Expression of MCM-3 and MCM-7 in Primary Cutaneous T-cell Lymphomas

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Abstract. *Background/Aim: Primary cutaneous T-cell lymphomas is a group of rare non-Hodgkin lymphomas, originally affecting the skin. Increased proliferation activity is a hallmark of diverse tumors and the proliferation rate, measured by the expression of various markers has a predictive value regarding the malignancy course. The aim of the present study was to evaluate the prognostic value and the potential correlation between the expression of proliferation markers Ki-67, MCM-3 and MCM-7, and clinicopathological data for different types of primary cutaneous T-cell lymphomas. Materials and Methods: Immunohistochemical reactions were performed on paraffin blocks obtained from 90 patients with mycosis fungoides (MF) and 21 patients with other CTCL (oCTCL), in comparison to 19 patients with benign inflammatory dermatosis (lichen planus, eczema), serving as control. Results: Statistically significant differences in the expression of Ki-67, MCM-3 and MCM-7 were observed between oCTCL vs. the control group (29% vs. 5%; 17% vs. 5%; 13% vs. 1.5%, respectively, ANOVA with Scheffé post-hoc test: p<0.01). In both, MF and oCTCL Ki-67 expression highly correlated with the expression of MCM-3 (r=0.83; p<0.001 and r=0.91; p<0.001, respectively) and MCM-7 (r=0.84; p<0.001 and r=0.87; p<0.01, respectively; Pearson correlation test). Similarly, a strong positive correlation was observed between MCM-3 and MCM-7 (r=0.81, p<0.001 and r=0.85, p<0.001). Regarding the MF group, Ki-67 and MCM-3 expression was significantly higher in advanced compared to early stages (11% vs. 3% and 15.5% vs. 5.0%, respectively, Student's t-test: p<0.05). Advanced MF had also significantly higher labeling indexes for Ki-67, MCM-3 and MCM-7 compared to benign inflammatory dermatoses (Student's t-test: p<0.01, p<0.001 and p=0.02, respectively). Considering skin involvement in MF, T1b had a significantly higher expression of Ki-67, MCM-3 and MCM-7 than T1a (p<0.001 for all comparisons) with similar observations between T2b and T2a (p=0.02; p<0.01; p=0.01, respectively, Student's t-test test). Regarding extracutaneous involvement, only MCM-3 expression in MF showed a positive relationship with both nodal and distant metastases (ANOVA with Scheffé post hoc test: p<0.01, p<0.01, respectively). Higher Ki-67 and MCM-3 expression correlated with shorter survival in MF, although the latter did not reach statistical significance (10-year survival 0.38 vs. 0.82, p=0.02, and 0.46 vs. 0.81, p=0.06, respectively; log rank test). Conclusion: All studied proliferation markers may had predictive values regarding the disease severity and prognosis. Further studies are required to analyze their implementation into patient stratification and treatment process such that will improve prognosis in CTCL.*

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Primary cutaneous T-cell lymphomas (CTCL) is a heterogeneous group of non-Hodgkin lymphomas (NHL) characterized by malignant T-cell proliferation originally limited to the skin. The CTCL group can be divided into three main sub-groups: classical CTCL, comprising mycosis fungoides (MF) and Sezary Syndrome (SzS), a spectrum of primary cutaneous CD30+ lymphoproliferative disorders (CD30+ LPD) and a group of rare T/NK lymphomas, characterized by distinct, usually aggressive behavior and rapid course (1, 2). Individual entities have been included in recent classification systems (WHO-EORTC and WHO 2008) (Table I) (2). The most common CTCL, accounting for approximately 65% of all disease cases is MF, characterized by malignant proliferation of mature T lymphocytes with Th2 phenotype and indolent course, clinically manifested by prevalent skin involvement,

and slow evolution of skin lesions from patches and plaques to tumors (1, 2). In a certain subset of patients, the leading symptom is erythroderma (involvement of more than 90% body surface). Extracutaneous spread is observed in less than one-third of patients, and significantly worsens prognosis (3). A staging system based on the TNMB classification enables for assignment of disease advancement, which is important due to the distinct disease course, prognosis and therapy according to disease stage. Early MF (eMF, stages IA-IIA) is characterized by the sole skin involvement (limited patches (T1a) and plaques (T1b) covering <10% of the body surface, or patches and plaques covering 10% or more of the body surface (T2a and T2b, respectively) (4-7). The cutaneous lesions in the course of eMF are non-specific and commonly mimic other inflammatory skin disorders, *i.e.* eczema or psoriasis, and the differential diagnosis of the disease is a major challenge for both, clinicians and pathologists (8). eMF is characterized by excellent prognosis with a median survival of more than 12 years (3, 4). Advanced MF (aMF) stages: IIB (presence of tumors, T3), III (erythroderma) and IV (IVA – nodal involvement; IVB – visceral involvement) have a generally poor prognosis and median survival of 2.5-5 years (3-7). The most common aggressive type of CTCL is Sezary Syndrome (SzS) that is characterized by triad of symptoms: erythroderma, generalized lymphadenopathy and the presence of malignant T-cells in the skin, lymph nodes and blood (2). The second most common group of CTCL are CD30+ LPD, characterized by malignant proliferation of CD30+ lymphocytes primarily in the skin and, clinically, by spontaneous regression of various skin lesions, mainly papules and tumors. In addition, fairly well-defined CTCL, there is also a group of rare T/NK lymphomas, characterized by heterogeneous clinical picture, distinct, usually aggressive behavior and rapid course (Table I) (2).

Ki-67 is a non-histone nuclear protein, that has been routinely used as a marker of proliferation in various malignancies over two decades. Ki-67 is expressed in G^1, S and G^2 phase of cell cycle and during mitosis, and recent studies indicate that it may be involved in the heterochromatin organization surrounding rRNA, after the exit of the cell from the G0 phase (9, 10). Since new proliferation markers have been implemented in cancer diagnostics over the past years, comparative studies of selected markers are constantly conducted.

Minichromosome maintenance proteins (MCM) are a family of six highly conservative, homogenous proteins (MCM-2–7) that play an active role in initiation of DNA synthesis and prevent re-replication during the same cell cycle (11). At the early G1 phase MCM-2–7 proteins interact with each other and form stable heterohexamers with a core represented by proteins MCM-4, -6 or -7 with a helicase activity. Moreover, particular subunits show different functions, *i.e.* MCM-2 facilitates histone re-deposition during DNA synthesis or MCM-3 acts after post-translational modification by inhibition of the cell entry into the S phase (12, 13). MCM are abundantly expressed during the whole cell cycle and their amount decreases during differentiation or quiescence (14, 15). Results of many studies suggest that MCM antigens are of potential value in proliferation rate assessment in malignant processes. Additionally, it was pointed-out that they may have a similar expression pattern as Ki-67 and may be useful as the diagnostic and prognostic marker (16, 17).

In the present study we aimed to evaluate the prognostic value and the potential correlation between the expression of proliferation markers Ki-67, MCM-3 and MCM-7, and clinicopathological data in different types of primary cutaneous T-cell lymphoma.

Materials and Methods

Patients. The study was performed on archival paraffin blocks obtained from the 90 patients with MF, 21 other subtypes of cutaneous T-cell lymphomas (oCTCL) and 19 benign inflammatory dermatosis (16 cases with lichen planus, 3 with eczema) serving as controls, collected between 1994-2015 in the Department of Dermatology, Venereology and Allergology of Wroclaw Medical University. Clinical and pathological characteristics of MF and oCTCL group are presented in Table II. Since the oCTCL group is small and heterogeneous in terms of biological behavior and prognosis, additional characteristics of individual cases are shown in Table III. For the purpose of statistical analysis, isolated cases of subcutaneous panniculitis-like T-cell lymphoma and primary cutaneous aggressive epidermotropic CD8+ cytotoxic T-cell lymphoma were incorporated in the primary cutaneous peripheral T-cell lymphoma not otherwise specified subgroup due to the similar biological behavior. The diagnosis of MF and oCTCL was based on clinical, histopathological and immunohistochemical examinations, according to the WHO classification (2008) (2). Staging was assessed according to TNMB system (ISCL/EORTC revision) (5). The study was approved by the Ethics Committee of the Wroclaw Medical University (decision no. KB 574/2011).

Immunohistochemistry (IHC). All samples were fixed in 4% buffered formalin, de-hydrated and embedded in paraffin in a typical manner. Subsequently, 7-μm sections were cut and stained with hematoxylin and eosin (Figure 1A, B). For IHC reactions, 4-μm and- thick sections were prepared. The sections were de-paraffinized, re-hydrated and the epitopes were exposed using Pre-Treatment Link Rinse Station and Target Retrieval Solution (pH 6 for Ki-67; pH 9 for MCM-3 and MCM-7; 97˚C, 20 min) (DakoCytomation, Glostrup, Denmark). Activity of endogenous peroxidase was blocked by 5 min exposure to Peroxidase-Blocking Reagent (DakoCytomation). The sections were then rinsed with wash buffer and incubated for 20 min at room temperature with the following primary antibodies directed against: MCM-3 (clone 101, 1:50, DakoCytomation), MCM-7 (clone DCS-141.1, 1:50; Leica, Weltzar, Germany), and Ki-67 (clone MIB1, RTU, DakoCytomation). Secondary goat anti-mouse antibody coupled to a dextran core, linked to horseradish peroxidase, was

Table I. *World Health Organization – European Organization for Research and Treatment of Cancer (WHO-EORTC) classification of cutaneous lymphoma with primary cutaneous manifestations (2, 4-7).*

Primary cutaneous T/NK cell lymphoma
Classical CTCL
Mycosis fungoides (classic type)
Mycosis fungoides (rare types)
Folliculotropic variant
Pagetoid reticulosis
Granulomatous slack skin
Sezary Syndrome
Primary cutaneous CD30+ lymphoproliferative disorders
Lymphomatoid papulosis
Primary cutaneous anaplastic large cell lymphoma
Rare T/NK lymphomas
Adult T-cell Leukemia/Lymphoma
Subcutaneous panniculitis-like T-cell lymphoma
Extranodal NK/T-cell lymphoma, nasal type
Primary cutaneous peripheral T-cell lymphoma, rare types
Primary cutaneous aggressive epidermotropic cytotoxic CD8+ T-cell lymphoma
Primary cutaneous T-cell lymphoma γ/δ
Primary cutaneous small/medium-sized T-cell lymphoma

CTCL, Primary cutaneous T-cell lymphoma.

applied and subsequent visualization was performed using the EnVisionTM FLEX+ system (DakoCytomation), according to the manufacturer's 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 counter-stained with Mayer's hematoxylin.

Evaluation of IHC reaction. For the evaluation of MCM-3, MCM-7 and Ki-67 in each paraffin section three fields with the highest number of neoplastic cells yielding a positive nuclear reaction (hot spots) were selected. The percentage of positive cells in each hot spot was evaluated under ×400 magnification, scoring the brown-labeled cell nuclei of neoplastic cells (BX-41 light microscope equipped with Cell^D software for computer-assisted image analysis; Olympus, Tokyo, Japan). The final result for every sample was an average of the three hot spot percentages. The intensity of the IHC reactions in coded preparations were independently evaluated by two pathologists. Moreover in doubtful cases, re-evaluation with a double-headed microscope was performed until a consensus was reached.

Statistical analysis. All data were analyzed using Statistica10.0 (Statsoft, Cracow, Poland). The possible differences between analyzed patient groups were evaluated using analysis of variance (ANOVA) with Scheffé post hoc test and χ^2 test. Relationships between quantitative data were verified with Pearson correlation test. Kaplan-Meier overall survival was calculated from the date of the start of therapy until the last follow-up or death. The differences between the curves were assessed by log rank test. *p*-Values <0.05 were considered significant.

Table II. *Patients' clinicopathological characteristics*.

	oCTCL (n=21)		MF (n=90)	
Mean age (range)	56.8±19.1 (14-91)		59.±13.8 (19-81)	
Parameters	N	%	N	%
Gender				
Male	10	47.6	57	63.3
Female	11	52.4	33	36.7
Tumor (skin involvement)				
T1	0	0	32	35.6
T1a	0	0	23	25.6
T1b	0	0	9	10.0
T2	7	33.3	29	32.2
T2a	0	0	23	25.5
T2b	7	33.3	6	6.7
T3	10	47.6	12	13.3
T4	4	19.1	17	18.9
Lymph nodes				
N0	8	38.1	54	60.0
N1, N2, N3	13	61.9	36	40.0
Metastasis				
M0	17	81.0	89	98.9
M1	4	19.0	1	1.1
Blood involvement				
B0	16	76.2	85	94.4
B1, B2	5	23.8	5	5.6
pTNMB				
1A	0	0	27	30.0
1B	6	28.6	21	23.3
2A	1	4.8	12	13.3
2B	5	23.8	7	7.8
3A	0	0	14	15.6
3B	0	0	2	2.2
4A	5	23.8	6	6.7
4B	4	19.0	1	1.1
Stage				
Early (IA-IIA)	7	33.3	60	66.7
Advanced (IIB-IVB)	14	66.7	30	33.3

MF, Mycosis fungoides; oCTCL, other primary cutaneous lymphoma.

Results

Evaluation of proliferation marker expression. The expression of Ki-67 was evaluated with immunohisto-chemistry in 90 MF, 21 oCTCL and 19 control cases. A positive nuclear reaction was observed in 76, 17, 14 of subjects, respectively (Figure 1B and C). Post-hoc analysis revealed significantly higher Ki-67 expression in oCTCL than in MF and control group ($p<0.001$ for both; Scheffe test). The difference between MF and control group was statistically insignificant ($p=0.74$), however when comparing advanced MF (aMF) to the control groups a significantly higher expression of Ki-67 was observed in the first group ($p=0.01$).

The expression of MCM-3 and MCM-7 was evaluated in 87 MF, 15 oCTCL and 18 control cases. Positive nuclear

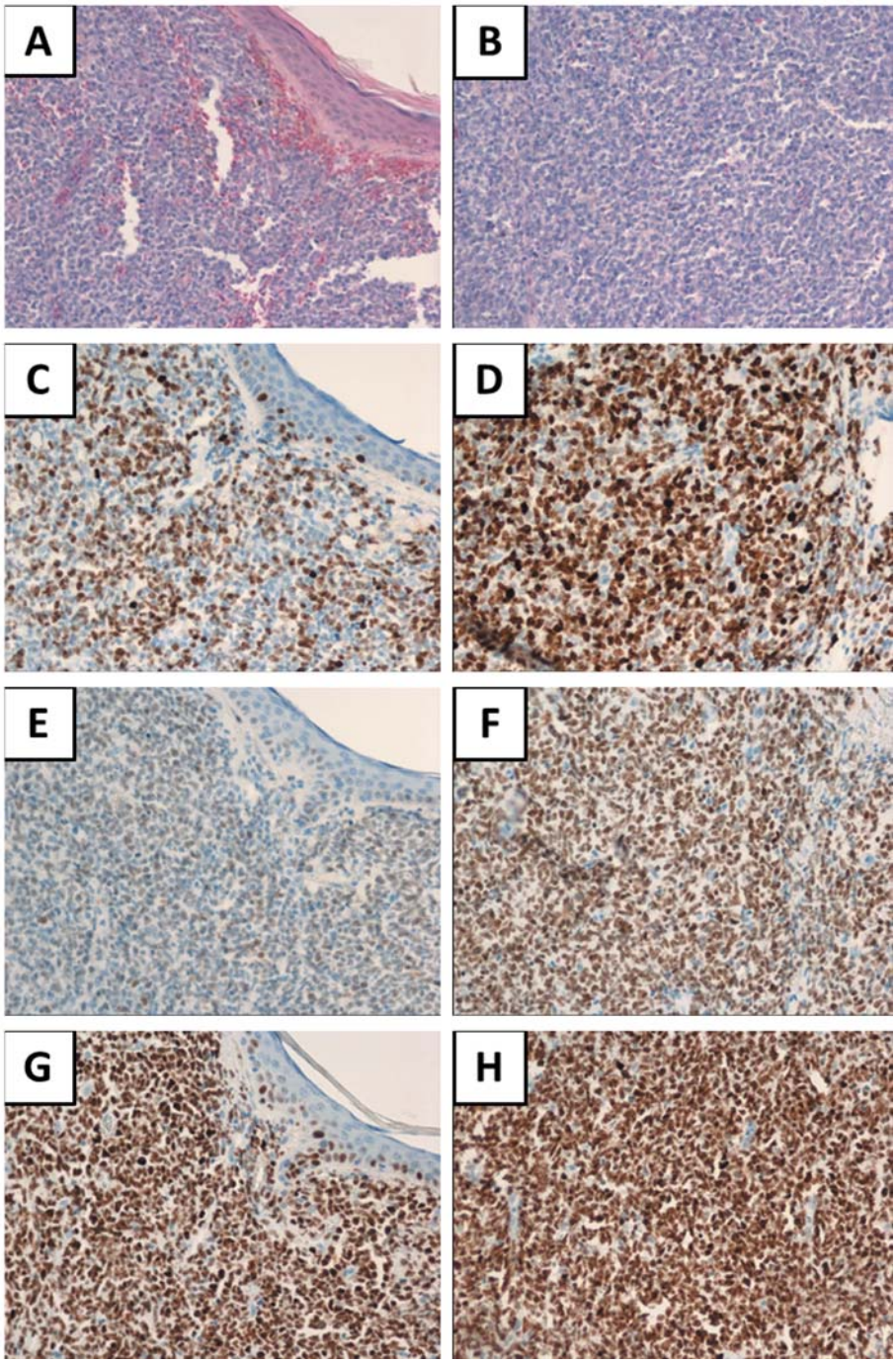

Figure 1. *Hematoxylin and eosin staining of epidermis (A) and dermis (B). Epidermal (C, E, G) and dermal (D, F, G) expression of the analyzed markers. Nuclear expression of Ki-67 antigen (C, D), minichromosome maintenance protein (MCM)-3 (E, F) and MCM-7 (G, H) in primary cutaneous T-cell lymphomas. Magnification ×200.*

reaction for MCM-3 was shown in 77, 13 and 14 cases, respectively (Figure 1D and E). Statistical analysis revealed a significantly higher expression of MCM-3 in oCTCL than in MF (p=0.02) and the control group (p<0.001). No significant differences were observed between MF and the control group (p=0.1), however taking into account only aMF cases a significantly higher MCM-3 expression was observed (p<0.001).

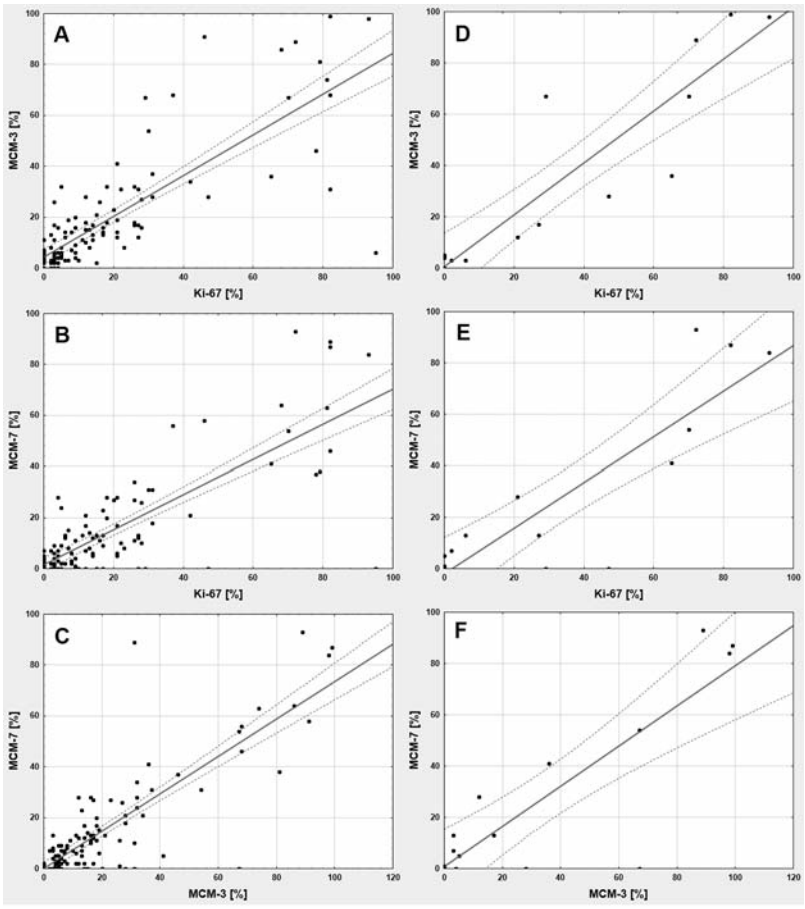

Figure 2. *Pearson correlation test revealed significant positive associations between expression of minichromosome maintenance protein (MCM)-3 and Ki-67 (A; r=0.83, p<0.001; D; r=0.91, p<0.001), MCM-7 and Ki-67 (B; r=0.84, p<0.001; E; r=0.87, p<0.001) and MCM-3 and MCM-7 (C; r=0.81, p<0.001; F; r=0.85, p<0.001) in patients with mycosis fungoides (A-C) and other CTCL (D-F).*

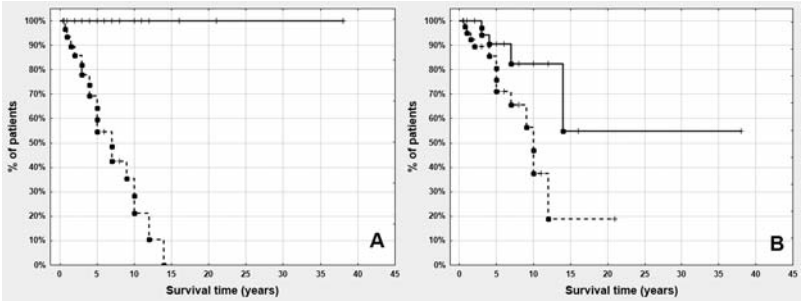

Figure 3. *Kaplan-Meier survival curves of patients with mycosis fungoides (MF) based on the: disease advancement (A; — early MF, --- advanced MF; p<0.001), and Ki-67 antigen expression (B; — Ki-67≤10%, --- Ki-67 >10%; p=0.02). Analysis revealed that patients with weak Ki-67 expression as well with early MF lived longer (log rank test).*

Regarding MCM-7 expression, a positive reaction was observed in 67, 11 and 10 cases, respectively (Figure 1F and G). Similarly to MCM-3, analysis of MCM-7 expression revealed significantly higher expression in oCTCL than in MF (p=0.02) and in the control group (p<0.01). Differences between MF and control group were insignificant (p=0.26), however taking into account only aMF cases, a significantly higher MCM-7 expression was observed (p=0.02).

Table III. *Characteristics of oCTCL patients.*

Patient no.	Stage	TNMB	Age	Gender	Survival status (1–alive, 0–dead)	Disease duration (years)	Type of CTCL
1	IIB	3 0 0 0	55	F	1	0.5	c-ALCL
2	IB	2 0 0 0	91	F	1	2	c-ALCL
3	IVA	3 3 0 0	78	F	0	4	c-ALCL
4	IIA	2 1 0 0	70	M	1	0.5	c-ALCL
5*	IB	2 0 0 0	64	M	1	1	c-ALCL
6*	IB	2 0 0 0	59	M	1	1	c-ALCL
7*	IB	2 0 0 0	27	M	1	1	PCSM-TCL
8*	IB	2 0 0 0	14	F	1	0.8	PCSM-TCL
9	IVB	3 3 1 0	55	F	0	1.5	PCSM-TCL
10	IVA	4 3 0 0	67	M	1	2	PCSM-TCL
11	IB	2 0 0 0	29	M	1	1.5	PCSM-TCL
12	IIB	3 0 0 0	79	M	1	3	PCSM-TCL
13*	IIB	3 1 0 0	37	F	1	5	PTCL-NOS/SPTCL
14	IVA	3 3 0 0	80	F	0	10	PCAE-TCL
15	IIB	3 1 0 0	42	F	0	4	PTCL-NOS
16	IIB	3 1 0 0	63	M	0	0.5	PTCL-NOS
17	IVB	4 3 1 1	63	M	0	0.5	PTCL-NOS
18	IVB	4 2 1 1	49	F	0	1	PTCL-NOS
19	IVB	3 1 1 1	67	F	0	0.5	PTCL-NOS
20*	IVB	4 2 0 1	53	F	0	0.4	PTCL-NOS
21	IVA	3 3 0 0	51	F	1	1	PTCL-NOS

c-ALCL, Primary cutaneous anaplastic large cell lymphoma; PCSM-TCL, primary cutaneous CD4+ -small/medium sized pleomorphic T-cell lymphoma; PTCL-NOS, primary cutaneous peripheral T-cell lymphoma, not otherwise specified; SPTCL, subcutaneous panniculitis-like T-cell lymphoma; PCAE-TCL, primary cutaneous aggressive epidermotropic CD8+cytotoxic TCL. *only Ki-67 expression was assessed.

The statistical analysis conducted with Person correlation test revealed very strong relationships between all studied markers in both, MF and oCTCL groups (Figure 2).

Early vs. advanced MF. Patients with aMF showed a significantly higher expression of Ki-67 (median=15.5%, quartiles: 6-27%) and MCM-3 (median=16%, quartiles: 11-31%) compared to eMF Ki-67 (median=5%, quartiles: 2-16.5%) and MCM-3 expression (median=8%, quartiles: 3-18%) ($p<0.01$ for both). There was no significant difference ($p=0.37$) between in expression of MCM-7 aMF (median=9.5%, quartiles: 2-14%) and eMF (median=5%, quartiles: 1-18%). In view of TNMB status of MF, T1b stage showed significantly higher expression of Ki-67, MCM-3 and MCM-7 than T1a stage ($p<0.001$ for all). Similar differences were found between stages T2a *vs.* T2b considering all studied proliferation markers ($p<0.01$ for all) (Table IV). Patients with MF without lymph node involvement (N0) showed a significantly lower expression of MCM-3 than the rest of the patients ($p<0.01$). In contrast, patients with distant metastases (M1) showed higher expression of MCM-3 than those without disseminated disease (M0) ($p=0.01$). All tested associations between TNMB status and proliferation markers in MF patients are shown in Table IV.

Expression of proliferation markers and disease severity in other CTCL types. Analyzing proliferation markers and disease advancement in other CTCL types (TNMB status, disease stage) we did not find any significant relationships regarding studied parameters, neither in the whole other-CTCL group, nor in the particular CTCL sub-types.

Analysis of survival. Patients with eMF (stage IA to IIA) showed an excellent overall survival (OS) ratio. In contrast, less than 60% of patients with aMF (stage IIB to IV) lived longer than 6 years (Figure 3A). Regarding studied markers, patients with low proliferation activity of neoplastic cells (Ki-67 ≤10%) had a significantly better prognosis ($p=0.02$) (Figure 3B). Patients with higher expression of MCM-3 also showed a poorer survival ratio, however, the difference was not significant ($p=0.06$), while the expression of MCM-7 did not influence the survival ratio ($p=0.48$). The multivariative regression analysis showed that among the studied parameters only disease advancement was an independent prognostic parameter. In addition, analysis showed no significant difference in the OS regarding gender and age.

Regarding oCTCL types we did not find any relationship between the expression of studied proliferation markers and survival ratio neither for the whole group nor for a particular

Table IV. *Relationship between analyzed proliferation markers and TNMB classification in patients with mycosis fungoides.*

TNMB	Ki-67 (%)		MCM-3 (%)		MCM-7 (%)	
	Median	Quartiles	Median	Quartiles	Median	Quartiles
T1	5.5	2.5-16.5	7.5	2-18	4.5	1-18
T1a	**4***	**0-8***	**4***	**2-8***	**3***	**0-7***
T1b	**26***	**14-46***	**28***	**16-32***	**28***	**18-58***
T2	4	2-16	11	3.5-22	5.5	0.5-22
T2a	**3****	**2-16****	**5.5****	**3-17****	**2****	**0-11****
T2b	**13.5****	**7-31****	**24.5****	**19-37****	**18****	**13-31****
T3	19	6-33.5	18	10-47.5	8	1-22
T4	14	6-22	15	11-26	10	2-13
	p=0.18		*p*=0.17		*p*=0.92	
	***p*<0.001**		***p*<0.001**		***p*<0.001**	
	****p*=0.02**		****p*<0.01**		****p*=0.01**	
N0	4.5	2-15	6	3-18	4	0-11
N1	17	6-22	16	12-31	12	5-28
N2	12	6-42	15	14-34	12	0-21
N3	15	0-56	30.5	3.5-61	19	4.5-38.5
	p=0.06		***p*<0.01**		*p*=0.18	
M0	12	3-26	**15**	**5-28**	7	1-23
M1	24.5	9-58	**48**	**21-74.5**	29.5	26.5-47
	p=0.18		***p*=0.01**		*p*=0.08	
B0	8	3-18	12	4-21	6	1-14
B1	21	12-42	28	19-34	21	6-21
	p=0.07		*p*=0.4		*p*=0.57	

MCM, Minichromosome maintenance protein. Statistically significant associations are given in bold.

lymphoma type. In addition, no association between gender and OS was observed. This phenomenon could be explained by the small patient populations in the particular lymphoma type.

Discussion

Over recent decades the proliferation rate assessment has been recognized as an important element of cancer diagnostics. Intensive research has been conducted on different proliferation markers and their expression during the cell cycle in various malignancies, however, little is known on their role in MF and oCTCL.

In our study we demonstrated significant differences in the expression of Ki-67, MCM-3 and MCM-7 between studied groups. First, the expression of all proliferation markers was the highest in oCTCL and significantly differed from the control group confirming that immunopositivity of proliferation markers are higher in malignant lymphoproliferative disorders than in benign inflammatory dermatoses. We also observed a significantly higher proliferation rate of all studied markers in oCTCL than in MF. Little is known about the Ki-67, MCM-3 and MCM-7 expression in this heterogeneous group of lymphomas, however studies on NHL suggest that high labeling indexes for Ki-67 and MCMs are usually associated with a more aggressive behavior and poor survival and indolent lymphomas tend to have lower proliferation indexes (18-20). Certain reports indicate that proliferation activity in MF is lower compared to other T-cell lymphomas and most probably reflects indolent nature of this lymphoma (21). In a study that aimed at identifying diagnostic markers that might differentiate Sezary Syndrome (SzS; an aggressive erythrodermic CTCL), from other erythrodermic CTCL with more indolent behavior, expression of Ki-67 was significantly higher in the first group (22). On the other hand, another study on heterogeneous group of CTCL revealed no differences in Ki-67 labeling index between different disease entities, regardless of their histological type and grade (23).

In our analysis we did not find significant difference between MF and benign inflammatory dermatoses. However, knowing that the eMF differ from aMF in terms of disease course and prognosis, we compared the expression level between benign inflammatory dermatoses and the early and advanced stages of MF. In the present analysis we found a significantly higher expression of Ki-67, MCM-3 and MCM-7 in aMF compared to the control group, whereas there were no such associations between eMF and control. The expression of Ki-67 and MCM-3 were also significantly higher in aMF with respect to early disease stages. Interestingly, the expression of MCM-7 did not differ between early and advanced stages. Our observations are consistent with previously published reports. Gamblicher *et al*. evaluated immunohistochemical expression of Ki-67 in MF, parapsoriasis en plaque (PP; reactive T-cell dermatosis) and lymphomatoid papulosis (LyP; indolent CD30+ CTCL), and found significantly higher expression of Ki-67 in aMF compared to PP, however similar correlation between eMF and PP was not observed (24). Similarly, Dummer *et al*. revealed higher labeling indices for Ki-67 in aMF compared to early stages (25). By contrast, Gamblicher *et al*. have noted more an abundant expression of MCM-7 in aMF compared to eMF and PP, whereas we haven't seen different MCM-7 expression levels within MF group (24). Recent reports indicate significant difference in terms of prognosis between the disease stages T1a *vs*. T1b and T2a *vs*. T2b (5). In our analysis we found significantly higher expression of all studied markers in relation T1b *vs*. T1a as well T2b *vs*. T2a.

Furthermore, we were also interested to see whether there were differences in the expression of the investigated markers and extracutaneous involvement in CTCL. In MF we

found that only expression of MCM-3 positively correlated with extracutaneous involvement, both nodal and distant, whereas either Ki-67 or MCM-7 expression did not show any great association. We also examined the predictive value of Ki-67, MCM-3 and MCM-7 and we found that high labeling indexes of Ki-67 and MCM-3 correlated with shorter survival of patients with MF, while the intensity of MCM-7 expression had no effect on survival in MF. A number of studies have provided data showing associations between increased expression of Ki-67 and MCM and advanced disease stage or clinical outcome in human malignancies, although the published results are not always unequivocal (16, 18, 26, 27). High expression of Ki-67 has been shown to correlate with advanced disease and metastatic status in ovarian and gastric cancer (28, 29). It has been associated with disease progression in chronic lymphocytic leukemia and has been correlated with shorter survival in NHL (18, 30). Immunohistochemical expression of MCM has been reported to affect clinical outcome in breast cancer, and has been related to poor prognosis in gliomas (31, 32). Regarding CTCL, abundant dermal Ki-67 expression was a strong negative predictive marker of survival in MF and SzS (33, 34). On the other hand, several studies have presented divergent results regarding diagnostic and prognostic value of discussed markers. Higher expression levels of MCM-2 and MCM-7 were associated with earlier stages in Hodgkin lymphoma (HL) and inversely correlated with adverse prognostic factors, such as B-symptoms, anemia or lower serum albumin levels (35). In colorectal cancer more abundant MCM-2 and MCM-7 expression was observed in Dukes' stage B than in Dukes' stage C (36). Werynska *et al*. did not find any significant association between Ki-67 or MCM and clinical outcome in non-small cell lung cancer as well as Fujioka *et al*. (17, 37).

In our analysis, no significant correlation between gender and survival in MF and other CTCL has been found, what is in agreement with previous studies (38-40). However, there are reports suggesting that both, male and female sex can be a negative prognostic marker in MF (3, 41). There are divergent results regarding the association between the age of the patient at the time of diagnosis and the survival. In one study performed on a large population of SzS Japanese patients, the age >70 years significantly correlated with poorer prognosis (33). Similar associations were also found in populations of MF patients (3, 39, 42). By contrast, Grzanka *et al*. reported a better prognosis for patients >60 years compared with middle aged patients with MF (38).

Although we found a strong correlation between the expression level of all studied markers, certain discrepancies between expression of Ki-67, MCM-3 and MCM-7 were also demonstrated. Ki-67 and MCM-3 are reliable parameters for the correlation with clinical stage of MF. MCM-3 is predictive for extracutaneous involvement and Ki-67 and MCM-3 expression are of prognostic value in MF. These diversities in the expression level of discussed markers clearly demonstrate separate functions of Ki-67 and MCMs, as well as different roles of individual proteins within the MCM family.

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