FOXP3 Predicts Response to Treatment in Mycosis Fungoides

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Received: January 2, 2021
Accepted: June 15, 2021

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ABSTRACT

Background: The role of the T-regulatory cells (Tregs) marker forkhead box Protein 3 (FOXP3) in mycoses fungoides (MF) pathogenesis is unclear and the results of previous studies are inconclusive.

Objective: We aimed at ascertaining the possibility that FOXP3 expression may serve to predict MF stage and response to therapy.

Patients and methods: Immunohistochemistry staining for FOXP3 was performed on 30 skin biopsies from patients with MF, and FOXP3 expression level was quantitatively graded. Disease stage, progression, and response to treatment were determined based on clinical and imaging evidence, and association with FOXP3 expression was assessed.

Results: FOXP3 expression in the dermis correlated with poor response to treatment (P=0.047). A negative non-significant relationship between epidermal FOXP3 expression and clinical stage severity was observed (P=0.17).

Conclusions: Dermal FOXP3 expression in MF lesions could be used to predict response to treatment in patients with MF.

KEY WORDS: MF, mycosis fungoides, CTCL, cutaneous T-cell lymphomas, Tregs, T regulatory cells, FOXP3, forkhead box P3, prognostic factor

INTRODUCTION

Mycosis fungoides (MF) is a primary cutaneous lymphoma that is characterized by uncontrolled growth of T-cells and is the most common skin lymphoma. MF has an overall favorable prognosis; however, a small subset of patients can present with a more severe disease progression leading to advanced stages and even death.

Prognostic tools capable of predicting MF progression include age, sex, functional status, type of lesions (patch, plaque, tumor), body surface area involved, lymph node involvement, extra-cutaneous disease, transformation to large cell disease and laboratory findings such as elevated C-reactive protein (CRP), lactate dehydrogenase (LDH), erythrocyte sedimentation rate (ESR), clusterin, and β2 microglobulin (1-6).

The pathophysiological mechanisms responsible for progression of early-stage MF to tumor and advanced stages remain elusive.

Acta Dermatovenerol Croat
2021;29(2):67-71

T-regulatory cells (Tregs) may play a major role in the development and progression of both solid and hematologic tumors (7,14). Forkhead box protein 3 (FOXP3) is a major transcription factor and a specific marker of Tregs. Berger *et al*. showed *in vitro* that Tregs ability to block anti-tumor immune responses plays a key role in the pathogenesis of cutaneous T-cell lymphoma (CTCL) (13). Thus, we hypothesized that FOXP3 may predict disease course in patients with MF.

Gjerdum *et al*. found a significantly higher number of FOXP3 positive Tregs in early-patch stage MF in comparison with late-plaque and tumor stages of the disease. They therefore concluded that the existence of such cells predicts a better prognosis (8). In contrast, Hallermann *et al*. found an association between FOXP3 expression and a more aggressive disease course in a limited number of patients with large cell transformation (9). Finally, Klemke *et al*. (10) as well as Fried and Cerroni (11) did not find any difference in the number of FOXP3-positive Tregs between different MF stages. The latter study evaluated sequential biopsies of patients at various stages of disease.

Fried and Cerroni (11) showed that while Tregs percentage increased from 10% in the patch stage to 15% in the tumor stage (in sequential biopsies of one patient), the prognosis was better and the patient remained alive for 11 more years, in contrast to the average survival seen in tumor-stage MF.

Herein, we evaluated the predictive value of FOXP3 expression in MF lesions for a number of disease characteristics.

PATIENTS AND METHODS

Study population

The study was conducted in a series of patients treated at the Cutaneous Lymphoma Clinic at the Tel Aviv Sourasky medical center between 2007 and 2014 according to a protocol reviewed and approved by the institutional ethics committee. We analyzed 30 patients who were diagnosed according to the WHO-ISCL\EORTC classification of cutaneous lymphomas (15). Data were retrieved retrospectively from the patients' medical records. Patient characteristics recorded included: demographic data (age, sex) as well as clinical data (age of onset, age at diagnosis, clinical stage according to the EORTC criteria). Stage severity was graded on a scale of 1-3: mild (1) – stages IA-IIA; moderate (2) – stages IIB-IIIB; severe (3) – stages IVA1-IVB.

A response to treatment scale was defined, as described below (Table 1), taking into consideration the following elements: complete or partial remission, time period until reaching remission, elapsed time until disease recurrence, change in clinical stage during therapy.

Immunohistochemistry

Paraffin-embedded sections were stained using an anti-FOXP3 specific monoclonal antibody (236\E7 specific anti FOXP3 antibody, Serotec, Kidlington, UK). FOXP3 expression was assessed separately in the dermis and epidermis along a semi quantitative scale

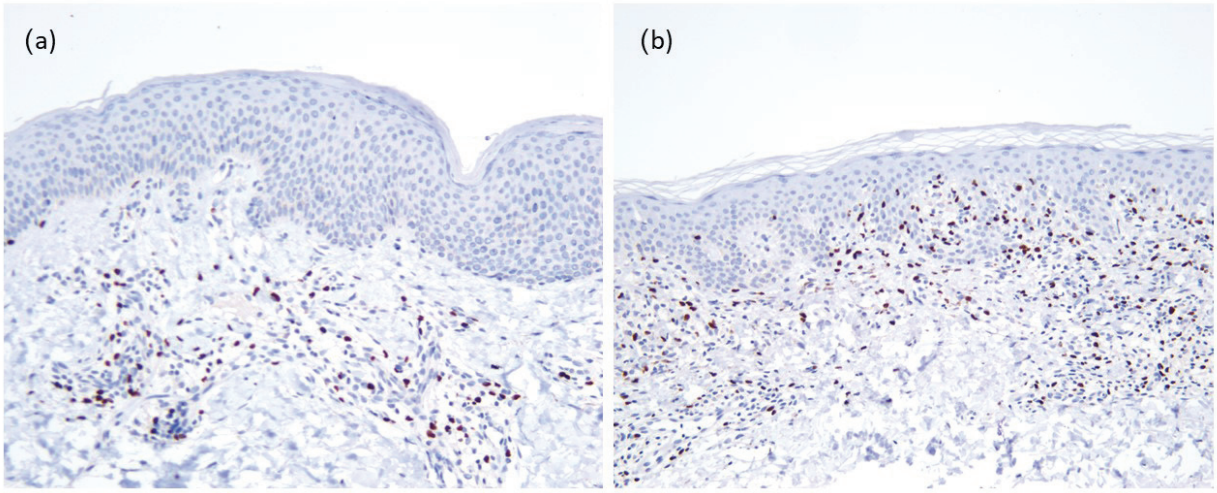

Figure 1. Histopathologic findings of MF skin biopsies, showing superficial and deep skin infiltrates of FOXP3-positive T lymphocytes. (a) FOXP3 positive T lymphocyte infiltrates of the dermis with no staining in the epidermis; (b) Combined positive epidermal and dermal FOXP3-positive T lymphocyte infiltrates. (Staining with 236\E7 specific anti FOXP3 antibody, Serotec, Kidlington, UK, original magnification ×10)

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Table 1. Response to treatment scale

Complete Response (CR) and no relapse:	
1	Did not progress to a more advanced stage, reached CR within 3 months, no relapse
2	Did not progress to a more advanced stage, reached CR within 3-12 months, no relapse
3	Did not progress to a more advanced stage, reached CR within more than 12 months, no relapse
Complete Response (CR), with relapse after more than 12 months:	
4	Did not progress to a more advanced stage, reached CR within 3 months, relapse after more than 12 months
5	Did not progress to a more advanced stage, reached CR within 3-12 months, relapse after more than 12 months
6	Did not progress to a more advanced stage, reached CR within more than 12 months, relapse after more than 12 months
Complete Response (CR), with relapse after 3-12 months:	
7	Did not progress to a more advanced stage, reached CR within 3 months, relapse after 3-12 months
8	Did not progress to a more advanced stage, reached CR within 3-12 months, relapse after 3-12 months
9	Did not progress to a more advanced stage, reached CR within more than 12 months, relapse after 3-12 months
Complete Response (CR), with relapse after less than 3 months:	
10	Did not progress to a more advanced stage, reached CR within 3 months, with relapse after less than 3 months
11	Did not progress to a more advanced stage, reached CR within 3-12 months, with relapse after less than 3 months
12	Did not progress to a more advanced stage, reached CR within more than 12 months, with relapse after less than 3 months
Did not reach CR	
13	Did not progress to a more advanced stage, reached PR only
14	Progressed to a more advanced stage (PD), but not to large cell transformation or to Sézary syndrome
15	Progressed to a more advanced stage, to Sézary syndrome, or to large-cell transformation
16	Sézary syndrome was the initial diagnosis
17	Died from MF/SS, or from related complications

Abbreviations: CR – complete response; PR – partial response; PD – progressive disease

ranging 1-5 (Figure 1). Positive cells were counted over the entire lymphoid infiltrate.

Statistical methods

Correlations between FOXP3 expression levels in the dermis and epidermis, disease stage, and response to treatment were examined using the Spearman correlation test – using SAS 9.2 for Windows.

RESULTS

We studied 30 patients (Table 2) including16 male individuals (53.3%), with an average age of 51.5 years. Age of onset ranged from 5-87 years, and the average time from onset of symptoms to diagnosis was 18.75 months for men and 54.86 months for women.

Most patients were diagnosed at an early stage of MF. FOXP3 expression levels in the dermis and epidermis of patients with MF was on average 2.23 and 1.43, respectively. Data analysis revealed a statistically significant positive correlation between FOXP3 expression in the dermis and response to treatment score (r=0.36, *P*=0.047) (Figure 2). Epidermal FOXP3 expression was not associated with response to treatment. We found that the higher the FOXP3 level in the dermis, the longer it took to achieve remission, with relapse occurring earlier. Furthermore, we identified a non-significant trend for negative correlation between FOXP3 expression in the epidermis and stage severity (r=-0.25, *P*=0.17) and a non-significant correlation with the clinical stage (*P*=0.66). No significant correlation was found between dermal FOXP3 expression levels and stage severity or clinical stage at the time of diagnosis.

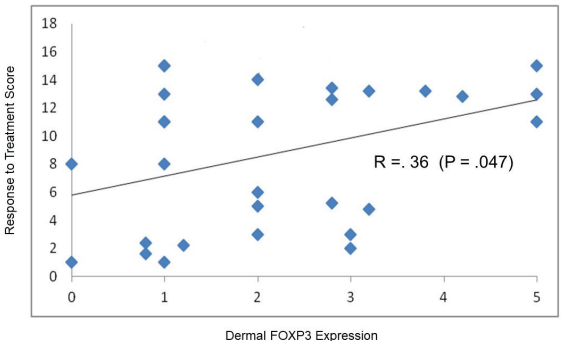

Figure 2. Correlation between dermal FOXP3 expression and response to treatment score.

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Table 2. Demographic and clinical features of the cohort

Case num	Sex	Symptoms duration prior to diagnosis (y)	Age at disease presentation	Age at diagnosis	FOXP3 epidermis	FOXP3 dermis	Stage by EORTC	Stage severity	Response to treatment score
1	F	1	81	82	0	2	IIB	Moderate	14
2	F	2	18	20	2	3	IA	Mild	2
3	M	0	12	12	1	3	IA	Mild	3
4	M	1	55	56	3	4	IIA	Mild	13
5	F	0	81	81	2	3	IIIB	Moderate	17
6	F	0	50	50	1	1	IA	Mild	2
7	M	2	66	68	1	3	IIIA	Moderate	13
8	F	3	37	40	2	3	IA	Mild	13
9	F	0	54	53	2	3	IA	Mild	13
10	F	0	58	58	1	5	IIIA	Moderate	13
11	M	1	55	56	3	2	IA	Mild	5
12	F	23	63	86	2	2	IB	Mild	11
13	F	21	5	26	1	1	IIA	Mild	13
14	M	1	54	55	2	2	IIB	Moderate	6
15	F	4	52	56	0	1	IVA1	Severe	15
16	M	2	30	32	1	1	IA	Mild	8
17	M	4	55	59	1	1	IA	Mild	2
18	M	3	37	40	2	3	IA	Mild	5
19	M	0	54	54	1	1	IVA1	Severe	15
20	M	1	64	65	0	1	IA	Mild	1
21	F	8	9	17	1	4	IB	Mild	13
22	F	1	47	48	0	0	IA	Mild	1
23	M	0	53	53	3	1	IB	Mild	11
24	F	1	18	19	0	1	IA	Mild	2
25	M	3	86	89	1	2	IB	Mild	3
26	M	3	44	47	1	1	IIA	Mild	11
27	M	2	63	65	1	3	IA	Mild	5
28	M	1	47	48	2	0	IA	Mild	8
29	M	1	23	24	5	5	IB	Mild	11
30	F	0	87	87	1	5	IVA1	Severe	15

DISCUSSION

Previous studies which assessed the prognostic value of FOXP3 staining in skin biopsies yielded contradictory results (9,11). In the present study, we detected a positive and statistically significant correlation between FOXP3 expression in the dermis and response to treatment score. Higher levels of FOXP3 in the dermis predicted a more severe course of the disease and poorer response to therapy, including longer periods for achieving remission and higher chance for recurrence and shorter remission. Furthermore, a negative non-significant correlation between FOXP3 expression level in the epidermis and stage severity was found.

The small number of patients with severe stage disease was a limitation of this study. Our conclusions are valid for patients with early stage disease, but no firm conclusions can be drawn from the expression of FOXP3 in patients with advanced stage MF.

There could be several explanations for the contradicting results in the different studies which investigated the expression of FOXP3 in MF, including: varying antibody specificity (9); the fact that neoplastic and reactive cells could not be separated in these studies (FOXP3-positive malignant cells seem to loose expression of the protein during disease progression (9)); the fact that several types of Tregs have been recognized with different effect on disease

prognosis (8-11): (a) suppressor Tregs; (b) malignant Tregs; (c) direct tumor-killing Tregs; and (d) incompetent Tregs (16). Tumor-killing Tregs and incompetent Tregs are associated with better prognosis, while the opposite is true for suppressor Tregs and malignant Tregs. FOXP3 promoter demethylation may underlie the role of malignant Tregs in MF (17).

In summary, FOXP3 expression in skin biopsies of patients with MF might serve as an adjunct prognostic tool. The present study suggests the need for confirmatory larger and prospective studies using series of sequential biopsies.

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