Expression of SATB1, MTI/II and Ki-67 in Mycosis Fungoides

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Abstract. *Background/Aim: A genome organizer protein, special AT-rich sequence binding protein 1, (SATB1), was recently shown to play an important role in the development and spread of various malignancies. Metallothioneins I and II (MTI/II) are multifunctional proteins involved, among others, in cell proliferation and apoptosis resistance in tumors. The role and relevance of these factors in mycosis fungoides (MF), the most common primary cutaneous T-cell lymphoma, is not fully understood. The aim of the present analysis was to evaluate the expression and potential correlation of SATB1, MTI/II and Ki-67 with clinicopathological data in MF. Materials and Methods: We performed immunohistochemical analysis for SATB1, MTI/II and Ki-67 on 90 cases of MF and 19 controls (chronic benign dermatoses). The expression of SATB1 and Ki-67 was analyzed in cancer cell nuclei, whereas nuclear and cytoplasmic expressions of MTI/II were scored separately (nMT, cMT; respectively). Results: We recorded a significantly higher expression of SATB1 and cMT in MF compared to the control group ($p<0.002$, $p=0.04$, respectively, Student's t-test). We also noted significant differences in the mean (±SD) expression of nMT and cMT in advanced MF, compared to early MF, (1.4±1.3 vs. 0.9±0.9, 4.1±3.8 vs. 2.5±2.9 , respectively; $p=0.04$ for both). Similarly, Ki-67 expression was significantly higher in advanced MF ($p<0.01$). The expression of SATB1, cMT and Ki-67 was significantly higher in more infiltrating skin lesions ($p<0.001$, $p=0.08$ and $p<0.001$, respectively). Regarding extracutaneous involvement, a higher expression of SATB1, nMT, cMT and Ki-67 was found in patients with clinical or histological involvement of lymph nodes (N1-3 vs. N0) ($p<0.001$, $p=0.002$, $p<0.001$ and $p=0.1$, respectively). A marked correlation was observed between SATB1 and Ki-67 (Spearman correlation test: $r=0.53$, $p<0.001$). No associations between SATB1, nMT and cMT expression and demographic data nor overall survival were found. Conclusion: Our study provides data on the differences in the expression of SATB1 and cMT regarding differential diagnosis of MF and tumor-node-metastasis-blood staging. Additionally, our report documented significantly different expression levels of MTI/II and Ki-67 according to the advancement of the disease. In view of these data, the role of studied factors in the development of this type of cutaneous T-cell lymphoma is postulated. Our results indicate that both SATB1 and MTI/II may be of diagnostic value, but our study revealed no prognostic significance; however, given the small number of reports focusing on this topic, further studies are required.*

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Mycosis fungoides (MF) is the most common type of primary cutaneous lymphomas, accounting for approximately 50% of all cases with an incidence rate ranging from one per 1,000,000 inhabitants in Japan to five per 1,000,000 in Brazil (1, 2). In the US, the disease incidence accounts for four per 1,000,000 person-years (3). The Polish or Europe specific data are unattainable, however, according to the UK's Haematological Malignancy Research Network analysis, in 2004-2012, MF accounted for 0.7% of all non-Hodgkin lymphomas (4). MF is characterized by malignant proliferation of $CD4^+CD45R0^+$ memory T-cells originally in the skin, with subsequent extracutaneous involvement in some cases. The disease hallmark is evolution of skin lesions from itchy patches and papules through the plaques to the tumors, often ulcerated. The disease staging is based on TNM system (Tables I and II) (5). Early diagnosis is crucial for the implementation of proper treatment and enables inhibition of disease progression, however, it is difficult due to the overlapping clinicopathological features of skin lesions in MF and benign dermatoses, *i.e.* eczema. The prognosis in

Table I. *Tumor-node-metastasis-blood (TNMB) classification (International Society for Cutaneous Lymphomas/European Organization of Research and Treatment of Cancer revision) (5).*

TNMB classification		
Skin	T_1	Patches, papules with/without plaques <10% of body surface (T_{1a}: patch, T_{1b}: plaque with/without patch)
	T_2	Patches, papules with/without plaques >10% of body surface (T_{2a}: patch, T_{2b}: plaque with/without patch)
	T_3	One or more tumors (>1 cm diameter)
	T_4	Confluent erythema >80% of body surface
Node	N_0	No clinically abnormal peripheral lymph nodes
	N_1	Clinically abnormal peripheral lymph nodes; histopathology Dutch grade 1 or NCI LN_{0-2} (N_{1a}: clone$^-$, N_{1b}: clone$^+$)
	N_2	Clinically abnormal peripheral lymph nodes; histopathology Dutch grade 2 or NCI LN_3 (N_{2a}: clone$^-$, N_{2b}: clone$^+$)
	N_3	Clinically abnormal peripheral lymph nodes; histopathology Dutch grades 3 and 4 or NCI LN_4; clone$^+$ or $^-$
Visceral	M_0	No visceral organs involved
	M_1	Viscera involved (pathological confirmation – imaging for spleen/liver)
Blood	B_0	<5% of peripheral blood lymphocytes are Sézary cells (B_{0a}: clone$^-$, B_{0b}: clone$^+$)
	B_1	>5% of peripheral blood lymphocytes are Sézary cells (B_{1a}: clone$^-$, B_{1b}: clone$^+$)
	B_2	High tumor burden: ≥1000/μl Sézary cells with clone$^+$

NCI: National Cancer Institute classification.

MF is highly dependent on disease advancement. In the early stage (IA), life expectancy does not differ from that of the age-, sex- and race-matched population, whereas in advanced stages, the median survival decreases to 5 and 2.5 years for those with stages IIB and IVB, respectively (6-8). Long-term follow-up studies have revealed a number of factors affecting the prognosis in MF, *e.g.* demographics, clinical staging, increased serum lactate dehydrogenase level, eosinophilia and clusterin expression (8-13), but novel diagnostic and prognostic tools in MF assessment are still required.

Special AT-rich sequence-binding protein 1 (SATB1) is a genome organizer protein that due to its three-dimensional, cage-like structure surrounds, folds and remodels chromatin and enables tethering of multiple genes by binding to special DNA sequences. SATB1 acts as a docking-site for chromatin-remodeling complexes and regulates the expression of more than 1,000 genes, including those involved in tumorigenesis. A number of studies have documented an altered expression of SATB1 in various malignancies, including solid tumors such as breast, lung, colorectal, liver, urinary, endometrial cancer, as well as hematological malignancies, *e.g.* acute myeloid leukemia, but only few analyzed the role of SATB1 in primary cutaneous T-cell lymphomas (CTCL) including MF (14-23).

Metallothioneins (MTs) are a group of highly conserved, low-molecular weight intracellular proteins involved in zinc and copper ion homeostasis and regulation of metallo-enzymes and transcription factors. Four members of the MT family have been described in mammals (MTI to MTIV). Common features of MTI and MTII are similar biological roles, high structural homology and, on the protein level, reaction with the same antibody, therefore in many studies they are discussed together. Among the numerous functions attributed to MTI/II, many result from its metal-binding ability. MTI and II are able to up-regulate a variety of oncogenes, and mitogenic, angiogenic and growth factors, and through them are involved in cell survival, angiogenesis, cell proliferation, differentiation and inhibition of apoptosis. By binding heavy metals, MTs play a role in detoxification and protection against cytotoxicity induced by alkylating agents such as cisplatin. They may also protect cell DNA against oxidative stress, triggered by radiation and other chemotherapeutic drugs *e.g.* bleomycin or etoposide (24). Cytoprotective functions of MTs and their involvement in cell proliferation may also contribute to tumorigenesis (25). In fact, increased MT expression at both mRNA and protein levels was observed in various human malignancies, such as lung, breast, ovarian, urinary bladder, pancreatic and skin cancer, as well in acute lymphoblastic leukemia, diffuse large B-cell lymphoma and primary central nervous lymphoma (26-29). On the other hand, certain types of neoplasms, such as gastric and colorectal cancer exhibited decreased MT expression (30). The prognostic significance of MTI and II has been studied on a variety of neoplasms, however, little is known on their role in MF.

To the best of our knowledge, the present study is the first one assessing the expression of MTI/II in MF, as well comparing the expression of MTI/II and STAB1 in relation to proliferation status and clinicopathological data in MF.

Materials and Methods

Patients. The study was performed on archival paraffin blocks obtained from 90 patients with MF (57 men and 33 women, mean age 59.2±13.8 years, range 19-81 years), diagnosed and treated between 1994 and 2015 at the Department of Dermatology, Venereology and Allergology of Wroclaw Medical University. The diagnosis of MF was

Table II. *Staging of mycosis fungoides (International Society for Cutaneous Lymphomas/European Organization of Research and Treatment of Cancer revision) (5).*

Stage	T	N	M	B
IA	1	0	0	0, 1
IB	2	0	0	0, 1
IIA	1, 2	1, 2	0	0, 1
IIB	3	0-2	0	0, 1
IIIA	4	0-2	0	0
IIIB	4	0-2	0	1
IVA1	1-4	0-2	0	2
IVA2	1-4	3	0	0, 2
IVB	1-4	0-3	1	0, 2

based on clinical, histopathological and immunohistochemical examinations, according to the WHO classification (2008) (31). Staging was assessed according to the TNMB system (International Society for Cutaneous Lymphomas/European Organization of Research and Treatment of Cancer revision) (5). Sixty patients had early-stage (IA-IIA) disease and 30 were classified as having advanced-stage (IIB-IVB). The control group consisted of 19 paraffin blocks from aged- and sex-matched patients with benign inflammatory dermatoses (16 with lichen planus, three with eczema). Routine hematoxylin and eosin staining, as well analysis of proliferative activity, were performed by the Authors in a previous study (32). The experiment was approved by the Ethics Committee of Wroclaw Medical University (decision no. KB 574/2011).

Immunohistochemistry (IHC). The reactions were conducted on 4 μm-thick paraffin sections. Deparaffinization, re-hydration and exposition of the epitopes were performed with use of Pre-Treatment Link Rinse Station and Target Retrieval Solution (pH 9, 97°C, 20 min). Activity of endogenous peroxidase was blocked by 5 min exposure to Peroxidase-Blocking Reagent. All sections were rinsed with wash buffer and incubated for 20 min at room temperature with the primary antibodies directed against: MTI/II (clone E9; 1:700; DakoCytomation, Glostrup, Denmark), and SATB1 (clone EPR3895; 1:100; Abcam, Cambridge, UK). Secondary goat anti-mouse antibodies coupled to a dextran core, linked to horseradish peroxidase were applied. Next, visualization was performed using the EnVision™ FLEX+ system according to the manufacturer instructions. All IHC reactions were performed in an automated staining platform Autostainer Link48 (DakoCytomation). The reactions were visualized using 3,3'-diaminobenzidine tetrachlorohydrate (DAB+ Chromogen). All slides were counterstained with Mayer's hematoxylin. All reagents were obtained from DakoCytomation.

Evaluation of IHC reaction. Expression of MTI/II was analyzed in two different fractions: cytoplasmic and nuclear. The evaluation of cytoplasmic MTI/II (cMT) reactions intensities were conducted using the semi-quantitative immunoreactive score (IRS) of Remmele and Stegner, which is based on the intensity of the color reaction and the percentage of positive cancer cells in the whole tissue sections (33). The scale takes into account the percentage of cells with positive reaction (0 points: absence of cells with positive reaction, 1 point: 1-10% cells, 2 points: 11-50%, 3 points: 51-80%, 4 points: over 80% cells with positive reaction) as well as intensity of the reaction (0 points: no reaction, 1 point: low, 2 points: moderate, and 3 points: strong intensity of color reaction). The product of both these parameters comprises the final score, ranging from 0 to 12 points. Nuclear expression of MTI/II (nMT) was analyzed according to a semiquantitative five-grade scoring scheme in whole tissue sections according to tumor cell positivity. The results were encoded as follows: 0: 0% cells stained, 1: 1-10% cells stained, 2: 11-25% cells stained, 3: 26-50% cells stained, 4: 51-100% cells stained. For the evaluation of SATB1 in each paraffin section, three fields with potentially the highest number of tumor cells yielding a positive nuclear reaction (hot spots) were selected. The general result for every sample was an average of the three hot-spot percentages evaluated under ×400 magnification, scoring the brown-labeled cell nuclei of cancer cells (BX-41 light microscope equipped with Cell^D software for computer-assisted image analysis (Olympus, Tokyo, Japan). The intensity of the IHC reactions in coded preparations were independently evaluated by two pathologists. Moreover in doubtful cases, a re-evaluation with a double-headed microscope was performed until a consensus was achieved.

Statistical analysis. All data were analyzed using Statistica10.0 (Statsoft, Cracow, Poland). Means, standard deviations (SD), medians and minimum and maximum values were calculated. Differences between analyzed groups were verified with unpaired Student's *t*-test, Mann–Whitney *U*-test, χ^2 test with Yates correction, and analysis of variance (ANOVA) with Scheffé *post hoc* test, where appropriate. Relationships between quantitative data were verified with Spearman rank correlation test. Kaplan–Meier overall survival was calculated from the date of the start of therapy until the latest follow-up or death. The differences between the curves were assessed by log rank test. *p*-Values less than 0.05 were considered to be significant.

Results

Expression of SATB1. A positive reaction for SATB1 was shown in 86/90 MF cases and 18/19 control cases (p=0.65) (Figure 1A and B). The mean expression of the protein was significantly higher in MF (48.6±33.9) compared to that in controls (23.4±15.4, p=0.002). In MF, slightly higher expression of SATB1 was shown in advanced MF compared to early-stage MF (52.1±35.3 *vs.* 46.9±33.4, respectively; p=0.36). SATB1 was also significantly more highly expressed in more infiltrative MF (plaques with/without patches *versus* patches only) and in patients with clinical or histological involvement of lymph nodes (N1-3 *vs.* N0) (Table III).

Expression of MTI/II. nMT and cMT expression was observed in 58/87 and 63/87 MF cases and 9/18 and 8/18 control cases, respectively (p=0.28 and p=0.04, respectively) (Figure 1C and D). The mean expression of nMT was 1.1±1.1 points in MF group and 0.6±0.7 in the control group (p=0.06). Regarding cMT, the mean expression was 3.1±3.3 in MF and 0.9±1.5 in the control group and the difference was statistically significant (p<0.01). Patients with advanced

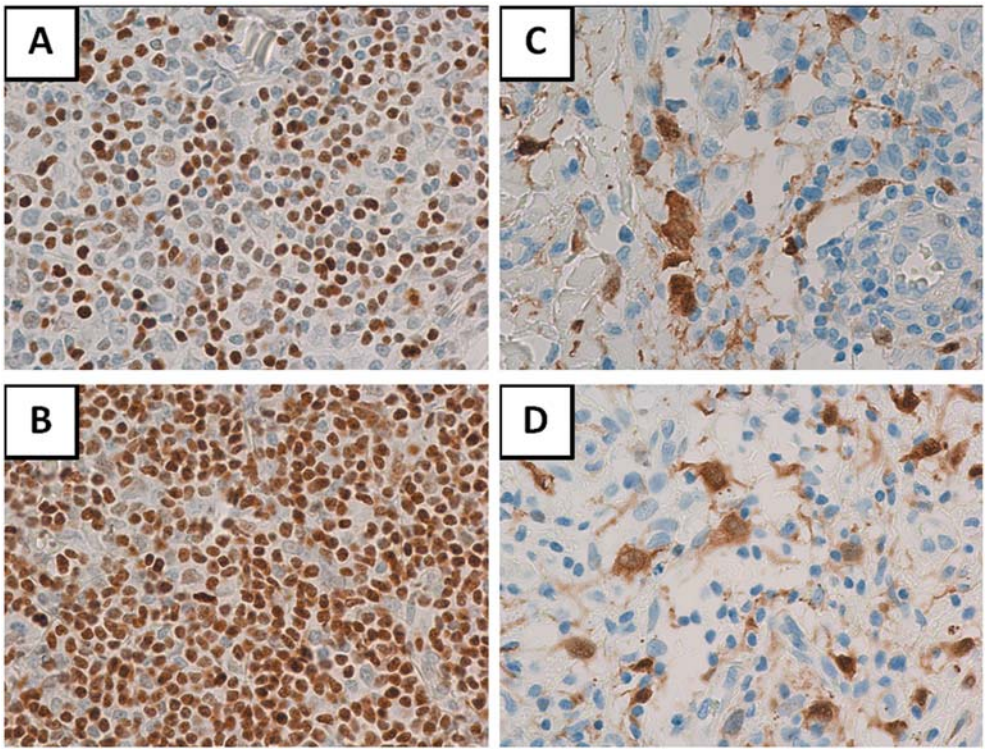

Figure 1. *Nuclear expression of special AT-rich binding protein 1 (SATB1) (A, B) and cytoplasmic expression of metallothionein I/II (MTI/II) (C, D) in primary cutaneous T-cell lymphomas. Magnification ×400.*

MF had significantly higher expression of the studied protein (1.4±1.3 and 4.1±3.8, respectively) compared to those with early-stage MF (0.9±0.9 and 2.5±2.9, respectively, p=0.04 for both; Mann–Whitney *U*-test) (Table IV). Regarding skin and extracutaneous involvement, "plaque with/without patches" stage exhibited significantly higher expression of studied marker than "patches only" stage, as well as nodal involvement (N1-N3) being correlated with higher expression of nMT and cMT in comparison to the N0 patients. Similarly, the expression of nMT and cMT was significantly higher in patients with distant metastases than in patients with M0 disease (Table III).

Expression of Ki-67. Analysis of Ki-67 in MF regarding TNMB staging and advancement of disease was reported in a previous study (33). A statistically significant correlation was found between Ki-67 and SATB1 expression (r=0.51, p<0.001; Spearman rank correlation test) (Figure 2).

Analysis of survival. The detailed analysis of survival was documented in our previous study on the expression of selected proliferation markers in CTCL, including the MF sub-group (33). Regarding SATB1 and MTI/II, no significant differences were found in overall survival of patients with MF (Table V).

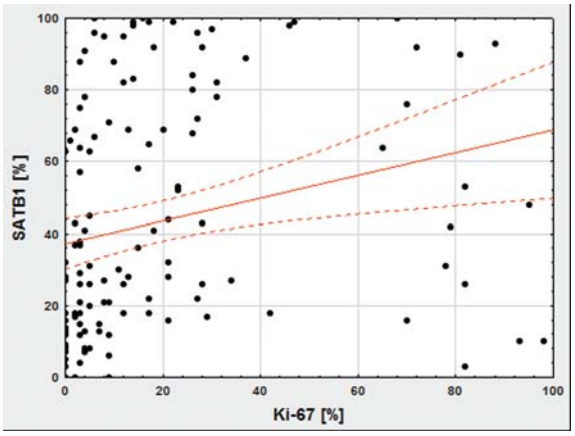

Figure 2. *Spearman rank correlation test revealed moderate positive correlation between Ki-67 and special AT-rich sequence binding protein 1 (SATB1) (r=0.51, p<0.001) in patients with mycosis fungoides.*

Discussion

Accumulated evidence has indicated a number of important diagnostic and prognostic factors in MF. Clinicopathological data, *i.e.* advanced age at the time of diagnosis, male sex,

Table III. *Mean±SD, range and median values of the expression intensities of metallothionein I/II (nuclear, nMT and cytoplasmic, cMT), special AT-rich sequence binding protein 1 (SATB1) and Ki-67 in mycosis fungoides (analysis of variance with Scheffé post hoc test and Student's t-test). Statistical significance accepted at p<0.05.*

TNM	cMT (points)			nMT (points)			SATB1 (%)			Ki-67 (%)		
	Mean±SD	Range	Median	Mean±SD	Range	Median	Mean±SD	Range	Median	Mean±SD	Range	Median
T1	2.6±3.1	0-12	1	0.9±1.0	0-4	1	47.1±33.6	0-98	42	14.0±20.7	0-82	5.5
T2	2.5±2.7	0-9	2	0.9±0.9	0-4	1	48.0±33.8	0-100	43	10.7±14.3	0-68	4
T3	5.7±4.8	0-12	4.5	1.9±1.6	0-4	1	42.0±39.5	0-97	23	23.2±22.5	0-79	19
T4	2.8±2.5	0-9	2	1.1±0.9	0-3	1	57.4±31.9	16-100	65	21.1±23.6	0-82	14
p-Value		0.13			0.15			0.41			0.18	
Stage a (patch only)	2.0±2.6	0-9	1	0.7±0.9	0-4	1	38.1±31.6	0-100	23.5	6.6±7.7	0-28	3
Stage b (plaques±patches)	3.4±3.3	0-12	3	1.1±1.0	0-4	1	57.5±36.5	0-100	68	34.6±31.8	0-98	27
p-Value		0.008			0.06			<0.001			<0.001	
N0	2.0±2.6	0-9	1	0.8±0.9	0-4	1	38.7±30.3	0-100	27.5	11.3±16.7	0-82	4.5
N1-3	4.5±3.7	0-12	3	1.5±1.2	0-4	1	63.5±34.0	0-100	76.5	21.9±22.9	0-82	15.5
p-Value		<0.001			0.002			<0.001			0.01	
M0	3.6±3.3	0-12	2	1.2±1.1	0-4	1	56.6±33.3	0-100	64	18.1±21.2	0-82	12
M1	7.2±4.3	2-12	7.5	2.5±1.3	1-4	2.5	78.2±25.3	42-100	85.5	33.5±33.2	6-79	24.5
p-Value		0.04			0.03			0.18			0.18	
B0	3.0±3.3	0-12	2	1.1±1.1	0-4	1	49.6±34.1	0-100	43	14.6±19.1	0-82	8
B1	3.2±2.8	0-6	3	1.0±0.7	0-2	1	31.8±29.0	12-82	18	31.2±29.9	3-78	21
p-Value		0.72			0.8			0.28			0.08	

Table IV. *Expression of nuclear and cytoplasmic metallothionein (nMT and cMT, respectively), special AT-rich binding protein 1 (SATB1) and Ki-67 in early and advanced mycosis fungoides (MF) (Mann-Whitney U-test). Data are median and mean±SD values. Statistically significant results are given in bold.*

Parameter	Early MF Median; mean±SD	Advanced MF Median; mean±SD	*p*-Value
cMT (0-12 points)	2; 2.5±2.9	3; 4.1±3.8	**0.04**
nMT (0-4 points)	1; 0.9±0.9	1; 1.4±1.3	**0.04**
SATB1 (0-100%)	42; 46.9±33.4	39.5; 52.1±35.3	0.36
Ki-67 (0-100%)	5; 12.5±18.0	15.5; 21.6±22.5	**<0.01**

Table V. *Analysis of overall survival (OS) in patients with mycosis fungoides (MF) in regard to cytoplasmic metallothionein (cMT), nuclear metallothionein (nMT), special AT-rich binding protein 1 (SATB1). Grouping was according to median values; p-values are according to log-rank test.*

Parameter	15-Year OS
cMT (<3 *vs.* ≥3 points)	0.3 *vs.* 0.38, *p*=0.65
nMT (<2 *vs.* ≥2 points)	0.43 *vs.* 0.21, *p*=0.08
SATB1 (<50% *vs.* ≥50%)	0.61 *vs.* 0.31, *p*=0.9

TNMB classification, large cell transformation, eosinophilia, increased serum concentrations of lactate dehydrogenase and β_2-microglobulin, are considered to be the most important, however, studies on determination of new parameters of diagnostic and prognostic value in MF are ongoing (7-9, 11, 34, 35). Results of recent research have pointed to SATB1 as being a novel potential marker of development and spread of various human malignancies, therefore, in the present work, we aimed to investigate the potential significance of SATB1 in MF biology.

Our analysis demonstrates the higher expression of SATB1 in MF compared to chronic benign dermatoses. These results are consistent with previous reports that revealed significant differences in the SATB1 expression patterns between prostate, esophageal, colon and ovarian cancer, benign lesions and normal control tissues. (16, 36-38). Given that early-stage MF commonly mimics other types of skin lesions in the course of non-malignant processes, *e.g.* psoriasiformis, eczematous or lichenoid disorders, differential diagnosis of skin lesions in MF, especially in the early stages of the disease, remains a challenge for both dermatologists and pathologists (39).

Recently, an algorithm for the diagnosis of early MF has been proposed (40), however, additional, novel, easily applicable diagnostic tools are still required, and based on the results of our analysis, SATB1 and MTI/II seem to be promising candidates.

In our study, we documented the different SATB1 expression levels according to skin and extracutaneous involvement. Regarding tumor stage, we found a significantly higher expression of SATB1 in more infiltrating skin lesions (patches and plaques *vs*. patches only). Our results indicate that thicker skin lesions are characterized by higher intensity of SATB1 expression, however, this phenomenon applies only to the early lesions. Considering stage T3 disease, we herein did not observe similar associations. Recently published reports indicate that patients with patches alone (T1a, T2a) have better prognosis in terms of overall survival compared with patients with patches and plaques (T1b, T2b) (11, 34, 41). Our observations provide new arguments on the stratification of early lesions in MF. Additionally, we also revealed that higher SATB1 expression is associated with increased risk of extracutaneous spread. Patients with nodal involvement (N1-3) were characterized by a higher intensity of SATB1 expression compared to patients with N0 stage. The ability of SATB1 to regulate gene expression allows tumor cells to make significant changes in the gene expression pattern and to alter their phenotype. In recent *in vitro* and *in vivo* studies, SATB1 overexpression was shown to correlate with malignant growth and metastasis. In human breast cancer, studies on cancer cell cultures and xenograft mice revealed the capacity of SATB1 to promote metastasis (42). In colorectal cancer, the high expression of SATB1 was correlated with advanced TNM stage and distant metastases (43). Regarding SATB1 expression in CTCL, only few studies concerning this problem have been published (21-23). In recent reports, divergent expression of SATB1 in different T-cell lymphoproliferative disorders was found, which is not surprising given the heterogeneity of T-cell malignancies. Focusing on the Sézary syndrome, an aggressive variant of CTCL, Wang *et al*. examined SATB1 expression pattern in Sézary cell lines obtained from patients and commercially available CTCL lines (Hut-78 and HH), finding a down-regulation of SATB1 at both the mRNA and protein level (22). Differences in SATB1 expression in MF and Sézary syndrome, considered to be a leukemic counterpart of MF, are worth emphasizing, as they provide additional data confirming the distinct origin of malignant T-cells in these two disease entities and suggests different mechanisms involved in the development of various tumors. In the study of Wang *et al*., the restoration of SATB1 expression induced spontaneous cell death in Sézary cell lines and these results led the authors of the report to the conclusion that SATB1 down-regulation is the main cause of apoptosis resistance in Sézary syndrome (22). Similarly, in their study on Jurkat cells, Grzanka *et al*. confirmed that SATB1 down-regulation using siRNA allows the cells to escape from activation-induced cell death (23). On the other hand, high expression of SATB1 mRNA and protein was documented in the second most common subgroup of CTCL, cutaneous $CD30^+$ lymphoproliferative disorders, including lymphomatoid papulosis, primary cutaneous anaplastic large cell lymphoma and MF with large cell transformation, and the overexpression of was suggested to induce $CD30^+$ T-cell proliferation *via* p21-repressing mechanisms (21).

Due to the different functions that SATB1 may have, its altered expression may be of different prognostic value in different CTCL sub-types. In our analysis we did not find any association between overall survival and SATB1 expression. Divergent results were obtained by Grzanka *et al*., who recorded significant positive correlation between higher SATB1 expression and better prognosis in terms of slow progression, overall survival and disease-free survival in MF (44). On the contrary, in cutaneous $CD30^+$ lymphoproliferative disorders, SATB1 up-regulation was associated with the disease progression (21).

Given the fact that genetic instability and uncontrolled proliferation are key features of the malignant process, we analyzed the association between expression of SATB1 and the well-known proliferative marker Ki-67 and revealed the moderate correlation. Our observations are consistent with the results of Kobierzycki *et al*. who disclosed a similar association between SATB1 and Ki-67 in an immuno-histochemical study on ductal breast carcinoma (14). The discussed correlation may mirror the role of SATB1 in malignant cell proliferation.

To the best of our knowledge, there have been no reports in the literature focusing on the MTI/II expression in MF, however, several lines of evidence imply the role of MTI/II in the pathogenesis of other hematological malignancies. Our study analyzed the intensity of MTI/II expression in MF compared to chronic benign dermatoses, as well as in relation to clinicopathological data. We performed separate analysis of MT expression regarding its localization (nuclear *vs*. cytoplasmic). Firstly, our analysis revealed elevated levels of cMT in MF compared to chronic benign dermatoses. Several studies reported data that MTI/II expression assessment may be useful in discriminating benign and malignant lymphoid neoplasms (29, 45). According to our data, MTI/II may be a novel useful, easily applicable marker in differential diagnosis of MF.

Secondly, we examined MTI/II expression in relation to disease advancement and found significantly higher expression, regardless of cell localization in advanced MF compared with early MF. We also studied MTI/II expression in comparison to the TNMB classification, and, similarly to SATB1 expression, noting significantly higher cMT

expression in more infiltrating lesions. Additionally, the expression of MTI/II, regardless of localization was also associated with nodal involvement and distant metastases. Other reports have also documented a correlation between higher MTI/II expression and clinical progression of the neoplasm (27).

Our study has revealed discrepancies in the nuclear and cytoplasmic expression of MTI/II regarding differential diagnosis, tumor stage and the presence of distant metastases. Recently, Kobierzycki *et al*. immunohistochemically evaluated the expression of MTI/II expression in both localizations in a series of 103 patients with ovarian cancer (27). Similarly to our report, the authors found disparity of nMT and cMT expression according to the clinicopathological data (different histological malignancy grade and proliferative activity). The results shown herein clearly imply the significance of separate analysis of MT localization in differential diagnosis and tumor staging in MF.

Considering the potential role of MTI/II in cell proliferation, we examined the association between MTI/II and the marker of proliferation Ki-67, and found no significant correlation between the studied factors. Recent reports provide divergent data on the correlation of MTI/II and Ki-67 expression. In the immunohistochemical analysis on 145 patients with non-small lung cancer, a significant correlation between the analyzed markers was noted in adenocarcinoma, whereas no similar association was observed in squamous cell carcinoma (26). Subsequently, in another study concerning gastrointestinal stromal tumors, a moderate positive correlation was shown for benign lesions, but not for their malignant counterparts (46). On the other hand, immunohistochemical study on ovarian cancer disclosed differences in the correlation between Ki-67 and MTI/II expression, depending on the topography of the latter (27).

Prognostic and predictive value of MTI/II has been deeply studied in solid tumors. In contrast, limited evidence is available for the significance of MTI/II expression in hematological malignancies. Increased MTI/II expression at both mRNA and protein levels have been found to be an independent risk factor in diffuse large B-cell lymphoma, and in acute myeloid leukemia has been suggested to correlate with poor prognosis (29, 47). In acute lymphoid leukemia, divergent results regarding the prognostic value of MTs have been observed. Tsangaris *et al*. noted correlation between MT expression and chemotherapy resistance, whereas Sauerbrey *et al*. reported no association between MT staining positivity and other prognostic factors, *e.g*. sex, age, immunological subtype (47, 48). Our results are consistent with this recent report as we did not find any correlation between MTI/II expression and sex, age or overall survival.

In conclusion, our study provides data on the differences in the expression of SATB1 and cMT regarding MF differential diagnosis and TNMB staging. Additionally, our report documented significantly different expression of MTI/II and Ki-67 according to the advancement of the disease. In view of these data, a role for SATB1 and MTI/II in the development of this type of CTCL is postulated. Our results indicate that both SATB1 and MTI/II may be of diagnostic value, but our study revealed no prognostic significance, however, given the small number of reports focusing on this topic, further studies are required.

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