Diagnostic and prognostic markers in tumor stage mycosis fungoides and Sézary syndrome

Boonk, S.E.

4

Differential expression of TOX by skin-infiltrating T cells in Sézary syndrome and erythrodermic dermatitis

SE Boonk[1*], F Çetinözman[1*], MH Vermeer[1], PM Jansen[2] and R. Willemze[1]

Departments of Dermatology[1] and Pathology[2], Leiden University Medical Center, Leiden, the Netherlands

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[*]These authors contributed equally to this study.

ABSTRACT

Background The histopathologic differentiation between Sézary syndrome (SS) and erythrodermic dermatitis may be extremely difficult. In this immunohistochemical study, it was investigated if thymocyte selection-associated high mobility group box protein (TOX) and C-MYC can be used as additional diagnostic markers to differentiate between SS and erythrodermic dermatitis.

Method Paraffin-embedded skin biopsies from 15 SS patients and 17 erythrodermic dermatitis patients were stained and scored for TOX or C-MYC expression.

Results Strong nuclear staining for TOX in more than 50% of skin-infiltrating T cells was observed in 13 of 15 (87%) SS cases, whereas erythrodermic dermatitis cases showed weak nuclear staining in 11–50% (median = 25%) of the T cells; strong nuclear staining as found in SS was never observed in erythrodermic dermatitis. No significant differences in C-MYC expression between SS and erythrodermic dermatitis were found. In most patients of both groups, percentages of C-MYC positive cells varied between less than 10 and 25% of skin-infiltrating T cells.

Conclusion Our results suggest that strong expression of TOX in more than 50% of skin-infiltrating T cells in erythrodermic skin is a useful marker in the differentiation between SS and erythrodermic dermatitis, whereas staining for C-MYC does not contribute to differential diagnosis.

INTRODUCTION

Sézary syndrome (SS) is a rare and aggressive type of cutaneous T-cell lymphoma (CTCL), characterized by a pruritic erythroderma and the presence of clonal neoplastic T cells (Sézary cells) in skin and peripheral blood.[1]

The microscopic differentiation between SS and erythrodermic dermatitis may be extremely difficult.[2;3] The histopathologic findings of SS are similar to that of mycosis fungoides (MF), but more commonly the entity shows a monotonous band-like or perivascular infiltrate in the papillary dermis that is mainly composed of lymphocytes with atypical or cerebriform nuclei. Epidermotropism is present and Pautrier microabscesses may be found. However, in up to one third of SS cases, microscopic sections may show non-specific features of chronic dermatitis.[4;5]

In a recent study, blind evaluation of hematoxylin and eosin-stained sections from skin biopsies of 18 patients with a CTCL, including 14 SS patients and 29 patients with erythrodermic dermatitis, correct differentiation between CTCL and erythrodermic dermatitis was made in approximately 50% of the cases.[3] These observations indicate that there is an urgent need for diagnostic biomarkers for SS.

In a recent study of our group both programmed death-1 (PD-1; CD279) and CD7 proved valuable immunophenotypic markers in the differentiation between SS and erythrodermic dermatitis.[6] Expression of PD-1 by more than 50% of the skin-infiltrating T cells was found in 23 of 25 (92%) SS cases and only in 4 of 30 (13%) erythrodermic dermatitis cases. In SS, PD-1 was expressed by the neoplastic CD4+ T cells, whereas in erythrodermic dermatitis PD-1 was predominantly expressed by dermal and epidermal CD8+ T cells. Loss of CD7 by more than 50% of the skin-infiltrating T cells was found in 16 of 24 (66%) SS cases but also in 4 of 30 (13%) erythrodermic dermatitis cases. However, expression of CD7 by 20% or less of infiltrating T cells was found only in SS (13 of 24 cases).

In this study, we used the same cohort of erythrodermic patients to investigate the differential diagnostic value of two other potentially useful biomarkers, namely thymocyte selection-associated high mobility group box protein (TOX) and C-MYC.

TOX belongs to a large family of chromatin-associated proteins. In T-cell development, TOX is highly expressed in the thymus during the transition of CD4+CD8+ precursors to CD4+ T cells but is normally not expressed by mature CD4+ T cells once they leave the thymus.[7;8]

Recent studies have reported increased TOX expression by malignant CD4+ T cells in MF and SS skin biopsies, but not in benign inflammatory dermatoses.[9-12] In addition, Huang et al showed that TOX mRNA expression is significantly enhanced in primary CD4+CD7– cells from peripheral blood of SS patients, as compared to those cells from patients with benign inflammatory dermatosis.[13] The increase of TOX mRNA expression was correlated with increased risk of disease progression and disease-specific mortality. It was suggested that TOX might contribute to the development of CTCL.[13] However, its biological effects on CTCL pathogenesis have not been explored. Studies focusing on TOX expression in skin of patients with extensive erythrodermic dermatitis have not been performed.

The rationale to consider C-MYC as a potential diagnostic marker results from previous studies showing a gain of 8q24 harboring the *MYC* gene in 41–75% of SS patients.[14;15] Immunohistochemical studies for C-MYC expression in MF or SS are limited. Kanavaros et al reported expression of C-MYC by 5–25% of the skin-infiltrating lymphoid cells in cases of early stage MF and SS, whereas higher percentages (25–50%) were found in one-third of patients with advanced MF.[16] In a recent study, 12 of 13 (92%) patients with erythrodermic MF/SS showed positive staining for C-MYC in at most 15% of the dermal lymphocytic infiltrate cells.[17]

In this study, we therefore investigated if TOX and C-MYC can be used as additional markers to differentiate between SS and erythrodermic dermatitis.

METHODS

PATIENTS

Paraffin-embedded skin biopsies from 15 patients with SS and 17 patients with erythrodermic dermatitis were selected for this study. The diagnosis of SS was based on the recent criteria of the World Health Organization – European Organization of Research and Treatment of Cancer classification.[1] The diagnosis of erythrodermic dermatitis in each patient was based on clinical and histopathological criteria, supplemented by immunophenotyping and clonality analysis of peripheral blood to exclude peripheral blood involvement by CTCL. The erythrodermic dermatitis group included four patients with atopic erythroderma, three patients with erythrodermic psoriasis, nine patients with idiopathic erythroderma, and one patient with paraneoplastic erythroderma. Review of clinical records revealed that none of the patients developed a lymphoma after a median follow-up of 46 months (range = 7–332 months). The study complied with the Declaration of Helsinki and was performed in accordance with the Dutch Code and Leiden University Medical Center guidelines on leftover material.

HISTOPATHOLOGY AND IMMUNOHISTOCHEMISTRY

Sections from all skin biopsies had routinely been stained with hematoxylin-eosin and with monoclonal antibodies against T cell-associated antigens (CD2, CD3, CD4, CD5, CD7, CD8), B cell-associated antigens (CD20 and/or CD79a), and CD68 and CD1a to differentiate between CD4+ T cells and CD4+ histiocytes and Langerhans cells/dendritic cells, respectively. For the purpose of this study, sections from all patients were stained for TOX and C-MYC.

Immunohistochemical staining was performed on 4-µm sections using standard procedures. After antigen retrieval by heating for 10–12 minutes in 1.0 mmol/L ethylenediaminetetraacetic acid (pH 8.0) or 10 mmol/L citrate buffer (pH 6.0), tissue sections were incubated overnight with antibodies against TOX (Sigma-Aldrich, Zwijndrecht, The Netherlands) and C-MYC (Abcam, Cambridge, UK). Sections were then incubated for 30 min with BrightVision Poly-horseradish peroxidase, and subsequently incubated with diaminobenzidin (DAB) solution (Sigma-Aldrich) for 10 min. Finally, all slides were counterstained with Mayer hematoxylin.

The percentages of T cells expressing TOX or C-MYC were scored, as less than 10, 11–25, 26–50, 51–75 and more than 75%. These percentages had been estimated independently by three observers (FC, PMJ and RW). In the few cases in which there was disagreement, sections were viewed jointly by all authors and consensus was reached.

RESULTS

SÉZARY SYNDROME

The results of the immunohistochemical stainings are summarized in **Table 1**. This table also contains the results of PD-1 staining and the presence or absence of antigen loss from our previous study.[6]

Skin biopsies from patients with SS characteristically showed perivascular to band-like infiltrates in the papillary dermis. More diffuse infiltrates extending into the reticular dermis were observed in four cases. Epidermotropic neoplastic CD4+ T cells with (n = 5) or without (n = 5) Pautrier microabscesses were found in 10 cases. The dermal infiltrates were predominantly composed of small to large atypical CD4+ T cells with hyperconvoluted nuclei (Sézary cells) and variable numbers of blast cells. In 2 of 15 cases (nos. 6 and 15) the cellular atypia was minimal, and a diagnosis of CTCL was at most suspected. Percentages of admixed CD8+ T cells varied between less than 5% and almost 50%, but a percentage more than 30% was observed in only two cases (nos. 6 and 15).

Strong nuclear staining for TOX was observed in more than 50% of the skin-infiltrating T cells in 13 of 15 (87%) with percentages more than 75% in 10 of them (**Table 1**). Intraepidermal neoplastic T cells consistently showed strong nuclear staining for TOX (**Figure 1**). In two cases (nos. 6 and 15), weak nuclear staining in 25–30% of the skin-infiltrating T cells was found (**Figure 2**). C-MYC was expressed by less than 10% of the skin-infiltrating T cells in 5 of 15 cases, by 11–25% in eight cases, and in 26–50% in two cases. Strong nuclear C-MYC staining was particularly observed in large Sézary cells and blast cells (**Figure 1**). In addition, epidermal basal cells showed strong nuclear C-MYC staining, serving as a useful internal control.

ERYTHRODERMIC DERMATITIS

Biopsy specimens of erythrodermic dermatitis generally showed a sparse to moderately dense perivascular to band-like infiltrate in the superficial dermis, and the infiltrate was generally much less pronounced than observed in SS. Intraepidermal T cells were few or absent, and in some cases could only be recognized in immunostained sections. The superficial dermal infiltrate was mainly composed of small lymphocytes admixed with variable numbers of histiocytes, and in cases of atopic and idiopathic erythroderma with eosinophils. In three cases of atopic erythroderma (nos. 16, 18 and 19), the dermal infiltrate showed a considerable number of slightly atypical small to medium-sized pleomorphic T cells and scattered blast cells, and a diagnosis of suspected CTCL had initially been made. Percentages of CD8+ T cells in the dermal infiltrate varied between 15 and 75% (median = 30%) of the dermal CD3+ T cells.

Weak nuclear staining for TOX was observed in 11–25% of the infiltrating T cells in

Table 1. Summary of the immunohistochemical stainings.

	Diagnosis	TOX (%)	C-MYC (%)	PD-1 (%)	Antigen loss
1	SS	++++	+	++++	–
2	SS	++++	+	++++	CD7, CD2
3	SS	++++	+	++++	CD7
4	SS	+++	–	++++	CD7
5	SS	++++	+	++++	CD7
6	SS[1]	++ (w)	–	+++	–
7	SS	++++	++	++++	CD7
8	SS	+++	–	+++	–
9	SS	++++	++	++++	CD7
10	SS	++++	+	++++	CD7
11	SS	++++	+	+	–
12	SS	++++	+	+++	CD7, CD2
13	SS	+++	–	++++	CD7
14	SS	++++	+	++++	CD7
15	SS[1]	++ (w)	–	+++	CD7
16	AE[2]	+ (w)	–	+	–
17	AE	+ (w)	–	++	–
18	AE[2]	+ (w)	–	+++[#]	–
19	AE[2]	++ (w)	–	+++[#]	CD7
20	PSOR	+ (w)	+	++	–
21	PSOR	+ (w)	–	+	–
22	PSOR	+ (w)	–	–	–
23	IE	+ (w)	+	–	–
24	IE	++ (w)	–	–	–
25	IE	++ (w)	+	–	–
26	IE	+ (w)	+	–	–
27	IE	++ (w)	–	–	–
28	IE	+ (w)	+	+	–
29	IE	+ (w)	+	+	CD7
30	IE	+ (w)	+	+	–
31	IE	+ (w)	–	+	–
32	PARA	+ (w)	+	+	–

AE, atopic erythroderma; IE: idiopathic erythroderma; PARA: paraneoplastic erythroderma; PD-1, programmed death-1; PSOR: psoriatic erythroderma; SS: Sézary syndrome; TOX, C-MYC and PD-1: –, <10%; +, 11–25%; ++, 26–50%; +++, 51–75%; ++++, >75%. Antigen loss: loss of CD2, CD3, CD4, CD5 or CD7 expression by more than 50% of the (neoplastic) T cells; –: no loss

[1] SS cases showing no or minimal cellular atypia.

[2] Cases showing considerable numbers of small to medium-sized atypical T cells.

[#] CD8+ T cells; (w): weak staining.

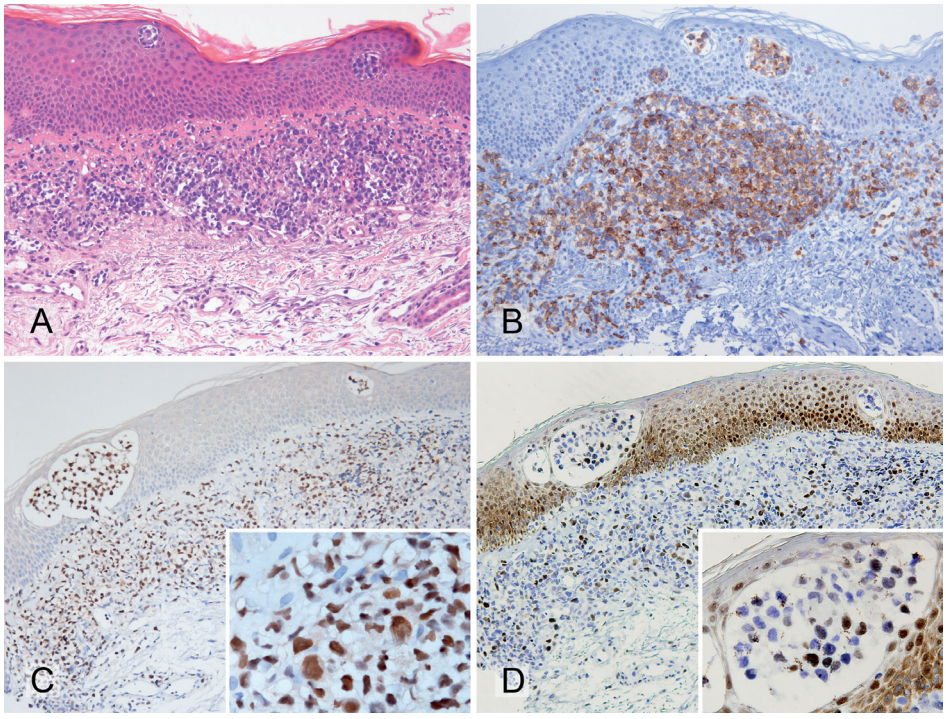

Figure 1. Histopathologic features of a representative Sézary syndrome patient with strong TOX expression.
A) Hematoxylin-eosin staining showed an infiltrate in the superficial dermis with epidermal Pautrier microabscesses. B) Expression of CD3. C) More than 50% of the skin-infiltrating T cells show strong nuclear staining for TOX, particularly in large Sézary cells and blast cells. D) Less than 25% of the skin-infiltrating T cells expressed C-MYC. The insets in (C) and (D) show higher magnifications of corresponding areas. (A – D: ×100, inset in C: ×630, inset in D: ×400).

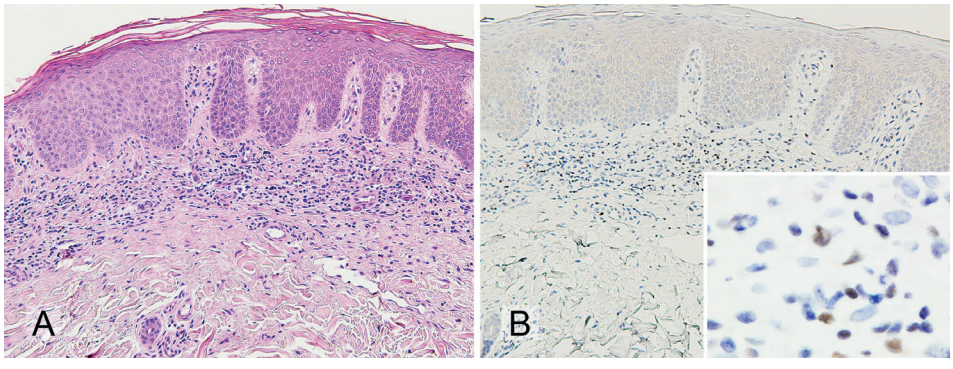

Figure 2. Histopathologic features of a Sézary syndrome patient with weak TOX expression.
A) Hematoxylin-eosin staining showed a perivascular to band-like infiltrate in the papillary dermis. B) Weak expression of TOX by 25–30% of the skin-infiltrating T cells. (A and B: ×100 and inset in B: ×630).

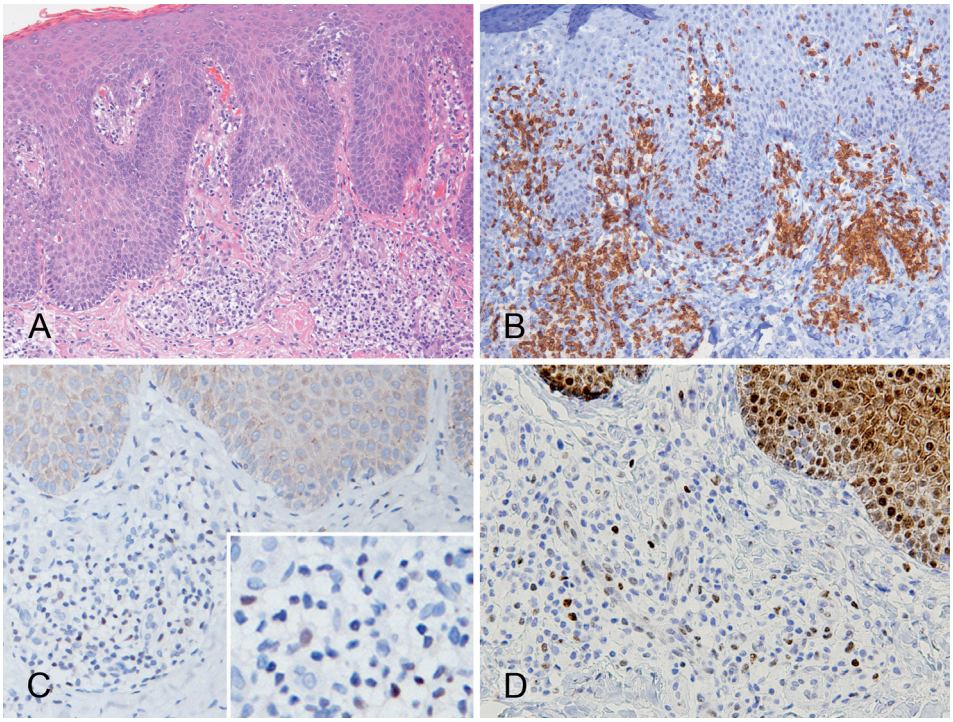

Figure 3. Histopathologic features of a representative patient with erythrodermic psoriasis.
A) The hematoxylin-eosin staining of the lesion showed acanthosis and a dermal lymphocytic infiltrate. B) Expression of CD3. C) Weak nuclear staining for TOX in a small proportion of the infiltrating T cells. D) Nuclear staining for C-MYC in scattered infiltrating T cells. (A and B: ×100, C and D: ×200, inset in C: ×630).

13 of 17 cases, and in 26–50% in four cases (**Figure 3**). Strong nuclear staining for TOX as found in SS was never observed in erythrodermic dermatitis.

Nuclear staining for C-MYC was detected in 11–25% of both small and scattered larger T cells in 8 of 17 cases and by less than 10% in nine cases. In three cases, they were completely lacking (nos. 17, 18 and 19), whereas the epidermal basal cells still showed strong nuclear staining.

DISCUSSION

In this study, strong nuclear staining for TOX by more than 50% of the skin-infiltrating T cells was found in 13 of 15 (87%) Sézary cases, whereas all erythrodermic dermatitis cases showed weak nuclear staining of TOX varying between 11 and 50% of the T cells. The two remaining SS cases (nos. 6 and 15) showed weak nuclear TOX expression at a similar level as seen in erythrodermic dermatitis patients. Interestingly, both cases showed minimal atypia and had high numbers of admixed CD8+ T cells. It should however be noted that in our previous study both cases had shown expression of PD-1 by more than 50% of the skin-infiltrating T cells. Alternatively, in one SS patient with expression

of PD-1 in only 10% of neoplastic T cells (no. 11), more than 75% of the neoplastic T cells showed strong nuclear TOX staining, suggesting that combining both markers may be diagnostically helpful.

In a previous study, we observed that – in contrast to SS – PD-1 is uncommonly expressed by the neoplastic T cells in skin biopsies from erythrodermic MF. Expression of PD-1 by more than 50% of the neoplastic T cells was found in only one of eight cases.[18] Examination of skin biopsies from seven of these cases showed strong nuclear staining for TOX by more than 50% of the malignant T cells in six of them (Willemze et al; unpublished observations 2015). These results are similar to those of this study in SS and indicate that TOX is a useful biomarker to differentiate erythrodermic dermatitis from both SS and erythrodermic MF.

Our results are consistent with recent studies showing strong nuclear staining for TOX by atypical CD4+ T cells, both in dermis and epidermis, in SS and MF skin biopsies compared to benign inflammatory dermatoses and normal skin.[9;10;12] In addition, previous studies have shown increased mRNA expression levels for TOX in both SS and MF as well.[9-13]

The high expression of TOX in CTCL is as yet unexplained. In normal T-cell development mature CD4+ T cells do not express TOX after leaving the thymus. Whether the TOX expression found in SS and other types of CTCL may be due to an impaired regulated maturation status or to re-expression during the formation into memory T cell has yet to be determined.[7]

No significant differences in C-MYC expression between SS and erythrodermic dermatitis were found. In both groups about half of the cases showed C-MYC positivity in 11–25% of the skin-infiltrating T cells. Higher percentages (25–50%) were found in only two cases of SS. Similarly, Kavanos et al reported percentages of 11–25% in three of three (100%) SS cases.[16]

Previous studies reported a gain of 8q24 harboring the *MYC* gene in 41–75% of SS patients.[14;15] In a recent European multicenter study on 59 Sézary patients gain for *MYC* was found in 23 of 58 (40%) SS cases as well (Boonk et al. 2015, submitted manuscript). However, using the quantitative PCR technique, no differences in *MYC* gene expression levels were found between Sézary cells and CD4+ T cells from erythrodermic dermatitis patients. Three of the 15 SS cases (nos. 2, 10 and 11) from this study had been included in the previous study and showed a gain for *MYC*. However, all of them showed C-MYC staining in only 11–25% of the skin-infiltrating T cells, similar to the other SS cases. Why the gain for *MYC* does not lead to increased gene and protein expression is as yet unexplained.

In conclusion, strong expression of TOX, but not C-MYC, can be another useful adjunct in the differentiation between SS and erythrodermic dermatitis.

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