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ORIGINAL ARTICLE

Histology-based classifier to distinguish early mycosis fungoides from atopic dermatitis

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Abstract

Background: Histopathological differentiation of early mycosis fungoides (MF) from benign chronic inflammatory dermatoses remains difficult and often impossible, despite the inclusion of all available diagnostic parameters.

Objective: To identify the most impactful histological criteria for a predictive diagnostic model to discriminate MF from atopic dermatitis (AD).

Methods: In this multicentre study, two cohorts of patients with either unequivocal AD or MF were evaluated by two independent dermatopathologists. Based on 32 histological attributes, a hypothesis-free prediction model was developed and validated on an independent patient's cohort.

Results: A reduced set of two histological features (presence of atypical lymphocytes in either epidermis or dermis) was trained. In an independent validation cohort, this model showed high predictive power (95% sensitivity and 100% specificity) to differentiate MF from AD and robustness against inter-individual investigator differences.

Limitations.: The study investigated a limited number of cases and the classifier is based on subjectively evaluated histological criteria.

Conclusion: Aiming at distinguishing early MF from AD, the proposed binary classifier performed well in an independent cohort and across observers. Combining this histological classifier with immunohistochemical and/or molecular techniques (such as clonality analysis or molecular classifiers) could further promote differentiation of early MF and AD.

INTRODUCTION

Mycosis fungoides (MF) is the most common primary cutaneous T-cell lymphoma (CTCL), arising from skin-tropic memory T lymphocytes.[1] The incidence of CTCL is about 1 per 100,000 people and 40%–70% of cutaneous lymphomas fall within the diagnosis of MF.[2–4]

The diagnosis of early MF is a challenge. Clinical and histopathological findings of early MF can be very subtle and often mimic those of benign chronic inflammatory dermatoses like atopic dermatitis (AD), but also many other dermatoses, such as pityriasis lichenoides chronica, drug eruptions and vitiligo.[5–7]

This diagnostic gap is highly relevant for MF and AD, as their treatment regimens fundamentally differ. In patients with early MF, the policy of 'wait-and-see' or skin-directed therapy is recommended, mainly to avoid unnecessary side-effects of systemic therapies. In contrast, nowadays, AD is often treated early with systemic drugs such as Janus kinase inhibitors or dupilumab. Recently, several cases reporting the 'unmasking' of CTLC during treatment of assumed cases of AD with dupilumab have been published.[8–12] Histopathologically, progressive increase in the densities of atypical lymphoid infiltrates, the presence of atypical epidermotropic lymphocytes and papillary dermal fibrosis were registered in a small cohort.[13] This further corroborates the necessity of early and precise diagnosis of MF versus AD.

The International Society for Cutaneous Lymphoma developed an algorithm for the diagnosis of early MF involving a holistic integration of clinical, histopathologic, immunopathologic and molecular biological characteristics.[5,6,14] The first study evaluating this algorithm emphasized CD5 and CD7 deficiency (cut-off value of CD7 expression <22.5%) as mandatory immunopathologic criteria and PCR-based testing for TCR-γ and β chains as required molecular criteria.[15]

However, molecular techniques such as clonality assessment do not show a high specificity for MF.[16,17] Prospectively, the convergence of machine learning and next-generation sequencing represents a golden opportunity to advance precision dermatology.[18,19]

To date, technical validity and clinical utility of the numerous suggested histopathologic criteria rely on subjective evaluation. This leaves a substantial gap and uncertainty in the differential diagnosis of early MF, especially versus AD.

To address this unmet need, we trained a disease classifier to identify the minimal number of histological attributes needed to differentiate early MF from AD. Using machine learning, a two-item histological classifier performed with a sensitivity and specificity of >95% in an independent validation cohort (VC) and across observers.

METHODS

Samples

Fifty haematoxylin and eosin (H&E) stained slides of MF patients were retrospectively collected from the departments of dermatology of three different university hospitals, namely Zurich (23 patients), Münster (20 patients) and Munich (7 patients).

Patients from Zurich and Munich represent the training cohort (TC) ($n = 30$). Münster patients ($n = 20$) built up the VC. Both cohorts were investigated by independent dermatopathologists.

Skin punch biopsies were obtained from patients who presented with clinical findings suspicious for an early form of MF (time point of the study specimen). Final clinical diagnosis was unequivocally confirmed after a median follow-up period of several years (TC: 77 ± 88 months; VD: 42 ± 43 months). Here, only patients with persistent patches or clear progression into plaque stage MF were included into the study. The course of the disease was documented in clinical records, clinical images and further skin biopsies that confirmed the initial diagnosis of MF. In the training cohort, the diagnosis of MF was also verified by clonality analysis.[20]

Moreover, biopsies of 50 patients with the diagnosis of AD were included in the study (30 patients from Munich and 20 patients from Münster). Inclusion criteria were diagnosis of AD as confirmed by typical patient's medical history, clinical manifestation and the presence of atopy stigmata clearly documented in clinical records and clinical images.

Histological annotation

Out of the many criteria which have been proposed to be specific—or at least indicative clues for the diagnosis of MF (cf. Table 1)—a list containing 33 histological attributes with corresponding scores was created for annotating the slides (see Table 2). Each H&E slide was evaluated for all of the 33 attributes. The slides were evaluated blinded, in a cohort-dependent manner, by two independent dermatopathologists. Corresponding scores were filled in an excel sheet, which was the source for further statistical analysis. Histological clues were defined as follows:

- *Epidermotropism*: Presence of a disproportional amount of epidermal lymphocytes—with slightly larger nuclei compared to dermal lymphocytes—within only little spongiotic epidermis.
- *Atypical lymphocytes* in the epidermis or within the dermal infiltrate, respectively: Lymphocytes with irregular shaped, enlarged, hyperchromatic nuclei, but also clearly larger nuclei and larger lymphocytes with hyperchromatic nuclei compared to dermal lymphocytes.
- *Disproportional epidermotropism*: Epidermotropism of lymphocytes with a paucity of spongiosis.
- *Basilar epidermotropism*: Presence of four (or more) contiguous lymphocytes at the basal layer of the epidermis.
- *Wiry collagen bundles*: Presence within the papillary dermis, right below the rete ridges.
- *Interface dermatitis*: Interface dermatitis sensu *strictu*, lichenoid or vacuolar.

TABLE 1 The most predictive histological attributes for the diagnosis of MF.

Histopathological criteria	(20)	(21)	(22)	(23)	(7)
Epidermis					
Epidermotropism of lymphocytes	+	+	+	+	+
Intra-epidermal collections of more than 4 lymphocytes	+	−	±	±	±
Perilymphocytic halo	0	+	+	+	+
Atypical lymphocytes	+	0	+	+	+
Spongiosis	0	+	0	−	0
Dermis					
Patchy lichenoid infiltrate	0	+	+	+	+
String of beads/basilar epidermotropism	+	+	+	0	+
Wiry collagen bundles	0	+	0	0	+
Atypical lymphocytes	+	+	±	+	0
Interface dermatitis	0	+	0	+	+
Pigment incontinence	0	+	0	0	0

Association analysis and classification model

To analyse the association of the 33 histological features (mixed ordinal and nominal variables) with the diagnosis of MF versus AD, Cramér's V analysis including bias correction for Cramér's V according to Bergsma was performed.[21] The feature 'leukocytoclastic vasculitis' was excluded from the analysis as this feature was not detected in any of the slides. For the disease classification model, Python 3.6 with the machine-learning module 'scikit' based on linear SVC was used to extract the most discriminative subset of features from the 32 features of the data set (http://www.python.org). The word 'classifier' was interpreted in its mathematical definition being 'an algorithm that sorts data into labeled classes'. The Brier score was applied to assess the performance of the classifier. As a training set, the training cohort was used to obtain the most discriminative model by optimising the hyperparameters of the linear SVC classifier supported by 10-fold CV. The performance of the classifier was tested with the VC as independent test set.

RESULTS

Baseline and follow-up demographics

30 MF patients (18 males and 12 females) served as TC. Gender distribution was identical in the VC (12 males and 8 females). Mean age of MF patients in the VC was 54 years—a little lower than in the training cohort (mean age 62 years). All MF patients had a long follow-up period after the initially taken biopsy to ensure the diagnosis MF (TC: 77 ± 88 months; VC: 42 ± 43 months).

In the AD patients' group, the number of female patients was higher (12 females out of 20 patients in the VC and 14 females out of 30 patients in the TC). Mean age was 49 years for the VC's eczema patients and 47 years for TC's AD patients, respectively.

Histological attributes

The majority of MF patients showed little (63% TC, 75% VC) or moderate (33% TC, 25% VC) *epidermotropism* while *intraepidermal collections of more than four lymphocytes* was only seen in 8 out of 30 patients (26.7%) within the TC and 25% of the VC's patients, respectively. Even if the majority of the AD patients (63% TC, 60% VC) showed mild-to-moderate presence of intraepidermal lymphocytes, the MF-typical accumulation in 'microabscesses' of more than four lymphocytes was only seen in 2 out of 30 patients within the TC and none of the VC's patients.

Almost all of the MF patients showed *atypical epidermal* (80% within TC, 95% within VC) and *atypical dermal* (90% within TC, 80% within VC) *lymphocytes*. In contrast, '*atypical epidermal lymphocytes*' were rarely observed in the epidermis of AD patients' samples (TC 13,3%, VC 0%).

Basilar epidermotropism was seen in about half of the MF patients (TC 56.7%, VC 45%), but only in a minority of AD lesions (TC 10%, VC 0%).

Wiry collagen bundles were observed in 76.7% of TC's MF patients, but in less than half of the VC's MF patients (45%). More consistent were the results of AD patients within the TC and VC, showing absence of wiry collagen in 80% and 95% of the cases, respectively.

Patchy-lichenoid distribution of lymphocytes was observed in two-thirds of the TC's MF patients. A *predominantly perivascular distribution* was very rare (6.7%), whereas it was the most common distribution pattern within the TC's AD patients (63.3%).

Association of histological features with the diagnosis of MF versus AD

To assess the relevance of individual histological parameters for differentiating MF from AD, we performed an association analysis using Cramér's V method (Figure S1). We found that the features '*atypical dermal lymphocytes*' and '*atypical epidermal lymphocytes*' showed the highest association with Cramér's coefficient $V = 0.84$ for '*atypical dermal lymphocytes*' and $V = 0.82$ for '*atypical epidermal lymphocytes*' which means that these features showed the highest discriminative power to differentiate MF from AD.

Disease classification model

Next, we followed a machine learning approach based on linear SVC to establish a disease classification. In line with

TABLE 2 33 histological attributes with definition and corresponding scores: This list had been created prior to the study and served as a basis for annotating the slides.

	MFTC $n = 30$ (100%)[a]							MF VC $n = 20$ (100%)[a]							AD TC $n = 30$ (100%)[a]							AD VC $n = 20$ (100%)[a]						
	0	1	2	3	4	5	6	0	1	2	3	4	5	6	0	1	2	3	4	5	6	0	1	2	3	4	5	6
Suprapapillary epidermis[b]		28 (93)	2 (7)					2 (10)	17 (85)		1 (5)					28 (93)		2 (7)					17 (85)	2 (10)	1 (5)			
Hyperkeratosis[c]	13 (43)	13 (43)	3 (10)	1 (3)				9 (45)	6 (30)	5 (25)					2 (7)	16 (53)	12 (40)					4 (20)	10 (50)	6 (30)				
Parakeratosis[c]	14 (47)	12 (40)	3 (10)	1 (3)				12 (60)	7 (35)		1 (5)				5 (17)	15 (50)	10 (33)					6 (30)	10 (50)	3 (15)	1 (5)			
Parakeratosis[d]	9 (30)	6 (20)	8 (27)	6 (20)	1 (3)			12 (60)		5 (25)	1 (5)	1 (5)		1 (5)	5 (17)		7 (23)	4 (14)	6 (21)		8 (27)	6 (30)		6 (30)		7 (35)		1 (5)
Orthokeratosis[c]		22 (73)	7 (23)	1 (3)				1 (5)	14 (70)	5 (25)					8 (27)	13 (43)	9 (30)					1 (5)	14 (70)	5 (25)				
Acanthosis quantitative[e]	5 (17)	17 (57)	7 (23)	1 (3)					15 (75)	5 (25)						13 (43)	15 (50)	2 (7)					6 (30)	12 (60)	2 (10)			
Acanthosis qualitative[f]	5 (17)	17 (57)	5 (17)	3(10)				15 (75)		3 (15)	2 (10)				2 (7)	11 (37)	13 (43)	4 (14)				6 (30)		14 (70)				
Granulosis[g]		1 (3)	6 (20)	19 (63)	4 (13)				2 (10)	1 (5)	1 (5)	15 (75)	1 (5)			1 (3)	5 (17)	19 (63)	5 (17)						7 (35)	11 (55)	2 (10)	
Scale crust[h]		3 (10)	27 (90)						2 (10)	18 (90)						8 (28)	22 (73)						6 (30)	14 (70)				
Spongiosis (1)[c]	10 (33)	15 (50)	5 (17)					17 (85)	1 (5)	2 (10)					8 (27)	15 (50)	6 (21)	1 (3)				2 (10)	10 (50)	6 (30)	2 (10)			
Dilated capillaries in papillary dermis[c]	19 (63)	11 (37)						14 (70)	2 (10)	3 (15)	1 (5)				9 (30)	19 (63)	2 (7)					15 (75)	4 (20)	1 (5)				
Lymphocytes dermal[c]		13 (43)	11 (37)	6 (20)				1 (5)	5 (25)	11 (55)	3 (15)					24 (80)	6 (21)						14 (70)	6 (30)				
Allocation of lymphocytes (2)[i]		2 (7)	4 (13)			20 (67)	4 (13)		7 (35)		3 (15)	3 (15)	7 (35)			19 (63)	1 (3)	9 (30)		1 (3)			15 (75)		5 (25)			
Depth extention of lymphocytes (3)[j]		7 (23)	21 (70)	2 (7)					14 (70)	5 (25)	1 (5)					4 (14)	16 (53)	10 (33)					10 (50)	9 (45)	1 (5)			
Dermal lymphocytes qualitative[k]		2 (7)	1 (3)	27 (90)					7 (35)		9 (45)	4 (20)					1 (3)	3 (10)	26 (87)							20 (100)		
Size of dermal lymphocytes[l]		18 (60)	12 (40)						15 (75)	4 (20)	1 (5)					25 (83)	5 (17)						19 (95)	1 (5)				
Atypical mitosis[h]		1 (3)	29 (97)							20 (100)						1 (3)	29 (97)							20 (100)				
Basilar epidermotropism (5)[h]		17 (57)	13 (43)						9 (45)	11 (55)						3 (10)	27 (90)							20 (100)				
Lymphocytes epidermal quantitative[c]	1 (3)	19 (63)	10 (33)						15 (75)	5 (25)					11 (37)	15 (50)	4 (14)					8 (40)	11 (55)	1 (5)				
Perilymphcytic clear halo[m]	5 (17)	9 (30)	16 (53)					1 (5)	1 (5)	18 (90)					14 (47)	10 (33)	6 (21)					8 (40)	10 (50)	2 (10)				
Intraepidermal collections of more than 4 lymphocytes (6)[h]		8 (27)	22 (73)						5 (25)	15 (75)					2 (7)		28 (93)							20 (100)				
Atypical lymphocytes epidermal (7)[h]		24 (80)	6 (20)						19 (95)	1 (5)						1 (3)	29 (97)							20 (100)				
Neutrophil granulocytes quantitative[c]	25 (83)	5 (17)						20 (100)							26 (87)	2 (7)	2 (7)					16 (80)	4 (20)					
Neutrophil granulocytes qualitative[n]	25 (83)	4 (13)	1 (3)					20 (100)							26 (87)	3 (10)	1 (3)					16 (80)	4 (20)					
Eosinophils[c]	19 (63)	11 (37)						19 (95)	1 (5)						17 (57)	10 (33)	1 (3)	2 (7)				12 (60)	8 (40)					

TABLE 2 (Continued)

Interface-dermatitis quantitative (8)[c]	10 (33)	18 (60)	2 (7)			11 (55)	9 (45)		26 (87)	4 (14)			19 (95)	1 (5)		
Suprabasal dyskeratosis[h]	12 (40)	17 (57)	1 (3)			15 (75)	5 (25)		26 (87)	4 (14)			19 (95)	1 (5)		
Number of dyskeratoses per hotspot (x40)	12 (40)	8 (27)	6 (20)	2 (7)	2 (7)	11 (55)	9 (45)			2 (7)	2 (7)		19 (95)	1 (5)		
Distribution of dyskeratosis[o]	10 (33)	17 (57)	2 (7)	1 (3)		12 (60)	7 (35)	1 (5)		4 (14)				1 (5)		
Interface dermatitis qualitative[p]	10 (33)	17 (57)		3(10)		11 (55)	9 (45)			3 (10)	1 (3)		19 (95)	1 (5)		
Leucocytoclastic vasculitis[c]	30 (100)					20 (100)			30 (100)				20 (100)			
Wiry collagen papillary dermis[h]	13 (43)	7 (23)				9 (45)	11 (55)			5 (17)	25 (83)			1 (5)	19 (95)	
Pigment incontinence[c]	19 (63)	10 (33)	1 (3)			18 (90)	2 (10)		22 (73)	7 (23)	1 (3)		18 (90)	2 (10)		

Note: (1) the most spongiotic area of the epidermis should be evaluated.(2) predominant allocation of the majority (>80%) of dermal lymphocytes.

(3) deepest part of the dermis where >95% of lymphocytes are allocated above.

(4) lymphocytes of a conspicuous form, non-round contour.

(5) presence of 4 (or more) contiguous lymphocytes within the basal layer of the epidermis.

(6) formerly referred to as Pautrier microabscesses.

(7) lymphocytes with clearly cerebriform nuclei, but also clearly larger nuclei and larger lymphocytes with hyperchromatic nuclei compared to dermal lymphocytes.

(8) any form of vacuolar alterations within the basal epidermis.

[a]For better readability percentage in columns are rounded off to the nearest full number, therefore the sum of percentages might be beyond 100 %.

[b]0 = none, 1 = normal, 2 = thickened 3 = thinned out.

[c]0 = none, 1 = little, 2 = moderate, 3 = strong.

[d]0 = no cornified layer, 1 = orthokeratosis, 2 = predominantly (pred.) orthokeratosis, 3 = pred. parakeratosis, 4 = equably parakeratosis and orthokeratosis, 5 = checker-board pattern, 6 = parakeratosis.

[e]0 = atrophy 1 = normal epidermis, 2 = moderate acanthosis, 3 = strong acanthosis.

[f]0 = atrophy, 1 = normal thickness of squamous layer 2 = unregular, 3 = regular.

[g]1 = hypergranulosis, 2 = pred. hypergranulosis, 3 = hypogranulosis/hypergranulosis alternating, 4 = pred. hypogranulosis, 5 = agranulosis.

[h]1 = present, 2 = not present.

[i]1 = pred. perivascular, 2 = pred. interstitial, 3 = interstitial and perivascular 4 = lichenoid, 5 = perivascular and lichenoid ("patchy-lichenoid"), 6 = patchy-lichenoid+interstitial.

[j]0 = no lymphocytes, 1 = papillary dermis, 2 = reticular dermis superficial 3 = reticular dermis deep, 4 = subcutis.

[k]0 = none, 1 = smaller than epidermal lymphocytes 2 = cerebriform nucleus, 3 = atypical (4), 4 = normal.

[l]1 = small, 2 = moderate, 3 = big.

[m]0 = no lymphocytes epidermal 1 = within spongiosis, 2 = without spongiosis.

[n]0 = none 1 = pred. dermal, 2 = pred. cornified layer.

[o]1 = focal, 2 = intermittent, 3 = continuous.

[p]0 = none, 1 = non sensu strictu (8), 2 = vacuolar 3 = lichenoid.

the correlation analysis, '*atypical epidermal lymphocytes*' and '*atypical dermal lymphocytes*' were found to be the most discriminative features for the classification model (see Figure 2a,b). According to the model, five MF samples (of 30) were misclassified as AD, and two AD samples (of 30) were misclassified as MF (Figure 1a), resulting in a Brier score of 0.078, a sensitivity of 0.83 (0.93) and specificity of 0.93 (0.83) for the diagnosis of MF (AD). On the independent VC, this model misclassified one MF (of 20) as AD, whereas all AD samples were correctly classified (Figure 1b) resulting in a Brier score of 0.044, a sensitivity of 0.95 (1.00) and a specificity of 1.00 (0.95) for MF (AD).

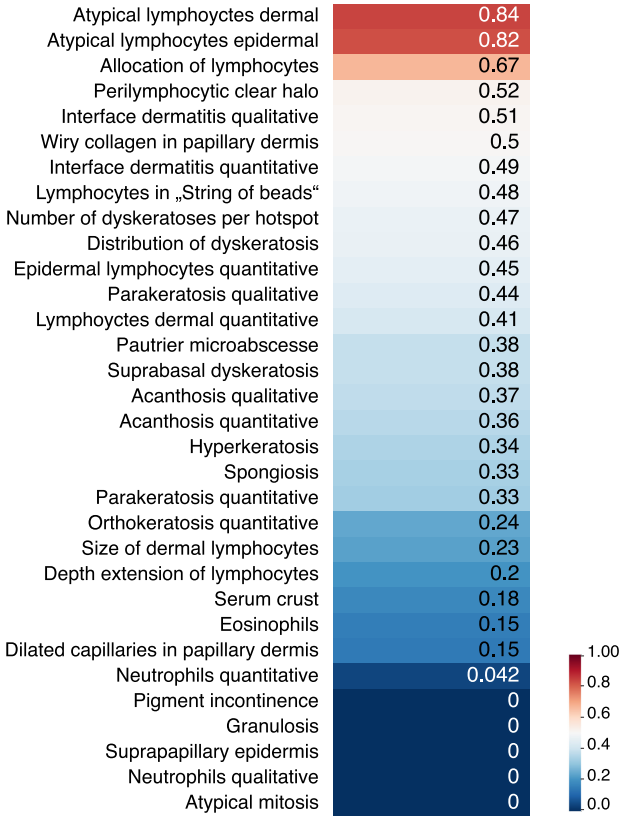

FIGURE 1 Correlation of histological parameters with the diagnosis of MF. Shown are the correlation coefficients τ_b of each feature with the diagnosis of MF. For positive (negative) correlations between the respective parameter and MF: $0 < \tau_b \leq 1$ ($-1 \leq \tau_b < 0$).

Inter-observer differences

To prove the inter-observer robustness of the proposed binary classifier, 13 clonality-analysed MF samples were scanned and validated by the two dermatopathologists according to the established 32 histological attributes independent from each other.

However, the difference of individual interpretation was lower if only the two attributes used for the binary classifier were regarded. Regarding the feature '*atypical epidermal lymphocytes*', only 1 out of 13 cases (7.7%) was differently assessed by the two dermatopathologists. The remaining samples (92.3%) were evaluated in the same way.

Even if in 4 out of 13 (30%) MF cases (Figure 3a–c) '*atypical dermal lymphocytes*' were absent, the majority of scanned slides were evaluated consistently by both pathologists. Finally, the classification model was applied to the second pathologist's evaluation. While the evaluation of histological features of Observer 1 resulted in 4 out of 13 misclassified MF samples, the evaluation of histological features of Observer 2 resulted in 2 misclassified MF patients.

DISCUSSION

In the last decades, a plethora of histological clues has been suggested by expert dermatopathologists to allow differentiation of early MF from benign chronic inflammatory

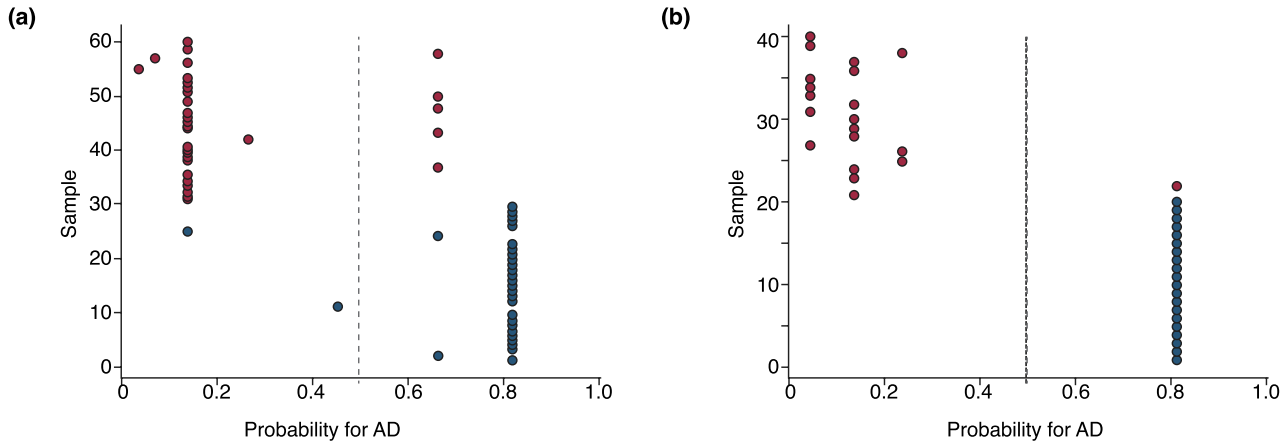

FIGURE 2 (a and b) Disease classification model for MF and eczema (AD) using 32 histological parameters. (a) The model was trained and tested on patients with MF (each red circle indicates a single MF patient) and eczema (each blue circle indicates a single eczema patient). (b) The model was then validated in an independent cohort of patients with MF (each red circle indicates a single MF patient) and eczema (each blue circle indicates a single eczema patient). Prediction probabilities for MF are indicated on lower panels of each graph.

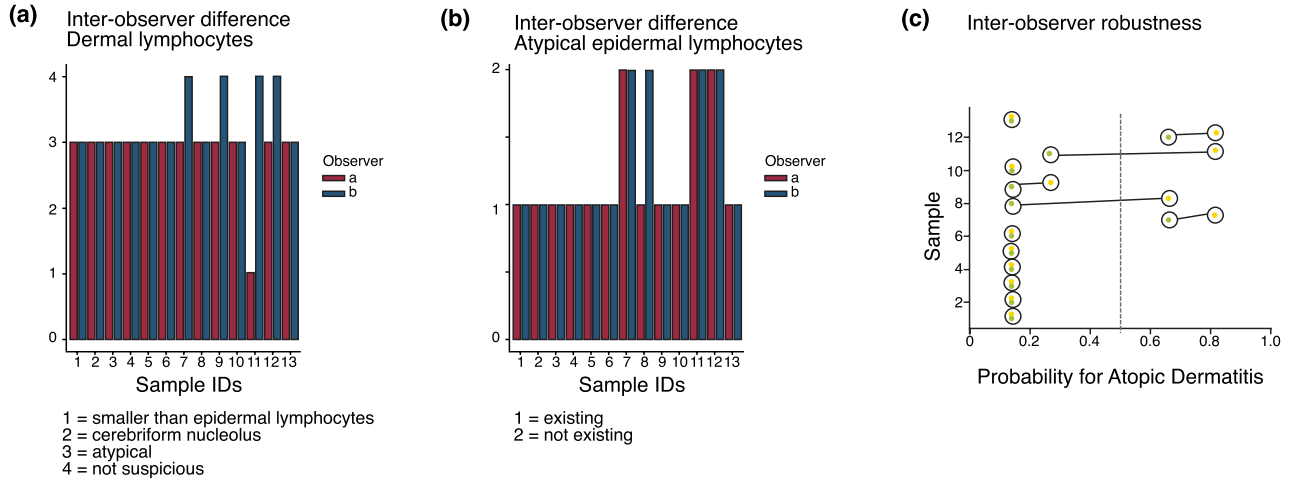

FIGURE 3 (a–c) Inter-observer differences and inter-observer robustness of the disease classification model. (a,b) Inter-observer differences of the two attributes used for the binary classifier. (c) Two independent dermatopathologists reviewed 13 MF cases from the training cohort based on the 32 parameters. The disease classification model was applied to both datasets. Each orange circle indicates a single MF patient evaluated by Observer 1, each green circle indicates a single MF patient evaluated by Observer 2. Black circles and interconnecting lines indicate corresponding observer evaluations for one MF case, respectively. Prediction probabilities for AD are indicated on lower panels of each graph.

diseases, such as AD.[5–7,22–28] However, the assessment of these attributes is based on subjective evaluation.

The principle to design a classifier—based on a minimal number of clues that would be robust if different experts assess the clues—differs from the trend to design automated machine-learning classifiers based on image data.[29] Our classifier is a diagnostic aid for a dermatopathologist that can be easily implemented in daily clinical routine and might be developed for further difficult differential diagnoses such as pyoderma gangrenosum versus ulcer or benign nevus versus melanoma.

We identified the annotation of only two features, namely '*atypical epidermal lymphocytes*' and '*atypical dermal lymphocytes*' was robust in terms of technical validity and clinical utility. These features are consistent in between cohorts, robust versus inter-observer differences and show a high difference in between early MF and eczema.

The first feature that builds our histologic classifier is the presence of **atypical lymphocytes within the epidermis** and has previously been described.[7,22,23,26,30] While *epidermotropism of atypical lymphocytes* was observed in 80% or 95% of all MF patients in our TC and VC, respectively, the formation of the pathogenicnomonic *intraepidermal collections of more than four lymphocytes (formerly referred to as 'Pautrier's microabscesses' or 'Darier's nests'*) was less common. This is in line with previous reports of high specificity, but low sensitivity of '*microabscesses*'.[23,27,28,30,31]

Also, the second feature of our classifier, '***atypical dermal lymphocytes***', is well-studied in the literature.[22–24] Interestingly, the WHO/EORTC classification reports atypical (dermal) cells with small-to-medium-sized, highly indented (cerebriform) and sometimes hyperchromatic nuclei as a rare finding in early MF, where atypical lymphocytes seem to be confined to the epidermis (epidermotropism).[30] However, in both our TC and VC, the vast majority of MF patients showed suspicious lymphocytes within the dermis that were annotated as *atypical lymphocytes* or *smaller than the epidermal lymphocytes*. These criteria were virtually absent in eczema samples, thus opening a large diagnostic window.

Of note, using the clear definition, both features were evaluated by independent dermatopathologists in a blinded and very consistent manner despite their subjective nature.

Limitations of this study are the small number of samples and the subjective evaluation by the dermatopathologists. Nevertheless, the classifier performed robustly in an independent VC (sensitivity 0.95, specificity 1.0) and the inter-observer variability is low.

Diagnosis of early MF requires subjective evaluation of a dermatopathologist as alternative, objective diagnostic methods alone are currently insufficient: Beyond classical histology, two of the most popular diagnostic tools to differentiate MF from AD are T-cell clonality analysis and immunohistochemistry. Even if the typical immunohistochemical findings seem to be specific (elevation of the CD4/CD8 ratio from 2 to 7,[32–34] loss of CD5 and/or CD7 on epidermotropic CD3+CD4+ T cells), they have only low sensitivity and can also be found physiologically and in inflammatory disorders.[33] Also, T-cell receptor sequencing has its limitations, as it lacks specificity and dominant T-cell clones are frequently found in non-malignant skin diseases[16] with some studies even reporting TCR clonality to be more common in benign dermatoses than in CTCL.[17] To advance precision dermatology, high-throughput TCR sequencing is a promising method to differ MF from benign inflammatory skin diseases, such as AD.[19] Nevertheless, limitations of this method such as PCR bias, DNA degradation after formalin fixation and—to now—a laborious and rather costly procedure, must be considered.

Hence, only 2.3% dermatologists order TCR clonality assays as part of the initial diagnostic evaluation and only 3.7% of pathologists rely on them to diagnose cutaneous lymphoproliferative disorders.[35,36]

Disease classifiers should be evaluated regarding technical validity, clinical utility, cost-effectiveness, and feasibility. Taking all these criteria into account, our classifier based on the two features '*atypical epidermal lymphocytes*' and '*atypical dermal lymphocytes*' is a valuable innovation in the clinically relevant differential diagnosis of early MF and AD. Further studies are needed to investigate synergistic effects of a combination of this histological classifier with immunohistochemical and molecular techniques such as clonality analysis or molecular classifiers.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest in relation to this article.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICAL APPROVAL

IRB approval status: The study was approved by the local ethics committee (Klinikum Rechts der Isar, 5590/12).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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