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Original Article

CD7 Expression in Differentiating Mycosis Fungoides from Benign Cutaneous Lymphocytic Infiltrates

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Abstract

Background: Diagnosis of the early phase of mycosis fungoides (MF) is sometimes difficult. Loss of CD7 expression is considered a distinguishing characteristic of MF. The aim of this study was to determine the range of CD7 expression in MF and compare the results with benign inflammatory dermatosis and equivocal cases of possible MF.

Methods: During a period of 30 months, we examined 15 patients with MF, 12 patients suspicious for MF, and 15 patients with benign inflammatory dermatosis. The slides stained by H&E were reviewed by two pathologists. Immunostaining was done for CD43, CD3, CD5, CD7, and CD20 on paraffin embedded tissues.

Results: All the patients in MF group showed absence of CD7 expression in epidermotropic mycosis cells. Compared with benign inflammatory dermatosis, patients with MF had significantly lower CD7 expression in the dermal infiltrate (P< 0.0001). In patients with MF, the mean CD7 was significantly lower than CD43, CD3, and CD5 (P=0.001). The mean CD7 count of parapsoriasis was significantly higher than MF (P= 0.01). The mean CD7 count of parapsoriasis was significantly lower than benign inflammatory dermatosis (P=0.016). The lowest mean CD7 counts were found in patch stage of MF. Low CD7 expression < 10 % lymphocytes had sensitivity and positive predictive values of 75% and 100% and specificity and negative predictive values of 100% and 83.3% for the diagnosis of patch stage of MF.

Conclusion: Minimal expression of CD7 is a specific finding for patch stage of MF. Benign inflammatory dermatosis can also show low expression of this marker, but rarely matches that of patch stage of MF.

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Keywords ● Mycosis fungoides ● CD7 ● parapsoriasis ● cutaneous t cell lymphoma

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Introduction

Mycosis fungoides (MF) is the most common form of cutaneous T-cell lymphoma. It is a clinically and pathologically distinct form of cutaneous lymphoma characterized by an epidermotropic infiltrate of small to medium sized T-lymphocytes.[1] An accurate diagnosis of early MF may be difficult because of the various clinical and histological

expressions of the disease.[2] At early stage, atypical cells are few in number and the diagnosis of MF is sometimes difficult. Many studies were done to detect the early phase of MF.

MF is a cutaneous lymphoma in which the tumor cells express a mature T-helper memory phenotype, i.e. CD3+, CD4+, CD8-, and CD45RO+. In MF, it is suspected that loss of expression of CD7 is a marker of neoplastic progression that allows for immunophenotypic differentiation from benign inflammatory histologic mimics.[3,4] Studies have also demonstrated normal CD7+ population in patients with early MF.[4,5]

Immunophenotyping and T-cell receptor (TCR) gene rearrangement analysis have been performed repeatedly by many investigators. Clonal TCR gene rearrangement has been identified in MF. However, it is related to the stage of disease and monoclonality is not always synonymous with malignancy.[6] Benign inflammatory infiltrates can also show low CD7 expression, which rarely matches that of early stage of MF. Progressive loss of CD7 expression in benign inflammatory dermatosis (BID) is the likely consequence of expansion of antigen selected CD3+, CD4+, and CD7- T cells.[7] In this study we analyzed the expression of CD43, CD3, CD5, CD7, and CD20 on paraffin embedded samples of benign, malignant, and equivocal cases.

Materials and Methods

This study was conducted from December 2004 to May 2007 in the dermatology and pathology departments of hospitals affiliated to Shiraz University of Medical Sciences. All the patients referred to dermatology clinic were suspected to have mycosis fungoides or premycosis were selected for skin biopsy. The biopsy specimens were transferred to the pathology laboratory in 4% formalin. After evaluation by light microscopy, those cases which were diagnosed definitely as MF and also equivocal cases of possible MF were selected for immunophenotyping.

Patients

A total of 15 cases of MF were diagnosed in slides stained by H&E. These samples included 12 patch and 3 plaque stages. The criteria for diagnosis of patch stage MF were based on those reported by Santucci et al.[8]

Clinically suspicious cases of MF (≥3 year history of eczematous patches unresponsive to treatment), without fulfilling the histological criteria for MF or an inflammatory skin disease, were labeled as parapsoriasis.

Twelve patients who were biopsied with impression of parapsoriasis or possible MF were chosen for histological examination and immunophenotyping. These cases were considered as equivocal or inconclusive group including 10 parapsoriasis and 2 poikilodermatous reaction. Poikilodermatous reaction is characterized by hyperkeratosis, epidermal atrophy, basal cell liquefactive degeneration, pigmentary incontinence, telangiectasia, and a variable superficial dermal lymphohistiocytic infiltrate.[9] Fifteen cases of BID were also selected for histopathological examination and immunohistochemical staining. These cases include 11 eczematous dermatitis (nine subacute and two chronic), two lichenoid eczema, and two pityriasis rosea. All the slides were seen by two pathologists independently.

Immunostaining and Evaluation

Tissue sections of 5 micron thickness were cut from representative paraffin embedded block in each case, placed on charged glass slides, deparaffinized in xylene, and rehydrated through graded alcohol and stained with the following antibodies: CD43 (DAKO Denmark, DF-T1, 1:80), CD3 (Signet USA, polyclonal, prediluted), CD5 (Novocastra England, 4C7, prediluted), CD7 (DAKO, CBC. 37, 1:50), CD20 (DAKO, L26, 1:200). For CD3, CD5, CD7, and CD20, the slides were pretreated for antigen retrieval by steaming in 10 mmol/l sodium citrate buffer (pH = 6.0) for one hour, immediately followed by 30 minutes of cooling. Manual immunohistochemistry testing was performed using positive controls (lymph nodes) and negative controls (without primary antibodies). Avidin-biotin peroxidase was used to localize the antigen antibody complex in all cases. All the slides were lightly counterstained with hematoxylin. Immunohistochemistry testing for CD7 was repeated in 10 samples after EDTA antigen retrieval to determine whether greater CD7 antigen expression could be elicited. Two pathologists performed a quantitative analysis independently. At least five randomly selected high power (X400) fields, including epidermis and dermis, were examined. Percentages of mononuclear cells labeled with each antibody were estimated by each observer and averaged. Interobserver estimates did not vary by more than 10%. The presence or absence of staining was evaluated in both epidermal and dermal lymphocytic infiltrate and the percentage of positivity was noted for each case. The cases were categorized into five groups according to the percentage of positivity as negative, <10%, 10-25%, 26-50%, and >50%.

Statistical Analysis

Statistical analysis was carried out using SPSS software version 16.0.1. Differences in immunolabeling between three groups were tested by the Kruskal-Wallis test for means and between two groups by Mann-Whitney test. Wilcoxon Signed Ranks test was used to

determine the correlation between variables (CD markers) in each diagnostic category of MF, equivocal, and BID. The criterion for significance for all tests was P< 0.05%.

Results

Immunohistochemical results for all skin samples are summarized in table 1 and for CD7 graphically showed in figure 1. Examination for equality of populations among three diagnostic groups by Kruskal-Wallis test showed that the mean CD7 counts were significantly different across the diagnostic categories of MF, spongiotic dermatitis (eczema), spongiotic, and lichenoid reaction (lichenoid eczema), pityriasis rosea, parapsoriasis, and poikilodermatous reaction (P< 0.0001).

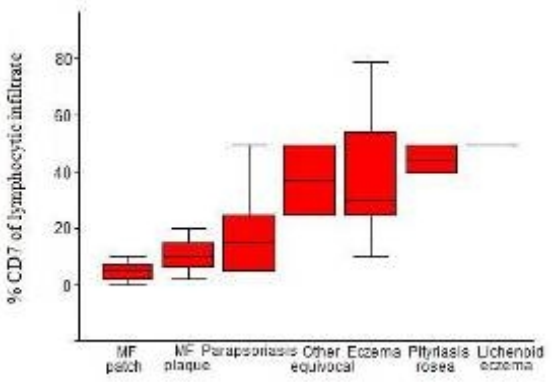

Figure 1: Comparison of CD7 expression in the diverse cutaneous benign and malignant lymphocytic infiltrates. The Patch stage of mycosis fungoides (MF) showed the lowest and least variable expression of CD7 compared with other groups of equivocal and benign inflammatory infiltrate (eczema and pityriasis rosea). There is lack of significant overlap between patch stage of MF and BID group, and also between parapsoriasis and BID. There is a significant overlap between plaque stage of MF and parapsoriasis which could be differentiated by cytomorphologic features. The line in the middle of the box represents the median.

Mycosis Fungoides

A total of 15 patients with MF were diagnosed. There were nine male and six female patients with male to female ratio of 1.5. The age ranged from 22-75 years and duration of the disease prior to diagnosis varied between 2 months to 10 years. The cases included 12 patch stages (figure 2a, 2b), three cases with poikilodermatous features (figure 3a, 3b, 3c) and 3 plaque stage. All (100%) cases had complete absence of CD7 expression in the epidermotropic mycosis cells (figure 2b, 3c), but CD7 was expressed in the dermal lymphocytes of seven (46.6%) cases ranging between 5% up to 30%. Three of 12 (25%) cases of patch stage were also negative for CD7 expression in the dermis and other nine cases had less than 10% (six cases), 10% (one case), and 30% (two cases) positive staining for CD7. Of the three patients with plaque stage, one expressed less than 10%, and two others showed 10% and 20% CD7 positive cells in the dermal infiltrates. This significant difference was also true for the patch stage of MF compared with BID (Mann-Whitney test, P< 0.0001). Compared with plaque, patch stage had lower CD7 counts (figure 1).

Compared with the other T- cell markers, the mean CD7 counts were significantly lower than the mean CD43, CD3, and CD5 (Wilcoxon Signed Ranks test, P=0.001). CD20 levels were non-existent or lower than CD7 counts in all the cases (Wilcoxon Signed Ranks test, P=0.002). CD5 counts closely matched CD3 levels in all the cases. CD43 had the most populated and strongest expression among dermal and epidermal cells.

The lowest mean CD7 counts were found in patch stage of MF. Low CD7 expression < 10 % lymphocytes had sensitivity and positive predictive values of 75% and 100% and specificity and negative predictive values of 100% and 83.3% for the diagnosis of patch stage of MF.

Parapsoriasis and Poikilodermatous Reaction (Clinically Suspicious, but not Histologically Diagnostic for MF)

This group included 10 cases of parapsoriasis, and two poikilodermatous reaction. Their age ranged from 17- 79 years with female to male ratio of 3:1. The mean CD7 count of this group was significantly higher than that of MF (Mann-Whitney test, P =0.01) and also lower than BID group (Mann-Whitney test,P=0.016). In addition, the mean CD7 counts were significantly lower than the CD3, CD5, and CD43 and significantly higher than CD20 counts (Wilcoxon Signed Ranks test, P =0.002). A wide range for CD7 expression that overlapped

Table1: Immunophenotype of diverse benign and malignant cutaneous infiltrate

STUDY GROUP		CD43*	CD3*	CD5*	CD7*	CD20*	D7:CD3*
MF (patch stage)	Mean	7.000	90.000	66.670	8.170	1.420	0.0918
N:12	Std. D	1.477	4.264	31.067	10.513	2.234	0.121
Equivocal	Mean	97.100	88.000	77.000	20.000	1.700	0.229
(parapsoriasis) N:10	Std. D	1.449	5.869	10.853	17.321	1.494	0.194
BID	Mean	95.870	88.000	76.670	42.330	2.730	0.482
N:15	Std. D	4.912	7.270	9.759	20.948	5.509	0.228
Total	Mean	96.570	88.650	73.510	25.220	2.030	0.287
N:37	Std. D	3.304	5.969	19.610	22.493	3.775	0.252

*Percentage of both epidermal and dermal lymphocytic infiltrate labeling with specific CD antibody: mean and SD.

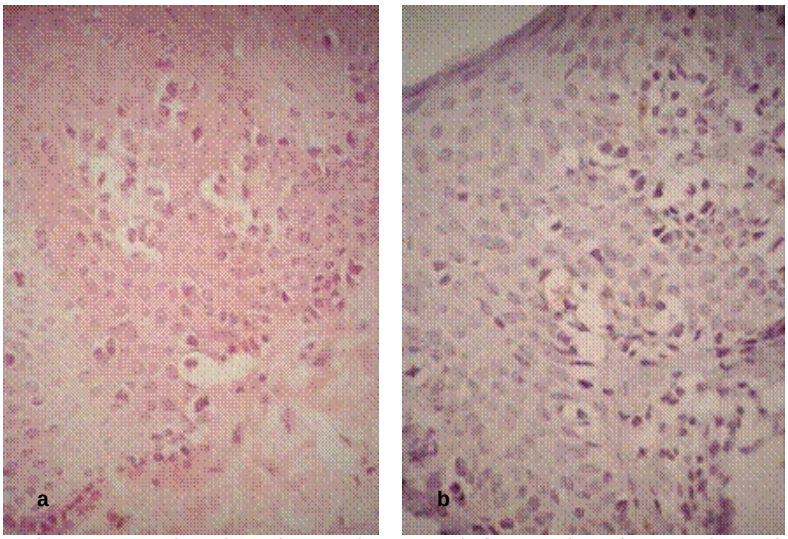

Figure 2: Patch stage of MF, **a.** Pautrier micro abscess (H&E X250). **b.** Complete absence of CD7 immunoreactivity in the dermal and epidermal lymphocytes.

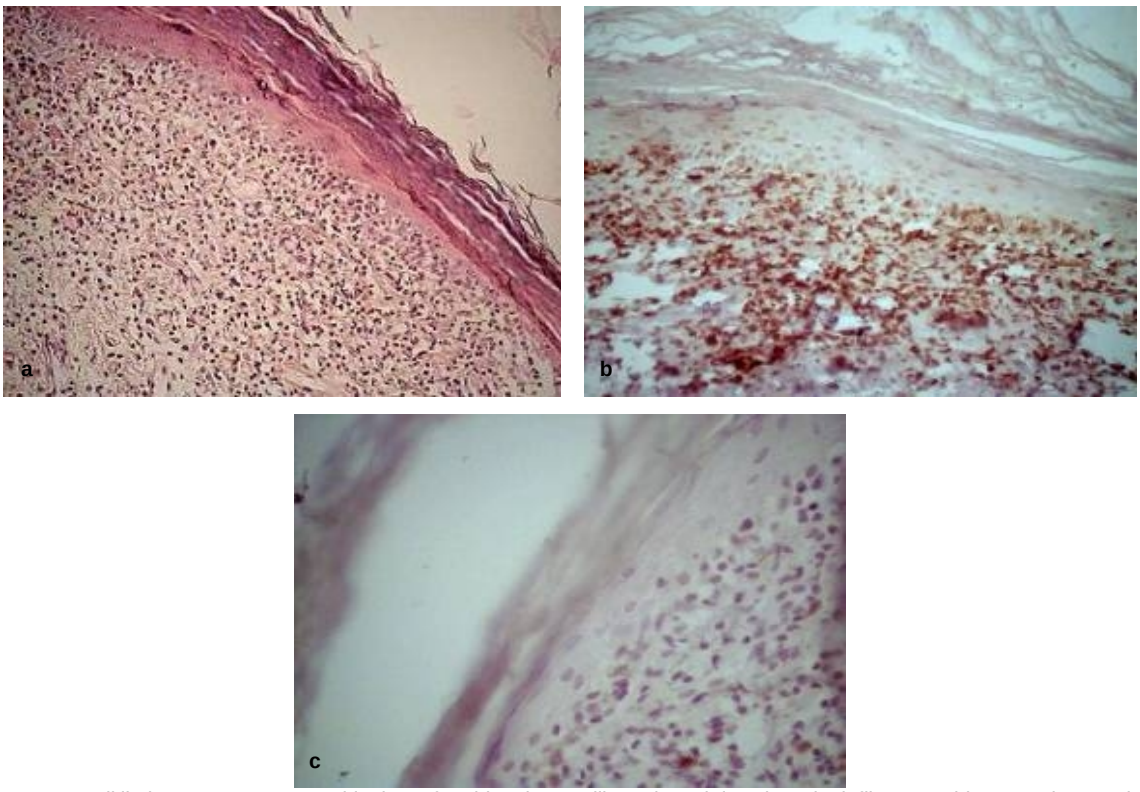

Figure 3: Poikilodermatous MF, **a.** Thinning of epidermis, papillary dermal lymphocytic infiltrate, epidermotropism and increased vascular channels (H&E X100). **b.** Large dermal aggregates of CD3+ lymphocytes. **c.** Complete absence of CD7 immunoreactivity in the epidermal infiltrate.

with MF was found for cases we classified as parapsoriasis (figure 1). Dermal lymphocytes were positive in various percentages for CD7 in 12 cases and ranged from <10% up to 50%.

Of the ten cases of parapsoriasis, four had no CD7 expression, but the other six cases showed 10-25% CD7 positive cells in the epidermal lymphocytes. All of the ten cases presented CD7 in their dermal lymphocytes (<10% up to 50%), three less than 10 %, and two had 50%. Two cases of poikilodermatous reaction presented CD7 in 25% of dermal lymphocytic infiltrates. Four cases of parapsoriasis were negative for CD7 expression in the epidermal lymphocytic infiltrate.

Benign Inflammatory Dermatosis (Eczematous Dermatitis, Pityriasis Rosea, and Lichenoid Eczema)

Fifteen patients with clinical diagnosis of benign inflammatory dermatosis (13 eczema and 2 pityriasis rosea) were included in this study. Their ages ranged from 11-57 years with female to male ratio 2:1. CD7 was presented in all 15

patients in both epidermal and dermal infiltrates ranging from 10% up to 80%. CD20% was negative or presented from 2-20% of dermal infiltrate.

Chronic eczema cases included nine patients who had positive CD7 cells (figure 4) ranging from 10-80% in both epidermal and dermal lymphocytes. Two cases of subacute dermatitis and two lichenoid eczema and also two cases of pityriasis rosea showed CD7 positive cells in 50% of their lymphocytic infiltrates.

Similar to the equivocal group, patients with BID showed a significantly higher mean CD7 count than MF group (Mann-Whitney test, P <0.0001). However, at its lowest range, this group contained a few cases that overlapped with the CD7 counts in MF group. Of the 15 cases of this group, 12 (80%) had a CD7 count of 50% or less (10%-25%, n=3, 26-50%, n=9), and three (20%) cases had CD7 count of >50%. CD7 counts in this group were significantly lower than CD3 and CD5 counts and higher than CD20 counts (Wilcoxon Signed Ranks test, P=0.001).

Two out of the 15 cases of BID group showed both spongiotic and lichenoid reaction diagnosed as lichenoid eczema, which had 50% CD7 count. The two other cases from this group with a diagnosis of pityriasis rosea had CD7 count of 50%.

Patch stage of MF showed the lowest and least variable expression of CD7 compared with equivocal and BID groups. But there was a significant overlap between parapsoriasis and plaque stage of MF.

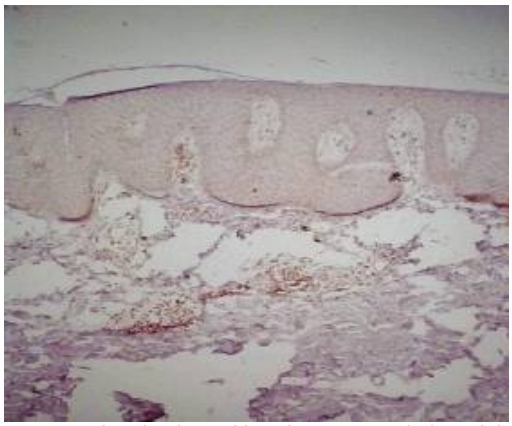

Figure 4: Chronic dermatitis, about 50% of dermal lymphocytes are positive for CD7.

Discussion

The clinical and histological diagnosis of MF is often difficult. Even with histologic evaluation, early stage MF can be difficult to distinguish from various benign inflammatory dermatoses such as lymphomatoid drug reaction, contact dermatitis, and parapsoriasis, particularly when mixed cell populations with only occasional atypical cells are observed.[10]

Because of limitations of histomorphology in the difficult cases, assessment of abnormal T-cell immunophenotype such as CD7 deletion and a monoclonal TCR gene rearrangement is increasingly used to help differentiate MF from reactive benign dermatosis.[10] CD7 can be effectively evaluated on formalin- fixed, paraffin embedded biopsy specimens obtained in community clinical practice.[11]

We analyzed the expression of CD7 in paraffin embedded specimens of MF, BID, and equivocal cases of possible MF. Our results showed that MF exhibits little or no CD7 expression, whereas BID can show a broad range of CD7 expression that overlaps with the limited range found for MF in a few cases.

The present study showed that all 15 cases of MF were negative for CD7 in the epidermotropic atypical lymphocytes and CD7 did not express in three cases in the dermal lymphoid infiltrate. All the 15 patients with benign inflammatory dermatosis were positive for CD7 in their epidermal and dermal infiltrates.

Murphy, et al,[7] in a study consisting of 22 cases of MF and 61 controls showed minimal CD7 expression by lymphocytes in MF and a few cases of benign inflammatory dermatosis (BID).

The results of their study revealed that the lowest mean CD7 counts as a percentage of total lymphocytes were found in MF patch stage, range of 0-10, plaque and tumor stages with a range of 5-25. These counts were significantly lower than those of BID with a range of 5-80 (P=0.001). Logistic regression analysis in the study showed that low CD7 expression (<10 lymphocytes labeling) had sensitivity and positive predictive values of 80% and 72%, respectively and specificity and negative predictive values of 93% and 96%, respectively for the diagnosis of patch stage MF.

Of the 12 equivocal cases of possible MF, four were negative for CD7 expression in the epidermal lymphocytes, but all cases had CD7 expression in their dermal infiltrates. For better evaluation, we recommend follow up re-biopsy from proper site.

Some inflammatory non-neoplastic T-cell hyperplasia including cutaneous drug reactions show histological features resembling patch stage of MF (epidermotropism). In some skin drug reactions, atypical cells, or even immunophenotypic changes of MF and also monoclonal TCR gene rearrangement are seen, which are reversible after cessation of the drugs.[11] Careful consideration of clinical factors should be undertaken before definitively excluding a lymphomatoid drug eruption in the differential diagnosis of epidermotropic T-cell malignancies.[12] On occasion, subacute to chronic spongiotic or lichenoid dermatitis may be associated with lymphoid

epidermotropism in the relative absence of intercellular edema or apoptosis.[11]

Ormbsy, et al,[6] in their study on 17 cases of MF and 27 cases of lichen planus (LP), found no CD7 deletion in any case of LP. The sensitivity and specificity of CD7 deletion for MF was 81% and 100%, respectively.

Ormbsby, et al,[6] who used the EDTA method of antigen retrieval, found no CD7 deletion in their BID cases, but in 81% of their MF cases CD7 deletion was observed. Murphy, et al,[7] used sodium citrate buffer (pH = 6.0) for antigen retrieval. They repeated IHC testing for CD7 in a subset of cases after EDTA buffer (pH = 8.0) antigen retrieval. They found that although EDTA antigen retrieval resulted in a small increase in CD7 in some samples, CD7 counts remained lower than CD3 counts in all cases. This minor increase in CD7 antigen expression can affect the results.

Benign inflammatory dermatosis can harbor monoclonal T-cell populations (so-called clonal dermatitis), with consequence of clonal expansion of antigen selected T-cells. Thus neither monoclonal T-cell populations nor CD7-T populations are diagnostic for MF. Because BID can show CD7 loss similar to that of MF and MF can show the gain of CD7, CD7 expression is best assessed in conjunction with other broad panel lymphoid markers.

Minimal CD7 expression in equivocal cases can be considered as a high risk to develop MF, so follow up biopsy and TCR gene rearrangement should be recommended. The results of a similar study performed by Murphy, et al on 22 cases of MF and 61 controls showed the lowest mean CD7 counts in MF. The counts were significantly lower than those for BID (P= 0.001). Low CD7 expression (<10% lymphocytes) had sensitivity and positive predictive values of 80% and 72%, respectively, and specificity and negative predictive values of 93% and 96%, respectively, for the diagnosis of patch stage MF. They concluded that minimal CD7 expression was a specific finding for MF.

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Conflict of Interest: None declared

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