

ORIGINAL ARTICLE: CLINICAL

Prognostic factors and risk stratification in early mycosis fungoides

Eric C. Vonderheid¹, Igor Pavlov², Julio C. Delgado², Thomas B. Martins², Gladys H. Telang³, Allan D. Hess¹ & Marshall E. Kadin⁴

¹Department of Oncology and The Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins Medical Institutes, Baltimore, MD, USA, ²ARUP Institute for Clinical and Experimental Pathology, Department of Pathology, University of Utah School of Medicine, Salt Lake City, UT, USA, ³Department of Dermatology, Brown University, Providence, RI, USA and ⁴Department of Dermatology, Boston University and Roger Williams Medical Center, Providence, RI, USA

Abstract

Available demographic, clinical, histologic, immunohistochemical and laboratory findings, including serum cytokine/cytokine receptor levels, obtained at initial evaluation in a cohort of 33 patients with mycosis fungoides (MF) at stages I–IIA who had subsequent progression of disease were compared against 70 stage-matched cases of MF without observed progression. Significant factors that correlated with both disease progression and overall survival were: (1) presence of large Pautrier microabscesses (10 or more atypical lymphocytes), (2) presence of atypical lymphocytes with hyperchromatic or vesicular nuclei in the dermal infiltrate, (3) less than 20% CD8+ cells in the dermal infiltrate and (4) above normal (> 122 U/mL) serum immunoglobulin E (IgE) level. Combination of these factors was used to construct prognostic groupings which, if validated, might be useful to identify patients with clinically early MF at highest risk for disease progression and poor outcome.

Keywords: T-cell mediated immunity, lymphoma and Hodgkin disease, prognostication, cytokine production

Introduction

Mycosis fungoides (MF) is the most common form of cutaneous T cell lymphoma (CTCL). It typically presents as one or more persistent patches or infiltrated plaques on skin areas not habitually exposed to ultraviolet radiation (sunlight) [1]. Once the diagnosis of MF has been established, the risk of subsequent disease progression and disease-specific survival pattern depends on the magnitude of tumor infiltrate in the skin and extracutaneous sites at diagnosis (estimated by clinical stage), the innate biologic aggressiveness of the neoplastic T cells, the effectiveness of the anti-tumor host response and response to treatment. For most patients with clinically early MF, the prognosis is favorable, with 10-year disease-specific survival rates of more than 95% for stage

IA, 80–85% for stage IB and about 70% for stage IIA [2–4]. Nevertheless, a proportion of these patients develop progressive disease with poor outcomes. Accordingly, our aim is to identify risk factors for those patients at highest risk for progressive disease and poor outcome.

A number of clinical, pathologic and laboratory findings in patients with MF have been reported to have adverse prognostic implications when examined independently in prognostic models (univariate analysis) such as the Cox proportional hazards regression model. In many instances, the prognostic variable strongly correlates with skin T rating or clinical stage, and consequently no longer retains significance in models that include clinical stage as a covariate (multivariate analysis).

Pertinent to this study, examples of variables in patients with MF/CTCL previously reported to correlate with poor prognosis in models that include skin T rating or clinical stage include: advanced age [2,4–8], male gender [4], thickness of dermal infiltrate [9], follicular mucinosis/folliculotropic MF variant [3,4], diminished numbers of dermal CD8+ cells [10,11], increased numbers of dermal CD30+ cells [12], low absolute CD8+ counts in the blood [13], blood eosinophilia [14] and increased serum lactate dehydrogenase (LDH) levels [4,5]. Other stage-independent markers not addressed in this study are malaise [15], low numbers of epidermal Langerhans cells [16], increased erythrocyte sedimentation rate [17], low sensitization rate to dinitrochlorobenzene testing [18] and complete response to initial treatment [3].

Recently the clinical staging system used for CTCL has been revised to incorporate blood findings (B rating) [19], and its relationship to disease progression and survival patterns has been confirmed by several groups [4,8,20]. To identify prognostic factors independent of clinical stage, we compared findings obtained at initial evaluation in a cohort of patients with clinically early MF (stages I–IIA) who had

subsequent progression of disease to a stage-matched MF control group without observed progression. Based on these results, we constructed prognostic categorizations based on widely available histo-immunopathologic findings that, if validated in larger studies, should be applicable to clinical practice.

Materials and methods

Case selection

To focus on the possible relationship of increased soluble CD30 (sCD30) serum levels with prognosis [21], we identified 33 cases with patch or plaque MF (12 at stage IA, 15 at stage IB and six at stage IIA) that had available stored sera obtained at initial evaluation and subsequent evidence of disease progression (P-MF group). For comparison, 70 stage-matched cases without disease progression were selected as controls (NP-MF group). This study was approved by the Institutional Review Board at Johns Hopkins Medical Institute.

Demographic and clinical variables, which were available for all patients, included patients' age, gender, race, duration of disease manifestations (time of onset of symptoms to time of evaluation), history of atopy (atopic dermatitis, allergic rhinitis or asthma), patch or plaque lesions of MF, extent of skin surface involvement ($< 10\%$, $10\text{--}50\%$, $> 50\%$), and currently recommended revised T-rating (T1a, T1b, T2a, T2b) and clinical stage (IA, IB, IIA) [22]. The response to initial skin-directed treatment was also recorded, but was not used for predictive purposes as this information is not known *a priori*.

For 100 of 103 cases, specimens of lesional skin were bisected, half processed for histopathology and half frozen in optimal cutting temperature (OCT) cryopreservative for immunohistochemistry (IHC). Specimens from two other patients were processed only for histopathology, and a biopsy was not performed in one patient. Specimens taken prior to referral or from other skin lesions were not reviewed.

Histopathologic variables included type of lesion biopsied (patch, thin plaque, thick plaque), Pautrier microabscesses (PMAs), defined as clusters of three or more atypical cells in the epidermis, pattern of dermal infiltrate, degree of dermal lymphocyte atypia (nuclear pleomorphism), the number (absent, less than 5% or 5–10%) of atypical lymphocytes with hyperchromatic or vesicular (open-chromatin pattern) nuclei, presence or absence of mitotic figures, eosinophils, plasma cells and multinucleated inflammatory giant cells in the dermal infiltrate, and infiltration of follicular epithelium with or without mucinosis.

By immunohistochemistry, the following T cell surface markers were routinely studied: CD2, CD3, CD4, CD5, CD7, CD8 and CD62L. CD30 and CD25 were studied in 68 and 20 cases, respectively. The level of surface marker expression by atypical cells in the epidermis was classified as positive, negative or partially expressed. The proportion of cells expressing T cell markers in the dermal infiltrate was visually estimated as a percentage relative to unlabeled cells.

Concurrent laboratory studies included a total leukocyte (WBC) count with differential count, number of Sézary cells per 100 lymphocytes, biochemical profile and serum immunoglobulin E (IgE) level (available in 96 cases). In addition, single parameter flow cytometry was utilized to measure percentage of lymphocytes expressing CD4, CD8, CD7 (101 cases) and activation markers human leukocyte antigen (HLA)-DR and CD25 and the natural killer cell marker CD16 (54 cases). Cytokine/cytokine receptor levels were measured at ARUP laboratories on stored frozen sera using multi-analyte fluorescent Luminex-based assays, as previously described [21,22].

Statistical analysis

Disease progression was defined as the development of cutaneous tumors or erythroderma (T3 or T4 skin rating) or pathologic evidence of extracutaneous involvement. For patients with P-MF, disease progression occurred within 5 years for 24 (73%) patients (median, 39 months; range, 3–123 months).

To ensure that the short follow-up of some patients with NP-MF did not affect the observed rates of disease progression associated for each variable, a separate analysis was performed using the 53 patients with NP-MF who were followed for at least 5 years.

Fisher's and Pearson's χ^2 exact tests were used to test for differences in categorized variables in 2×2 and $r \times c$ contingency tables, respectively. A Mann-Whitney rank-sum test was used to test for a difference in the median values between two groups. Spearman's rank-order correlation coefficient tested the strength of association between two variables. Overall survival and disease-specific survival were calculated from the date of initial evaluation to the date of death as a result of any cause or death caused by MF (deaths from advanced disease, infection or treatment toxicity), respectively. Patients who were alive at last contact or lost to follow-up were censored at the date of the last contact. The Kaplan-Meier method and Cox proportional hazards regression model were used to test for variables with prognostic significance. The log-rank test of Mantel and the multiple comparison procedure of Holm-Sidak were used to detect survival differences among groups. All tests were two-sided with significance level set at 0.05. SYSTAT 10, SigmaStat version 3 and SPSS 13.0 for Windows (SPSS Inc., Chicago, IL) and EGRET (Cytel Software Corporation, Cambridge, MA) were used for data analysis.

Results

Histopathologic observations

Epidermotropism by lymphocytes was observed in 99 (97%) specimens and varied from focal involvement, often with atypical cells aligning at the basal layer, to diffuse pagetoid infiltration of all layers of the epidermis. PMAs were present in 55 (54%) specimens, and these were further classified as small sized (3–10 atypical lymphocytes/cluster; 32 specimens) or large sized (> 10 atypical lymphocytes/cluster; 23 specimens, Figure 1). Analysis of different combinations of superficial and deep patterns of dermal infiltration indicated that perivascular or interstitial involvement of the reticular dermis,

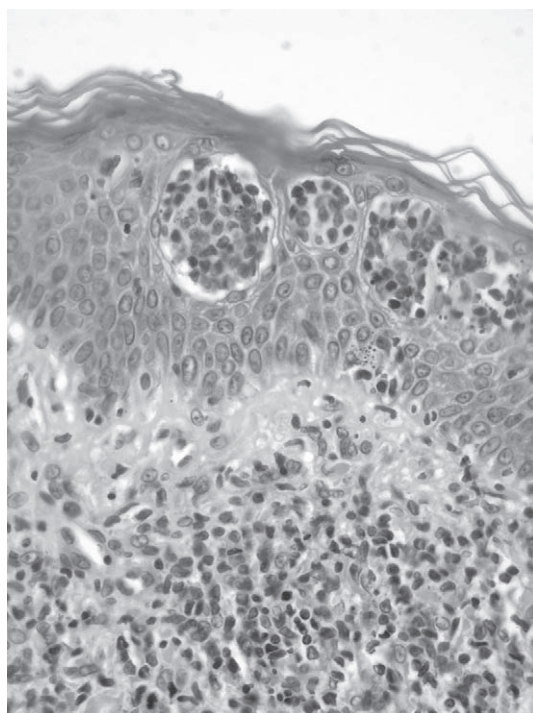


Figure 1. Photomicrograph of skin specimen obtained from a patient with progressive MF that shows large Pautrier microabscesses (clusters of more than 10 atypical cells within the epidermis), atypical lymphocytes aligning the basal layer and moderate lymphocyte atypia in the dermis (original magnification $\times 400$).

irrespective of pattern in the papillary dermis, was associated most strongly with P-MF, albeit marginally so ($p = 0.055$).

The degree of lymphocyte atypia in the dermal infiltrate was categorized as minimal (43 specimens), moderate (54 specimens) or marked (five specimens). Because assessment of atypia is subjective, we also recorded the number of easily identifiable atypical cell forms in the infiltrate. Atypical lymphocytes with hyperchromatic ("lumps of coal") or vesicular nuclei ("lymphoblasts") were identified in 31 (30%) and 46 (45%) specimens, respectively (Figure 2). Mitotic figures, eosinophils, plasma cells and multinucleated giant cells were seen in 17, 35, 32 and 11 specimens,

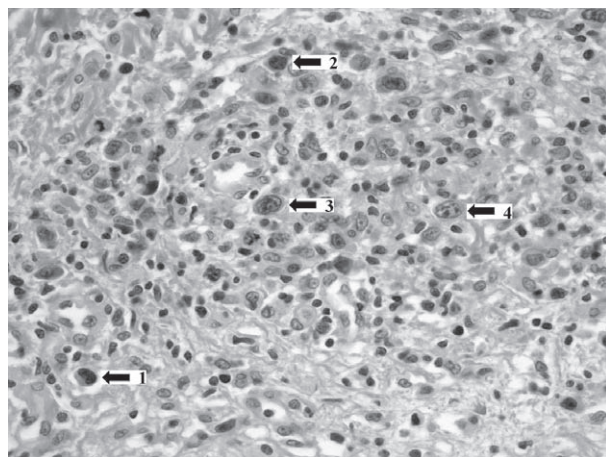


Figure 2. Photomicrograph of skin specimen obtained from a patient with progressive MF that shows severe lymphocyte atypia (nuclear pleomorphism) in the dermal infiltrate. The infiltrate contains both atypical cells with hyperchromatic nuclei (arrows 1 and 2) and vesicular nuclei (arrows 3 and 4) (original magnification $\times 400$).

respectively. Finally, a hair follicle was observed in 41 sections and lymphocytic infiltration of the follicular epithelium was present in 27 (66%) specimens, of which seven displayed follicular mucinosis.

Variables that correlated with both disease progression and overall survival

Not surprisingly, the 33 patients with P-MF experienced a significantly worse overall survival (death rate, 85%; median survival, 7.7 years) compared to the 70 controls with NP-MF (death rate, 37%; median survival, 13.6 years; $p < 0.001$; Supplementary Figure 1 to be found online at <http://informahealthcare.com/doi/abs/10.3109/10428194.2013.790541>). Disease-specific survival was also different ($p < 0.001$), but because details about the specific cause of death were not available for many deceased patients, we elected to use overall survival patterns for subsequent survival analysis. Also, overall survival patterns were compared in the Cox regression model with clinical stage entered as a covariate to adjust for the influence of stage.

Table I summarizes the variables that correlated with both disease progression and overall survival and therefore are considered to have high clinical relevance as potential prognostic indicators. The survival curves of variables used for multivariate analysis are shown in Supplementary Figures 2–6 to be found online at <http://informahealthcare.com/doi/abs/10.3109/10428194.2013.790541>. Although the presence of PMAs *per se* did not correlate with either disease progression or overall survival, the presence of large PMAs (> 10 atypical lymphocytes/cluster) was significantly associated with both P-MF and poor survival (Supplementary Figures 2 to be found online at <http://informahealthcare.com/doi/abs/10.3109/10428194.2013.790541>). Moderate (54 specimens) or marked (five specimens) lymphocyte atypia was more frequent in P-MF than in NP-MF cases ($p = 0.008$), and the survival patterns reflected this trend (Supplementary Figures 3 to be found online at <http://informahealthcare.com/doi/abs/10.3109/10428194.2013.790541>). Although lymphocytes with cerebriform nuclei (so-called MF cells) could be identified in the dermal infiltrate of almost all specimens, their numbers could not be readily quantified. Conversely, lymphoid cells with hyperchromatic or vesicular nuclei (LALs) could be quantified as absent, less than 5% or 5–10% of cells in the infiltrate. When analyzed separately, neither LAL type correlated significantly with P-MF, although the presence of lymphocytes with vesicular nuclei was suggestive ($p = 0.092$). However, when both atypical cell types were combined, a significant association with P-MF and poor overall prognosis became evident, and this provided a more objective assessment of atypia (Table I, Supplementary Figures 4 to be found online at <http://informahealthcare.com/doi/abs/10.3109/10428194.2013.790541>). Of note, increasing numbers of atypical cells with vesicular nuclei, but not cells with hyperchromatic nuclei, correlated with a worse prognosis, suggesting that these cells represent proliferating or transformed neoplastic cells (Supplementary Figures 7 to be found online at <http://informahealthcare.com/doi/abs/10.3109/10428194.2013.790541>).

Table I. Variables that significantly correlate with both progressive mycosis fungoides and poor overall survival.

	P-MF	NP-MF	Progression <i>p</i> -value [†]	Progression <i>p</i> -value [‡]	Survival <i>p</i> -value [§]	Survival <i>p</i> -value [¶]
Large PMAs (absent/present)*	17/14	59/9	0.002	0.001	0.012	0.044
Atypia (mild/moderate/marked)	8/21/4	35/33/1	0.008	0.002	0.002	0.005
LALs (absent/present)	7/26	32/37	0.017	0.007	0.001	0.001
Median % dermal CD8+ cells (range)**	10 (0–50)	20 (3–55)	0.003	0.021	–	0.035
Dermal CD8+ cells (<20/≥20%)*	17/11	21/39	0.037	0.049	0.026	0.045
Median % dermal CD7+ cells (range)	18 (2–60)	25 (0–90)	0.008	0.010	–	0.030
IgE (≤122/>122 U/mL)	15/18	48/15	0.003	0.001	0.046	0.034
Treatment response (CR/other) ^{††}	10/21	46/20	0.001	0.001	0.021	0.089

P-MF, progressive mycosis fungoides; NP-MF, non-progressive mycosis fungoides; PMA, Pautrier microabscess; LAL, atypical lymphocyte with hyperchromatic or vesicular nucleus; IgE, immunoglobulin E; CR, complete response.

*Three cases excluded because size of PMAs not determined.

[†]Comparison of P-MF and NP-MF cohorts by Mann-Whitney test (median values) or Fisher's or Pearson's χ^2 exact test (contingency tables).

[‡]Correlation with progression rates in Cox proportional hazards model with clinical stage as strata variable.

[§]Comparison of survival curves by log-rank method of Mantel.

[¶]Correlation with overall survival in Cox proportional hazards model with clinical stage as strata variable.

**CD8-expressing cases of MF excluded.

^{††}Response to initial skin-directed treatment.

The immunophenotype of neoplastic T cells was CD4+ CD8– for 92 cases (CD4 partially expressed in two cases), CD8+ CD4– for four cases, CD4+ CD8+ for three cases (confirmatory dual marker staining not performed), and one case that lacked expression of both CD4 and CD8 (staining for T cell receptor [TCR]- $\gamma\delta$ subset not performed). Of interest, the seven CD8-expressing cases of MF had a higher percentage of CD8+ cells in the dermal infiltrate (median, 50%, range, 8–75%) compared to 88 CD8-negative cases (median, 20%, range 0.1–55%; $p = 0.002$). No differences were seen for CD2, CD3, CD5, CD7 and CD62L. This observation suggests that the dermal infiltrate of some CD8+ MF cases comprises a proportion of CD8+ neoplastic T cells that express other pan-T markers. For this reason, subsequent analysis was performed on the 93 CD8-negative cases of MF.

Comparison of T-cell marker expression in the dermal infiltrate of P-MF and NP-MF cases revealed no significant differences in median percentages except for CD8 and CD7, which were significantly lower in the P-MF group than in the NP-MF group (Table I). The percentages were then dichotomized into two groups, specifically, CD8 < 20% and CD8 ≥ 20% according to Hoppe *et al.* [10] and CD7 ≤ 33% and CD7 > 33% according to Wood *et al.* [23]. For both variables, the lower percentage was associated significantly with P-MF. However, only a low percentage of dermal CD8+ cells significantly correlated with a worse prognosis (Supplementary Figures 5 to be found online at <http://informahealthcare.com/doi/abs/10.3109/10428194.2013.790541>). Because low CD8 percentage was associated with both disease progression and worse overall prognosis, it was chosen for multivariate analysis.

Finally, a significantly higher serum IgE level was found for MF cases that progressed (median, 146 U/mL) compared to cases that did not (median, 48 U/mL; $p = 0.016$). Moreover, when dichotomized into above normal (IgE > 122 U/mL) versus normal (IgE ≤ 122 U/mL), an abnormal IgE level occurred more than twice as often in the P-MF group than in the non-progressive group (Table I). In addition, an elevated IgE level was associated with a worse prognosis in the Cox model with clinical stage, and was therefore chosen for multivariate analysis.

Other prognostic variables

Variables that correlated with disease progression or overall survival but not both are listed in Supplementary Table I to be found online at <http://informahealthcare.com/doi/abs/10.3109/10428194.2013.790541>. Other variables of interest are listed in Supplementary Table II to be found online at <http://informahealthcare.com/doi/abs/10.3109/10428194.2013.790541>. Excluding the CD8-expressing MF cases, the percentage and absolute number of blood CD8+ cells correlated with prognosis. The percentage of blood CD8+ cells was then dichotomized into relatively low (<20%) and high (≥20%) groups, and a lower CD8+ cell percentage was significantly associated with a poor overall prognosis (Supplementary Figures 8 to be found online at <http://informahealthcare.com/doi/abs/10.3109/10428194.2013.790541>). Likewise, absolute CD8 counts were categorized into three groups according to Abeni *et al.* [13]. A relatively poor survival for our patients was associated with absolute CD8+ counts < 250 cells/mm³ (Supplementary Figures 9 to be found online at <http://informahealthcare.com/doi/abs/10.3109/10428194.2013.790541>).

Multivariate analysis

Categorized variables associated with progression and poor overall survival (Table I) were investigated in the Cox proportional hazards model with age (< 60, ≥ 60 years) entered as a strata variable and any cause of death as the event. To mitigate the effect of CD8+ neoplastic T cells on CD8+ cell numbers in skin lesions, analysis was restricted to 78 cases of CD4+ MF for which results on all variables were available. The strongest correlation with overall survival (lowest – 2 log likelihood score) was a model that included clinical stage, large PMAs, degree of atypia and < 20% CD8+ in the dermal infiltrate (– 2 log likelihood score: 216.22). Dichotomized IgE levels (≤ 122, > 122 U/mL) were not significant. If LALs in the dermal infiltrate were substituted for degree of atypia in the model, the correlation with survival was weaker but only slightly so (– 2 log likelihood score: 217.53), and all variables other than IgE remained significant. Skin T rating could be substituted for clinical stage with similar results, but the strength of correlation with overall survival was less

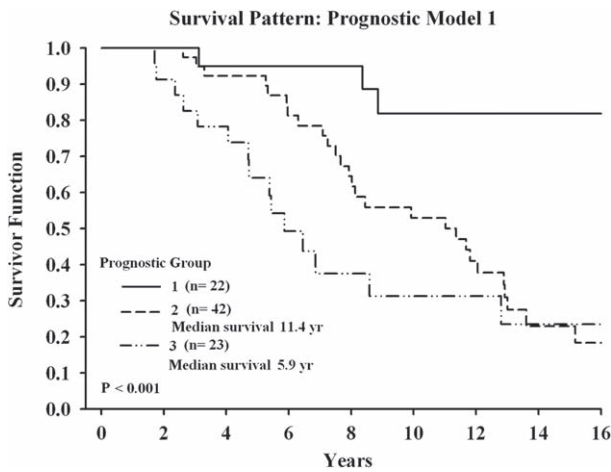


Figure 3. Overall survival patterns based on presence or absence of atypical lymphocytes with hyperchromatic or vesicular nuclei and percentage of CD8+ cells in the dermal infiltrate of CD4+ mycosis fungoides (stages IA-IIA). Prognostic group 1: atypical lymphocytes absent and dermal CD8+ cells $\geq 20\%$; group 2: either large atypical cells present or dermal CD8+ cells $< 20\%$; group 3: large atypical cells present and dermal CD8+ cells $< 20\%$.

than for clinical stage ($-2 \log$ likelihood score: 221.07), presumably due to the effect of stage IIA on survival.

A similar analysis in the Cox model using disease-specific death as the event confirmed the prognostic importance of large PMAs, lymphocyte atypia and $< 20\%$ dermal CD8+ cells in the dermal infiltrate, although substituting LALs for atypia weakened the model.

Prognostic groupings

Two three-tiered prognostic groupings were developed based on combinations of large PMAs and LALs with hyperchromatic or vesicular nuclei and CD8+ cells in the dermal infiltrate. Because we are uncertain about the frequency of large PMAs if multiple specimens or sections could be examined, the first model was constructed based on LALs and proportion of CD8+ cells: (1) absence of LALs in dermis and CD8+ cells $\geq 20\%$ ($n = 22$), (2) either LALs present or CD8+ cells $< 20\%$ ($n = 42$) and (3) LALs in dermis present and CD8+ cells $< 20\%$ ($n = 23$). Although the difference in overall survival between prognostic groups 2 and 3 was not significant (Figure 3), the difference in disease-specific survival curves was statistically significant among the three

groups (data not shown). A summary of the prognostic implications is provided in Table II.

The proposed model predicts that prognostic categorization will identify patients with CD4+ MF who are at risk of progression and subsequent poor prognosis at clinical stages IA, IB or IIA. Indeed, survival curves for subsets of our cases at each stage showed the expected trends. Because only 14 of our patients were at clinical stage IIA and 12 of these cases had T2 skin rating, we show the survival curves for T1 and T2 disease in Supplementary Figures 10 and 11 to be found online at <http://informahealthcare.com/doi/abs/10.3109/10428194.2013.790541>.

The second model was based on the frequency of abnormal findings: (1) no finding present ($n = 19$); (2) one finding present ($n = 26$); two findings present ($n = 28$); and (3) all three findings present ($n = 5$). Because the survival curves for patients with one and two findings were nearly identical, these were combined ($n = 54$). The overall and disease-specific survival curves differed significantly among the three groups (Supplementary Figures 12 to be found online at <http://informahealthcare.com/doi/abs/10.3109/10428194.2013.790541>). A summary of the prognostic implications is provided in Supplementary Table III to be found online at <http://informahealthcare.com/doi/abs/10.3109/10428194.2013.790541>.

Discussion

The factors that correlated most strongly with progression ($0.001 < p < 0.01$) and poor overall survival are the presence of large-sized PMAs, moderate to marked lymphocyte atypia in the dermal infiltrate which could be more objectively defined using the presence of LALs, diminished proportion of CD7+ and CD8+ cells in the dermal infiltrate and increased serum IgE (Table I). Multivariate analysis confirmed that these histologic and immunopathologic variables, but not IgE, were independently significant in a Cox model that included clinical stage as a covariate, and this enabled construction of stratified groups that, if confirmed with a larger patient population, might be used to predict prognosis for patients with clinically early MF. These factors are discussed in more detail below. A complete response to initial skin-directed treatment also seems to be important both in inhibiting disease progression and in improving survival as previously reported [3,24].

Table II. Prognostic implications based on atypical lymphocytes with hyperchromatic or vesicular nuclei and proportion of CD8+ cells in the dermal infiltrate of early CD4+ mycosis fungoides (stages I-IIA).

Prognostic group*	No.	No. P-MF (%)	Death rate		Survival [CI]		
			Any cause (%)	MF-related (%)†	Median (years)	5-year (%)‡	HR§
1	22	1 (5)	3 (14)	0 (0)	–	95.0 [69.5, 99.3]	1.00
2	42	15 (36)	27 (64)	7 (17)	11.4 [6.8, 15.9]	92.3 [78.0, 97.5]	4.27 [1.28, 14.17]
3	23	11 (48)	15 (65)	8 (35)	5.9 [4.4, 7.3]	64.1 [40.6, 80.2]	8.10 [2.30, 28.50]
All	87	27 (31)	45 (52)	15 (17)	11.7 [8.6, 14.8]	85.3 [75.6, 91.4]	

MF, mycosis fungoides; P-MF, progressive MF; CI, 95% confidence interval; HR, hazard ratio.

*Group 1: atypical lymphocytes absent and CD8+ cells $\geq 20\%$ in dermal infiltrate; group 2: either atypical lymphocytes present or CD8+ cells $< 20\%$ in dermal infiltrate; group 3: both atypical lymphocytes present and CD8+ cells $< 20\%$ in dermal infiltrate.

†May be underestimated because details about cause of death lacking in some patients.

‡10-year survival rates [CI], group 1: 81.9% [53.1, 93.9]; group 2: 53.0% [35.6, 67.7]; group 3: 31.3% [12.5, 52.3]; all patients: 54.0% [41.8, 64.8].

§HR relative to group 1 in Cox model with categorized age (< 60 , ≥ 60 years) entered as a strata variable and clinical stage as a covariate.

Other factors that correlated significantly ($0.01 < p < 0.05$) with progression but not overall survival may be a function of skin tumor burden and not the growth properties of neoplastic T cells *per se* (Supplementary Table I to be found online at <http://informahealthcare.com/doi/abs/10.3109/10428194.2013.790541>). It is possible that the slight increase in the median percentage of CD4+ cells and decrease in the median percentage of HLA-DR+ cells in the blood are the result of low levels of circulating CD4+ HLA-DR- neoplastic cells that are more apt to occur in P-MF cases. However, Sézary cell counts did not support this interpretation. Furthermore, because neoplastic T cells can secrete interleukin 6 (IL-6) and IL-10 [25,26], this could explain the increased median IL-6 and above normal IL-10 serum levels associated with P-MF.

The patients in our P-MF cohort had more aggressive disease compared to patients with MF at comparable stages in the general MF population. For example, 73% of patients with P-MF had progression to stage IIB or higher within the first 5 years of follow-up. By contrast, using the annual incidence data of progression to stage IIB or higher (our criterion of progression) that are provided in Table III in the article by Quaglino *et al.* [20], we calculate that a maximum of 24% of Italian patients at comparable clinical stages might have experienced progression to advanced disease by 5 years. This three-fold difference between actual and calculated progression rates was highly significant ($p < 0.001$). This conclusion was supported by other studies in which progression to a higher stage (a different criterion of progression from the one we utilized) after 5 years has been reported to be less than 10% for stage IA and about 20% for stage IB [3,4]. Therefore, factors identified in this study to be associated with both disease progression and poor overall survival should be considered to be markers of relatively aggressive MF.

We hypothesize that these prognostic variables reflect a diminished host response mediated by CD8+ cells coupled with an increased number of activated or transformed neoplastic T helper type 2 (T_H2) cells in skin lesions of P-MF compared to NP-MF. CD8+ cells in skin lesions of MF are activated (HLA-DR+) and express cytotoxic proteins and Fas ligand [11,27]. Low percentages of CD8+ cells in the dermal infiltrate are more apt to occur in MF tumors than in plaques, and as observed in this study, this finding signifies an adverse prognosis that is independent of clinical stage [10,11]. The poor prognosis associated with low absolute blood CD8+ lymphocyte count is also consistent with a diminished CD8+ host response [13,28]. It has been proposed that CD8+ cells in skin lesions are eliminated by Fas ligand expressed by neoplastic T lymphocytes [29]. Whether this mechanism contributes to diminished circulating CD8+ numbers or whether the low CD8+ levels in skin and blood reflect a relatively ineffective systemic host immune response is unknown.

The strong association of large-sized PMAs with disease progression and prognosis has not been reported before to our knowledge. Smoller *et al.* compared histopathologic findings, including number of PMAs observed per high magnification field, in 26 cases of P-MF vis-à-vis 21 cases of NP-MF and found no difference between the groups [30].

We also observed the presence of PMAs of any size not to be significantly different. Considering that PMAs are clusters of neoplastic T cells surrounding epidermal Langerhans cells and that Langerhans cells provide stimulatory signals to neoplastic T cells [31,32], we postulate that large-sized PMAs develop as a result of enhanced growth of neoplastic cells within the epidermal microenvironment and thereby unfavorable prognosis.

The degree of lymphocyte atypia as a prognostic marker also was not addressed in Smoller's article, although the number of cells with hyperconvoluted nuclei was slightly higher in P-MF cases than in NP-MF cases ($p < 0.1$). Hödl classified patients with CTCL (47 with MF, 13 with Sézary syndrome) according to cytomorphologic features in the dermal infiltrate, and observed a 10-year survival rate of 68% for the small lymphoid cell type and 30% for the mixed polymorphic cell type [33]. Brooks *et al.* observed that MF cases with larger nuclear volumes were associated with a worse prognosis [34]. Because the assessment of lymphocyte atypia is subjective, we categorized our cases based on the presence or absence of LALs, defined as atypical lymphocytes with hyperchromatic ("lumps of coal") or vesicular nuclei ("lymphoblasts"). The former cells presumably represent cells with hyperdiploid DNA content, whereas the latter cells are activated or transformed cells [35].

The association of increased total serum IgE levels with disease progression and poor survival is of interest. However, this prognostic variable did not retain significance when subjected to multivariate analysis in a model that included large PMAs, and the presence of LALs and a low proportion of CD8+ cells in the dermal infiltrate. The reason appears to be a significant correlation between the presence of LALs and increased IgE levels. Specifically, 23/53 (43%) cases with LALs had increased IgE levels compared to 6/32 (19%) without LALs ($p = 0.033$). This observation suggests that neoplastic T cells might be mediating IgE secretion.

IgE production by B cells requires two signals provided by the interaction of T cells via CD40L/CD40 (first signal) and cytokines IL-4 and IL-13 secreted by activated T cells (second signal) [36]. Although CD40L and IL-13 were detected in the sera of 65% and 46%, respectively, of patients with clinically early MF, their levels were, if anything, lower in P-MF cases than in NP-MF cases, whereas the converse might be expected if an increased number of activated CD40L-expressing neoplastic T cells secreting IL-13 were present in the skin lesions of cases prone to progression. Furthermore, no correlation was found between levels of these cytokines and IgE. In this study, a history of atopy or any of its components (atopic dermatitis, allergic rhinitis, asthma) was not associated with either disease progression or adverse prognosis. Finally, IgE levels were not found to have prognostic importance in an earlier study by the Mycosis Fungoides Cooperative Group (MFCG) [15]. Additional studies are needed to clarify the relationship between IgE levels and prognosis.

The present study confirmed our prior observation that above normal sCD25 and sCD30 levels connote a poor overall survival in clinically early MF when considered as univariates, but not in Cox models that include clinical stage. Contrary to the study of Edinger *et al.* [12], we and

others [8] found no association between a higher proportion of CD30+ cells in the dermal infiltrate and either disease progression or overall prognosis.

In summary, we identified practical biomarkers for prognostication of patients with early MF which should be confirmed in a larger retrospective or prospective study of patients from the general MF population.

Potential conflict of interest: Disclosure forms provided by the authors are available with the full text of this article at www.informahealthcare.com/lal.

References

- [1] Pimpinelli N, Olsen EA, Santucci M, et al. Defining early mycosis fungoides. *J Am Acad Dermatol* 2005;53:1053–1063.
- [2] Kim YH, Liu HL, Mraz-Gernhard S, et al. Long-term outcome of 525 patients with mycosis fungoides and Sézary syndrome: clinical prognostic factors and risk for disease progression. *Arch Dermatol* 2003;139:857–866.
- [3] van Doorn R, Van Haselen CW, van Voorst Vader PC, et al. Mycosis fungoides: disease evolution and prognosis of 309 Dutch patients. *Arch Dermatol* 2000;136:504–510.
- [4] Agar NS, Wedgeworth E, Crichton S, et al. Survival outcomes and prognostic factors in mycosis fungoides/Sézary syndrome: validation of the revised International Society for Cutaneous Lymphomas/European Organisation for Research and Treatment of Cancer staging proposal. *J Clin Oncol* 2010;28:4730–4739.
- [5] Diamandidou E, Colome M, Fayad L, et al. Prognostic factor analysis in mycosis fungoides/Sézary syndrome. *J Am Acad Dermatol* 1999;40:914–924.
- [6] Jones GW, Wilson LD. The changing survival of patients with mycosis fungoides: a population-based assessment of trends in the United States. *Cancer* 1999;86:191–193.
- [7] Suzuki SY, Ito K, Ito M, et al. Prognosis of 100 Japanese patients with mycosis fungoides and Sézary syndrome. *J Dermatol Sci* 2010;57:37–43.
- [8] Talpur R, Singh L, Daulat S, et al. Long-term outcomes of 1,263 patients with mycosis fungoides and Sézary syndrome from 1982 to 2009. *Clin Cancer Res* 2012;18:5051–5060.
- [9] Martí RM, Estrach T, Reverter JC, et al. Prognostic clinicopathologic factors in cutaneous T-cell lymphoma. *Arch Dermatol* 1997;127:1511–1516.
- [10] Hoppe RT, Medeiros LJ, Warnke RA, et al. CD8-positive tumor-infiltrating lymphocytes influence the long-term survival of patients with mycosis fungoides. *J Am Acad Dermatol* 1995;32:448–453.
- [11] Vermeer MH, van Doorn R, Dukers D, et al. CD8+ T cells in cutaneous T-cell lymphoma: expression of cytotoxic proteins, Fas Ligand, and killing inhibitory receptors and their relationship with clinical behavior. *J Clin Oncol* 2001;19:4322–4329.
- [12] Edinger JT, Clark BZ, Pucevich BE, et al. CD30 expression and proliferative fraction in nontransformed mycosis fungoides. *Am J Surg Pathol* 2009;33:1860–1868.
- [13] Abeni D, Frontani M, Sampogna E, et al. Circulating CD8+ lymphocytes, white blood cells, and survival in patients with mycosis fungoides. *Br J Dermatol* 2005;153:324–330.
- [14] Tancrede-Bohin E, Ionescu MA, de La Salmonière P, et al. Prognostic value of blood eosinophilia in primary cutaneous T-cell lymphomas. *Arch Dermatol* 2004;140:1057–1061.
- [15] Green SB, Byar DP, Lamberg SI. Prognostic variables in mycosis fungoides. *Cancer* 1981;47:2671–2677.
- [16] Meissner K, Löning T, Reppenning W. Epidermal Langerhans cells and prognosis of patients with mycosis fungoides and Sézary syndrome. *In Vivo* 1993;7:277–280.
- [17] Hallermann C, Niermann C, Fischer RJ, et al. Erythrocyte sedimentation rate as an independent prognostic factor in mycosis fungoides. *Br J Dermatol* 2012;166:873–874.
- [18] Vonderheid EC, Ekbot SK, Kerrigan K, et al. The prognostic significance of delayed hypersensitivity to dinitrochlorobenzene and mechlorethamine hydrochloride in cutaneous T cell lymphoma. *J Invest Dermatol* 1998;110:946–950.
- [19] Olsen E, Vonderheid E, Pimpinelli N, et al. Revisions to the staging and classification of mycosis fungoides and Sézary syndrome: a proposal of the International Society for Cutaneous Lymphomas (ISCL) and the cutaneous lymphoma task force of the European Organization of Research and Treatment of Cancer (EORTC). *Blood* 2007;110:1713–1722.
- [20] Quaglino P, Pimpinelli N, Berti E, et al. Time course, clinical pathways, and long-term hazards risk trends of disease progression in patients with classic mycosis fungoides: a multicenter, retrospective follow-up study from the Italian Group of Cutaneous Lymphomas. *Cancer* 2012;118:5830–5839.
- [21] Kadin ME, Pavlov IY, Delgado JC, et al. High soluble CD30, CD25, and IL-6 may identify patients with worse survival in CD30+ cutaneous lymphomas and early mycosis fungoides. *J Invest Dermatol* 2012;132:703–710.
- [22] Martins TB, Pasi BM, Pickering JW, et al. Determination of cytokine responses using a multiplexed fluorescent microsphere immunoassay. *Am J Clin Pathol* 2002;118:346–353.
- [23] Wood GS, Abel EA, Hoppe RT, et al. Leu-8 and Leu-9 antigen phenotypes: immunologic criteria for the distinction of mycosis fungoides from cutaneous inflammation. *J Am Acad Dermatol* 1986;14:1006–1013.
- [24] Vonderheid EC, Tan ET, Kantor AF, et al. Long-term efficacy, curative potential, and carcinogenicity of topical mechlorethamine chemotherapy in cutaneous T cell lymphoma. *J Am Acad Dermatol* 1989;20:416–428.
- [25] Buhl L, Søgaard H. Immunohistochemical expression of IL-10 in mycosis fungoides. *Exp Dermatol* 1997;6:195–198.
- [26] Nielsen M, Nissen MH, Gerwien J, et al. Spontaneous interleukin-5 production in cutaneous T-cell lymphoma lines is mediated by constitutively activated Stat3. *Blood* 2002;99:973–977.
- [27] Wood GS, Edinger A, Hoppe RT, et al. Mycosis fungoides skin lesions contain CD8+ tumor-infiltrating lymphocytes expressing an activated, MHC-restricted cytotoxic T-lymphocyte phenotype. *J Cutan Pathol* 1994;21:151–156.
- [28] Asadullah K, Friedrich M, Döcke WD, et al. Enhanced expression of T-cell activation and natural killer cell antigens indicates systemic anti-tumor response in early primary cutaneous T-cell lymphoma. *J Invest Dermatol* 1997;108:743–747.
- [29] Ni X, Hazarika P, Zhang C, et al. Fas ligand expression by neoplastic T lymphocytes mediates elimination of CD8+ cytotoxic T lymphocytes in mycosis fungoides: a potential mechanism of tumor immune escape? *Clin Cancer Res* 2001;7:2682–2692.
- [30] Smoller BR, Detwiler SP, Kohler S, et al. Role of histology in providing prognostic information in mycosis fungoides. *J Cutan Pathol* 1998;25:311–315.
- [31] Schmitt D, Thivolet J. Lymphocyte-epidermis interactions in malignant epidermotropic lymphomas: I. Ultrastructural aspects. *Acta Derm Venereol* 1980;60:1–11.
- [32] Edelson RL. Cutaneous T cell lymphoma: the helping hand of dendritic cells. *Ann NY Acad Sci* 2001;941:1–11.
- [33] Hödl S [Mycosis fungoides and Sézary syndrome. A retrospective study of 60 patients with correlation between histologic classification and survival time.] *Z Hautkr* 1986;61:993–1013.
- [34] Brooks B, Sørensen FB, Thestrup-Pedersen K. Estimates of nuclear volume in plaque and tumor-stage mycosis fungoides. A new prognostic indicator. *Am J Dermatopathol* 1994;16:599–606.
- [35] van Vloten WA, van Duijn P, Schaberg A. Cytodiagnostic use of Feulgen-DNA measurements in cell imprints from the skin of patients with mycosis fungoides. *Br J Dermatol* 1974;91:365–371.
- [36] Poulsen LK, Hummelshøj L. Triggers of IgE class switching and allergy development. *Ann Med* 2007;39:440–456.

Supplementary material available online

Supplementary Tables I–III and Figures 1–12 showing further results

Supplementary Tables I–III

Supplementary Table I. Variables that significantly correlate with either progressive mycosis fungoides or overall survival, but not both.

	P-MF	NP-MF	Progression P-value [†]	Progression P-value ^{††}	Survival P-value [‡]	Survival P-value [§]
Age (< 60/≥ 60 yrs)	15/18	31/39	1.000	0.636	< 0.001	< 0.001
Male/Female	16/17	49/21	0.049	0.141	0.464	0.963
Duration symptoms (< 60/≥ 60 mos)	18/15	35/35	0.679	0.552	0.016	0.025
T-rating (1a/1b/2a/2b)	4/8/2/19	10/22/2/36	0.756	0.278	0.026 [¶]	–
Stage (IA/IB/IIA)	12/15/6	30/32/8	0.650	–	0.012 [¶]	–
Dermal CD7+ cells (≤ 33/> 33%)	26/3	42/24	0.010	0.018	0.272	0.129
Median % CD4+ cells in blood (range)	48 (23–68)	42 (14–69)	0.013	0.023	–	0.932
Median % HLA-DR+ cells in blood (range)	17 (8–26)	20 (12–39)	0.017	0.044	–	0.473
% CD8+ cells in blood (< 20/≥ 20%) [*]	11/20	19/44	0.624	0.049	0.002	0.013
Absolute CD8+ cells in blood (< 250/≥ 250 /mm ³) [*]	10/21	19/43	1.000	0.695	0.002	0.011
Median serum IL-6, pg/mL [*] (range)	0.9 (0–104)	0.4 (0–9.6)	0.034	0.037	–	0.336
Serum IL-10* (normal/ increased)	22/11	62/8	0.013	0.013	0.301	0.126
Median serum albumin, gm/ dl (range)	4.6 (3.6–5.6)	4.6 (3.6–5.5)	0.530	0.260	–	0.001
Median A/G (range)	1.5 (0.8–2.6)	1.5 (0.9–2.4)	0.991	0.015	–	0.044

Abbreviations: P-MF and NP-MF, progressive and non-progressive mycosis fungoides; A/G, albumin/globulin ratio.

*Normal ranges established at ARUP laboratories are as follows: IL-6 (0–5 pg/mL) and IL-10 (0–18 pg/mL).

[†]Comparison of P-MF and NP-MF cohorts by Mann-Whitney test (median values) or Fisher's or Pearson's chi-squared exact test (contingency tables).

^{††}Correlation with progression rates in Cox proportional hazards model with clinical stage as strata variable.

[‡]Comparison of survival curves by log-rank method of Mantel.

[§]Correlation with overall survival in Cox proportional hazards model with clinical stage as strata variable.

^{*}CD8-expressing MF cases excluded.

[¶]No significant difference among survival curves for T-ratings and between stage IB versus IIA.

Supplementary Table II. Selected laboratory studies of interest but without apparent prognostic importance for patients with progressive and non-progressive mycosis fungoides (stages I to IIA).

	P-MF	NP-MF	Progression P-value [†]	Progression P-value ^{††}	Survival P-value [‡]	Survival P-value [§]
Median lymphocyte count/mm ³ (range)	1416 (580–3168)	1633 (496–3248)	0.170	0.120	–	0.092
Median eosinophil count/mm ³ (range)	152 (0–2821)	120 (0–1390)	0.171	0.238	–	0.425
Sézary cell count# (< 5/≥ 5%)	22/3	44/4	0.685	0.811	0.314	0.632
Percent CD30+ cells In dermal infiltrate	3 (0–10)	3 (0–25)	0.387	0.749	–	0.888
Serum sCD25* (normal/increased)	25/8	61/9	0.163	0.274	0.032	0.285
Serum sCD30* (normal/increased)	22/11	53/17	0.352	0.336	0.031	0.301
sCD25 or sCD30* (normal/increased)	20/13	49/21	0.375	0.379	0.007	0.122
Serum IL-17A* (normal/increased)	28/5	60/10	1.000	0.534	0.518	0.185
Serum CD40L* (normal/increased)	23/10	37/33	0.135	0.107	0.397	0.959
Serum IL-1β* (normal/increased)	31/2	66/4	1.000	0.858	0.485	0.650
Alkaline Phosphatase (normal/ increased)	29/4	62/7	0.744	0.476	0.035	0.091
Uric acid, mg/dl (normal/increased)	31/1	62/5	0.661	0.352	0.012	0.058
LDH, U/L (normal/increased)	20/12	51/16	0.232	0.188	0.066	0.220

Abbreviations: P-MF and NP-MF, progressive and non-progressive mycosis fungoides, LDH, lactate dehydrogenase.

*Normal ranges established at ARUP laboratories are as follows: sCD25/IL-2R (0–1033 pg/mL), sCD30 (1–29 ng/mL), sCD40L (0–244 pg/mL), IL-1β (0–36 pg/mL). IL-17A is normally undetectable.

[†]Comparison of P-MF and NP-MF cohorts by Mann-Whitney test (median values) or Fisher's exact test (contingency tables).

^{††}Correlation with progression rates in Cox proportional hazards model with clinical stage as strata variable.

[‡]Comparison of survival curves by log-rank method of Mantel.

[§]Correlation with overall survival in Cox proportional hazards model with clinical stage as strata variable.

[#]Sézary cells per 100 lymphocytes on blood smear.

Supplementary Table III. Prognostic implications based on number of prognostic histo-immunopathologic findings in early CD4 + mycosis fungoides (stages I to IIA).

Prognostic Group*	No.	No. P-MF (%)	Death Rate		Survival [CI]		
			Any cause (%)	MF-specific (%) [‡]	Median (yr)	5-year (%) [§]	HR [†]
1	18	0 (0)	2 (11)	0 (0)	–	100	1.00
2	55	21 (38)	33 (60)	10 (18)	8.6	84.2	6.33
					[5.0, 12.2]	[70.8, 91.8]	[1.51, 26.54]
3	5	4 (80)	5 (100)	4 (80)	5.4	60.0	21.59
					[2.5, 8.2]	[12.6, 88.2]	[4.06, 114.891]
All	78	25 (32)	40 (51)	14 (18)	11.4	86.3	–
					[7.8, 14.9]	[75.9, 92.4]	

Abbreviations: MF, mycosis fungoides, P-MF, progressive MF, CI, 95% confidence interval, HR, hazard ratio.
*Group 1: CD8+ cells <20%, atypical lymphocytes with hyperchromatic or vesicular nuclei in dermal infiltrate, large Pautrier microabscesses absent; Group 2: any two criteria present; Group 3: all three criteria present.
[†]HR relative to group 1 in Cox model with categorized age (<60, ≥60 years) entered as a strata variable and clinical stage as a covariate.
[‡]May be underestimated because details about cause of death lacking in some patients.
[§]10-year survival rates [CI], group 1: 83.9 [49.4, 95.7]; group 2: 47.0 [31.7, 60.8]; group 3: 20.0 [0.8, 58.2]; all patients: 53.6 [40.5, 65.0].

Supplementary Figures 1–12

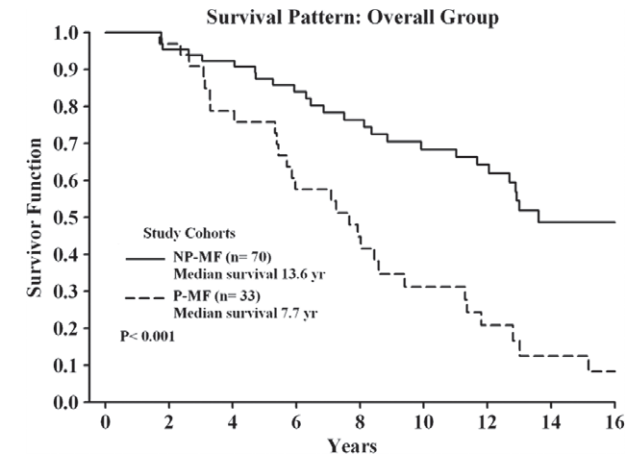


Figure 1. Supplementary Overall survival patterns for selected patients with progressive and non-progressive mycosis fungoides (stages IA to IIA).

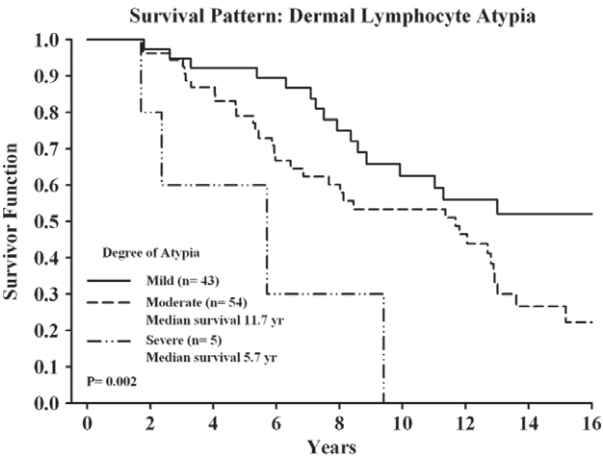


Figure 3. Supplementary Overall survival patterns according to degree of lymphocyte atypia (nuclear pleomorphism) in the dermal infiltrate of selected patients with mycosis fungoides (stages IA to IIA).

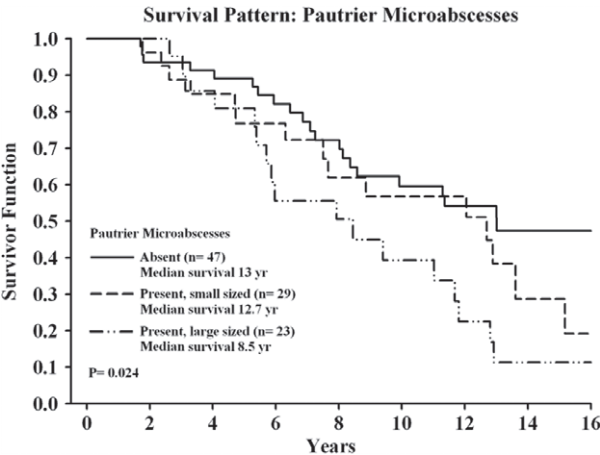


Figure 2. Supplementary Overall survival patterns according to presence or absence of Pautrier microabscesses in selected patients with mycosis fungoides (stages IA to IIA). The presence of large sized Pautrier microabscesses (>10 atypical cells/cluster) was associated with poor outcome.

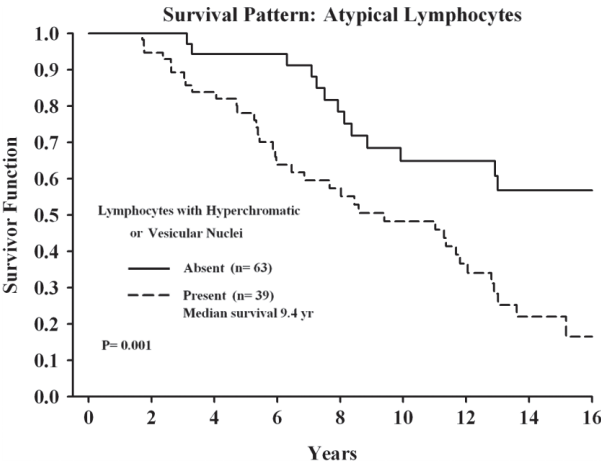


Figure 4. Supplementary Overall survival patterns according to presence or absence of atypical lymphocytes with hyperchromatic or vesicular nuclei in the dermal infiltrate of selected patients with mycosis fungoides (stages IA to IIA).

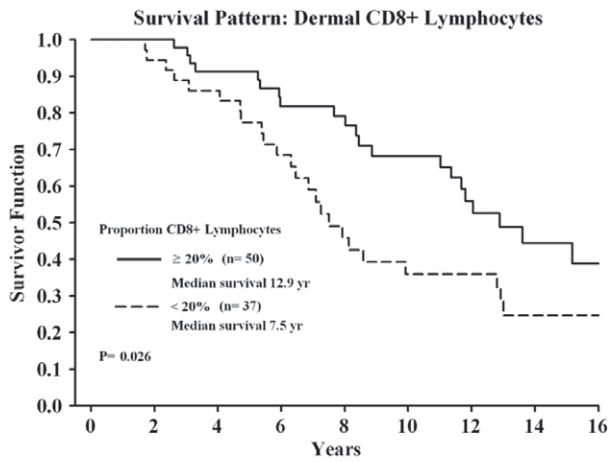


Figure 5. Supplementary Overall survival patterns according to relatively low ($<20\%$) versus high ($\geq 20\%$) proportion of CD8+ lymphocytes in the dermal infiltrate of selected patients with CD4+ mycosis fungoides (stages IA to IIA).

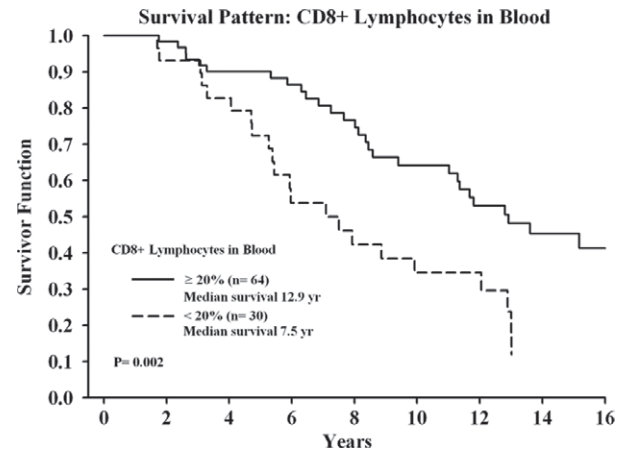


Figure 8. Supplementary Overall survival patterns according to relatively low ($<20\%$) versus high ($\geq 20\%$) CD8+ cells in the blood of selected patients with CD4+ mycosis fungoides (stages IA to IIA).

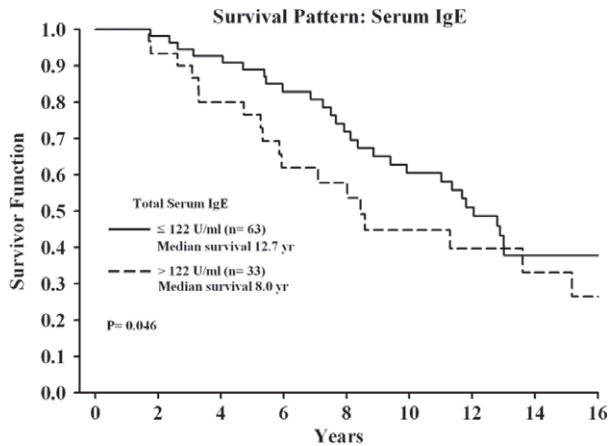


Figure 6. Supplementary Overall survival patterns according to normal or increased levels of total IgE in the blood of selected patients with mycosis fungoides (stages IA to IIA).

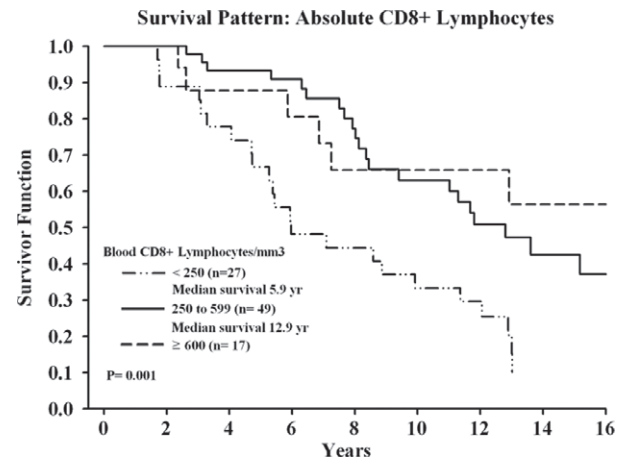


Figure 9. Supplementary Overall survival patterns according to absolute number of CD8+ cells in the blood of selected patients with CD4+ mycosis fungoides (stages IA to IIA).

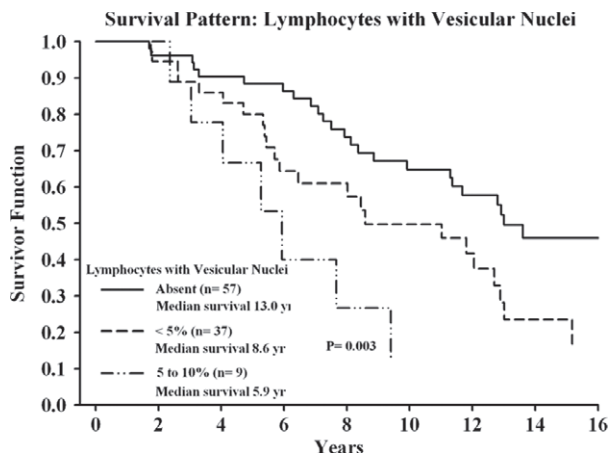


Figure 7. Supplementary Overall survival patterns according to number of large atypical cells with vesicular nuclei in the dermal infiltrate of selected patients with mycosis fungoides (stages IA to IIA).

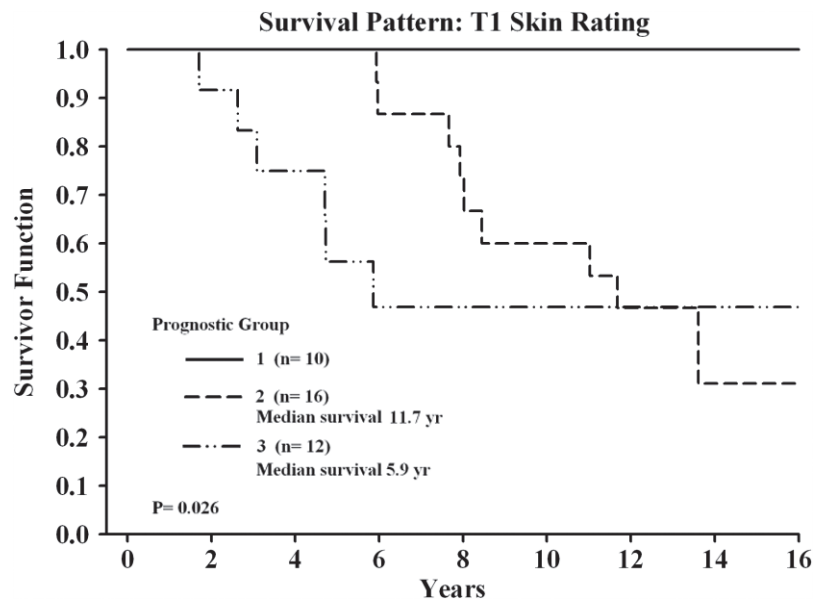


Figure 10. Supplementary Overall survival patterns based on presence or absence of large atypical lymphocytes and percentage of CD8+ cells in the dermal infiltrate of CD4+ mycosis fungoides (skin rating T1). Prognostic Group 1: LALs absent and dermal CD8+ cells $\geq 20\%$; Group 2: Either LALs present or dermal CD8+ cells $< 20\%$; Group 3: LALs present and dermal CD8+ cells $< 20\%$.

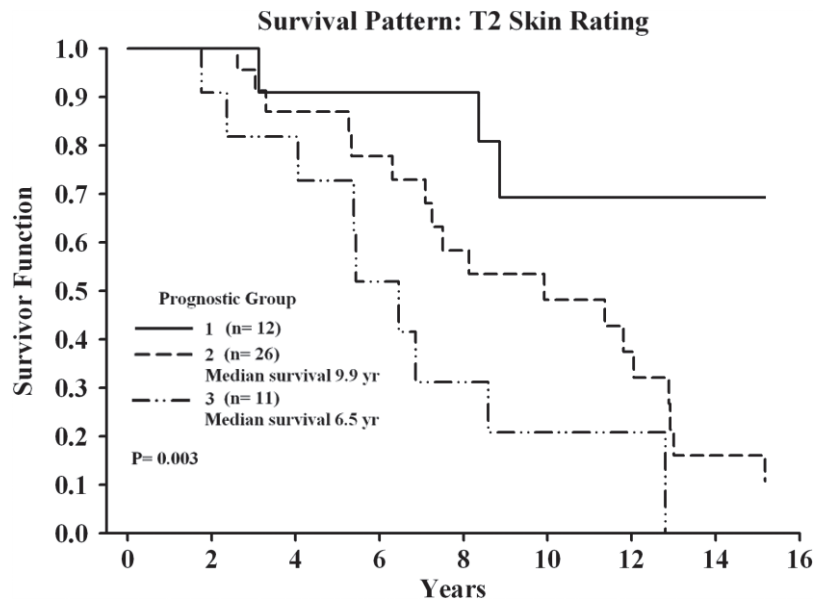


Figure 11. Supplementary Overall survival patterns based on presence or absence of large atypical lymphocytes and percentage of CD8+ cells in the dermal infiltrate of CD4+ mycosis fungoides (skin rating T2). Prognostic Group 1: LALs absent and dermal CD8+ cells $\geq 20\%$; Group 2: Either LALs present or dermal CD8+ cells $< 20\%$; Group 3: LALs present and dermal CD8+ cells $< 20\%$.

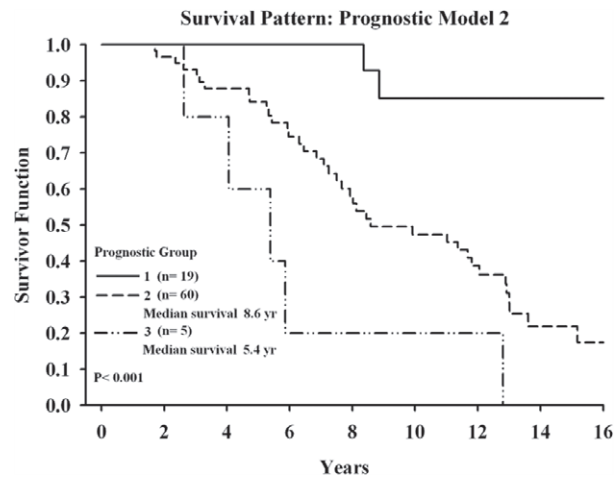


Figure 12. Supplementary Overall survival patterns based on presence or absence of large Pautrier microabscesses (PMAs) in the epidermis, atypical lymphocytes with hyperchromatic or vesicular nuclei (LALs) and percentage of CD8+ cells in the dermal infiltrate of CD4+ mycosis fungoides (stages IA to IIA). Prognostic Group 1: Large PMAs absent, LALs absent, CD8 $\geq$ 20%; Group 2: One or two variables present; Group 3: All three variables present.