

EBV-associated, extranodal NK-cell lymphoma, nasal type of the breast, after heart transplantation

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Post-transplantation lymphoproliferative disorders (PTLDs) are predominantly Epstein–Barr virus (EBV)-associated B-cell lymphoproliferations. PTLDs of T-cell lineage are rare, mostly reported after renal transplantation and show less frequent association with EBV. NK-cell lymphomas after transplantation (NK-cell PTLDs) are very rare; only five cases are reported so far in the English literature, all developed after renal transplantation. We describe a case of EBV-associated, extranodal NK-cell lymphoma of nasal type, involving the breast in a cardiac allograft recipient 5 years after transplantation. The neoplastic cells are positive for CD2, cytoplasmic CD3, CD7, CD43, CD56, TIA-1 and p53; and negative for surface CD3 and CD57. Analysis of T-cell receptor beta and gamma genes fails to show clonal rearrangement. EBV studies show clonal episomal integration of EBV and latency II pattern (EBER-1+, LMP-1+, EBNA-1+, EBNA-2-). In conclusion, NK-cell PTLDs are rare complications that arise relatively late after solid organ transplantation, show strong association with EBV, and can follow an aggressive clinical course. To the best of our knowledge, we present the first reported case of NK-cell PTLT after cardiac transplantation and the unifying clinical and diagnostic features of NK-cell PTLTs occurring after solid organ transplantation.

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Post-transplantation lymphoproliferative disorders (PTLDs) are a well-recognized complication of solid organ and bone marrow transplantation^{1–3} that arise secondary to immunosuppression, and are associated with significant morbidity and mortality. PTLTs are a clinically and morphologically heterogeneous group of diseases that account for up to 25% of post-transplantation malignancies.⁴ Most PTLTs are of B-cell lineage and show strong association with EBV. PTLTs of T-cell lineage are more commonly seen in Asia⁵ and are rarely reported in Western countries.^{6,7} NK-cell PTLTs are a very rare entity with only a handful of reported cases in the literature. Under the current WHO classification,³ T- and NK-cell PTLTs are considered under the monomorphic lesions and classified similar to T- and NK-cell lymphomas arising in immunocompetent patients. T- and NK-cell lymphomas can have overlapping features, including the immunophenotype. In fact, some T-cell lymphomas

can upregulate NK-cell-associated markers (CD56 and CD57) and downregulate surface CD3.^{8,9} However, while T-cell lymphomas usually show clonal T-cell receptor (TCR) gene rearrangements, the TCR gene in NK-cell lymphomas is usually in germline configuration.^{9–11} Although clonal chromosomal abnormalities (deletions of 6q and trisomy 7) have been recently described in a few cases of NK-cell lymphoma,^{12–15} these are not specific for NK-cell lymphomas.

Generally, NK-cell neoplasms, including those occurring in immunocompetent individuals, are relatively rare. The most common and well characterized are the nasal/nasal-type NK-cell lymphomas. These lymphomas are typically clinically aggressive and show an angiocentric/angiodestructive growth pattern with zonal necrosis^{8,10} and the following immunophenotype: CD2+, cytoplasmic CD3ε+, CD56+, surface CD3-, TIA-1+ and frequently CD7+. TCR proteins are not expressed on the cell surface and p53 protein is frequently overexpressed.¹⁶ EBV infection is also present in the vast majority of cases.^{3,17,18} Clinically, two-thirds of patients with localized disease can be successfully treated, but many (50%) relapse and some (25%) progress to a highly fatal disseminated systemic disease.⁸ As a subtype, nasal-type NK-cell lymphomas have a worse prognosis.⁸

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In this report, we describe a case of EBV-associated, extranodal NK-cell lymphoma of nasal-type, involving the breast after cardiac transplantation. NK-cell PTLDs are extremely rare in the literature. Our review of the literature reveals only five reported cases, all developing after renal transplantation.^{4-7,19} The first reported case is from the United States in 1998.⁷ Since then, only four other cases of NK-cell PTLDs have been reported (three in Asia and one in Austria). To the best of our knowledge, our case is the first NK-cell PTLT to be described after cardiac transplantation.

Case report

The patient was a 20-year-old female of Korean descent with a history of orthotopic heart transplant for anthracycline-induced heart disease, secondary to chemotherapy for a Burkitt lymphoma. She presented with a right breast mass approximately 5 years post-transplantation.

The patient was diagnosed with a Burkitt lymphoma at the age of 5 years in an outside institution, where she presented with a large intestinal mass. At the time, bone marrow involvement was seen on biopsy (we could not review the pathology of Burkitt lymphoma and the patient's EBV status was not documented). She was treated with a chemotherapy regimen, which included adriamycin, and subsequently developed progressively worsening heart failure secondary to the chemotherapy, eventually requiring cardiac transplantation. At this time, serological studies for EBV showed that the patient had already been infected with EBV based on serum levels of IgG. Post-transplantation, her immunosuppressive therapy included cyclosporin A, prednisone and azathioprine. Serial EBV-enzyme immunoassays for IgG immediately after transplant showed increasing titers from 1:640 to 1:2560. Her post-transplantation course was relatively uncomplicated with minimal to no chronic rejection of the heart (ISHLT Grade 0-1A).

At 5 years after transplantation, she presented with a right breast mass, bilateral axillary lymphadenopathy, and a focal lesion in the right lobe of the liver on MRI. An excisional biopsy of the breast mass was performed, but the axillary lymph nodes and liver lesion were not biopsied. After diagnosis of PTLT was made on the breast biopsy, the patient was treated with reduction of immunosuppression and chemotherapy. However, there was no clinical response and the patient expired approximately 1 month after admission. Limited autopsy of the heart revealed cardiac involvement by lymphoma.

Materials and methods

Morphological analysis was performed on standard H&E-stained sections from formalin-fixed and paraffin-embedded tissue. Immunohistochemical ana-

lysis (IHC) was performed on formalin-fixed and paraffin-embedded sections using the Dako Envision plus system for detection (DAKO, Carpinteria, CA, USA) and the immunostains listed in Table 1. Molecular analysis of the TCR beta gene (TCR β gene) was performed on frozen tissue by Southern blot (SB) analysis using the J β -2 primer and restriction enzymes *Bam*HI, *Hind*III. Paraffin-embedded tissue was analyzed for TCR gamma (TCR γ) gene rearrangement using PCR-heteroduplex analysis with polyacrylamide gel electrophoresis using the V2 V9, V10/11, J1/2, JP and JP1/P2 primers as reported by Bottaro *et al.*²⁰ EBV infection status was detected by IHC (Table 1) and by *in situ* hybridization (ISH) for EBV-encoded RNAs (EBER 1-2, Ventana INFORM EBER, Tucson, AZ, USA) and by SB analysis of EBV episomal integration, using a probe for EBV termini. Formalin-fixed, paraffin-embedded tissue was cut to 5 μ m thick sections and processed for fluorescence *in situ* hybridization (FISH) using a paraffin pretreatment kit supplied by Vysis (Downers Grove, IL, USA). The pretreated tissue sections were hybridized using spectrum orange-labeled alpha satellite CEP7 probe (Vysis, Downers Grove, IL, USA) by standard methods. Images were captured using Cytovision probe capturing system (Applied Imaging, Santa Clara, CA, USA).

Results

The breast biopsy showed an angiocentric and angiodestructive infiltrate of atypical mononuclear cells in a background of zonal necrosis (Figure 1a). The cells were medium to large with vesicular nuclei and variably prominent nucleoli. Numerous apoptotic bodies and mitotic figures were present (Figure 1b). The complete results of the immunophenotyping are shown in Table 1. The neoplastic cells expressed CD2, CD3 (faint cytoplasmic), CD7, CD43, CD45, CD56 and TIA-1 (Figure 1c, d). EBER by ISH was strongly positive in the vast majority of neoplastic cells (Figure 1f) and by IHC, the neoplastic cells expressed LMP-1 and EBNA-1, but not EBNA-2. Approximately 70% of the neoplastic cells were positive for MIB-1 and over 50% showed nuclear staining for p53 (Figure 1e). The molecular analysis by SB showed germline configuration of the TCR β gene (Figure 2a), and PCR of the TCR γ gene demonstrated a polyclonal pattern (data not shown). The SB analysis for EBV clonality showed monoclonal episomal integration of EBV in the neoplastic cells (Figure 2b). The cytogenetic analysis by FISH showed normal complement of chromosome 7.

Discussion

In this report, we describe a case of NK-cell lymphoma of nasal-type that occurred in the breast

Table 1 Immunophenotyping results

<i>Antibody</i>	<i>Source</i>	<i>Major specificity</i>	<i>Reactivity</i>
CD2	Novocastra, Burlingame, CA, USA	Pan T	+
CD3	DAKO, Carpinteria, CA, USA	Pan T	+ (cytoplasmic, weak)
CD4	Novocastra	T	—
CD5	Novocastra	T	—
CD7	Novocastra	Pan T	+
CD8	DAKO	T	—
CD20	DAKO	B	—
CD30	Novocastra	Activated T, B	—
CD34	Biogenex, San Ramon, CA, USA	Immaturity	—
CD43	Biogenex	Pan hematopoietic	+
CD45	DAKO	Pan hematopoietic	+
CD56	Novocastra	NK, T	+
CD57	Novocastra	NK, T	—
CD68	DAKO	Histiocytic	—
CD79a	DAKO	B	—
MIB-1	DAKO	Proliferation marker	+ (70%)
TIA-1	Immunotech, Westbrook, ME, USA	NK, T	+
p53	Immunotech	Tumor suppressor gene	+ (50%)
TdT	Supertechs, Bethesda, MD, USA	Immaturity	—
CK	Cocktail: Chemicon, Temecula, CA, USA	Epithelial	—
	Immunotech		
LMP-1	DAKO	Latent membrane protein 1	+
EBNA-1	Chemicon	EBV-associated nuclear antigen 1	+
EBNA-2	DakoCytomation, Carpinteria, CA, USA	EBV-associated nuclear antigen 2	—

of a cardiac transplant patient with the typical features of a NK-cell lymphoma of nasal type, including clinical presentation (aggressive course and extranodal location), morphology (angiocentric growth with zonal necrosis), immunophenotype (ie CD2 +, cytoplasmic CD3 ϵ +, CD7 +, CD56 +, TIA-1 +), a high proliferation rate (Ki-67 labelling index of 70%) and TCR gene rearrangement profiles (germline TCR β by SB and polyclonal TCR γ by PCR). Although the SB analysis of TCR β was performed using only the J- β 2 primer without the C β probe, the TCR γ gene, generally believed to rearrange prior to TCR β ,²¹ is polyclonal by PCR,²² suggesting a true NK-cell lineage. In addition, the neoplastic cells in our current case show evidence of EBV infection and overexpression of the p53, both frequently seen in nasal/nasal-type NK-cell lymphoma.^{3,16–18}

Reports of NK-cell PTLDs are extremely rare in the literature, all (6/6) developed relatively late after transplantation (1–8 years) and most (5/6) presented predominantly at extranodal sites. The vast majority are positive for EBER (5/6) and LMP-1 (4/5). This is in contrast to T-cell PTLDs, as was shown in a recent review of 76 cases, the vast majority (61/76) were negative for EBV.²³ Of the cases with EBNA-2 studies, the majority (3/4) of cases showed lack of EBNA-2 expression consistent with a latency II pattern. The expression of EBNA-2 is studied in three reports.^{4–6} Stadlmann *et al*⁶ (Table 2, Case 2) and Mukai *et al*⁴ (Table 2, Case 4) both report lack of EBNA-2 expression consistent with latency II. Hoshida *et al*⁵ report EBNA-2 expression in two cases consistent with latency III. However, one of

the two cases appears to be from Mukai *et al*⁴ (Table 2, Case 4) with some immunophenotypic discrepancies. Nevertheless, the latency II pattern, also reported in Hodgkin's lymphoma and nasopharyngeal carcinoma,^{24,25} has been reported in NK-cell PTLDs.^{4,6} Although the exact role of EBV infection in the pathogenesis of NK-cell PTLDs is unknown, the strong association (5/6) suggests a possible pathogenic role for EBV in NK-cell PTLDs. In addition, the strong EBV association suggests that, similar to bone marrow transplant patients, early prophylactic treatment of EBV infection may help prevent the development of NK-cell PTLDs.²⁶

As with nasal/nasal-type NK-cell lymphoma occurring in immunocompetent individuals, p53 overexpression/mutation is also commonly (3/4) seen in NK-cell PTLDs. Hoshida *et al*²⁷ reports two cases (Table 2, Cases 4 and 5) of NK-cell PTLDs in which mutations of the p53 tumor suppressor gene are studied using PCR. Of the two cases, only one (Table 2, Case 4) contains mutations in the p53 gene. In addition, in two of two cases (Table 2, Cases 2 and 6) studied, p53 overexpression has been demonstrated by IHC. Unfortunately, we were unable to study the p53 gene by PCR due to limitations of available tissue. However, since p53 protein overexpression may not be associated with p53 gene mutations,²⁸ future studies of NK-cell PTLDs should evaluate both p53 gene mutations and protein overexpression.

Nasal-type NK-cell lymphoma is more commonly seen in Asia and Latin America.⁵ Although the ethnicity of the patients developing NK-cell PTLDs in the reported cases is not known, three are from

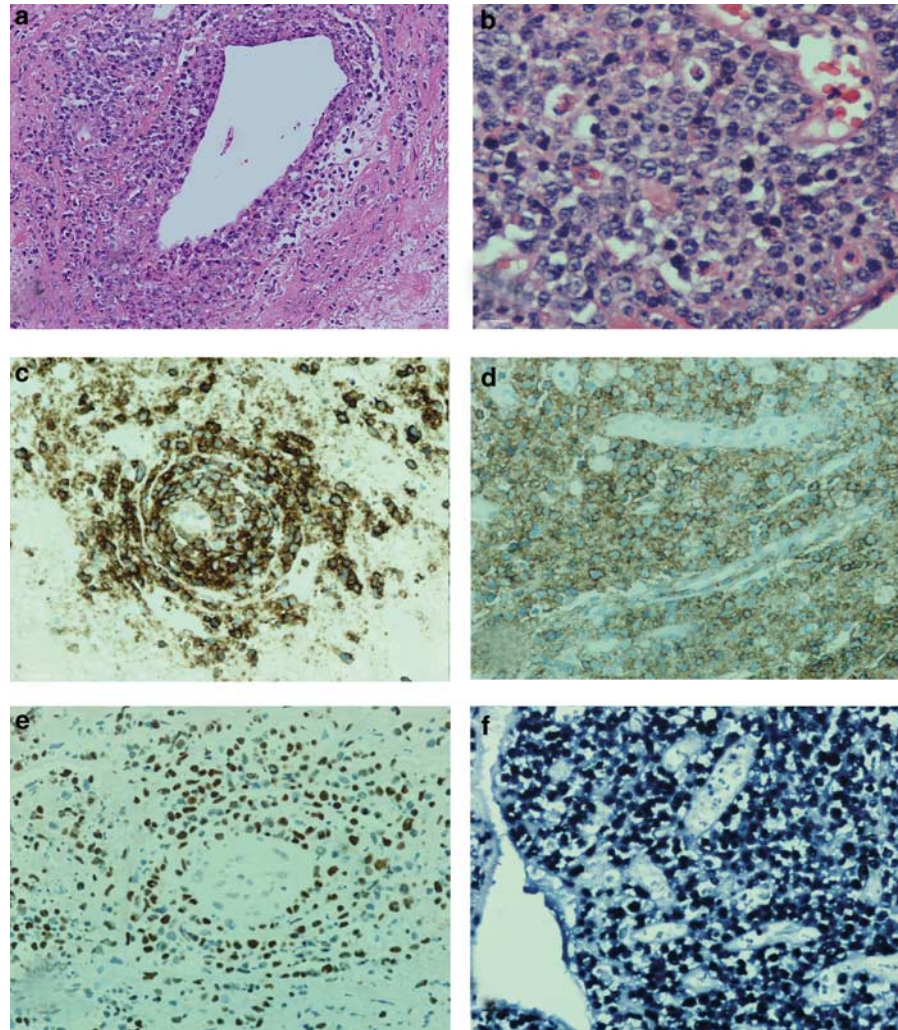


Figure 1 Excisional breast biopsy showing NK-cell lymphoma in a patient after cardiac transplant. (a) Low magnification ($\times 100$) of angiocentric pattern of infiltration. (b) Higher magnification ($\times 400$) of angiodestructive infiltration with numerous mitotic figures and apoptotic bodies. (c–e) Immunohistochemistry showing membranous staining for CD2 (c) and CD56 (d), and aberrant expression of p53 protein (e). (f) ISH with EBER 1-2 showing strong positive reaction in almost all the neoplastic cells.

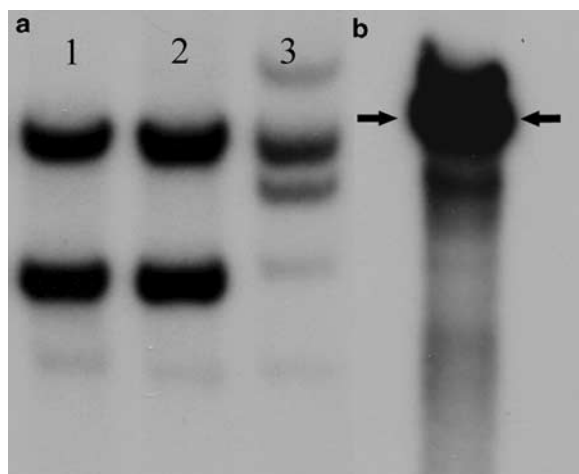


Figure 2 (a) SB analysis using TCR $J\beta$ -2 probe showing germline configuration (lane 1, patient; lane 2, negative control; lane 3, positive control). (b) SB analysis of EBV episomal integration showing monoclonal band (arrows).

Asian countries (Japan and Hong Kong) and are likely patients of Asian ethnicity. Our patient is also of Asian ethnicity. Thus, NK-cell PTLs, like NK-cell lymphoma, may show a racial predisposition (4/6) in Asian patients.

Clinically, the course of NK-cell PTLs varies from complete remission after chemotherapy (3/6) to death due to disease or complications of disease within months of diagnosis (3/6) (Table 2). Those without systemic dissemination (Cases 2, 4 and 5, Table 2) seem to have the best outcome. Thus, early detection and intervention is necessary.

In conclusion, NK-cell neoplasms are very rare forms of monomorphic PTLs that can arise late, not only after renal transplantation, but after cardiac transplantation as well, and present predominantly at extranodal sites. Although not entirely clear, a racial predisposition may exist in Asians. Diagnosis of a true NK-cell neoplasm is difficult and immunophenotypic and molecular genetic studies are

Table 2 Summary of clinicopathological characteristics of previously reported cases and current case of NK-cell PTLDs

Case	Age (years) sex	Organ transplant	Yrs. Post-transplant	Lesion	Clinical outcome	EBER	LMP-1	EBNAs 1/2	p53	Reference
1	42/M	Cadaveric renal	8	Bilateral renal masses	NR, DOD (4 weeks)	-	-	Not reported	Not reported	Hsi, ED
2	65/M	Cadaveric renal	1	Hypopharynx	Chemo, CR (3+ years)	+	+	?/-	Overexpression by IHC	Stadlmann, S
3	43/M	Cadaveric renal	1	Bone marrow	DOC (10 weeks)	+	Not reported	Not reported	Not reported	Kwong, YL
4	27/M	Cadaveric renal	5	Nasal cavity mass	Chemo, CR (3 years)	+	+	?/-	Mutation by PCR	Mukai, HY
5	35/M	Renal	Not specified	Cervical lymph node	Chemo, CR (1 year)	+	+	?/+	Wild type by PCR	Hoshida, Y
6	20/F	Cardiac	5	Breast	NR, DOD (4 weeks)	+	+	+/-	Overexpression by IHC	Index case

NR = no response, DOD = dead of disease, CR = complete response, DOC = Dead of complication.

essential. In addition, in contrast to T-cell PTLDs, the vast majority of these lesions are EBV associated with LMP-1 expression, suggesting a role for EBV infection in the pathogenesis of NK-cell PTLDs. Mutations in the p53 tumor suppressor gene can be seen, but its role in NK-cell PTLDs is unclear. Lastly, while most NK-cell PTLDs follow an aggressive clinical course with poor response to therapy, cases with limited disease may respond well to aggressive intervention. Therefore, early detection and aggressive intervention is essential.

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