EBV Viral Load in Tumor Tissue Is an Important Prognostic Indicator for Nasal NK/T-Cell Lymphoma

Pin-Pen Hsieh, MD,[1,6] Chun-Liang Tung, MD,[2] Amy Bik-Wan Chan, MD,[3] Jia-Bin Liao, MD,[1] Jyh-Seng Wang, MD, PhD,[1] Hui-Hwa Tseng, MD,[1] Hsing-Hao Su, MD,[4] Kun-Chao Chang, MD,[5] and Chung-Che Chang, MD, PhD[6,7]

Key Words: Epstein-Barr viral load; Nasal NK/T-cell lymphoma; Prognostic factor

DOI: 10.1309/MN4Y8HLQWKD9NB5E

Abstract

We retrospectively studied 19 cases of nasal NK/T-cell lymphoma for various potential prognostic factors and performed real-time quantitative polymerase chain reaction for Epstein-Barr virus (EBV) viral load in tumor tissue. Patients with a low EBV viral load (<1 copy per cell) more frequently survived for more than 2 years compared with patients with a high EBV viral load (≥1 copies/cell) (7/7 vs 3/9; P = *.014; Fisher exact test). Furthermore, the patients with low EBV viral loads had a better overall survival than patients with high viral loads (50% accumulative survival: not reached vs 4-5 months; Kaplan-Meier survival analysis;* P = *.049). In contrast, the overall survival of the patients did not correlate with the extent of lesion, age, stage, necrosis, histologic subtypes, CD56 expression, or angiocentric or angiodestructive growth pattern. Our findings suggest that the EBV viral load in tumor tissues is a useful indicator for predicting outcome of nasal NK/T-cell lymphoma.*

Nasal NK/T-cell lymphoma (NNTCL), defined as extranodal NK/T-cell lymphoma, nasal type, occurring in nasal areas, is a rare lymphoma in the United States and Europe but is relatively common in Asia and Latin America.[1-3] Although most sinonasal lymphomas in the Western population are diffuse large B-cell lymphomas, 40% to 74% of sinonasal lymphomas are NNTCL in Asia.[4,5] NNTCLs usually occur in middle-aged patients, and their presenting features are characterized by localized disease in the majority of patients, frequent adjacent tissue invasion, a high frequency of B symptoms, and rare bone marrow involvement.[6-8] Pathologically, NNTCL has a broad cytologic spectrum varying from small, medium, and large cells to anaplastic cells. The tumor cells have been characterized positively for CD2, cytoplasmic CD3 (CD3ε), CD56, and T-cell intracellular antigen-1 (TIA-1) by immunophenotyping and positively for Epstein-Barr virus–encoded RNA (EBER) by in situ hybridization.[9-11]

Because NNTCL is relatively a newly recognized, distinctive clinicopathologic entity in the World Health Organization (WHO) classification, prognostic factors have not been fully defined. Although validated in many types of lymphomas ranging from low to high grades, the prognostic impact of the International Prognostic Index has been controversial in NNTCL, with some studies yielding a positive correlation[6,8] and some a negative correlation.[12-14] Similarly, the Ann Arbor Staging System has only limited value in predicting prognosis. Patients with nasal stage IIIE/IVE had been reported to exhibit more aggressive tumor behavior and poorer prognosis than patients with nasal stage IE/IIE NNTCL.[15] However, the Ann Arbor stage failed to predict the survival difference between stage IE and stage IIE,[16] which included most of the cases (82%) at presentation.[17]

A few studies have evaluated the relationship between patient survival and disease extent,[12,16,18,19] histologic subtype, or plasma EBV viral load.[9,20] A recent study suggested that local tumor invasiveness is more predictive of patient survival than the International Prognostic Index in stage IE/IIE NNTCL.[16] Kuo et al[9] noted no statistical difference in patient survival by histologic subtype. Lei et al[20] stated that patients with high baseline plasma Epstein-Barr virus (EBV) DNA levels (≥600 copies/mL) demonstrated a significantly inferior survival to patients with low baseline plasma EBV DNA levels (<600 copies/mL) in extranodal NK/T-cell lymphoma.

The lack of prognostic markers has created significant challenges in treatment selection for the very heterogeneous clinical behavior of NNTCL—particularly for patients with stage IE/IIE disease (ie, paranasal extension, bone destruction, skin or regional lymph node involvement).[21-24] The goal of the present study was to identify prognostic markers for NNTCL by comprehensively evaluating the prognostic significance of the following factors: extent of lesion, histologic subtypes, age, CD56 expression, stage, necrosis, angiocentric and angiodestructive growth patterns, and EBV viral load in tumor tissue. To the best of our knowledge, the last 2 factors have never been well studied in NNTCL.

Materials and Methods

Cases

We retrospectively searched for NNTCL cases in the pathology files of the Veterans General Hospital-Kaohsiung, Kaohsiung, in southern Taiwan and the Prince of Wales Hospital, Shatin, Hong Kong, from October 1990 to September 2004. We excluded cases that were too small for performing further immunophenotyping and EBER in situ hybridization studies. A total of 19 cases were included in the study. The history of each patient was reviewed and included physical examination, computed tomography scanning, and bone marrow biopsy results for evaluation of the extent of disease. All cases were staged using the Ann Arbor Staging System. The overall survival time was calculated by determining the time from the date of diagnosis to the date of death or last follow-up.

Histomorphologic and Immunohistochemical Evaluation

The specimens from all cases were fixed in 10% buffered formalin, processed by routine methods, and embedded in paraffin. An immunohistochemical study was performed for each case using the BioGenex Super Sensitive Non-Biotin HRP Detection System (BioGenex, San Ramon, CA). An antigen-retrieval technique was applied as needed for each specific antibody. The antibodies used were CD3 and CD20 (DAKO, Glostrup, Denmark), CD5 and CD56 (Novocastra Laboratories, Newcastle upon Tyne, England), and TIA-1 Immunotech, Marseille, France). EBER in situ hybridization was performed using an in situ hybridization detection kit (Novocastra Laboratories).

The H&E-stained sections, immunohistochemical stains, and EBER studies for each case were reviewed. The diagnosis of NNTCL in all cases was reconfirmed according to the criteria of the WHO classification.[11] According to the predominant population of the lymphoma cells, NNTCL was further classified into small, medium, large, and anaplastic cell (or pleomorphic large cell) subtypes as described in the recent WHO classification[11] and by Kuo et al.[9] The growth patterns, such as angiocentricity (more condensed lymphoid infiltration around vessels) and angiodestructive growth patterns, and necrosis were also recorded.

Viral Load Detection

The slides from each case containing more than 80% of tumor cells were used for DNA extraction. The formalin-fixed, paraffin-embedded tumor tissue was scraped from the glass slides and transferred to a sterile 1.8-mL screw top microcentrifuge tube and digested with Proteinase K at 55°C for 2 nights. DNA was then extracted using the DNeasy Tissue Kit (Qiagen, Valencia, CA). The DNA quantity for each case was measured by using the real-time polymerase chain reaction (PCR) assay using the primers and probes for the *tPA* gene (a housekeeping gene) included in the LightCycler t(14;18) Quantification Kit (Roche Applied Science, Mannheim, Germany). A conversion factor of 6 pg of DNA per diploid cell was used for estimating the number of total cells in each sample. The EBV copy number in each sample was determined by the LightCycler EBV Quantification Kit (Roche Applied Science). The amplification efficiencies were similar between the tPA and the EBV PCR assays, with both assays showing a 3.3 PCR cycle difference for samples containing 10-fold different amounts of DNA targets. The EBV viral load in tumor tissue was then calculated as the ratio of copies of EBV DNA to cell numbers (copies per cell). This EBV viral load quantitation method is similar to one previously reported in the literature.[25]

Results

Patient Characteristics

The clinical findings for 19 NNTCL cases are summarized in **Table 1**. Of the patients, 13 were men and 6 were women (M/F ratio, 2.2:1) with a median age of 52 years (mean, 55.0 years; range, 30-90 years). Of 19 patients, 3 (16%) had a history of different malignant neoplasms after chemotherapy several years before the onset of NNTCL. Of 18 cases with complete staging information, 3 (17%) had a

DOI: 10.1309/MN4Y8HLQWKD9NB5E

Table 1
Clinicopathologic Features and Outcome of Nasal NK/T-Cell Lymphoma

Case No./ Sex/Age (y)	Stage	Involved Site	EBV Load*	Histologic Type	AC	AD	NE	Follow-up	OS (mo)†	Treatment Method
1/M/73	IIE	NC, NLN	NA	Small	+	+	–	AWD	7	C and R
2/M/68	IE	NC, SP, SS	NA	Medium	+	+	+	DOD	2	C
3/M/52	IE	NC, ES, MS	0.11	Medium	+	+	+	DOD	31	C and R
4/F/49	IE	NC, ES, MS, SS	0.12	Small	–	–	+	AWOD	100	C
5/M/70	IE	NC, ES, MS, FS	1.90	Medium	+	+	+	AWOD	96	C
6/M/65	IE	NC, NP	NA	Small	+	–	–	AWD	1	Biopsy
7/M/53	IE	NC, MS	2.685	Small	+	+	+	DOD	2	C
8/M/76	IE	NC	9.34	Medium	+	–	+	DOD	5	C
9/F/30	UN	NC	7.91	Small	+	+	+	DOD	4 d	Biopsy
10/F/36	IE	NC, ES, MS	477.15	Medium	+	+	+	DOD	4	C and R
11/F/45	IE	NC, MS	12.28	Large	+	+	–	AWD	67	Excision
12/M/70	IE	NC	53.55	Medium	+	+	–	AWOD	24	C
13/M/47	IE	NC, BU, UP, UL	0.38	Anaplastic	+	+	+	AWOD	43	C and R
14/M/48	IIE	NC, TO, NLN	17.75	Medium	–	+	+	DOD	4	C and R
15/F/34	IE	NC, NP	0.953	Large	–	–	+	AWOD	120	C and R
16/M/54	IE	NC, NP	0.757	Medium	+	+	–	DOD	36	C and R
17/M/34	IE	NC	0.338	Medium	+	+	+	AWOD	48	C and R
18/M/51	IV	NC, ES, MS, NP, NLN, T	0.757	Medium	+	+	+	DOD	24	C and R
19/F/90	IE	NC, cheek	1.945	Large	+	+	+	DOD	5	C and R

AC, angiocentric; AD, angiodestructive; AWD, alive with disease; AWOD, alive without disease; BU, buccal mucosa; C, chemotherapy; DOD, died of disease; ES, ethmoid sinus; MS, maxillary sinus; NA, not available (no amplifiable DNA); NC, nasal cavity; NE, necrosis; NLN, neck lymph node; NP, nasopharynx; OS, overall survival; R, radiotherapy; SP, soft palate; SS, sphenoid sinus; T, testis; TO, tonsil; UL, upper lip; UN, unknown; UP, upper palate; +, positive; –, negative.
* Copies per cell.
† Unless otherwise indicated.

single lesion limited to the nasal cavity and the other 15 (83%) had more extensive lesions beyond the nasal cavity that included paranasal sinus extension, nasopharyngeal involvement, neck lymph node involvement, or distant metastasis. The staging results were as follows: stage IE, 15 cases (83%); stage IIE, 2 cases (11%); and stage IV, 1 case (6%). Ten patients received combined chemotherapy and radiotherapy; 6 received chemotherapy alone. The median length of follow-up was 24 months (range, 4 days to 120 months) in all cases and 48 months (range, 1 to 120 months) in surviving patients. The overall 1- and 5-year survival rates were 52.6% and 21.1%, respectively.

Histomorphologic and Immunohistochemical Findings

Histologically, 5 tumors (26%) were the small cell type, 10 (53%) were the medium-sized cell type, 3 (16%) were the large cell type, and 1 (5%) was the anaplastic cell type. Necrosis was noted in 14 cases (74%). Angiocentric and angiodestructive patterns were noted in 16 cases (84%) **Image 1** and 15 cases (79%) **Image 2**, respectively; 14 (74%) cases had both patterns, and 2 cases (11%) were without either pattern. Immunohistochemical studies in all cases were positive for TIA-1 and by EBER in situ hybridization but negative for CD20. Cytoplasmic CD3ε was expressed in all cases except 1 (95%); however, this single case expressed CD56 and TIA-1 and was positive in EBER in situ hybridization, confirming the diagnosis of NNTCL. CD56 expression was noted in 16 cases (84%).

EBV Viral Load

Of the 19 samples, 16 had amplifiable DNA for determining the EBV viral load in tumor tissue (Table 1). The median viral load was 1.923 copies per cell (range, 0.11-477.15 copies per cell). Patients with a low EBV viral load (<1 copy per cell) were more likely to survive for more than 2 years than patients with a high EBV viral load (≥1copies per cell; 7/7 vs 3/9; P = .014; Fisher exact test). Furthermore, patients with a low EBV viral load had better overall survival than patients with high viral load (50% accumulative survival: not reached vs 4-5 months; Kaplan-Meier survival analysis; P = .049; Breslow-Gehan-Wilcoxon test) **Figure 1**.

Other Parameters

In contrast with the EBV viral load, overall survival did not correlate with the extent of lesion, age, stage, necrosis, histologic subtype, CD56 expression, or angiocentric or angiodestructive growth pattern **Table 2**. The 2 patients (cases 4 and 15) without an angiocentric or angiodestructive pattern (both had low EBV viral loads) survived more than 8 years, in contrast with only 1 patient with either pattern (2/2 vs 1/17, P = .0175; Fisher exact test).

Discussion

In this retrospective study, we investigated the prognostic impact of EBV viral load on the tumor of NNTCL and other potential prognostic factors. Our results suggest that the tumor

DOI: 10.1309/MN4Y8HLQWKD9NB5E

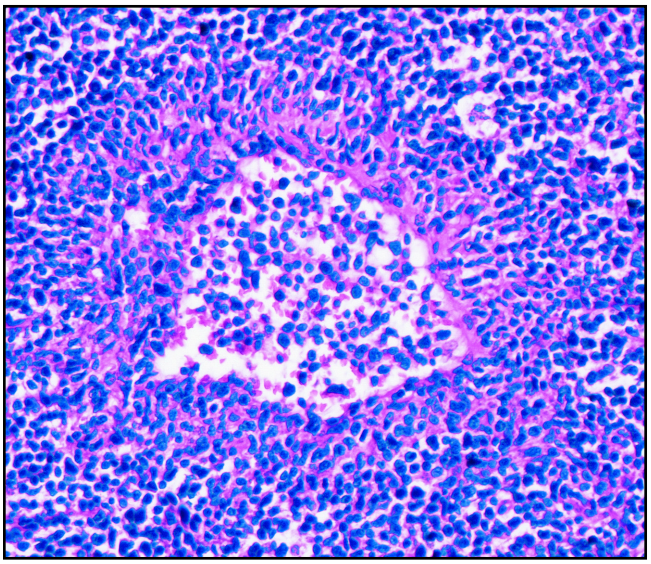

Image 1 (Case 10) Dense, medium-sized lymphoid cells infiltration with angiocentric pattern (H&E, ×200).

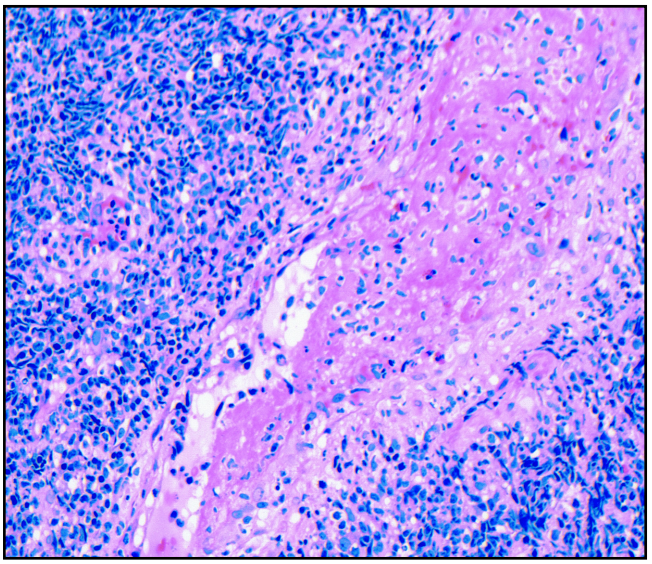

Image 2 (Case 18) Medium-sized lymphoid cells infiltration with angiodestruction and necrosis of vascular wall (H&E, ×200).

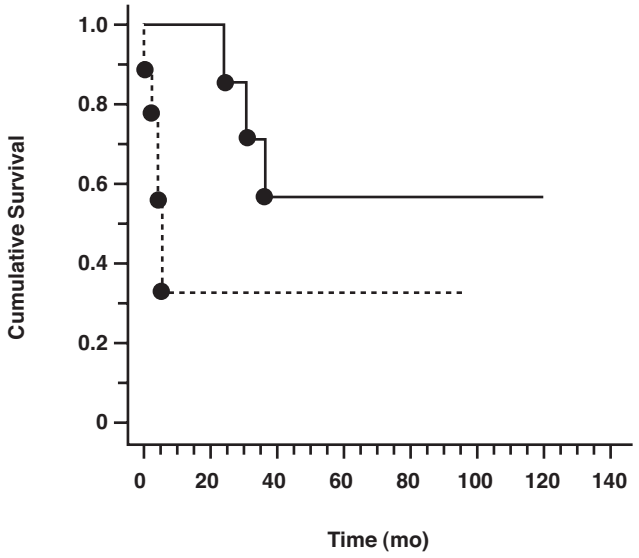

Figure 1 Kaplan-Meier survival analysis of the Epstein-Barr virus (EBV) viral load in tumors of nasal NK/T-cell lymphoma. Patients with a low EBV viral load (solid line, EBV viral load <1 copy per cell) had better overall survival than patients with a high EBV viral load (dotted line, EBV viral load ≥1 copies per cell) (P = .049; Breslow-Gehan-Wilcoxon test).

Table 2
Statistical Analysis Results for the Impact of the Evaluated Parameters on Overall Survival in Nasal NK/T-Cell Lymphoma*

Parameter	Values Compared	*P*
Viral load (copies per cell)	≥1 vs <1	.049
Age (y)	≥45 vs <45	.6350
Extension	ELBN vs LINC	.7244
Stage	I vs II and IV	.5667
Angiocentric	+ vs –	.5442
Necrosis	+ vs –	.1255
Angiodestructive	+ vs –	.3854
CD 56	+ vs –	.3395
Histologic type	Small vs nonsmall	.3847

ELBN, extensive lesion beyond the nasal cavity; LINC, lesion limited to nasal cavity; nonsmall, medium, large, or anaplastic cell type; small, small cell type; +, positive; –, negative.
* Kaplan-Meier survival analysis; *P* value calculated by using the Breslow-Gehan-Wilcoxon test.

EBV viral load of NNTCL is an important prognostic indicator in this relatively large cohort of patients. In contrast, angiocentric or angiodestructive growth pattern, extent of the lesion, histologic subtype, age, necrosis, CD56 expression, and Ann Arbor stage were unable to identify prognostic subgroups in our patients.

The prognostic significance of EBV viral load in NNTCL tumor tissue has not been well documented, although virtually all NNTCL lesions are associated with the presence of EBV, suggesting a significant pathogenic role of EBV in NNTCL. The quantitation of the EBV viral load in the tumor was not possible until development of the real-time quantitative PCR method. Similar to our approach, Ryan et al[25] recently reported a method for the quantitation of EBV in paraffin-embedded tissue samples of EBV-associated malignant neoplasms. They validated quantitative PCR assays targeting highly conserved segments of the EBV genome and using the *ApoB* gene as a normalizer by which to control the number of cells tested and to check for inhibitors of amplification or failed extraction. The range of EBV viral load in their study in EBV-associated malignant neoplasms was 0.00095 to 14.3 copies per cell; however, they did not include any NNTCL and did not

DOI: 10.1309/MN4Y8HLQWKD9NB5E

compare the prognosis and the quantities of the viral load in their study. We used real-time PCR assays with the LightCycler EBV Quantification Kit to detect the EBV copies and the *tPA* gene as a normalizer to calculate the EBV viral load. The quantities of EBV viral load in NNTCL in the present study are similar to the range in the study by Ryan et al.[25] Of note, the EBV viral load does not correlate with the percentages of cells showing positive staining for in situ hybridization for EBER in our cases (data not shown). In addition, the percentages of cells positive for EBER in situ hybridization do not correlate with patients' overall survival.

Our results demonstrate that a high EBV viral load in NNTCL tumor tissue suggests an adverse prognosis. Recently, Lei et al[20] reported that patients with high baseline plasma EBV DNA levels (≥600 copies/mL) demonstrated a significantly inferior survival to patients with low baseline plasma EBV DNA levels (<600 copies/mL) in extranodal NK/T-cell lymphoma (66.7% of their cases were NNTCL). They also showed that all patients who responded to treatment showed a marked reduction of plasma EBV DNA to low or undetected levels. In contrast, patients in whom treatment failed had a rapid increase in plasma EBV DNA levels. A similar prognostic impact of plasma EBV viral load has also been observed in patients with other EBV-associated lymphoid malignancies, including Burkitt lymphoma, posttransplantation lymphoproliferative disorders, and Hodgkin lymphoma.[26-28]

Our results further indicate that in addition to the plasma EBV viral load, the EBV viral load in tumor tissue is also an important prognostic factor. It would be of interest to study the plasma EBV viral load in our patients to see if the plasma viral load correlates with viral load in tumor tissue. However, we do not have the plasma of the patients at the time of their presentations. Prospective studies are needed to evaluate this correlation. The biologic mechanisms for a high plasma EBV viral load leading to an aggressive clinical course are unclear. One possibility is that the high plasma EBV viral load serves as a surrogate marker for high tumor burden. The other possible mechanism is that a high plasma EBV viral load indicates a lytic phase of EBV infection leading to the proliferation of EBV and the overexpression of EBV-associated oncoproteins. These oncoproteins may, thus, result in further transformation of the tumor cells into more aggressive clones.

Angiocentric and angiodestructive growth patterns are characteristic findings in NNTCL; however, to the best of our knowledge, no studies have focused on correlating these growth patterns with prognosis. Our results suggest that these 2 growth patterns evaluated separately do not correlate with the overall survival ($P = .5442$ and $P = .3854$, respectively). We acknowledge that the adequacy of the statistical analysis is somewhat compromised owing to few cases lacking these features in this cohort of patients. Nevertheless, the only 2 patients, both with a low EBV viral load (0.12 and 0.953 copies per cell), without an angiocentric or angiodestructive growth pattern survived for more than 8 years without evidence of disease. The low EBV viral load may, at least partially, contribute to the excellent prognosis for these 2 patients. Further studies are warranted to further evaluate the correlation of absence of both growth patterns and prognosis.

The Ann Arbor stage of the disease (IE vs other stages) did not correlate with the prognosis of NNTCL in this study ($P = .5667$). This finding agrees with a previous study reporting that stage was unable to dissect prognostic subgroups between stage IE and IIE disease.[16] However, of note, only 3 patients had greater than stage I disease in this cohort of patients. This may contribute to the failure of staging as a prognostic marker. Future studies with a larger number of patients with high-stage diseases are warranted to further evaluate the prognostic significance of staging. Interestingly, the only patient with stage IV disease with a low EBV viral load (0.757 copies per cell) survived for 2 years, the same as the median survival of this cohort. The reason this patient with high-stage disease had a relatively good prognosis remains unclear, but this could be attributed at least partially to the lower EBV viral load. In agreement with our results, Cheung et al[12] reported no prognostic significance of the extent of the disease, including paranasal extension and bony invasion, in their NNTCL cases. However, the extent of nasal lymphoma was considered a prognostic factor in other studies.[14,18,19] The reason for the discrepancy among these studies is unclear. It would be of interest to study EBV viral load to see if the differences among studies are caused by the inclusion of patients with different EBV viral loads.

Other parameters evaluated, including necrosis, CD56 expression, age, and histologic subtypes, showed no significant prognostic impact in our cohort. Our findings for age and histologic subtypes are consistent with those in previous studies.[9,15,29] Necrosis and CD56 expression, 2 findings present in most NNTCLs, to the best of our knowledge, have never been studied for their prognostic significance. However, some of the prognostic factors, such as CD56 positivity, angiocentricity, and necrosis, evaluated in this study also serve as diagnostic features of this lymphoma. This may reflect the small number of cases lacking such features and may result in insufficient statistical power to thoroughly evaluate their prognostic significance.

Our results suggest that the EBV viral load in tumor tissue is a better prognostic indicator than the other factors evaluated for predicting the outcome of NNTCL. Patients with low EBV viral loads in the tumor tissues have a favorable prognosis. Further studies with a larger number of NNTCL cases are needed to confirm our observations.

From the [1]Department of Pathology & Laboratory Medicine and [4]Department of Otorhinolaryngology, Veterans General Hospital-Kaohsiung, Kaohsiung, Taiwan; [2]Department of Pathology, Chia-Yi

DOI: 10.1309/MN4Y8HLQWKD9NB5E

Christian Hospital, Chia-Yi, Taiwan; [3]Department of Anatomical and Cellular Pathology, Chinese University of Hong Kong, Hong Kong; [5]Institute of Clinical Medicine, College of Medicine, National Cheng Kung University; [6]Department of Pathology and Laboratory Medicine, the Methodist Hospital and the Methodist Hospital Research Institute, Houston, TX; and [7]Department of Pathology, Weill Medical College of Cornell University, New York, NY.

Supported in part by grant VGHKS94-049 from Veterans General Hospital-Kaohsiung, Kaohsiung, Taiwan.

Address correspondence to Dr C.-C. Chang: Dept of Pathology and Laboratory Medicine, the Methodist Hospital, 6565 Fannin St, MS205, Houston, TX 77030.

References

1. Aviles A, Rodriguez L, Guzman R, et al. Angiocentric T-cell lymphoma of the nose, paranasal sinuses and hard palate. *Hematol Oncol.* 1992;10:141-147.
2. Ferry JA, Sklar J, Zukerberg LR, et al. Nasal lymphoma: a clinicopathologic study with immunophenotypic and genotypic analysis. *Am J Surg Pathol.* 1991;15:268-279.
3. Robbins KT, Fuller LM, Vlasak M, et al. Primary lymphomas of the nasal cavity and paranasal sinuses. *Cancer.* 1985;56:814-819.
4. Ko YH, Kim CW, Park CS, et al. REAL classification of malignant lymphomas in the Republic of Korea: incidence of recently recognized entities and changes in clinicopathologic features. *Cancer.* 1998;83:806-812.
5. Au WY, Ma SY, Chim CS, et al. Clinicopathologic features and treatment outcome of mature T-cell and natural killer–cell lymphomas diagnosed according to the World Health Organization classification scheme: a single center experience of 10 years. *Ann Oncol.* 2005;16:206-214.
6. Chim CS, Ma SY, Au WY, et al. Primary nasal natural killer cell lymphoma: long-term treatment outcome and relationship with the International Prognostic Index. *Blood.* 2004;103:216-221.
7. Kim K, Kim WS, Jung CW, et al. Clinical features of peripheral T-cell lymphomas in 78 patients diagnosed according to the Revised European-American Lymphoma (REAL) classification. *Eur J Cancer.* 2002;38:75-81.
8. Lee J, Park YH, Kim WS, et al. Extranodal nasal type NK/T-cell lymphoma: elucidating clinical prognostic factors for risk-based stratification of therapy. *Eur J Cancer.* 2005;41:1402-1408.
9. Kuo TT, Shih LY, Tsang NM. Nasal NK/T cell lymphoma in Taiwan: a clinicopathologic study of 22 cases, with analysis of histologic subtypes, Epstein-Barr virus LMP-1 gene association, and treatment modalities. *Int J Surg Pathol.* 2004;12:375-387.
10. Harris NL, Jaffe ES, Diebold J, et al. The World Health Organization classification of neoplastic diseases of the haematopoietic and lymphoid tissues: report of the Clinical Advisory Committee Meeting, Airlie House, Virginia, November 1997. *Histopathology.* 2000;36:69-86.
11. Chan JKC, Jaffe ES, Ralfkiaer E. Extranodal NK/T-cell lymphoma. In: Jaffe ES, Harris NL, Stein H, et al, eds. *Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues*. Lyon, France: IARC Press; 2001:204-207. *World Health Organization Classification of Tumours*.
12. Cheung MM, Chan JK, Lau WH, et al. Early stage nasal NK/T-cell lymphoma: clinical outcome, prognostic factors, and the effect of treatment modality. *Int J Radiat Oncol Biol Phys.* 2002;54:182-190.
13. Aviles A, Diaz NR, Neri N, et al. Angiocentric nasal T/natural killer cell lymphoma: a single centre study of prognostic factors in 108 patients. *Clin Lab Haematol.* 2000;22:215-220.
14. You JY, Chi KH, Yang MH, et al. Radiation therapy versus chemotherapy as initial treatment for localized nasal natural killer (NK)/T-cell lymphoma: a single institute survey in Taiwan. *Ann Oncol.* 2004;15:618-625.
15. Lee J, Suh C, Park YH, et al. Extranodal natural killer T-cell lymphoma, nasal-type: a prognostic model from a retrospective multicenter study. *J Clin Oncol.* 2006;24:612-618.
16. Kim TM, Park YH, Lee SY, et al. Local tumor invasiveness is more predictive of survival than International Prognostic Index in stage I(E)/II(E) extranodal NK/T-cell lymphoma, nasal type. *Blood.* 2005;106:3785-3790.
17. Siu LL, Chan JK, Kwong YL. Natural killer cell malignancies: clinicopathologic and molecular features. *Histol Histopathol.* 2002;17:539-554.
18. Li YX, Coucke PA, Li JY, et al. Primary non-Hodgkin's lymphoma of the nasal cavity: prognostic significance of paranasal extension and the role of radiotherapy and chemotherapy. *Cancer.* 1998;83:449-456.
19. Logsdon MD, Ha CS, Kavadi VS, et al. Lymphoma of the nasal cavity and paranasal sinuses: improved outcome and altered prognostic factors with combined modality therapy. *Cancer.* 1997;80:477-488.
20. Lei KI, Chan LY, Chan WY, et al. Diagnostic and prognostic implications of circulating cell-free Epstein-Barr virus DNA in natural killer/T-cell lymphoma. *Clin Cancer Res.* 2002;8:29-34.
21. Jaffe ES, Chan JK, Su IJ, et al. Report of the workshop on nasal and related extranodal angiocentric T/natural killer cell lymphomas: definitions, differential diagnosis, and epidemiology. *Am J Surg Pathol.* 1996;20:103-111.
22. Cheung MM, Chan JK, Lau WH, et al. Primary non-Hodgkin's lymphoma of the nose and nasopharynx: clinical features, tumor immunophenotype, and treatment outcome in 113 patients. *J Clin Oncol.* 1998;16:70-77.
23. King AD, Lei KI, Ahuja AT, et al. MR imaging of nasal T-cell/natural killer cell lymphoma. *AJR Am J Roentgenol.* 2000;174:209-211.
24. Koom WS, Chung EJ, Yang WI, et al. Angiocentric T-cell and NK/T-cell lymphomas: radiotherapeutic viewpoints. *Int J Radiat Oncol Biol Phys.* 2004;59:1127-1137.
25. Ryan JL, Fan H, Glaser SL, et al. Epstein-Barr virus quantitation by real-time PCR targeting multiple gene segments: a novel approach to screen for the virus in paraffin-embedded tissue and plasma. *J Mol Diagn.* 2004;6:378-385.
26. Lei KI, Chan LY, Chan WY, et al. Quantitative analysis of circulating cell-free Epstein-Barr virus (EBV) DNA levels in patients with EBV-associated lymphoid malignancies. *Br J Haematol.* 2000;111:239-246.
27. van Esser JW, Niesters HG, Thijsen SF, et al. Molecular quantification of viral load in plasma allows for fast and accurate prediction of response to therapy of Epstein-Barr virus–associated lymphoproliferative disease after allogeneic stem cell transplantation. *Br J Haematol.* 2001;113:814-821.
28. Solassol J, Kreuzer KA, Lass U, et al. Epstein-Barr virus DNA quantitation assessed by a real-time polymerase chain reaction in a case of Burkitt's lymphoma. *Leuk Lymphoma.* 2001;41:669-673.
29. Ko YH, Ree HJ, Kim WS, et al. Clinicopathologic and genotypic study of extranodal nasal-type natural killer/T-cell lymphoma and natural killer precursor lymphoma among Koreans. *Cancer.* 2000;89:2106-2116.

DOI: 10.1309/MN4Y8HLQWKD9NB5E

HOLOGIC®

First and Only FDA Cleared
Digital Cytology System

Genius™ Cervical AI

Genius™ Review Station

Genius™ Digital Imager

Empower Your Genius With Ours

Make a Greater Impact on Cervical Cancer
with the Advanced Technology of the
Genius™ Digital Diagnostics System

Click or Scan
to discover more

genius™
DIGITAL DIAGNOSTICS