# Peripheral T and NK Cell Non-Hodgkin Lymphoma a Challenge for Diagnosis

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**ABSTRACT**

*Background: Peripheral T and natural killer (NK) cell lymphomas represent a heterogeneous group of diseases with varied clinical features, prognosis and response to treatment.*

*Content and comments: Flow cytometric immunophenotyping facilitated the diagnosis and classification for T/NK-cell neoplasms, and is very useful in the identification of therapeutic target markers. Clinical, immunophenotypic, histopathological, immunohistochemical, molecular and genetic methods must be correlated because none of them is strong enough alone for diagnosis.*

*Conclusion: The low incidence of NK/T-cell lymphoma poses real difficulties for a complete and correct assessment. The unspecified lymphomas represent a heterogeneous group, which requires additional studies to elucidate their biological and genetic bases, to separate them. The group of NK/T-cell lymphomas remains a challenge for researchers.*

## BACKGROUND

Peripheral T and natural killer (NK) cell lymphoma represents a heterogeneous group of diseases with varied clinical features, prognosis and response to treatment. Underlying them is a mature, post-thymic T cell (1). Their incidence seems to have increased recently, also because of an improvement in diagnostic methods and of growing percentage of elderly people. Such conditions now account for approximately 20-30% in Asia (2-4) and 5-10% in Europe and North America (5, 6) of all lymphoid neoplasms. ❑

## CONTENT AND COMMENTS

### Classification

The current World Health Organisation (WHO) Classification, 4th edition (2008), includes 22 different types of T-cell and NK-cell lymphomas, seven more than in the previous classification, grouped into leukemic, extranodal, nodal and cutaneous type (7, 8). Among these, there are four provisional entities which required more data in order to be recognized as distinct entities. Predominantly nodal lymphoma subtypes include angioimmunoblastic T-cell lymphoma (AITL), anaplastic large-cell lymphoma (ALCL) anaplastic lymphoma kinase

(ALK) – positive, anaplastic large cell lymphoma (ALCL) ALK-negative (provisional entity) and peripheral T-cell lymphoma, not otherwise specified (PTCL-NOS). Predominantly extranodal lymphoma include extranodal NK/T-cell lymphoma nasal type, enteropathy-associated T-cell lymphoma (EATL), hepatosplenic T-cell lymphoma (HSTL), subcutaneous panniculitis-like T-cell lymphoma-alpha beta (SPTCL). Mature T-cell leukaemias include T-cell prolymphocytic leukaemia (T-PLL), T-cell large granular lymphocyte leukaemia (T-LGL), aggressive NK-cell leukaemia, chronic lymphoproliferative disorder of NK-cell (provisional entity), adult T cell leukemia/lymphoma-HTLV positive (ATLL), systemic EBV positive T-cell lymphoproliferative disease of childhood, Hydroa vacciniforme-like lymphoma. Cutaneous predominant subtypes include Mycosis fungoides (MF), Sezary syndrome (SS), primary cutaneous CD30-positive T-cell lymphoproliferative disorders (lymphomatoid papulosis and primary cutaneous anaplastic large cell lymphoma), primary cutaneous gamma-delta T cell lymphoma, primary cutaneous CD8 positive aggressive epidermotropic cytotoxic T-cell lymphoma (provisional entity), primary cutaneous CD4 positive small/medium T-cell lymphoma (provisional entity). The frequency of the different subtypes varies by geographical region. PTCL-NOS, ALCL and AILT are most commonly found in North America and Western Europe. In Asia ATLL and NK/T cell lymphoma, nasal-type, predominate. For ATLL HTLV-positive, Romania was cited as an endemic area, along with other recognized endemic areas (the Caribbean, Japan, Central Africa) (9, 10). The International T-Cell Lymphoma Project analysed data collected between 1990-2001 from 22 countries relating to 1314 cases of T-cell lymphoma. The most common subtypes were PTCL-NOS (29.5%), AITL (18.5%), ATLL (9.6%), ALCL - ALK positive (6.6%) and ALCL - ALK negative (5, 5%) (11).

## Immunophenotypic diagnosis

The diagnosis of peripheral NK and T cell lymphomas is very laborious. Clinical, immunophenotypic, histopathological, immunohistochemical, molecular and genetic findings must be correlated as none of them is strong enough to be used alone for diagnosis.

Immunophenotyping by flowcytometry facilitated diagnosis and classification for T/NK-cell neoplasms and is useful for the identification of therapeutic targets. There is no clear marker of T/NK-cell clonality, therefore the diagnosis is based on indirect data, such as lack of expression of T/NK cell associated antigens (12,13), altered intensity of staining for T/NK cell associated antigen (12,13) or abnormal expression of antigens that are not normally expressed in these cells (14-16). Altered expression of CD4/CD8 ratio does not establish the diagnosis of clonality, but draws attention to the possibility of the existence of abnormal T cell populations, for which investigations should be pursued (17). Analysis by flowcytometry of TCR V-β expression is a more sensitive and specific method for the detection of T cell clonality, but even in this case false positive or false negative results may sometimes occur (18).

Based on the expression of CD4 and CD8, immunophenotyping by flowcytometry may formulate a list of possible diagnoses.

The most common T cell lymphomas CD4 positive CD8 negative are:
- Sezary syndrome, cutaneous T cell lymphoma
- Adult T cell leukemia / lymphoma
- T-cell prolymphocytic leukaemia
- Anaplastic large cell lymphoma
- Angioimunoblastic T-cell lymphoma
- Peripheral T-cell lymphoma unspecified

Differential diagnosis between MF/SS and ATLL is based on the investigation of HTLV-I infection. Both forms may present with cutaneous involvement, similar morphology for lymphocytes on peripheral blood smear and CD4+ CD8- CD7- expression. It has been established that a more specific marker of clonality in MF/SS is the absence or reduction of CD26 expression on T cells, compared to the absence of CD7 expression (19). CD25 can be expressed variably and with different intensities in MF/SS, unlike in ATLL, where CD25 expression is intense and uniform.

Besides specific markers for T lineage (CD2+ CD5+ CD7+ CD3+low CD4+), AITL typically displays markers of T follicular helper cell (CD10, CXCL13, PD-1) (20). CXCL13 and PD-1 are of particular help in differential diagnosis with PTCL-NOS, as these two markers are expressed in a more constant pattern than CD10 in AITL. CXCL13 was also found to be positive on an exceptional basis in some forms of PTCL-NOS (21), with a Lennert lymphoma-

like histology (lymphoepitelioid), which raised the question whether this is a variant of angioimunoblastic lymphoma (21,22).

Diagnosis of ALCL is based on histopathology and immunohistochemistry, performed on the biopsy specimen, which highlights large pleomorphic lymphocytes, with characteristic intense and uniform expression for CD30 (Ki-1), with or without expression of the anaplastic lymphoma kinase (ALK+ and ALK-), along with one or more T-cell antigens. The role of flowcytometry occurs if there is peripheral blood involvement. Tariq Muzzafar et al analysed a group of 23 patients diagnosed with anaplastic lymphoma. Muzzafar's studies showed that most commonly ALK-positive ALCL expressed CD2 (67%), CD7 (60%), CD3 (45%), CD4 (33%), CD8 (14%) and ALK-negative ALCL expressed CD2 (100%), CD3 (50%) CD4 (40%), CD7 (40%), CD5 (25%) and CD8 (20%) (23). CD25 has also been identified in approximately 88% of cases in a study by Jonathan Juco et al of 19 patients diagnosed with anaplastic lymphoma, suggesting that it may be a useful marker in immunophenotypic diagnosis and a potential therapeutic target (24). Association with aberrant expression of myeloid markers (CD13, CD33) (23,16,24) which, in the absence of expression of markers of T-cell line (24), can lead to an incorrect interpretation as extramedullary myeloid tumour, has also been described. The expression of CD56, a marker of NK cells, can occur in some cases, conferring a poor prognosis.

Immunophenotypic examination has an important role in the diagnosis of T-PLL, along with clinical and morphologic features. The most frequent encountered phenotype in these entities is CD4+ CD8- CD3+ CD5+, and, unlike cutaneous T cell lymphoma and ATLL, CD7+ CD25-. Most cases are CD52+ (25) and do not express markers of NK cells and cytotoxic granule associated proteins (12).

PTCL-NOS comprise a heterogeneous group of entities with variable prognosis, and it therefore remains a diagnosis of exclusion for forms that cannot be classified in well-defined entities. The most frequently encountered immunophenotype is CD3+ CD4+ CD2+ CD5- CD7- CD8-. Based on the expression of cytokine receptor on Th1 and Th2 cells, immunophenotypic analysis has allowed the definition of phenotypes with different prognosis. Thus, Tsuchiya et al describe group 1 – positive for any of ST2 (L) (Th2marker, IL-1R family member), CCR5 (Th1), CXCR3 (Th1) and group 2 – negative for all these markers. Group 1 had a more favourable prognosis compared to group 2 (26).

Negative CD4 positive CD8 T cell lymphomas include:
- Large granular lymphocyte leukemia (T-LGL)
- Subcutaneous panniculitis-like T-cell lymphoma-alpha beta (SPTCL).
- Very rare - hepatosplenic T-cell lymphoma, cutaneous T lymphoma / Sezary syndrome, T-cell prolymphocytic leukaemia

The classic phenotype for T-LGL is most commonly a mature effector T cell CD3+ CD4- CD8+ CD16+ CD57+ TCRαβ+ (27-29). Rare cases of NK-LGL express CD2+ sCD3- cCD3+ TCRαβ- CD4- CD8- CD5+. CD57 is variably expressed (28, 30). The expression of cytotoxic granules associated protein is constant (TIA-1, granzime B, perforin).

CD4 CD8 coexpression is very rare in mature T-cell malignancies. Sometimes it is found in some forms of T-PLL, ATLL, PTCL-NOS.

CD4 negative CD 8 negative T cell lymphomas generally include extranodal lymphoma:
- Enteropathy associated T cell lymphoma
- Hepatosplenic T-cell lymphoma
- Nonhepatosplenice gamma delta T- cell lymphomas (cutaneous and mucosal)
- Extranodal NK/ T Cell Lymphoma, nasal type

Diagnosis of EATL is based on histological and immunohistochemical examination of small intestinal biopsies. Two different types of EATL are recognized: type 1, pleomorphic, associated with a history of celiac disease, which is more frequently meet than type 2, with CD3+ CD4- CD8- CD5- CD7+ CD103+ CD56- CD30+ TCRβ+/- phenotype, and type 2, monomorphic, not associated with history of celiac disease, with CD3+ CD4- CD8+ CD56+ CD30- TCRβ+ phenotype (31). Both express cytotoxic granule associated proteins TIA-1, perforin, granzime-B.

Extranodal NK/T-cell lymphoma, nasal type is an extremely aggressive upper airway lymphoma. It may affect other extranodal regions such as skin, soft tissue, gastrointestinal tract and testis. It is most commonly found in Asia. Lymphoma cells that typically invade the vascular wall are small, medium, large and pleo-

morphic cells and are always EBV positive, express CD56 and CD3, but are negative for CD4 and CD8. They express cytotoxic granule associated proteins TIA-1, granzyme B, perforin (32). There have also been cases with small cells, with no vascular invasion (32).

HSTL, the prototype of gamma delta T-cell lymphomas, represents another aggressive lymphoma. In this form, flowcytometric examination proves its usefulness by identifying the TCRγδ receptor, which cannot be easily revealed by immunohistochemical examination. Tumour cells express TCRγδ, CD2, CD3, CD7 and CD56, while CD4, CD5, CD8 are usually negative. There are forms that express TCRαβ, which are very difficult to distinguish from LGL (33). Most forms are CD57- CD25- CD30-. TIA-1 is the only cytotoxic granule associated proteins expressed (33).

In 2008 WHO classification, the subset of gamma delta subcutaneous panniculitis-like T cell lymphoma was reclassified as a provisional entity, under the name of primary cutaneous gamma-delta T cell lymphoma, which has a more aggressive clinical behaviour and a poorer prognosis than subcutaneous panniculitis-like alpha beta T-cell lymphomas. The phenotype of the two forms is different. In a study of patients diagnosed with the two forms of lymphoma, Willemze et al determined that the most frequent phenotypes for subcutaneous panniculitis-like T cell lymphoma were TCR αβ+ CD3+ CD8+ CD4- CD2- (10% of cases) CD5- (50% of cases) CD7- (44% of cases), CD56-, with expression of cytotoxic granule associated proteins (TIA-1, granzyme B, perforin), whereas for primary cutaneous gamma-delta T cell lymphomas, it was mainly TCRγδ+ CD3+ CD4- CD8- CD56+ (34). ❑

## CONCLUSION

The low incidence of NK/T-cell lymphoma poses real difficulties for a complete and correct assessment. Immunophenotyping by flowcytometry can identify an atypical population, specific for a known malignancy, and allows the classification and identification of therapeutic target markers. Some subtypes have a benign course, but most have an aggressive clinical behaviour and a dismal outcome. The unspecified group constitutes the most common subtype, and certainly, represent a heterogeneous group, which requires more data to separate them. For the moment, the immunophenotypic analysis of the expression of cytokine receptor on Th1 and Th2 cells, has allowed the definition of two phenotypes with different prognosis. The group of NK/T-cell lymphomas remains a challenge for researchers. ❑

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