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ORIGINAL PAPER

Clinical, Histopathological, Immunophenotypic and Molecular Analysis of 60 Patients with Cutaneous T-cell Infiltrates with Follow up of Indeterminate Cases to Identify T-cell Lymphoma

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Abstract Diagnosis of primary cutaneous T-cell lymphomas, especially of mycosis fungoides could be difficult in early stage due to clinical and histopathological similarity to reactive inflammatory dermatoses. To assess diagnostic value of complex histological, immunophenotypic and T-cell receptor γ gene rearrangement analysis, skin biopsy specimen and peripheral blood samples of 60 patients with suspected cutaneous T-cell lymphoma were analyzed. Our results indicate clear distinction between reactive dermatoses (*benign cases*, $n=31$) and cutaneous T-cell lymphomas (*lymphoma cases*, $n=17$). As definite diagnosis was not obtained in a smaller group of patients (*indeterminate cases*, $n=12$), these patients were followed up. Repeated skin biopsy confirmed mycosis fungoides in 6/12 cases, however in 6/12 patients the diagnosis remained *indeterminate*. We concluded that careful and complex clinical follow up and repeated histopathological, immunophenotypic and molecular analysis is needed for an appropriate diagnosis in the assessment of early stage mycosis fungoides and uncertain clinical cases.

Keywords Cutaneous T-cell lymphoma · Mycosis fungoides · Cutaneous T-cell infiltrate · Histopathology · Immunophenotype · Molecular biology

Abbreviations
PCR Polymerase chain reaction
TCR T-cell receptor

Introduction

Incidence of cutaneous T-cell lymphomas has been increased worldwide over last decades partly due to recent advances in diagnostic procedures. Accurate diagnosis could be established by simultaneously performed histological, immunophenotypic and gene rearrangement analysis, however diagnosis of early stage mycosis fungoides remained an important diagnostic problem in dermatopathology.

Many cutaneous lymphomas develop with rapidly growing skin tumors, so remarkable clinical presentation leads to early skin biopsy and histopathological examination. However mycosis fungoides has slow evolution from erythematous scaly patches resembling benign dermatoses such as eczema, psoriasis or fungal infections, so histopathological confirmation of the malignant process may delay even for years.

Histopathological findings in early patch stage of mycosis fungoides are often nonspecific. Disproportionate

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epidermotropism, Pautrier's microabscesses, band-like dermal infiltrate composed of atypical lymphoid cells and lymphocytes with medium to large sized cerebriform nuclei are highly characteristic findings for mycosis fungoides but mainly detected in later plaque stage [9]. Tumor cells have the phenotype of T-helper cells but detection of CD4 predominance is difficult also in the early stage due to low proportion of neoplastic cells [16, 17]. Demonstration of aberrant phenotypes such as CD4/CD8 coexpression and antigen deficiencies of CD2, CD3, CD5, CD7 antigens are useful markers in differentiation of mycosis fungoides from other inflammatory dermatoses, but they are related with advanced stage [2]. With application of T-cell receptor γ gene rearrangement analysis using polymerase chain reaction a malignant process can be detected at earlier stages when conventional morphologic and immunophenotypic studies are not diagnostic [8].

To assess diagnostic value of histopathological, immunophenotypic and molecular biological analysis of cutaneous T-cell infiltrates, we performed simultaneous histological, immunophenotypic and T-cell receptor γ gene rearrangement analysis of skin biopsy specimens and molecular analysis of peripheral blood samples of 60 patients with clinical suspicion of cutaneous T-cell lymphoma.

Material and Methods

Patients

Sixty patients were included prospectively in the study, 43 males and 17 females (male/female ratio=2.5:1), age ranged from 24 to 84 years (average age: 58.4 years). Twenty patients had mycosis fungoides-like eczematous patches, 9 erythroderma, 12 small plaque parapsoriasis, 5 large plaque parapsoriasis, 1 pityriasis lichenoides chronica, 9 therapy-resistant eczema, 2 actinic reticuloid, 1–1 granuloma annulare and papuloerythroderma of Ofuji (Table 1).

Histopathological, Immunophenotypic and Molecular Analysis

Skin biopsy specimens obtained from lesional skin were fixed in formalin, paraffin embedded and stained with hematoxylin and eosin for conventional histopathological analysis. Immunohistochemical studies were performed on paraffin sections using commercially available monoclonal CD3, CD4, CD5, CD7, CD8, CD20 and CD30 antibodies (DAKO Cytomation). TCR γ gene rearrangement analysis was performed on skin and peripheral blood samples. Polymerase chain reaction (PCR) amplification of the T-cell receptor (TCR) γ gene was performed by using a sense primer 5′-AGG GTT GTG TTG GAA TCA GG-3′ specific for Vγ gene and an antisense primer 5′-CGT CGA CAA CAA GTG TTG TTC CAC-3′ specific for Jγ gene. Linear amplification was performed with Jγ antisense primer for 25 cycles followed by further amplification with Vγ sense and Jγ antisense primers for 30 cycles. The following PCR conditions were used: denaturation at 94°C for 30 s, annealing at 55°C for 45 s and extension at 72°C for 45 s. PCR products were evaluated by polyacrylamide gel electrophoresis staining with ethidium bromide [13]. Positive controls were obtained from clonal skin samples previously analyzed with Southern blot technique, negative controls were obtained from peripheral blood DNA of healthy persons and distilled water. PCR result considered positive when a distinct band was detected, whereas smear-like pattern represented polyclonal population (negative result).

Follow-up

Clinical follow-up was available for all patients. In cases with uncertain diagnosis subsequent skin biopsy and peripheral blood analysis was performed during the follow-up period of 4–65 months (mean: 22 months) after the first biopsy.

Table 1 Clinical diagnosis of patients

Clinical diagnosis (cases)	Benign group I (n=31)	Indeterminate group II (n=12)	Lymphoma group III (n=17)
Mycosis fungoides-like patches (n=20)	5	7	8
Erythroderma (n=9)	6		3
Small plaque parapsoriasis (n=12)	7	4	1
Large plaque parapsoriasis (n=5)	2		3
Pityriasis lichenoides chronica (n=1)	1		
Eczema (n=9)	8		1
Actinic reticuloid (n=2)	1	1	
Granuloma annulare (n=1)	1		
Papuloerythroderma of Ofuji (n=1)			1

Table 2 Follow-up results of indeterminate cases (n=12)

Case (age/sex)	Clinical diagnosis	First biopsy				Follow-up period	Follow-up biopsy				Diagnosis
		Epid.	CD4+	Skin clone	Blood clone		Epid.	CD4+	Skin clone	Blood clone	
53/M	MF-like patches	−	+	−	−	26 mo	−	+	−	−	Suspected MF
50/M	MF-like patches	+	+	−	−	5 mo	−	+	−	−	Suspected MF
55/M	MF-like patches	+	−	−	0	10 mo	−	+	−	−	Suspected MF
59/M	MF-like patches	−	+	−	−	20 mo	−	+	−	−	Suspected MF
37/M	SPP	+	+	−	−	9 mo	+	+	−	−	Suspected MF
70/F	SPP	−	+	−	−	25 mo	+	+	−	+	Suspected MF
43/M	MF-like patches	+	+	−	−	65 mo	+	+	−	−	MF[a]
56/M	SPP	+	+	−	−	4 mo	+	+	−	−	MF[a]
30/M	MF-like patches	+	+	−	−	7 mo	+	+	+	−	MF
60/M	MF-like patches	−	+	−	−	27 mo	+	+	+	+	MF
62/M	Actinic reticuloid	−	+	−	−	25 mo	+ P	+	−	−	MF
57/F	SPP	+	−	−	0	46 mo	+ P	+	−	−	MF

M = male, F = female, MF = Mycosis fungoides, SPP = Small plaque parapsoriasis, Epid = Epidermotropism, CD4+ = CD4+ phenotype, mo = months, P = Pautrier's microabscess, + = positive result, − = negative result, 0 = not done
[a] Prominent band-like lymphoid infiltration in the papillary dermis with marked epidermotropism

Results

Based on the clinical findings and results of complex histological, immunophenotypic and TCR γ gene rearrangement analysis patients were divided into three groups.

Group I (n=31) consisted of patients in whom diagnosis of cutaneous T-cell lymphoma was excluded with certainty (*benign cases*). Clinical diagnosis of patients are listed in Table 1. Histopathological examination revealed only single cell lymphocytic exocytosis in the epidermis in 7 cases, moderate epidermotropism or Pautrier's microabscesses were not detected. Immunophenotypic analysis of the skin samples did not reveal aberrant phenotypes and polyclonal TCR γ gene rearrangement was detected in all skin and blood samples except of blood of a small plaque parapsoriasis case.

Group II (n=12) included patients who were not classified definitely as having benign or malignant disease despite the complex analysis (*indeterminate cases*). Clinical diagnosis of patients are listed in Table 1. Histopathology showed epidermal lymphocytic infiltrate in 8 cases with no Pautrier's microabscesses. Lymphoid infiltration in the epidermis and dermis composed of CD4+ T-cells in 10 cases, however T-cell clonality was not detected in the skin and blood. These cases had suspicion for having cutaneous T-cell lymphoma, but lacked sufficient criteria for definite diagnosis.

Group III (n=17) included patients in whom cutaneous T-cell lymphoma was detected (*lymphoma cases*). Clinical diagnosis of patients are listed in Table 1. Marked epidermotropism (13 cases) and Pautrier's microabscesses (6 cases) were found, dermal and epidermal lymphocytic infiltrate displayed CD4+ T-helper cell phenotype in all cases. Loss of CD7 surface marker was not detected in any cases. Clonal TCR γ gene rearrangement was detected in 7 skin and 10 blood samples, 5 patients had simultaneous skin and blood clonality. Based on our results 12 patients were identified as having mycosis fungoides, 1 patient had Sézary syndrome (erythroderma accompanied by lymph node enlargement and circulating atypical cells in the blood), 4 patients had peripheral T-cell lymphoma (atypical dermal lymphoid infiltration without epidermotropism).

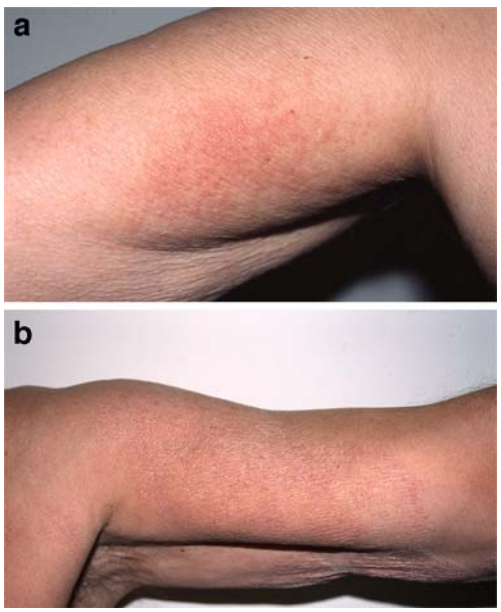

Fig. 1 Mycosis fungoides-like eczematiform patches are indistinguishable by the clinical picture. (**a**) Eczema (**b**) Patch stage mycosis fungoides

Patients of *indeterminate Group II* were followed and subsequent skin biopsy and peripheral blood analysis was performed at 4 to 65 months (average: 22 months) after first biopsy. Six patients (3 with mycosis fungoides-like patches, 2 with small plaque parapsoriasis and 1 with actinic reticuloid) were reclassified as mycosis fungoides cases because of evolution of prominent band-like dermal lymphocytic infiltrate, marked epidermotropism, Pautrier's microabscesses, CD4+ predominance, and/or clonal TCR γ gene rearrangement in the skin and blood samples (Table 2). Six patients lacked sufficient criteria for diagnosis of mycosis fungoides, they remained indeterminate cases.

Supported by the follow-up results finally 23 patients were diagnosed as having CTCL (mycosis fungoides: 18 cases, peripheral T-cell lymphoma: 4 cases, Sézary syndrome: 1 case).

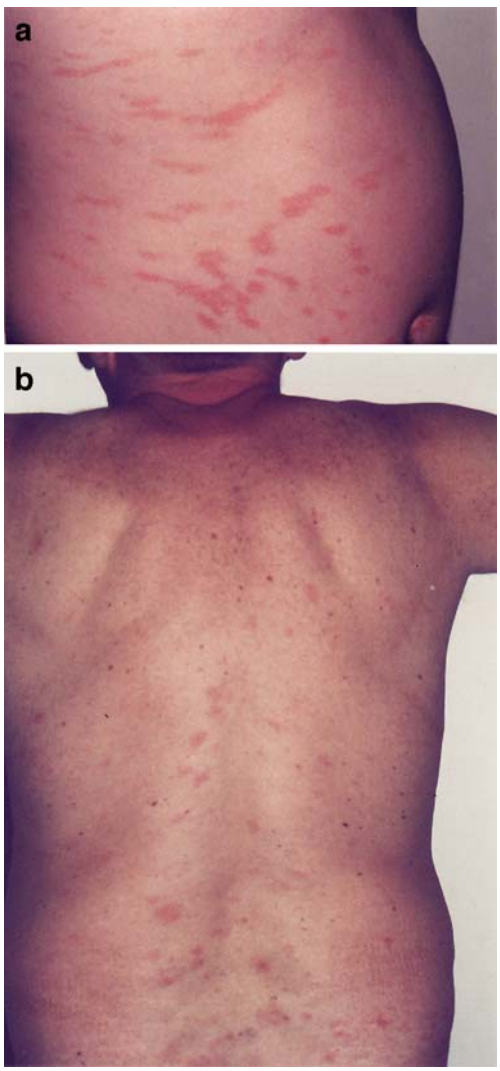

Fig. 2 Cases of small plaque parapsoriasis. (**a**) Indeterminate case (**b**) Mycosis fungoides

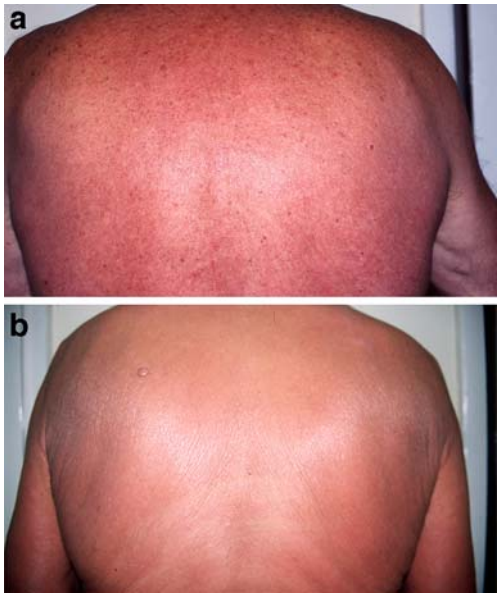

Fig. 3 Diffuse erythema and infiltration of the skin (erythroderma). (**a**) Benign inflammatory dermatitis (**b**) Erythrodermic mycosis fungoides

Discussion

Distinction between early stage of cutaneous T-cell lymphoma – especially of mycosis fungoides – and reactive cutaneous T-cell lymphocytic infiltrates may be difficult (Figs. 1, 2 and 3). We investigated 60 patients with clinical suspicion of cutaneous T-cell lymphoma to assess the diagnostic value of complex histological, immunophenotypic and molecular biological analysis.

Clear distinction was made between reactive lymphocytic infiltrations (n=31, 52%) and cutaneous T-cell lymphomas (n=17, 28%) by first skin biopsy. In 20% of patients (n=12) results were not sufficient to establish or exclude the diagnosis of lymphoma with certainty, these patients were followed and subsequent skin biopsy and blood sampling was performed. Evolution of mycosis fungoides was detected in 6 patients, while other 6 patients remained indeterminate.

PCR analysis revealed dominant T-cell clone in the skin of 9 lymphoma patients and not in indeterminate or benign

Table 3 Results of T-cell receptor γ gene rearrangement analysis of skin and blood (PCR method)

Group (cases)	Skin clonal TCR γ gene rearrangement	Blood clonal TCR γ gene rearrangement
Benign cases (n=31)	0	1
Indeterminate cases (n=6)	0	0
Lymphoma cases (n=23)	9	12

cases (Table 3). Our clonal results proved to be relatively low (39%) in comparison with the literature. Interestingly we detected more T-cell clones in the blood than in skin samples (1/31 patients of benign, 0/6 of indeterminate and in 12/23 lymphoma cases). Circulating T-cell clones were descibed in the literature in early stage cutaneous T-cell lymphoma, in precursor or inflammatory dermatoses as well as in healthy adults [12, 19, 20]. Clonality mostly observed in elderly patients and healthy individuals [4, 15], as we observed in our patients (average age: 61 years). Detection of blood T-cell clone in lymphoma patients does not prove extracutaneous dissemination or advanced stage, because malignant T-cells circulate between skin and lymph nodes, and detected in the blood occasionally [10, 11, 18–20]. T-cell clones are considered to be peripheral tumor cells with independent prognostic value when identical cutaneous clone could be demonstrated simultaneously [5]. We found identical cutaneous and blood clones in 6 patients as identical bands at same base pair level.

In summary, our results support findings from other reports, that diagnostic assessment of cutaneous lymphoproliferative disorders is usually possible with integration of clinical, histopathologic, immunophenotypic and molecular biological findings [1, 3, 6, 7, 14]. Our observations emphasize importance of careful clinical follow-up and repeated skin biopsy in patients with unusual cutaneous lesions, such as mycosis fungoides-like therapy-resistant eczematous patches, small plaque parapsoriasis and actinic reticuloid, which dermatoses are considered as precursor of mycosis fungoides.

References

1. Bachelez H (1999) The clinical use of molecular analysis of clonality in cutaneous lymphocytic infiltrates. Arch Dermatol 135:200–202
2. Bakels V, Oostveen JW, Putte SC, Meijer CJ, Willemze R (1997) Immunophenotyping and gene rearrangement analysis provide additional criteria to differentiate between cutaneous T-cell lymphomas and pseudo-T-cell lymphomas. Am J Pathol 150:1941–1949
3. Burg G, Kempf W, Dummer R (2001) Diagnostic signs of cutaneous lymphomas. J Eur Acad Dermatol Venereol 15:358–360
4. Delfau-Larue MH, Laroche L, Wechsler J, Lepage E, Lahet C, Asso-Bonnet M et al (2000) Diagnostic value of dominant T-cell clones in peripheral blood in 363 patients presenting consecutively with a clinical suspicion of cutaneous lymphoma. Blood 96:2987–2992
5. Dippel E, Klemke D, Hummel M, Stein H, Goerdt S (2001) T-cell clonality of undetermined significance. Blood 98:247–248
6. Flaig MJ, Schuhmann K, Sander CA (2000) Impact of molecular analysis in the diagnosis of cutaneous lymphoid infiltrates. Semin Cutan Med Surg 19:87–90
7. Holm N, Flaig MJ, Yazdi AS, Sander CA (2002) The value of molecular analysis by PCR in the diagnosis of cutaneous lymphocytic infiltrates. J Cutan Pathol 29:447–452
8. Li N, Bhawan J (2001) New insights into the applicability of T-cell receptor g gene rearrangement analysis in cutaneous T-cell lymphoma. J Cutan Pathol 28:412–418
9. Massone C, Kodama K, Kerl H, Cerroni L (2005) Histopathologic features of early (patch) lesions of mycosis fungoides: a morphologic study on 745 biopsy specimens from 427 patients. Am J Surg Pathol 29:550–560
10. Muche MJ, Lukowsky A, Asadullah K, Gellrich S, Sterry W (1997) Demonstration of frequent occurence of clonal T cells in the peripheral blood of patients with primary cutaneous T-cell lymphoma. Blood 90:1636–1642
11. Muche MJ, Lukowsky A, Heim J, Friedrich M, Audring H, Sterry W (1999) Demonstration of frequent occurence of clonal T cells in the peripheral blood but not in the skin of patients with small plaque parapsoriasis. Blood 94:1409–1417
12. Muche MJ, Sterry W, Gellrich S, Rzany B, Audring H, Lukowsky A (2003) Peripheral blood T-cell clonality in mycosis fungoides and nonlymphoma controls. Diagn Mol Pathol 12:142–150
13. Nagy M, Fehér K, László T, Szomor Á, Losonczy H, Kelényi G et al (1999) Analysis of the T-cell receptor g gene rearrangement in lymphoproliferative disorders using polymerase chain reaction. Orv Hetil 140:2441–2444
14. Pimpinelli N, Olsen EA, Santucci M, Vonderheid E, Haeffner AC, Stevens S et al (2005) Defining early mycosis fungoides. J Am Acad Dermatol 53:1053–1063
15. Posnett DN, Sinha R, Kabak S, Russo C (1994) Clonal populations of T cells in normal elderly humans: the T cell equivalent to “benign monoclonal gammopathy”. J Exp Med 179:609–618
16. Santucci M, Biggeri A, Feller AC, Massi D, Burg G (2000) Efficacy of histologic criteria for diagnosing early mycosis fungoides. An EORTC Cutaneous Lymphoma Study Group investigation. Am J Surg Pathol 24:40–50
17. Smoller BR, Bishop K, Glusac E, Kim YH, Hendrickson M (1995) Reassessment of histologic parameters in the diagnosis of mycosis fungoides. Am J Surg Pathol 19:1424–1430
18. Theodorou I, Delfau-Larue MH, Bigorgne C, Lahet C, Cochet G, Bagot M (1995) Cutaneous T-cell infiltrates: analysis of T-cell receptor g gene rearrangement by polymerase chain reaction and denaturing gradient gel electrophoresis. Blood 86:305–310
19. Wood GS (1998) Using molecular biologic analysis of T-cell receptor gene rearrangements to stage cutaneous T-cell lymphoma. Arch Dermatol 134:221–223
20. Wood GS (2001) Analysis of clonality in cutaneous T cell lymphoma and associated diseases. Ann N Y Acad Sci 941:26–30