Unusual histomorphologic and immunohistochemical features of mycosis fungoides in patch and plaque stages

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Background and Aim: Mycosis fungoides (MF) is the most common form of primary cutaneous lymphoma, resulting from the infiltration of malignant T cells into skin tissues. The disease has three distinct stages: patch, plaque, and tumor. In the patch and plaque stages, it can mimic the clinical features of benign dermatoses. However, two scoring systems facilitate diagnosis at these stages, which will be discussed in more detail in this study.

Methods: In this cross-sectional study, all formalin-fixed and paraffin-embedded skin specimens highly susceptible to MF based on clinical examination at the patch and plaque stages were collected from April 2017 to August 2019. They were subjected to H&E and IHC staining tests and examined according to Guitart and Pimpinelli criteria.

Results: Out of 78 samples, 76 had histological criteria for MF according to Guitart's criteria, 54 were immunologically significant according to Pimpinelli's criteria for MF, and 52 were classified as definitive MF according to both criteria. CD3 and CD4 markers were the most frequent markers, respectively. In contrast to previous studies, the CD7 marker was expressed at 10% or higher in 24 cases. In addition, 65 of 78 samples had a CD8 marker, and only 13 samples were CD8-.

Conclusion: In the early stages of MF, a single scoring system does not have sufficient sensitivity for the diagnosis. The triad of the patient's clinical presentation and histological and immunohistochemical features play a key role in achieving the correct diagnosis.

Keywords: Guitart's criteria, mycosis fungoides, patch and plaque stage, Pimpinelli's criteria

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INTRODUCTION

Cutaneous T-cell lymphomas (CTCLs) are a group of lymphomas with heterogeneous features. They may differ in appearance, clinical course, biological behavior, immunohistochemical features, prognosis, and survival rate [1-4].

Mycosis fungoides (MF) is an indolent type of CTCL and presents as patches, plaques, or tumors [5].

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The histopathology of MF in the early patch stage is nonspecific. The most common findings are epidermotropism of isolated cells with small round cerebriform nuclei and lymphocyte infiltration at the edges of the basal layer. These are usually associated with epidermal acanthosis or hyperkeratosis, papillary dermal fibrosis, and infiltration of other inflammatory cells. The lesions become more prominent in the plaque stage and turn purple to brown. At this stage, pruritis also occurs. They may also cause ulceration. Microscopic findings show frank epidermotropism and subepidermal and dermal lymphocyte infiltration. Pautrier's microabscesses are also present in many patients at the plaque stage. Finally, in the tumor stage of MF, epidermotropism disappears, and severe diffuse dermal infiltration of large lymphocytes with cerebriform nuclei is seen. Lymphonodal dissemination is also common at this stage. It is also possible that MF lesions progress to CD30+ or CD30- T-cell lymphoma at this stage [6,7]. Because of these nonspecific features of the disease and the necessity of early identification in improving MF prognosis, scoring systems are required.

Guitart *et al.* created the first scoring system for MF diagnosis. Their system consisted of the major criteria of dense lymphocyte infiltration, lymphocyte atypia, and epidermotropism, and minor criteria of lymphocytic infiltration without inflammatory features, reticular fibroplasia of papillary dermis, and intraepidermal atypical lymphocytes. This system was mostly based on histopathological characteristics of MF rather than immunological ones.

In 2006, Pimpinelli *et al.* conducted a comprehensive algorithm to identify MF based on clinical, histopathological, immunopathological, and molecular biological features. In this algorithm, additional items such as lesion distribution, shape variations, poikiloderma, clonal T-cell receptor gene rearrangement, and immunopathological criteria were added to the Guitart criteria for early diagnosis of MF. According to Pimpinelli's algorithm, a total of four points are required for the diagnosis of MF, which may be a combination of clinical, histopathological, immunopathological, or molecular biological features [8].

Also, in 2006, Cotta *et al.* performed a study on 42 patients with diffuse infiltration of lymphocytes in the dermis and epidermis. This study demonstrated a way to differentiate MF from other benign dermatoses in the early stages by measuring the CD7 marker. The CD7 marker may be absent in T cells of a malignant lesion and could be useful for the early diagnosis of MF [9].

We conducted this study at Razi Hospital to evaluate the histopathological and immunological features of Iranian patients based on the samples referred for MF to our center (based on clinical examinations with high probability) as a reference center for dermatological diseases in Iran.

METHODS

The study was designed as a cross-sectional study and was approved by the Tehran University of Medical Sciences Ethics Committee. All MF patients were informed about their participation in the study and signed an informed consent form. All formalin-fixed and paraffin-embedded skin specimens collected between April 2017 and August 2019 that were clinically indicative of patch or plaque stage MF were presented to the Department of Pathology at Razi Hospital and underwent H&E and IHC staining. Specimens with poor staining were excluded from the study. The samples with H&E staining were examined and categorized according to Guitart's criteria. Based on these criteria, samples with a score of 7 or more were diagnosed as definite MF [10].

A panel of monoclonal antibodies against CD3, CD4, CD5, CD7, and CD8 was used for IHC staining. The density of these markers in the epidermis was measured by counting the stained lymphocytes for each marker in 5 microscopic fields at 40x magnification and taking the average. In the next step, the CD4+/CD8+ ratio was calculated. In addition, the density of lymphocytes with CD3, CD4, CD5, CD7, and CD8 markers in the entire epidermis and dermis was measured and recorded by calculating the percentage of lymphocytes with a particular marker out of all T lymphocytes present in the epidermis and dermis. Subsequently, the ratio of staining of the epidermis and dermis for CD3, CD5, and CD7 markers was also recorded. The spectrum of staining ranges from 0 to 3. The immunohistochemical criteria for MF were derived from the Pimpinelli algorithm [8].

Finally, all data were collected and analyzed using IBM SPSS version 23 software.

Ethical consideration

All procedures followed were in accordance with the ethical standards of the Tehran University of Medical Sciences Ethics Committee and are compatible with the Helsinki Declaration, as revised in 2000. Informed consent was obtained from all patients to be included in the study.

RESULTS

We used H&E and IHC staining to examine 78 samples with a probable diagnosis of MF at the patch or plaque stage. Guitart's major and minor criteria were assessed microscopically. According to these criteria for MF, 76 samples (97.43%) scored 7 or more and were significantly suggestive of MF.

Infiltration density, epidermotropism, and lymphocyte atypia as major criteria, according to Guitart, were examined in all specimens, and the results are summarized in Table 1. The minor criteria were also assessed (Table 1).

The density of lymphocytes with CD3, CD4, CD5, CD7, and CD8 markers was measured in the epidermis in the manner already mentioned in the methods and summarized in Table 2. As expected, the CD3 and CD4 markers had the highest mean values. The CD7 marker was the least abundant in the tissues, with a mean value of 0.34.

We also examined the overall density of CD3, CD4, CD5, CD7, and CD8 markers throughout the dermis and epidermis and recorded their percentage (Table 3). Again, CD3 and CD4 markers were present in more than 50% of T cells, while the CD7 marker was least expressed by T cells. In most cases, CD7 was only present in 0-10%, while there were some cases where this marker was expressed in 50%. The markers CD5 and CD8 showed different densities in

Table 1. Guitart's major and minor criteria in the specimens

Major criteria	**Scale**			
	0	**1**	**2**	**3**
Infiltration density, n (%)	0 (0%)	1 (1.3%)	66 (84.6%)	11 (14.1%)
Epidermotropism, n (%)	0 (0%)	60 (76.9%)	18 (23.1%)	0 (0%)
Lymphocyte atypia, n (%)	0 (0%)	19 (24.4%)	47 (60.3%)	12 (15.4%)
Minor criteria	**Absent**		**Present**	
Reticular fibroplasia, n (%)	6 (7.7%)		72 (92.3%)	
Lymph infiltrate without inflammation features, n (%)	2 (2.6%)		76 (97.4%)	
Intraepidermal atypical lymphocytes, n (%)	2 (2.6%)		Low grade (Score = 1)	High grade (Score = 2)
			66 (84.6%)	10 (12.8%)

Table 2. The density of markers in the epidermis

	CD3	**CD4**	**CD5**	**CD7**	**CD8**
Mean	7.28	6.28	3.82	0.34	3.66
Std. Deviation	6.38	5.53	4.15	1.37	4.04
Minimum	0	0	0	0	0
Maximum	38	34	24.6	10	20

Table 3. The density of markers (%) in the whole epidermis and dermis

	Marker Density										
	0%	**10%**	**20%**	**30%**	**40%**	**50%**	**60%**	**70%**	**80%**	**90%**	**100%**
CD3, n	0	1	0	0	0	6	6	13	23	26	3
(%)	0	1.3	0	0	0	7.7	7.7	16.7	29.5	33.3	3.8
CD4, n	0	0	0	1	0	4	3	9	28	29	4
(%)	0	0	0	1.3	0	5.1	3.8	11.5	35.9	37.2	5.1
CD5, n	2	0	1	4	3	14	20	26	8	0	0
(%)	2.6	0	1.3	5.1	3.8	17.9	25.6	33.3	10.3	0	0
CD7, n	54	13	3	3	2	3	0	0	0	0	0
(%)	69.2	16.7	3.8	3.8	2.6	3.8	0	0	0	0	0
CD8, n	1	6	9	12	10	8	17	6	9	0	0
(%)	1.3	7.7	11.5	15.4	12.8	10.3	21.8	7.7	11.5	0	0

the different samples.

In addition, all samples were subjected to IHC assessment. Fifty-four samples (69.2%) were found to be "significant" for MF with a score of 1 or more, and 24 samples (30.8%) were found to be "not significant" for it. Of note, all specimens were classified as "significant" for MF by at least one of the histopathological (Guitart) or immunohistopathological criteria, and 52 specimens (66.67%) were classified as "significant" by both criteria.

In more detail, 54 cases (69.2%) had a density of less than 10% for the CD7 marker and scored in this immunological point. Only 10 samples (12.8%) had a density of less than 50% for CD3 and/or CD5 and scored on this point, and finally, none of our cases had a discordance of more than 2 in the ratio of epidermal/dermal staining intensity of the CD3, CD5 or CD7 markers.

It should be noted that we have two forms of density here. The density used for the Pimpinelli criteria is the percentage of lymphocytes with a particular marker in the entire epidermis and dermis (Table 3), while the density for a particular marker means the average of cells with that marker within five microscopic fields at 40x magnification and is measured only in the epidermis.

The CD4+/CD8+ ratio was also calculated and divided into eight groups, as shown in Table 4. We adopted group " > 20" for the CD8 samples that had no stained CD8 marker. As the results show, most of the cases (82.1%) in the epidermis were CD8+. In the CD8+ cases, the CD4+/CD8+ ratio was between 1 and 2 in most cases. There was also only one sample (1.3%) that did not express the CD4 marker. In this sample, other markers except CD3 and CD8 were also negative.

DISCUSSION

Among the three clinical stages of MF, early diagnosis during the patch or plaque stages is highly associated with a better prognosis and longer disease-free survival [11]. An organized and systematic scoring system based on histopathological and immunohistochemical characteristics may be useful in early diagnosis. Guitart's and Pimpinelli's scoring systems are two acknowledged and approved scoring systems used for disease diagnosis. They do, however, have flaws in some of their criteria. We aimed to evaluate our samples' histopathological and immunohistochemical features to obtain reliable data on early-stage MF features. Seventy-six specimens indicative of MF on clinical presentation met the Guitart criteria based on histopathological findings. None of the specimens scored 0 on any of the major Guitart criteria. However, 76 specimens (97.43%) had at least one of the inflammatory features that contradicted the commonly expected phenotype of MF [10]. The inflammatory features consisted of papillary dermal edema, infiltration of neutrophils and eosinophils, spongiosis, neutrophilic extravasation, and visible endothelial edema [12]. On the other hand, only 54 out of 78 specimens scored at least one according to the immunohistochemical criteria proposed by Pimpinelli. When calculating the density of markers, CD3 and CD7 were the most and least frequent markers, respectively.

As previous studies have shown, the CD3 marker is useful for distinguishing T cells from B cells. Since MF is a type of primary cutaneous T-cell lymphoma, the higher expression of the CD3 marker was in line with expectations. In addition, the expression of the CD7 marker was expected to be in contrast with the immunohistochemical features of MF. In our cases, 24 of 78 samples had a CD7 density of 10% or more, while 8 samples expressed the CD7 marker even more than 30%. According to Hristov *et al.*, the CD26- phenotype is more trustworthy than the previously accepted CD7- phenotype [13].

Another point regarding markers was the presence of CD8+ cells in 65 (83.3%) cases. Of these, 64 cases had CD4+/CD8+ immunophenotyping; even one sample was reported as CD4-/CD8+. The predominance of CD8+ cells over CD8- cells contrasts the general CD2+/ CD3+/ CD4+/ CD5/ CD8-/ CD20- phenotype reported for MF patients [14-16], indicating a novel, different immunological phenotype for MF. Further studies are needed to investigate whether there is a

Table 4. CD4+/CD8+ ratio

CD4+/CD8+ ratio	0	0-1	1-2	2-3	3-4	4-5	5-10	10-20	> 20
N (%)	1 (1.3%)	12 (15.4%)	22 (28.2%)	11 (14.1%)	4 (5.1%)	3 (3.8%)	7 (9%)	4 (5.1%)	14 (17.9%)

significant difference in the prognosis of the different immunological phenotypes of MF.

CONCLUSION

In this study, only 52 of our 78 specimens met both Guitart's and Pimpinelli's criteria for MF. Other specimens were classified as MF by only one of the two scoring systems. Moreover, inflammatory features were extremely common in the histological examination of the samples, and cells with the CD8+ phenotype clearly outweighed CD8- cells. In conclusion, especially in the early stages of the disease, a single scoring system does not have sufficient sensitivity for the diagnosis, and the triad of the patient's clinical presentation and histological and immunohistochemical features plays a key role in achieving the correct diagnosis.

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Authors' contributions

Mehri Maghsoodi: the corresponding author of the article and responsible for collecting samples and examining them, recording the characteristics of each and statistical analysis.

Alireza Ghanadan: Consultant for method of the study and selection of appropriate diagnostic algorithms for samples.

Kambiz Kamyab and Rokhsare Yadegar: Reviewing of samples for their characteritics.

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