Diagnostic and prognostic markers of cutaneous lymphomas

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CHAPTER

TOX EXPRESSION IN CUTANEOUS T-CELL LYMPHOMAS

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ABSTRACT

Background

Thymocyte selection-associated high-mobility group box (TOX) was shown to be aberrantly expressed in mycosis fungoides (MF) and Sézary syndrome (SS) and is suggested to have additional diagnostic value. However, data on expression in other types of cutaneous T-cell lymphoma (CTCL) are scarce and it is unknown whether TOX is expressed only by MF with a $CD4^+CD8^-$ phenotype.

Objectives

To investigate TOX expression in various types of CTCL with different T-cell phenotypes.

Materials and methods

Immunohistochemical expression of TOX was evaluated on 153 skin biopsies of 132 patients with CTCL and 60 patients with benign inflammatory dermatoses (BIDs).

Results

TOX was expressed by >50% of the neoplastic T cells in 49 of 59 (83%) patients with MF, and in 19 of 22 (86%) patients with SS. The TOX^+ cases of MF included 34 of 35 (97%) cases with a $CD4^+CD8^-$ phenotype, but also 5 of 8 (63%) cases with a $CD4^-CD8^+$ phenotype and 10 of 16 (63%) cases with a $CD4^-CD8^-$ phenotype. TOX expression in other types of CTCL was common but showed variable intensity. Although only 1 of 60 (2%) patients with BID expressed TOX in >50% of the skin-infiltrating T cells, some caution is warranted, as the majority of BIDs had TOX^+ T cells varying between 11% and 50%.

Conclusions

TOX expression is not tumour specific, is not restricted to CTCL with a $CD4^+CD8^-$ phenotype, and, on its own, is insufficient for diagnosis of CTCL. However, it may have an adjunctive diagnostic role in conjunction with other clinical and histological data.

INTRODUCTION

The major subtypes of cutaneous T-cell lymphoma (CTCL) are mycosis fungoides (MF) and Sézary syndrome (SS).[1] Both clinically and histologically, it can be difficult to distinguish early stages of MF and SS from benign inflammatory dermatoses (BIDs) such as atopic dermatitis and psoriasis, and the diagnostic process often leads to a delay that can extend up to many years.

Recently, gene expression profiling and additional immunohistochemistry have suggested that TOX (thymocyte selection-associated high-mobility group box) may be considered as a potential marker for the histological diagnosis of early-stage MF.[2] In 2002, TOX was firstly described by Wilkinson et al.[3] as part of the superfamily of high-mobility group box proteins that act as regulators of gene expression, mainly by modifying the chromatin structure.[3,4] In T-cell development, TOX is highly expressed in the thymus during β-selection and positive selection of $CD4^+CD8^+$ precursors to $CD4^+$ T cells, but it is downregulated in mature $CD4^+$ T cells once they leave the thymus.[3,5]

Following the first publication by Zhang et al.[2], several studies confirmed that TOX is aberrantly expressed in $CD4^+CD8^-$ neoplastic T cells in MF and SS, but not or only rarely by skin-infiltrating T cells in BIDs.[2,6-12] Data on expression in other types of CTCL are scarce, and it is unknown whether TOX can also be expressed by cases of MF with phenotypes other than $CD4^+CD8^-$. Therefore, in the present study, we performed immunohistochemistry for TOX on a large group of CTCLs to evaluate expression among various subtypes of CTCL with different T-cell phenotypes.

MATERIALS AND METHODS

Formalin-fixed and paraffin-embedded (FFPE) skin biopsies from 132 patients with different subtypes of CTCL were included in this study (Table 1). In all cases, diagnosis was made by an expert panel of dermatologists and pathologists at one of the regular meetings of the Dutch Cutaneous Lymphoma Group, according to the criteria of the World Health Organisation – European Organisation for Research

and Treatment of Cancer 2005 of cutaneous lymphomas.[1] In 21 patients, a second FFPE skin biopsy from a later date was available. As controls, FFPE skin biopsies from 60 patients with BID were selected (Table 1). All skin biopsies were collected from the archives of the Department of Pathology, Leiden University Medical Center (LUMC), Leiden, The Netherlands. The study was performed in accordance with the Declaration of Helsinki and the Dutch Code for Proper Secondary Use of Human Tissue, as approved by the medical ethics committee of the LUMC. Fifteen of the patients with SS and 17 patients with an erythrodermic BID were also included in a previous study by our group.[11]

Sections from all FFPE skin biopsies were routinely hematoxylin-eosin stained. Additionally, depending on the differential diagnosis, selections of the following immunostains were used: CD2, CD3, CD4, CD5, CD7, CD8, CD20, CD79a, PAX5, programmed-death (PD)-1, CD30, ALK, CD1a, CD56, CD68, Ki-67, T-cell receptor (TCR)-βF1, and TCR-γ. For the purpose of this study, sections of the biopsies were automatically stained using Dako Autostainer Link 48 (Dako, Grostrup, Denmark) with rabbit anti-human TOX antibodies of Sigma-Aldrich (HPA018322; St. Louis, MO, U.S.A.) at a dilution of 1:200. The 32 cases of SS and erythrodermic BID that had been included in a previous study by our group were re-stained automatically. Sections of reactive lymph nodes and tonsils were used as external controls. The sections were scored synchronously by all 3 authors until consensus was reached. The percentage of TOX^+ neoplastic T cells was estimated as <10%, 11-50%, or >50%. The intensity of the staining was scored as dim or strong.

Table 1. TOX expression in different types of cutaneous T-cell lymphoma and benign inflammatory dermatoses

	<10%, n (%)	11-50%, n (%)	>50%, n (%)
Mycosis fungoides (n=59)	3 (5)	7 (12)	49 (83)
early stage (n=41)	2 (5)	7 (17)	32 (78)
advanced stage (n=18)	1 (6)	0 (0)	17 (94)
Sézary syndrome (n=22)	0	3 (14)	19 (86)
CD30$^+$ LPDs (n=22)	5 (23)	2 (9)	15 (68)
C-ALCL (n=11)	4 (36)	1 (9)	6 (55)
lymphomatoid papulosis (n=11)	1 (9)	1 (9)	9 (82)
SPTCL (n=2)	1 (50)	1 (50)	0
PTCL, unspecified and rare subtypes (n=27)	2 (8)	1 (4)	23 (88)
PTCL-NOS (n=7)	0	1 (14)	6 (86)
CGDTCL (n=2)	0	0	2 (100)
AETCL (n=3)	2 (67)	0	1 (33)
PCSM-TCL (n=15)	0	0	15 (100)[a]
BPDCN (n=3)	3 (100)	0	0
Benign inflammatory dermatoses (n=60)	24 (40)	35 (58)	1 (2)
atopic dermatitis (n=12)	4 (33)	8 (67)	0
psoriasis (n=10)	5 (50)	5 (50)	0
drug-induced dermatitis (n=9)	5 (56)	3 (33)	1 (11)
idiopathic erythroderma (n=9)	3 (33)	6 (67)	0
lichen planus (n=7)	0	7 (100)	0
lupus erythemathosus (n=6)	4 (67)	2 (33)	0
other[b] (n=7)	3 (43)	4 (57)	0

Abbreviations: TOX, thymocyte selection-associated high-mobility group box; LPD, lymphoproliferative disorder; C-ALCL, primary cutaneous anaplastic large-cell lymphoma; SPTCL, subcutaneous panniculitis-like T-cell lymphoma; PTCL, peripheral T-cell lymphoma; PTCL-NOS, PTCL, not otherwise specified; CGDTCL, primary cutaneous γδ-positive T-cell lymphoma; AETCL, aggressive epidermotropic CD8$^+$ T-cell lymphoma; PCSM-TCL, primary cutaneous CD4$^+$ small/medium-sized pleomorphic T-cell lymphoma; BPDCN, blastic plasmacytoid dendritic-cell neoplasm.

[a]In CD4$^+$ medium/large-sized pleomorphic T cells.

[b]Patients with fungal infections (n=2), viral exanthema (n=1), graft-versus-host disease (n=1), panniculitis (n=1), inflammatory reaction to a basal cell carcinoma (n=1), and paraneoplastic erythroderma (n=1).

RESULTS

Mycosis fungoides

Seventy-three biopsies of 59 patients with MF were studied. In early-stage MF (stages IA-IB), staining of >50% of the neoplastic T cells was observed in 32 of 41 (78%) patients, including 23 of 24 (96%) patients with a $CD4^{+}CD8^{-}$ phenotype, 5 of 8 (63%) patients with a $CD4^{-}CD8^{+}$ phenotype (Figure 1), and 4 of 9 (44%) patients with a $CD4^{-}CD8^{-}$ phenotype. Of these cases, 29 of 32 (88%) expressed TOX in >75% of the neoplastic T cells. In advanced-stage MF (stages IIB-IV), 16 patients had tumour-stage, including 9 patients with a $CD4^{+}CD8^{-}$ phenotype and 7 patients with a $CD4^{-}CD8^{-}$ phenotype. Two patients had erythrodermic MF, both with a $CD4^{+}CD8^{-}$ phenotype. TOX was expressed by >75% of the neoplastic T cells in all patients with advanced-stage MF, except for 1 patient with a $CD4^{-}CD8^{-}$ phenotype. In 13 of 14 (93%) patients, second biopsy specimens were consistent in TOX expression. Overall, the intensity of staining of TOX^{+} neoplastic T cells was strong and comparable between all cases.

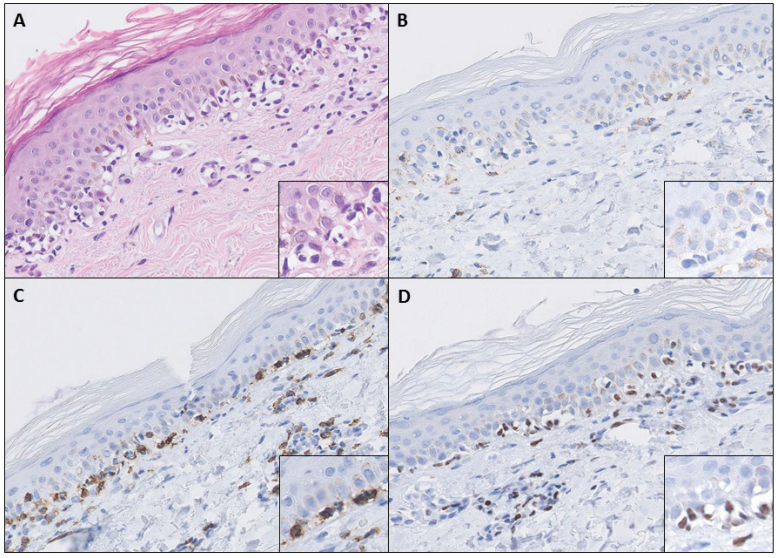

Figure 1. Histopathology of a patient with mycosis fungoides with a $CD4^{-}CD8^{+}$ phenotype. Hematoxilin-eosin staining (A) shows epidermotropism of $CD4^{-}$ (B) and $CD8^{+}$ (C) neoplastic T cells along the basal layer. TOX (D) is expressed in >75% of the neoplastic T cells. Magnification x 200; insets x 400.

Sézary syndrome

In 19 of 22 (86%) patients with SS, TOX was expressed in >50% of the Sézary cells, and in 17 of these 19 patients in >75%. Automatic re-staining of the cases that were manually stained in a previous study by our group[11] corresponded in 14 of 15 (93%) patients. TOX expression in SS was remarkably strong, especially in the Pautrier microabcesses and scattered epidermotropic T cells.

Primary cutaneous CD30$^+$ lymphoproliferative disorders

In cutaneous anaplastic large-cell lymphoma, TOX expression in >50% of the CD30$^+$ T cells was observed in 6 of 11 (55%) patients, including 5 of 7 (71%) with a CD4$^+$CD8$^-$ phenotype, 1 of 3 (33%) with a CD4$^-$CD8$^-$ phenotype, and none of 1 (0%) with a CD4$^+$CD8$^+$ phenotype. In lymphomatoid papulosis, staining in >50% of the CD30$^+$ T cells was noted in 9 of 11 (82%) patients, of whom 3 of 4 (60%) had a CD4$^+$CD8$^-$ phenotype, 1 of 1 (100%) had a CD4$^-$CD8$^+$ phenotype, 4 of 4 (100%) had a CD4$^-$CD8$^-$ phenotype, and 1 of 1 (100%) had a CD4$^+$CD8$^+$ phenotype. TOX expression was consistent in 4 of 5 (80%) patients with 2 biopsy specimens. The intensity of TOX expression in CD30$^+$ lymphoproliferative disorders was variable between dim and strong within and between the different cases.

Subcutaneous panniculitis-like T-cell lymphoma

Both patients with subcutaneous panniculitis-like T-cell lymphoma had TOX expression in <25% of the (mostly rimming) neoplastic CD4$^-$CD8$^+$ T cells.

Peripheral T-cell lymphoma, unspecified and rare subtypes

In cutaneous peripheral T-cell lymphoma, not otherwise specified (PTCL-NOS), TOX was expressed by >50% of the neoplastic T cells in 6 of 7 (86%) patients, including 5 of 6 with a CD4$^+$CD8$^-$ phenotype and 1 of 1 with a CD4$^-$CD8$^+$ phenotype. The intensity of TOX expression in PTCL-NOS was variable. Both patients with cutaneous γδ-positive T-cell lymphoma and 1 of 3 (33%) patients with aggressive epidermotropic CD8$^+$ T-cell lymphoma (AETCL) showed strong TOX expression in >75% of the neoplastic CD4$^+$CD8$^-$ T cells and CD4$^-$CD8$^+$ T cells, respectively. The 2 other cases of AETCL were completely negative with positive internal control. In all 16 biopsies of 15 patients with primary cutaneous CD4$^+$ small/medium-sized T-cell

lymphoma (PCSM-TCL), scattered medium/large-sized pleomorphic T cells stained strongly positive with TOX (Figure 2). Serial sections demonstrated that these were the same T cells expressing PD-1, as described in a previous study.[13]

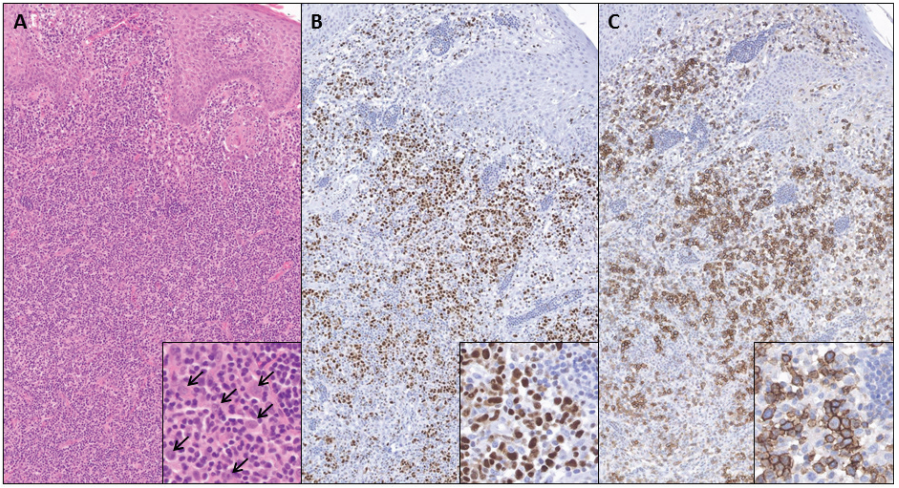

Figure 2. Histopathology of a patient with primary cutaneous $CD4^+$ small/medium-sized pleomorphic T-cell lymphoma. Hematoxilin-eosin staining (A) shows infiltration of the dermis by a mixed infiltrate in which scattered medium/large-sized pleomorphic cells (inset, arrows) stain strongly positive for TOX (B) and programmed death-1 (C). Magnification x 100; insets x 400.

Blastic plasmacytoid dendritic-cell neoplasm

In all 3 cases of blastic plasmacytoid dendritic-cell neoplasm, TOX was negative or weakly expressed by <10% of the neoplastic cells.

Benign inflammatory dermatoses

In BIDs, TOX was expressed by <50% of the inflammatory T cells in 59 of 60 (98%) cases, of which 24 of 59 (41%) were scored as <10%. Only 1 patient (2%), with a drug-induced dermatitis, showed staining in >50% of the inflammatory lymphocytes. In general, the intensity of TOX expression was dim. However, strong staining was observed in a few scattered blasts and epidermotropic lymphocytes, in particular in cases of atopic dermatitis. Additional immunophenotyping with

T-cell antigens showed that the TOX^+ epidermotropic T cells had mainly a $CD4^-CD8^+$ phenotype (Figure 3).

Unexpectedly, the reactive lymph nodes and tonsils that were included in every section as an external control showed TOX expression not only by scattered T cells in the interfollicular areas, but also in the reactive follicles. Additional studies showed that TOX has a similar distribution pattern as BCL6, a marker of germinal center cells, and is also expressed by the neoplastic B cells of primary cutaneous follicle center lymphoma and other B-cell lymphomas expressing BCL6.[14]

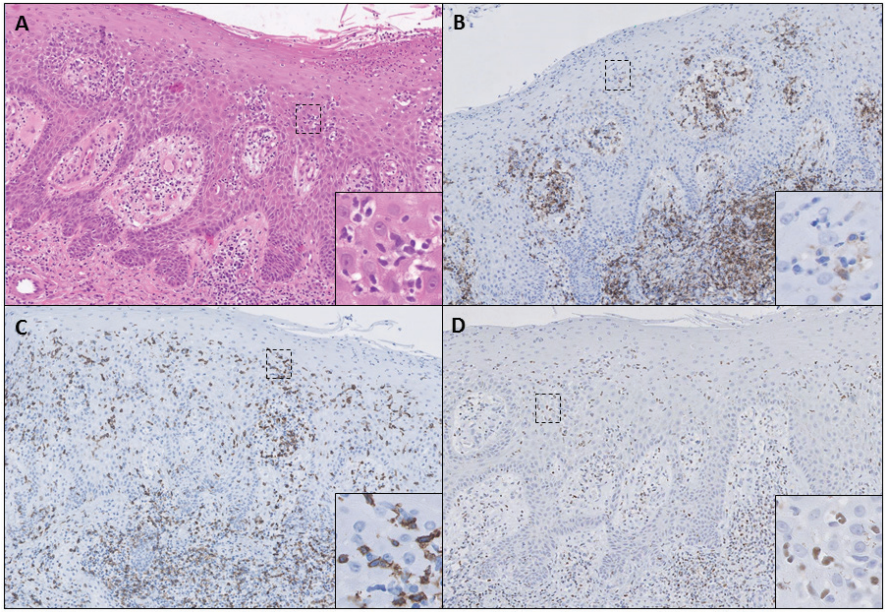

Figure 3. Histopathology of a patient with atopic dermatitis. Hematoxilin-eosin staining (A) shows superficial perivascular infiltrates and exocytosis of lymphocytes. These intraepidermal lymphocytes are predominantly $CD4^-$ (B) and $CD8^+$ (C), and the majority stains positive for TOX (D). Magnification x 100; insets x 400.

DISCUSSION

Consistently with previous studies, strong expression of TOX by >50% of the neoplastic T cells was found in most cases of MF (49/59; 83%) and SS (19/22; 86%), while expression of TOX by >50% of the skin-infiltrating T cells was found in only 1 of 60 (2%) patients with BIDs.[2,6-11] However, while other studies reported that TOX is expressed only by $CD4^+$ neoplastic T cells in MF and SS[2,6-11,15], in the present study, TOX was expressed not only in 35 of 36 (97%) cases with $CD4^+CD8^-$ MF, but also in 5 of 8 (63%) patients with $CD4^-CD8^+$ MF and 10 of 16 (63%) patients with $CD4^-CD8^-$ MF. In addition, TOX was expressed in other CTCLs with a $CD4^-CD8^+$ phenotype, including 1 patient with lymphomatoid papulosis, 1 patient with PTCL-NOS, and 1 patient with AETCL.

In BIDs, most previous studies reported no or few TOX^+ T cells and suggested that TOX expression may be a useful marker for early diagnosis of MF and SS. However, the present study indicates that some caution is warranted. Although TOX expression by >50% of the skin-infiltrating T cells was observed in only 1 of 60 (2%) cases of BID, 35 of 60 (58%) cases showed considerable numbers of TOX^+ T cells varying between 11% and 50%. TOX staining in these T cells was generally dim, but a few scattered blast cells and epidermotropic T cells, particularly $CD4^-CD8^+$ intraepidermal T cells in atopic dermatitis, showed strong expression of TOX. These observations suggest that the presence of TOX^+ T cells on itself is insufficient for a diagnosis of MF or SS and should always be considered in conjunction with other clinical and histological data.

TOX expression in reactive and neoplastic T cells and its biological significance are as yet unexplained. In CTCL, it has been suggested that it is a result of dedifferentiation.[16] However, the expression of TOX in a significant proportion of reactive T cells in BIDs argues against this suggestion. In PCSM-TCL, the overall expression of TOX was very similar to the expression of PD-1. PD-1 is a marker not only of follicular helper T cells, but also of activated T cells, and, just like TOX, it is involved in the development of thymocytes during both β-selection and positive selection.[3,17] The similar expression patterns of TOX and PD-1 and strong

expression of TOX in a few scattered blasts in reactive infiltrates suggest that TOX might also be a marker of activated T cells, although a previous study was unable to show this *in vitro*.[12] In both MF and SS, high levels of TOX have been associated with a worse prognosis.[7,12] Studies in CTCL cell lines also suggest a role for TOX in the pathogenesis of CTCL. Overexpression of TOX in an MF cell line increased proliferation and migration, while knockdown of TOX in CTCL cell lines induced apoptosis and decreased proliferation and tumour growth.[9,12]

In conclusion, TOX is expressed by various types of CTCL and is, therefore, not tumour-specific. Moreover, it is expressed not only by CTCL with a $CD4^+CD8^-$ phenotype, but also by CTCL with $CD4^-CD8^+$ and $CD4^-CD8^-$ phenotypes. Finally, as considerable amounts of reactive T cells in BIDs expressed TOX, although less strong and in lower frequencies than in CTCL, TOX expression in itself is insufficient for diagnosis of CTCL, but it may have an adjunctive diagnostic role in conjunction with other clinical and histological data.

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