T-Cell Lymphoma Presenting in the Breast: A Histologic, Immunophenotypic and Molecular Genetic Study of Four Cases

Nadine S.I. Aguilera, M.D., Fattaneh A. Tavassoli, M.D., Wei-Sing Chu, M.D., Susan L. Abbondanzo, M.D.

Departments of Hematopathology (NSIA, W-SC, SLA) and Gynecologic and Breast Pathology (FAT), Armed Forces Institute of Pathology, Washington, D.C.

Primary non-Hodgkin's lymphoma of the breast is uncommon. Most primary breast lymphomas are of B-cell phenotype, with only rare cases showing a T-cell phenotype. In this study, we report the clinicopathologic features of four cases of T-cell lymphoma in the breast. The patients all were female with a mean age of 48 years (range, 13 to 77 years). All cases showed immunoreactivity in paraffin-embedded tissue for T-cell markers CD3, CD45RO, and CD43. βF1 was positive in three of four cases. The four cases were further subclassified as anaplastic large cell lymphoma (CD30 positive) of T-immunophenotype; natural killer/T-cell lymphoma; peripheral T-cell (CD4 positive), large cell type; and peripheral T-cell (CD8 positive, T-cell intracellular antigen positive), medium cell type. Three of the four cases were monoclonal for T-cell receptor β and/or T-cell receptor γ. The one case of natural killer/T-cell lymphoma was negative for monoclonality with both T-cell receptor β and γ by molecular diagnostic studies. In all cases, IgH was negative. Follow-up was obtained in three cases. Two patients died within less than 1 year after the diagnosis. The third patient died within 18 months of the diagnosis. Our results suggest an aggressive clinical course for T-cell lymphomas that present in the breast.

KEY WORDS: Breast lymphoma, Natural killer/T-cell, T-cell lymphoma.

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Address reprint requests to: Nadine S.I. Aguilera, M.D., Department of Hematopathology, Armed Forces Institute of Pathology, Building 54, Washington, DC 20306-6000; Fax: 202-782-9157.

Non-Hodgkin's lymphoma (NHL) of the breast is uncommon, composing 0.04 to 0.5% of all malignant breast tumors (1–5). Most primary breast lymphomas are of B-cell phenotype or have not been immunophenotyped; only rare cases have been reported as T-cell in series of lymphomas of the breast (1–38). Single case reports of T-cell lymphoma of the breast also are found in the literature (39–47). Reports have included lymphoblastic lymphoma (1, 3, 9); anaplastic large cell (ALCL) (CD30 positive) (13, 14, 47); peripheral T-cell (PTCL; including large cell, pleomorphic, and high grade) (8, 14–17, 39, 40, 45, 46); adult T-cell, human T-cell lymphoma–related (41); multilobated (42); and mycosis fungoides (43, 44).

The pathobiology of T-cell lymphoma in the breast is poorly understood because of the limited number of cases available for study. With the increasing availability of T-cell markers for paraffin-embedded tissue, however, archival material can be more readily examined and subtyped. We present four cases of lymphoma with a T-cell and/or natural killer (NK) cell phenotype. All cases described in this article presented as mass lesions within breast parenchyma. The lymphomas followed an aggressive clinical course in the patients with available clinical follow-up.

MATERIALS AND METHODS

Four cases of T-cell lymphoma that occurred in the breast were retrieved from the files of the Armed Forces Institute of Pathology from 1990 to 1999. Blocks were available on all cases for immunohistochemical and genotypic studies.

Immunohistochemistry

Five-micron sections from paraffin-embedded tissue blocks were prepared for immunophenotypic analysis. Immunohistochemistry was performed according to the standard avidin-biotin complex

method of Hsu *et al.* (48). Sections were incubated at room temperature for 30 min with the following antibody panel for each case: CD45RB, CD20, CD45RO, CD3, CD43, βF1, and cytokeratin. CD30, anaplastic lymphoma kinase (ALK-1), T-cell intracellular antigen (TIA-1), CD4, CD8, CD56, T-cell receptor (TCR) δ-1, latent membrane protein for Epstein-Barr virus, and epithelial membrane antigen were performed in selected cases (see Table 1). Of the above antibodies, CD3, CD30, and βF1 required predigestion for 20 min with 0.4% Pepsin (#P-7000; Sigma Chemical Co., St. Louis, MO) in 0.1 M HCl buffer solution (pH 2.0) at 40 to 42° C. Microwave antigen retrieval, heated for 10 min in 10 mmol/L citrate buffer at pH 6.0, was necessary for CD56, ALK-1, TIA-1, CD4, CD8, and TCR δ-1 (49). All reactions were developed with 3,3-diaminobenzidine tetrahydrochloride using a 3,3′-diaminobenzidine chromogen kit (#K3466; DAKO, Carpinteria, CA).

Positive immunoreactivity for CD45RB and T-cell markers CD3, CD45RO, or βF1 with negative CD20 was used to determine T-cell immunophenotype.

Molecular Diagnostic Studies

Molecular diagnostic studies were performed by polymerase chain reaction (PCR) from formalin-fixed, paraffin-embedded tissue for Ig heavy chain (IgH), TCR β, and TCR γ by previously established methods (50).

In Situ Hybridization Studies

In situ hybridization for the presence of Epstein-Barr virus–encoded RNA was performed on sections of formalin-fixed paraffin-embedded tissue using an Epstein-Barr virus–encoded RNA *in situ* kit (DA160SS; BioGenex, San Ramon, CA) according to the manufacturer's instructions. Appropriate positive and negative controls were preformed simultaneously and stained according to the kit instructions.

TABLE 1. Antibodies

Antibody	Source	Dilution
CD45RB(LCA)[a]	DAKO, Carpinteria, CA	1:200
CD20(L26)[a]	DAKO	1:200
CD45RO(UCHL-1)[a]	DAKO	1:200
CD3[b,c]	DAKO	1:500
CD43(MT-1)[a]	Biotest, Denville, NJ	1:50
βF-1(8A3)[a,c]	Endogen, Woburn, MA	1:50
CD30(Ber H2)[a,c]	DAKO	1:100
Cytokeratin(AE1/AE3)[a,c]	Boehringer, Indianapolis, IN	1:400
TIA-1[a,d]	Coulter, Miami, FL	1:400
CD4[a,d]	Vector (Novocastra), Burlingame, CA	1:500
ALK-1[a,d]	DAKO	1:100
CD8[a,d]	DAKO	1:100
TCR δ-1(TCR1061)[a,d]	Endogen	1:10
LMP-1[a,c]	DAKO	1:50
EMA[a]	DAKO	1:100
CD56[a,d]	Caltag, Burlingame, CA	1:100

LCA, leukocyte common antigen; TIA, T-cell intracellular antigen; ALK, anaplastic lymphoma kinase; TCR, T-cell receptor; LMP, latent membrane protein; EMA, epithelial membrane antigen.

Clones are in parentheses.

[a] Mouse monoclonal.

[b] Rabbit polyclonal.

[c] Required predigestion for 20 minutes with 0.4% Pepsin.

[d] Microwave antigen retrieval.

RESULTS

Case 1

A 13-year-old Asian female who initially was believed to have a breast infection developed a fungating mass that was excised. The initial tissue diagnosis was an undifferentiated sarcoma, but immunohistochemical studies revealed a lymphoma, ALCL, CD30-positive, T-cell phenotype. The patient developed a lung mass within 5 months after excision of the mammary tumor. The lung mass was dissemination of the original lymphoma. The patient died shortly thereafter with diffuse pulmonary infiltrates and pleural involvement by the malignancy.

A 6.0 × 6.0-cm fungating mass involved the left breast with extensive epidermal necrosis. The cells infiltrating the tissue were large and monomorphic with focal areas of myxoid change in the background (Fig. 1). Rare Hodgkin's-like cells were present. Necrosis and inflammation were extensive. The cells were immunoreactive with CD45RB, CD30, CD45RO, CD43, ALK-1, TIA-1, epithelial membrane antigen, and βF1 (Fig. 2). CD3 was focally immunoreactive. CD20 and CD56 were negative. The malignant lymphoma was within the breast tissue near the epithelium, but cytokeratin did not show a lymphoepithelial (LE) component. The molecular genetic IgH assay was nonamplifi-

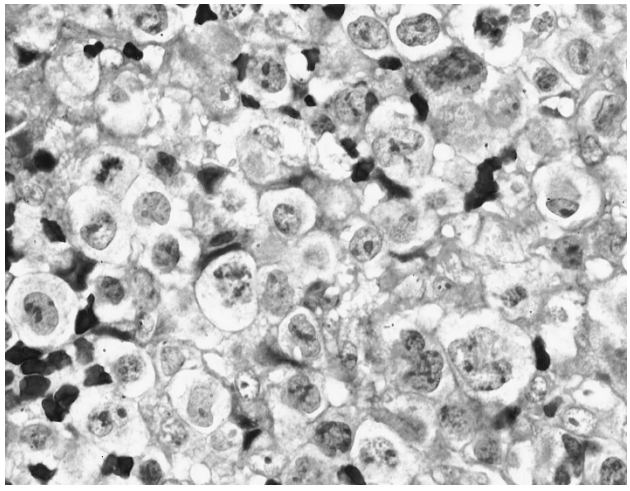

FIGURE 1. Case 1 of anaplastic large cell lymphoma showing numerous large cells with abundant cytoplasm. Many of the cells were monomorphic with scattered rare pleomorphic cells.

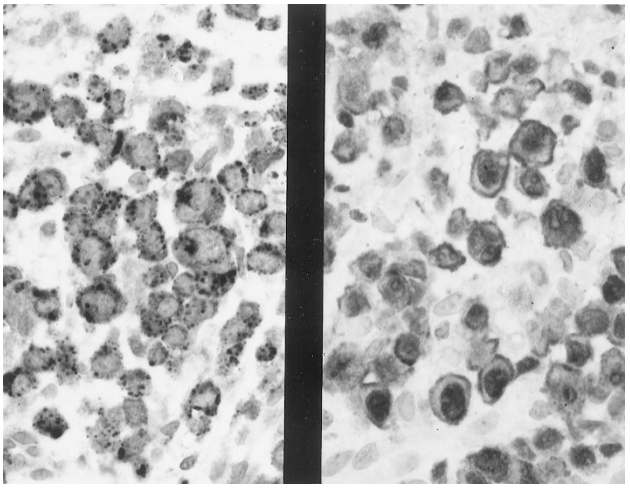

FIGURE 2. Case 1 showing anaplastic lymphoma kinase with nuclear and cytoplasmic immunoreactivity (***right***) and T-cell intracellular antigen (***left***) immunoreactivity.

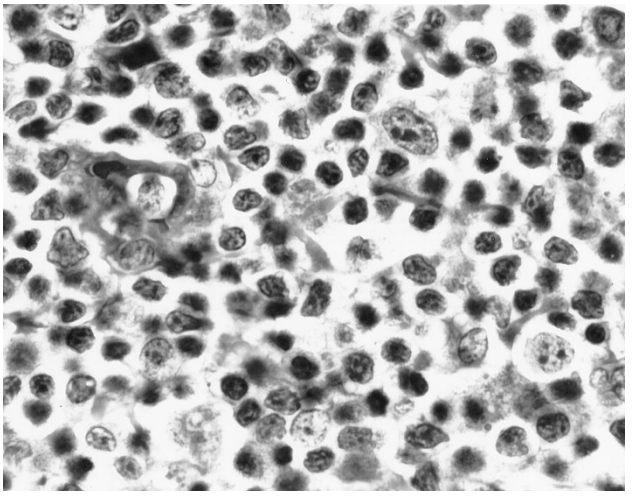

FIGURE 4. High-power field of Case 2 showing intermediate-sized lymphocytes with abundant cytoplasm and atypia.

able. TCR β and γ assays showed a monoclonal band.

Case 2

A 56-year-old Caucasian woman presented with a right breast mass and an enlarged axillary lymph node. The patient underwent a needle biopsy with a subsequent excisional biopsy, and a diagnosis of a T-cell lymphoma was rendered. The patient died with disease 3 months after the excisional biopsy.

A nodular proliferation of atypical clear cells of intermediate size with abundant interspersed histiocytes was present in the malignant T-cells and infiltrated the breast parenchyma and fat (Figs. 3 and 4). The lymphoma seemed to follow the lobular breast architecture and the associated fibrosis accentuated in the striking nodularity. The axillary lymph node showed effacement of the architecture by the same infiltrate seen in the breast tissue.

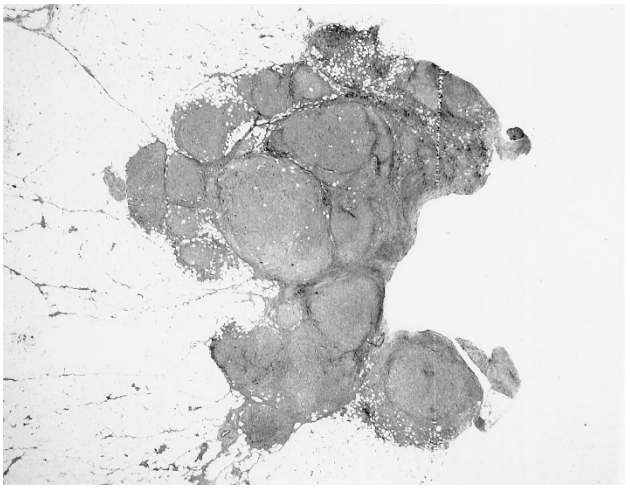

FIGURE 3. A low-power view of Case 2, which shows the nodular appearance of the lymphoma with extension into the fat.

Numerous epithelioid histiocytes were seen throughout the malignant cells of the lymph node. Because of the numerous epithelioid histiocytes, the case initially was diagnosed as a peripheral T-cell lymphoma, lymphoepithelioid cell ("Lennert's" lymphoma); we reclassified the case as PTCL, medium cell. Immunohistochemical studies showed that the malignant cells were immunoreactive with CD45RB, CD3, CD45RO, βF1, CD43, CD8, and TIA-1. Rare cells were immunoreactive with CD30. CD56 was weakly immunoreactive in 20% of the cells. The cells were negative with CD20, CD4, TCR δ-1, and cytokeratin. CD20 highlighted sparse B cells, which were found at the periphery of the nodules in the breast. No evaluation for LE components could be made in the breast mass because the unstained slides were limited. The TCR γ assay showed a monoclonal band. The IgH assay and TCR β assays showed no monoclonal band.

Case 3

A 44-year-old woman of unknown ethnic background presented with a left breast lump. A simultaneous lung lesion was detected. An excisional biopsy of the breast and a transbronchial biopsy of the lung were performed. The patient initially was believed to have a carcinoma of the breast with metastasis to the lung but on histologic examination was diagnosed as having a "peripheral T-cell lymphoma." The patient was given an autologous bone marrow transplant 2 months after the diagnosis but was subsequently found to have residual or recurrent lymphoma. Salvage treatment was given, but the patient died 18 months after the initial diagnosis of lymphoma. No autopsy was performed.

The breast lobules and soft tissue were infiltrated with a malignant population of medium-sized lym-

phocytes with abundant clear cytoplasm (Figs. 5 and 6). The lobular infiltrate gave the appearance of LE lesions (Fig. 7). The cells in the bronchial biopsy had a similar appearance. No necrosis or vascular invasion was present. The malignant cells were immunoreactive with CD45RB (weak), CD3, CD43, TIA-1, and CD56 (Fig. 8). The cells were negative with CD20, CD45RO, βF1, TCR δ, CD30, CD4, CD8, latent membrane protein for Epstein-Barr virus, and cytokeratin. The diagnosis was revised to NK/T-cell lymphoma. No monoclonal band was found in the IgH or the TCR β or γ assay. The *in situ* hybridization study for Epstein-Barr virus–encoded RNA was negative.

Case 4

A 77-year-old woman of unknown ethnic background presented with a right breast mass, which had been present for approximately 12 months. Several core biopsies of the mass were performed. At the time the mass was biopsied, it was noted that several 1-cm axillary lymph nodes were present but there was no history of lymphoma. The bone marrow was negative. Four months after diagnosis, widespread lymphadenopathy developed. The patient refused treatment, and no further follow-up could be obtained.

The core biopsies showed a vaguely nodular proliferation of small lymphocytes in adipose tissue. Elsewhere in the core, a small lymph node with a malignant population of T cells in a "T-zone" pattern was identified (Fig. 9). The malignant cells were intermediate in size with abundant clear cytoplasm (Fig. 10). Atretic germinal centers and sinuses also were present in these areas, confirming the impression of an intramammary lymph node. The lymphoid proliferation was CD45RB positive. The germinal centers were immunoreactive with CD20. The internodular areas were immunoreactive with CD3, CD45RO, CD4, and CD43, βF1 immunoreactive. CD30, CD8, CD56, and TIA-1 were negative. The cytokeratin did not show an LE component. The diagnosis was peripheral T-cell lymphoma, large cell type. The molecular genetic assay for IgH was negative. The assay for TCR β showed a monoclonal band with two DJ primers. TCR γ could not be performed because the DNA was nonamplifiable. Cases are summarized in Tables 2 and 3.

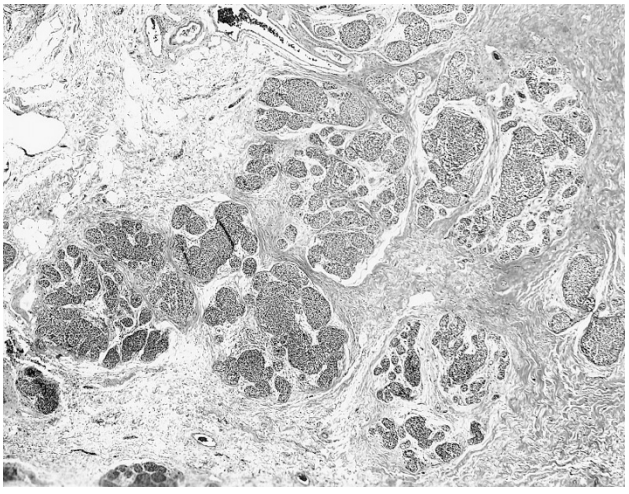

FIGURE 5. Case 3 of natural killer/T-cell lymphoma shows lobular pattern of infiltration of the lymphoma at low power.

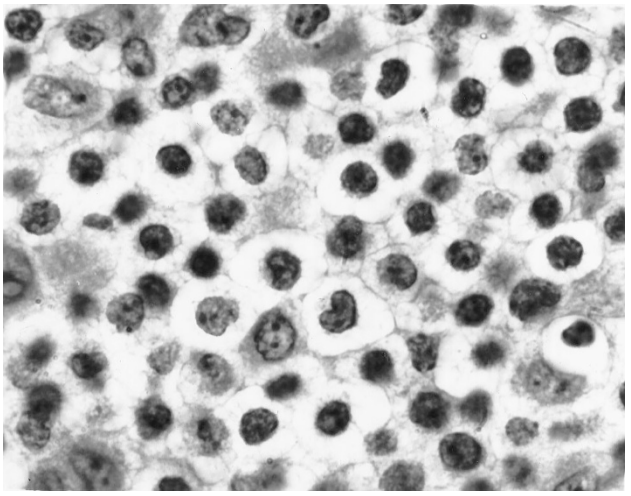

FIGURE 6. Case 3 at higher power showing lymphocytes with abundant clear cytoplasm and irregular nuclei.

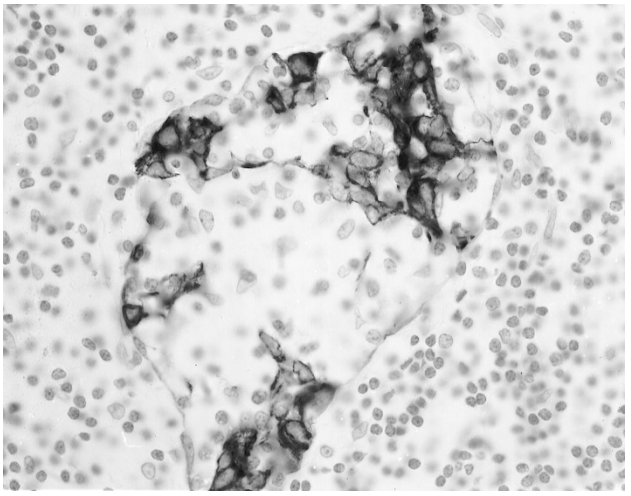

FIGURE 7. The pankeratin immunohistochemical maker highlights lymphoepithelial lesions in Case 3.

DISCUSSION

Peripheral T-cell lymphomas compose less than 15% of all non-Hodgkin's lymphomas (51) and are extremely rare in the breast, representing 0.04 to 0.5% of all breast malignancies (1–5). T-cell lymphomas, particularly nodal peripheral T-cell lymphomas, have an aggressive course and usually present with generalized disease, although some

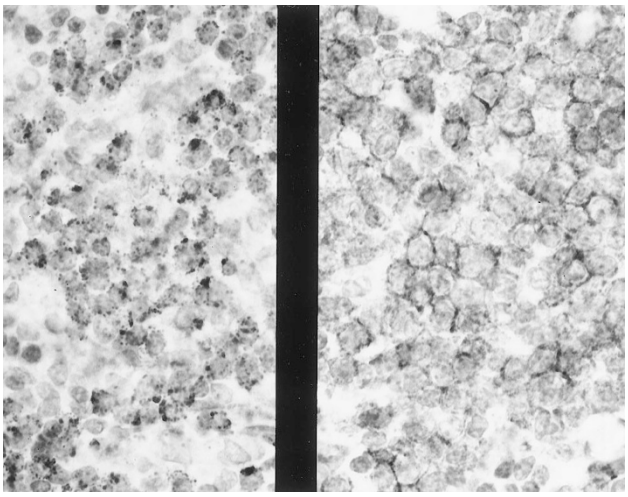

FIGURE 8. Case 3 showing immunoreactivity with CD56 (***right***) and T-cell intracellular antigen (***left***).

FIGURE 9. Low-power view of one of the core biopsies in Case 4 showing a lymph node surrounded by neoplastic cells.

subtypes may be less malevolent in behavior (51). Lymphomas such as ALCL and NK/T-cell type, which often are extranodal, have variable clinical behavior but in general have at least an intermediately aggressive clinical behavior. Little is known about nonpanniculitic lymphomas of $\alpha\beta$, CD8 phenotype, as described in Case 2. The T-cell lymphomas in the breast, which we present in this study, seem to represent the aggressive end of the spectrum in these neoplasms, regardless of subtype.

Anaplastic large cell lymphoma, as in Case 1, has been reported previously in two series (13, 14) and one case report (47). Whether, in our case, the skin involvement was primary or secondary to the underlying malignancy could not be determined with our specimen. No nodal disease was found. The neoplasm was aggressive and was immunoreactive with ALK-1 and epithelial membrane antigen, which indicates a systemic ALCL rather than a primary cutaneous ALCL (52).

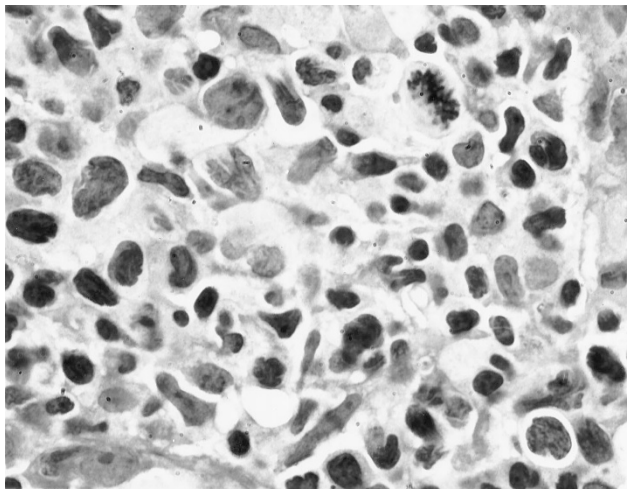

FIGURE 10. Case 4 showing atypical cells exhibiting mitoses and clear cytoplasm.

Previous cases of mammary T-cell lymphoma with CD8 immunoreactivity, as in Case 2, have been reported by Cohen and Brooks (2) and Vasei *et al.* (39). TIA-1 was not performed in the previous reports. Case 2 herein of CD8-positive and TIA-1 immunoreactive T-cell lymphoma is similar in immunophenotype to the so-called panniculitis-like T-cell lymphoma (53), but our case lacked several features of panniculitis-like T-cell lymphoma, namely necrosis and rimming of fat cells by the malignant T-cells. TIA-1 has been reported to be positive in nodal cases of PTCL of lymphoepithelioid type (54) (a diagnosis initially made in Case 2), but we are not aware of any documentation confirming the CD8 immunophenotype in cases of PTCL with a high content of epithelioid histiocytes. This particular type of T-cell may have a predilection for subcutaneous tissues, and the breast may be a natural haven for CD8-positive T-cells. However, this type of lymphoma is exceedingly rare and the present case is only the third such case reported. Further characterization of specific T-cell populations in the breast may be necessary to draw any conclusions.

To our knowledge, no cases of NK/T-cell lymphoma have been reported in the breast, making Case 3 unique. Case 3 showed extensive LE lesions, which has been reported in two other cases of mammary T-cell lymphoma, mycosis fungoides and an unspecified type (39, 43). Although we believed that this finding could be confused with marginal zone B-cell lymphoma, mucosa-associated lymphoid tissue type (MZBCL), in one previous study no LE lesions were seen in MZBCL (15) but germinal centers were present. Other studies have described less florid LE lesions in breast lymphoma including MZBCL (1, 2, 29). We did not see necrosis or vascular invasion characteristic of NK/T-cell lymphomas. The absence of germinal centers and

TABLE 2. Clinical Information

Case	Age/sex	Site	Diagnosis	Follow-Up
1	13/F	Lt breast	ALCL, CD30+, T-cell (ALK-1+, TIA-1+)	DOD 5 mo
2	56/F	Rt breast/axillary LN	PTCL, medium cell (βF1+, CD8+, TIA-1+)	DOD 3 mo
3	44/F	Lt breast/lung	NK/T (CD56+, TIA-1+, CD4−, CD8−)	DOD 18 mo
4	77/F	Rt breast	PTCL, large cell (CD4+, βF1+)	None

F, female; Lt, left; Rt, right; LN, lymph node; ALCL, anaplastic large cell lymphoma; PTCL, peripheral T-cell lymphoma; ALK, anaplastic lymphoma kinase; TIA, T-cell intracellular antigen; NK/T, natural killer cell/T-cell lymphoma; DOD, died of disease; +, immunoreactive; −, negative.

TABLE 3. Immunohistochemistry

Case	CD45RB	CD3	CD4	CD8	CD43	CD45RO	βF1	TCR δ-1	CD30	CD56	TIA-1	LMP-1	ALK-1	EMA
1	+	+ focal	Neg	Neg	+	+	+	ND	+	Neg	+	ND	+	+w
2	+	+	Neg	+	+	+	+	Neg	+ rare	+20%	+	ND	ND	ND
3	+	+	Neg	Neg	+	Neg	Neg	Neg	Neg	+90%	+	Neg	ND	ND
4	+	+	+	Neg	+	+	+	ND	Neg	Neg	Neg	ND	ND	ND

+, immunoreactive; Neg, negative; ND, not done; w, weak; TCR, T-cell receptor; TIA, T-cell intracellular antigen; LMP, latent membrane protein; ALK, anaplastic lymphoma kinase; EMA, epithelial membrane antigen.

the florid LE lesions in our case were helpful in distinguishing T-cell lymphoma from MZBCL.

Case 4 was a common type of PTCL with CD4 and βF1 immunophenotype. Although also rare in the breast, this type of lymphoma was reported in one series (40), but most series reporting breast lymphoma only rarely report T-cell immunophenotype and do not characterize the CD4 or CD8 reactivity of the lymphoma. The architecture of Case 4 suggests that it may have arisen in a mammary lymph node rather than in the breast tissue.

It is interesting that Cases 1, 2, and 3 were immunoreactive for TIA-1, a marker most often seen in extranodal T-cell lymphomas, commonly with a CD8 immunophenotype, of NK-cell origin or ALCL (52, 55). Therefore, it is not contradictory that in Case 4, a lymphoma most likely to be nodal, TIA-1 was absent.

The origin of T-cells, which give rise to lymphoma in the breast, remains unresolved. Examination of normal breast shows scattered histiocytes and T cells, which may be the origin of T-cell lymphoma (56). Mammary lymph nodes giving rise to T-cell lymphoma, and showing obliteration of the architecture or the overlying skin (particularly in regard to NK/T-cell and ALCL) also are plausible origins for this type of lymphoma. Although all of our cases presented in breast and involved the breast tissue, the site of inception is uncertain. Examination of normal T-cell populations and further study of these malignancies in the breast may help elucidate the origin and behavior of these exceedingly rare lymphomas.

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