphologic examination alone. However, morphologically normal marrow may harbor occult lymphoma cells revealed only by more sensitive techniques, such as immunohistochemistry for leukocyte markers or molecular techniques for immunoglobulin or T-cell receptor gene rearrangements.5-8 For example, in the study by Fraga et al,8 only 17% of patients with anaplastic large cell lymphoma had marrow involvement by conventional morphologic criteria, while immunostaining for CD30 or epithelial membrane antigen showed occult malignant cells in 23% of patients with negative marrow on histologic examination. Furthermore, patients with marrow involvement (including that demonstrated by routine histologic or immunohistochemical examination) had a worse prognosis than those without marrow involvement. It is understandable that morphologic identification of neoplastic involvement of marrow by nasal NK cell lymphoma can be difficult unless the involvement is extensive. Isolated tumor cells can be missed easily, especially since the neoplastic cells are often small to medium-sized.

In the present study, we determined the frequency of lymphoma involvement of the marrow by nasal NK cell lymphoma at diagnosis with the use of 2 fairly sensitive and specific techniques that can detect lymphoma cells in histologic sections while permitting simultaneous morphologic assessment. CD56 immunostaining is easy to interpret because the normal marrow contains few NK cells.15 The only other CD56+ cells are a subpopulation of plasma cells and osteoblasts lining the bony trabeculae, which are readily distinguishable from lymphoma cells (personal observation). EBER ISH is another sensitive technique because normal marrow rarely contains EBV-positive cells.13 To be considered positive, the labeled nuclei should exhibit some degree of atypia, ie, nuclei larger than small lymphocytes and showing some irregular foldings. The EBER ISH technique seems to be a more sensitive technique than CD56 immunostaining because the latter was positive in only 3 of 5 cases. The discrepancy may be related to heterogeneous expression of CD56 by the lymphoma cells.
The present study confirms that marrow involvement by nasal NK cell lymphoma is uncommon at diagnosis (2/25, 8%). One patient had disseminated disease at diagnosis, while the other patient showed occult marrow involvement and normal peripheral blood counts. On follow-up, only 3 additional patients (12%) developed marrow involvement, and this often was heralded by other sites of dissemination. The rare occurrence of marrow involvement in nasal NK cell lymphoma at diagnosis is intriguing, because disseminated disease with or without marrow involvement is a frequent finding in nasal-type NK cell lymphoma, with which the former shares many similarities. It will be important to study how the topographic site affects the biology of the lymphoma and vice versa and whether there are genetic differences between the 2 groups. Another study found differences in the loss of heterozygosity pattern between nasal and nasal-type NK cell lymphomas, such as a higher frequency of loss of heterozygosity at 6q in the former than in the latter (10/11 cases vs 2/4).16 Of interest, isochromosomes of 1q and 6p have been reported only in nasal NK cell lymphoma but not in nasal-type NK cell lymphoma.

It is difficult to establish the definite prognostic significance of marrow involvement in nasal NK cell lymphoma because of the aggressive nature of the disease and the small number of cases with marrow involvement. However, involvement of the bone marrow by nasal NK cell lymphoma, whether at diagnosis or during relapse, is associated with early death of the patients (a mortality of 100% vs 50% for patients without demonstrable marrow involvement). Bone marrow examination with special techniques may, therefore, have a role in defining a subset of patients with a particularly unfavorable prognosis and in excluding occult involvement of the marrow when autologous bone marrow or peripheral stem cell transplantation is contemplated.17 This is
important because the peripheral blood cell counts may be entirely normal despite marrow involvement by the NK cell lymphoma.

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References


