

## SINONASAL T-CELL LYMPHOMAS: A CLINICOPATHOLOGIC STUDY OF A POSSIBLY DISTINCT ENTITY

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**Background:** Most cases of sinonasal lymphomas reported in the literature which show positive expression for Epstein-Barr virus are CD2+, CD3-, CD43+ and CD56+, and also show a germ-line T-cell receptor genotype. Five-year survival is usually around 50%. We report a group of patients with T-cell sinonasal lymphoma that showed distinct immunophenotypic and molecular profiles and a more aggressive behavior.

**Patients and Methods:** Nineteen cases representing approximately 75% of sinonasal lymphoma diagnosed and treated at our institution between 1988 and 1997 were studied. They comprised 12 males and 7 females, with an age range of 10 to 73 years (median 46 years). The remaining cases (about 25%) were B-cell lymphomas. The morphology of the cases was evaluated together with a limited immunophenotyping. In situ hybridization for EBV mRNA was performed in 18 cases. Polymerase chain reaction (PCR) for T-cell receptor (TCR) gene rearrangement was performed in 15 cases. Clinical follow-up information was available on 14 patients. All cases showed a pattern of large-cell lymphoma, and three exhibited an immunoblastic morphology. The tumors showed extensive soft tissue invasion, necrosis and ulceration. While perineural invasion was a prominent feature, perivascular invasion was not noticed.

**Results:** Seventeen tumors (84%) were CD3 positive. PCR analysis showed TCR gene rearrangement in 7 of 15 cases (46%). Fifteen cases (79%) were positive for EBV. The 14 patients with available clinical information had extensive local diseases, with stages ranging from IE to IIIIE, where none showed positive bone marrow involvement. The 14 patients received chemotherapy with or without radiation therapy. Ten of the 14 patients (71%) died of the disease after a median of seven months, including all seven patients with positive TCR gene rearrangement.

**Conclusion:** Our findings suggest that sinonasal T-cell lymphoma represents a heterogeneous group of diseases with different phenotypic, genotypic and biological characteristics. Cases that show TCR gene rearrangement may represent a more aggressive subtype of the disease.

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**Key Words:** Sinonasal lymphoma, Epstein-Barr virus, phenotype, T-cell receptor gene rearrangement.

Sinonasal lymphomas are a heterogeneous group of neoplasms with different phenotypic, genotypic and biological characteristics<sup>1,2</sup> that are rarely seen in the West.<sup>3</sup> Of these lymphomas, the most frequently reported cases in the literature are those showing a CD56/CD57 (natural killer/NK) phenotype.<sup>4-6</sup> These cases are almost always positive for Epstein-Barr virus mRNA.<sup>7</sup> They are characteristically lacking expression of membranous CD3, but may occasionally show cytoplasmic expression of the antigen.<sup>6,7</sup> The tumors are also positive for the CD43

antigen.<sup>8</sup> Molecular studies have confirmed that almost all these lymphoma exhibit a germline configuration of T-cell receptors.<sup>9-11</sup> Clinicopathological studies of sinonasal lymphomas have shown a survival rate of around 50%.<sup>12,13</sup> We reviewed 19 cases of T-cell sinonasal lymphomas diagnosed in our institution, in order to compare their phenotypic, genotypic and biological characteristics with those reported in the literature.

### Patients and Methods

The files of the Department of Pathology at King Faisal Specialist Hospital and Research Centre (KFSH&RC) from 1988 to 1997 were reviewed for cases of primary malignant lymphomas of the nasal and paranasal sinuses diagnosed in our institution. Of the 25 cases identified, six cases (24%) had a confirmed B-cell phenotype. The remaining 19 cases,

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FIGURE 1. Case of sinonasal lymphoma showing an immunoblastic lymphoma pattern.



FIGURE 2. Case of large cell sinonasal lymphoma exhibiting perineural invasion.



FIGURE 3. Immunohistochemical staining of a case of large cell sinonasal lymphoma showing strong membranous/cytoplasmic expression of CD3 antigen.



FIGURE 4A. Case of sinonasal lymphoma showing weak and focal expression of CD56.



FIGURE 4B. Immunohistochemical staining of a case of large cell sinonasal lymphoma showing strong membranous/cytoplasmic expression of CD3 antigen.



FIGURE 5. In situ hybridization of a case of sinonasal lymphoma showing strong EBV mRNA expression in the lymphoma cells.

which were negative for B-cell markers, formed the basis of our study. They comprised 12 male and seven female patients, with a male to female ratio of approximately 1.5:1. Their ages ranged from 10 to 77 years, with a median age of 46 years. Clinical information was available on 14 patients. Four patients were lost to follow-up, and one patient who was recently diagnosed is still under treatment.

#### *Pathological Evaluation and Immunohistochemistry*

The slides pertaining to each case were retrieved and reviewed for pertinent histological features, including the presence or absence of necrosis, perineural and perivascular invasion, and the possible classification of the lymphoma according to the working formulation scheme. Immunohistochemical evaluation for CD3, CD20, CD45, CD56 and CD57 was performed on paraffin-embedded tissue using the microwave retrieval system.<sup>14</sup> Immunostaining for CD2 was not performed as it was not available.

#### *In-Situ Hybridization for EBV mRNA*

Representative sections of paraffin-embedded tissue were obtained in an attempt to identify EBV expression using in situ hybridization and the EBER-1 probe. The probe is a 30-base oligonucleotide complementary to a region of the EBER-1 gene of EBV base pairs 69-98. The probe was labeled with digoxigenin, and in situ hybridization was performed following procedures outlined in the literature.<sup>15,16</sup>

#### *Polymerase Chain Reaction (PCR) for the Identification of T-Cell Receptor Gene Rearrangement*

Material from formalin-fixed paraffin-embedded tissue was obtained for the polymerase chain reaction (PCR) in an attempt to identify T-cell receptor gene rearrangement. Enzymatic amplification was performed in a Perkin Elmer GenAmp PCR system 9600 using consensus primers for T-cell receptor  $\gamma$  chain  $V\gamma$  regions and a single consensus primer for the  $J\gamma$  region.<sup>17</sup> Ten microliters of PCR-amplified product were resolved by electrophoresis on a 6% polyacrilamide gel stained with ethidium bromide and visualized under ultraviolet light.

Three positive controls were used. The first control was obtained from the bone marrow of a patient with T-cell acute lymphoblastic leukemia that showed a positive band at the  $V\gamma$ 1-8 region (250 base pairs), and was negative at the  $V\gamma$ 9 and  $V\gamma$ 10-12 regions. The second was from another patient with T-cell acute lymphoblastic leukemia that showed a positive band at the  $V\gamma$ 9 region (190 base pairs) and was negative at the  $V\gamma$ 1-8 and  $V\gamma$ 10-12 regions. The third control was obtained from a patient with mycosis fungoides that showed a rearranged band at  $V\gamma$ 10-12 (210 base pairs) and was negative at the  $V\gamma$ 1-8 and  $V\gamma$ 9 regions. Discrete bands within the predicted size range were



FIGURE 6A. Electrophoretogram showing rearrangement at the V1-8 $\gamma$  region in samples from two other patients (lanes 2 and 4). Lane 1: normal DNA; lanes 3 and 5: samples from two patients with negative rearrangement; lane 6: positive control; lane 7: negative control; and lane 8: water.



FIGURE 6B. Electrophoretogram showing rearrangement of the V1-8 $\gamma$  region in samples from two patients (lanes 3 and 4). Lane 1: normal DNA; lane 2: patient's sample with negative rearrangement; lane 5: positive control; lane 6: negative control; and lane 7: water.

obtained by PCR amplifications with consensus primers for  $V\gamma$ 10-12 and  $J\gamma$  primer.

## Results

### *Pathologic Findings, Immunohistochemistry and EBV Expression*

All 19 patients showed lymphoma of the large cell type. Three cases showed an immunoblastic lymphoma morphology (Figure 1). The cases showed extensive invasion of the mucosa, adjacent soft tissue and bone. Necrosis was a prominent feature. One characteristic

TABLE 1. Clinical and pathological data of 19 patients with sinonasal T-cell lymphoma.

Age/Sex	Lymphoma type	Stage	Treatment	CD3	EBV	TCR	Follow-up	Time
42/M	LCL	III EAS	Chemotherapy	+	ND	ND	DOD	11
25/M	LCL	NA	No treatment	+	+	-	DOD	7
51/M	IBL	NA	RT + Chemotherapy	+	+	+	DOD	6
73/F	LCL	II EA	No treatment	+	+	-	NA	NA
10/M	IBL	II EA	RT + Chemotherapy	+	+	+	DOD	20
34/F	LCL	III EA	RT + Chemotherapy	+	+	-	DOD	12
30/M	LCL	II EA	Chemotherapy	+	+	-	DOD	3
65/F	LCL	III EA	Chemotherapy	+	+	+	DOD	6
24/F	IBL	II EA	Chemotherapy	+	+	+	DOD	3
73/M	LCL	NA	NA	+	+	ND	NA	NA
54/F	LCL	II EA	Chemotherapy	+	-	+	DOD	1
70/M	LCL	I EA	Chemotherapy	+	+	ND	AND	13
31/F	LCL	II EB	RT + Chemotherapy	+	+	+	DOD	2
36/M	LCL	I EA	RT + Chemotherapy	+	+	-	AND	36
65/M	LCL	II EA	RT + Chemotherapy	-	-	+	DOD	8
31/M	LCL	NA	NA	+	+	-	NA	NA
60/M	LCL	II EA	Chemotherapy	-	-	-	AND	30
70/M	LCL	III EA	Chemotherapy	+3	+	-	AND	67
47/F	LCL	NA	NA	+	+	ND	NA	NA

RT=radiation therapy; Stage=clinical stage; TCR=T-cell receptor gene rearrangement; DOD=dead of disease; NA=data not available; ND=not done; Time=follow-up in months; LCL=large cell lymphoma; AND=alive with no evidence of disease; IBL=immunoblastic lymphoma.

feature seen in almost all cases was perineural invasion (Figure 2). Immunohistochemical expression of membranous and/or cytoplasmic CD3 was seen in 17 cases (84%) (Figure 3). CD57 expression was seen in only one case and CD56 was seen in two cases. The expression was only focal (Figure 4). In situ hybridization for EBV mRNA expression was performed in 18 cases. Strong EBV mRNA expression was seen in the lymphoma cells in 15 cases (79%) (Figure 5).

*Molecular Studies*

Extractable DNA could be obtained from 15 cases. Seven of the 15 cases (46%) showed T-cell receptor  $\gamma$  gene rearrangement. The rearrangement was seen either in discrete bands or as double bands, and was seen more clearly in the V $\gamma$ 1-8 region (Figures 6A and B).

*Clinical Data*

One of the 15 patients with available clinical information refused any form of therapy and was lost to follow-up. The remaining 14 patients had mostly early stage disease, ranging from IE to IIIE. None of the patients showed positive bone marrow involvement. Only one patient exhibited B symptoms. The majority of the patients had extensive local disease manifesting by massive soft tissue and bone destruction and ulceration. All 14 patients received combination chemotherapy regimens with or without radiation therapy. Only four patients showed complete remission and are alive with no evidence of disease after a median follow-up period of 35 months. The remaining 10 patients died of the disease after a median follow-up of seven months. This included all seven patients with positive T-cell receptor gene rearrangement. Table 1

shows the clinical, morphological and molecular data pertaining to all 19 patients studied.

**Discussion**

Lymphomas of the sinonasal tract are known to represent a heterogeneous group of neoplasms.<sup>1,18</sup> The disease has been given several names, including midline granuloma syndrome, lethal midline granuloma, polymorphic reticulosis and midline destructive granuloma.<sup>2,19,20</sup> These terms are no longer acceptable, as most, if not all, of these lesions have been proven to be lymphomas. The classification of these lymphomas continues to defy the efforts of lymphoma experts.<sup>4,6</sup> The inclusion of all sinonasal lymphomas into one category has been attempted before.<sup>7,21</sup> A consensus meeting of lymphoma experts from around the world attempting to define lymphomas of the sinonasal tract concluded that these tumors have very characteristic morphological, immunophenotypic and molecular characteristics.<sup>4,7</sup> The study concluded that these lymphomas usually show a morphologic spectrum, ranging from small to mixed to large cell subtypes, with the chance of progression over time. The cells of these lymphomas, according to the study, are CD2+, CD3-, CD43+ and CD56/57+.

Most of the cases studied expressed Epstein-Barr virus mRNA, and almost all the cases exhibited a germline DNA pattern.<sup>21,22</sup> Our study showed several differences. All our cases were large cell lymphomas, which may suggest a more advanced phase of the disease. The cases in the consensus study showed a prominent angioinvasive pattern which was not obvious in our cases. The main differences are that most of our cases were strongly positive for CD3,

TABLE 2. Immunophenotypic profile of the 19 cases of sinonasal lymphoma.

Patient	CD3	CD43	CD56	CD57
1	+	+	-	+
2	+	+	-	-
3	+	+	-	-
4	+	+	-	-
5	+	+	-	-
6	+	+	-	-
7	+	+	-	-
8	+	+	-	-
9	+	+	-	-
10	+	+	-	-
11	+	+	-	-
12	+	+	-	-
13	+	+	-	-
14	+	+	-	-
15	-	+	-	-
16	+	+	+	-
17	-	+	+	-
18	+	+	-	-
19	+	-	+	-

and that close to half showed T-cell receptor  $\gamma$  gene rearrangement. These findings suggest that within the category of sinonasal lymphoma, more than one distinct disease pattern exists. One group of neoplasms represented by our patients showed CD3 expression and a high incidence of T-cell receptor gene rearrangement. Although some studies have suggested that rare cases of sinonasal T-cell lymphoma may show cytoplasmic CD3 expression and T-cell receptor gene rearrangement,<sup>9-11</sup> the rarity of this occurrence has led to their inclusion in the same category of T and NK cell lymphoma. A study describing flow cytometric and Southern blot analysis of 12 cases of large granular cell malignant lymphoma has concluded that CD3 expression and T-cell receptor gene rearrangement are sufficient evidence to rule out NK cell lymphoma.<sup>23</sup> This is more evidence that T and NK cell lymphoma of the sinonasal tract may even represent two distinct disease entities that should not be lumped in the same category.

A study has suggested that EBV expression is mostly seen in sinonasal lymphomas of the NK lineage and not other phenotypes.<sup>4,5,11</sup> This finding has also been challenged in our study. In spite of the strong CD3 expression in most of our cases, over 80% of the tumors showed strong EBV nuclear expression. It thus appears that although EBV may play a pathogenetic role in the development of sinonasal lymphoma, the final oncogenic event may lead to different disease categories.

Reports on the biological behavior of sinonasal lymphomas have shown that five-year survival is in the range of 50%.<sup>12,13</sup> A large study reporting on the effect of combined chemotherapy and radiation therapy showed that the use of combined treatment modalities significantly improved the five-year disease-free survival and overall survival rates.<sup>13</sup> Unfortunately, the study did not describe the different phenotypic characteristics of the lymphomas

studied. Our patients showed a completely different pattern of response. Only four of the 14 patients (28%) are alive 13, 30, 36 and 67 months, respectively, after treatment. Interestingly, the tumors in three of the four patients showed no evidence of T-cell receptor gene rearrangement. The remaining 10 patients showed no or partial response to therapy. The lymphoma in seven of these patients showed T-cell receptor gene rearrangement. Whether T-cell clonality has an impact on the biological behavior of these tumors or not is a matter of speculation. The findings may suggest, however, that clonal restriction could represent aggressive disease.

We thus conclude that sinonasal T-cell lymphomas may represent a distinct category of neoplasms of the sinonasal tract that perhaps shares a common pathogenetic pathway with NK cell lymphoma related to Epstein-Barr virus, but leading to two different disease processes. This category of lymphoma that is phenotypically and genotypically different from the more common NK cell subtype requires further investigation. Whether CD3 antigen expression and clonal restriction represent a more aggressive subtype of the disease remains to be seen.

## References

1. Cleary KC, Batsakis JG. Sinonasal lymphomas. *Ann Otol Rhinol Laryngol* 1994;103:911-4.
2. Weiss LM, Arber DA, Strickler JG. Nasal T-cell lymphoma. *Ann Oncol* 1994;5(Suppl):S39-S42.
3. Fellbaum C, Hansmann ML, Lennert K. Malignant lymphoma of the nasal cavity and paranasal sinuses. *Virchows Arch Pathol Anat Histopathol* 1989;414:399-405.
4. Ferry J, Sklar J, Zuckerberger L, Harris N. Nasal lymphoma: a clinicopathologic study with immunophenotypic and genotypic analysis. *Am J Surg Pathol* 1991;15:268-79.
5. Frierson HF, Innes D, Mills S, Wick M. Immunophenotype analysis of sinonasal non-Hodgkin's lymphoma. *Hum Pathol* 1989;20:636-42.
6. Jaffe ES, Chan JKC, Su I, Frizzera G, Mori S, Feller AC, 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;20:103-11.
7. Chan JKC, Yip TT, Tsang Wy, Ng CS, Lau WH, Poon YF, et al. Detection of Epstein-Barr viral RNA in malignant lymphomas of the upper aerodigestive tract. *Am J Surg Pathol* 1994;18:938-46.
8. Arber DA, Weiss LM, Albuja PF, Chen YY, Jaffe ES. Nasal lymphomas in Peru. High incidence of T-cell phenotype and Epstein-Barr virus infection. *Am J Surg Pathol* 1993;17:392-9.
9. Medeiros J, Peiper S, Elwood L, Yano T, Raffield M, Jaffe E. Angiocentric immunoproliferative lesions: a molecular analysis of eight cases. *Hum Pathol* 1991;22:1150-7.
10. Tao Q, Chiang AK, Srivastava G, Ho FC. TCR-CD56+CD2+ nasal lymphomas with membrane localized CD3 positivity: are CD3+ cells neoplastic or reactive? *Blood* 1995;85:2993-6.
11. Tao Q, Ho FC, Loke SL, Srivastava G. Epstein-Barr virus is localized in the tumor cells of nasal lymphomas of NK, T or B cell type. *Inter J Cancer* 1995;60:315-20.
12. Haraguchi H, Ebihara S, Saikawa M, Mashima K, Hameda T, Hirano K. Malignant tumors of the nasal cavity: review of a 60-case series. *Jap J Clin Oncol* 1995;25:188-94.
13. Logsdon MD, Ha CS, Kavadi VS, Cabanillas F, Hess MA, Cox JD. Lymphoma of the nasal cavity and paranasal sinuses. Improved outcome and altered prognostic factors with combined modality therapy. *Cancer* 1997;80:477-80.

14. Shi SR, Cote RJ, Taylor CR. Antigen retrieval immunohistochemistry: past, present and future. *J Histochem Cytochem* 1997;45:327-43.
15. Christiansen MS, Mourad WA, Hales ML, Oldring DJ. Spindle cell malignant lymphoepithelial lesion of the parotid gland: clinical, light microscopic and in situ hybridization findings in one case. *Mod Pathol* 1995;8:711-5.
16. Weiss LM, Arber DA, Strickler JG. Nasal T-cell lymphoma. *Ann Oncol* 1994;5(Suppl):S39-S42.
17. Kneba M, Bolz I, Linke B, Bertram J, Rothaupt D, Hiddemann W. Characterization of clone-specific rearranged T-cell receptor  $\gamma$ -chain genes in lymphomas and leukemias by the polymerase chain reaction and DNA sequencing. *Blood* 1994;84:574-81.
18. Abbondanzo S, Wenig B. Non-Hodgkin's lymphoma of the sinonasal tract. *Cancer* 1995;75:1281-91.
19. Chan J, Ng C, Lau W, Lo S. Most nasal/nasopharyngeal lymphomas are peripheral T cell neoplasms. *Am J Surg Pathol* 1987;11:418-29.
20. Chott A, Rappersberger K, Schlossarek W, Radaszkiewicz T. Peripheral T cell lymphoma presenting primarily as lethal midline granuloma. *Hum Pathol* 1988;19:1093-101.
21. Ho FCS, Choy D, Loke SL. Presence of Epstein-Barr virus DNA in nasal lymphomas of B and T cell type. *Haematol Oncol* 1990;8:271-81.
22. Wong KF, Chan JKC, Ng CS, Lee KC, Tsang WYW, Cheung MMC. CD56 (NKH1)-positive hematolymphoid malignancies: an aggressive neoplasm featuring frequent cutaneous/mucosal involvement, cytoplasmic azurophilic granules and angiocentricity. *Hum Pathol* 1992;798-804.
23. Nichols GE, Normansell DE, Williams ME. Lymphoproliferative disorders of granular lymphocytes: nine cases including one with features of CD 56 (NKH-1) positive natural killer cell lymphoma. *Mod Pathol* 1994;7:819-24.