Clinical staging and prognostic factors in folliculotropic mycosis fungoides

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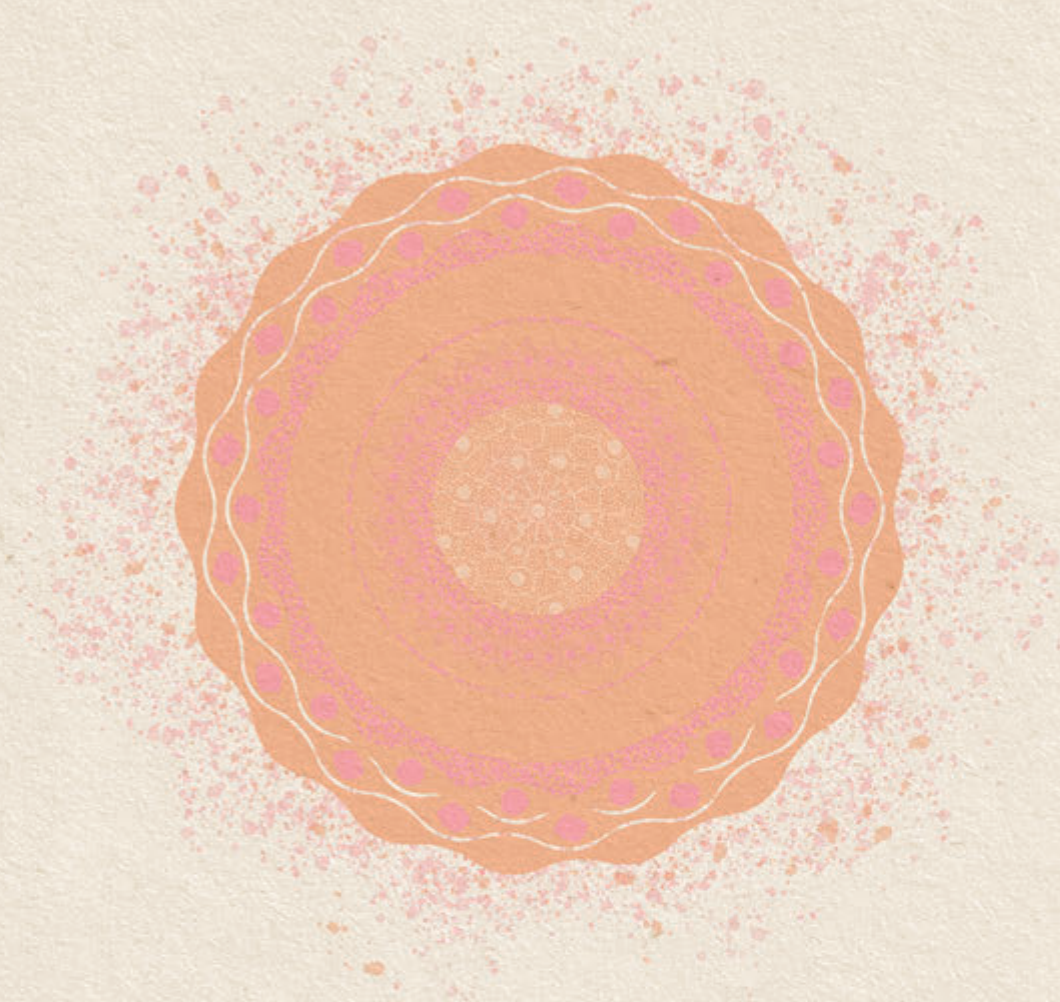

CLINICAL STAGING AND PROGNOSTIC FACTORS IN FOLLICULOTROPIC MYCOSIS FUNGOIDES

Suzanne van Santen

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CLINICAL STAGING AND PROGNOSTIC FACTORS IN FOLLICULOTROPIC MYCOSIS FUNGOIDES

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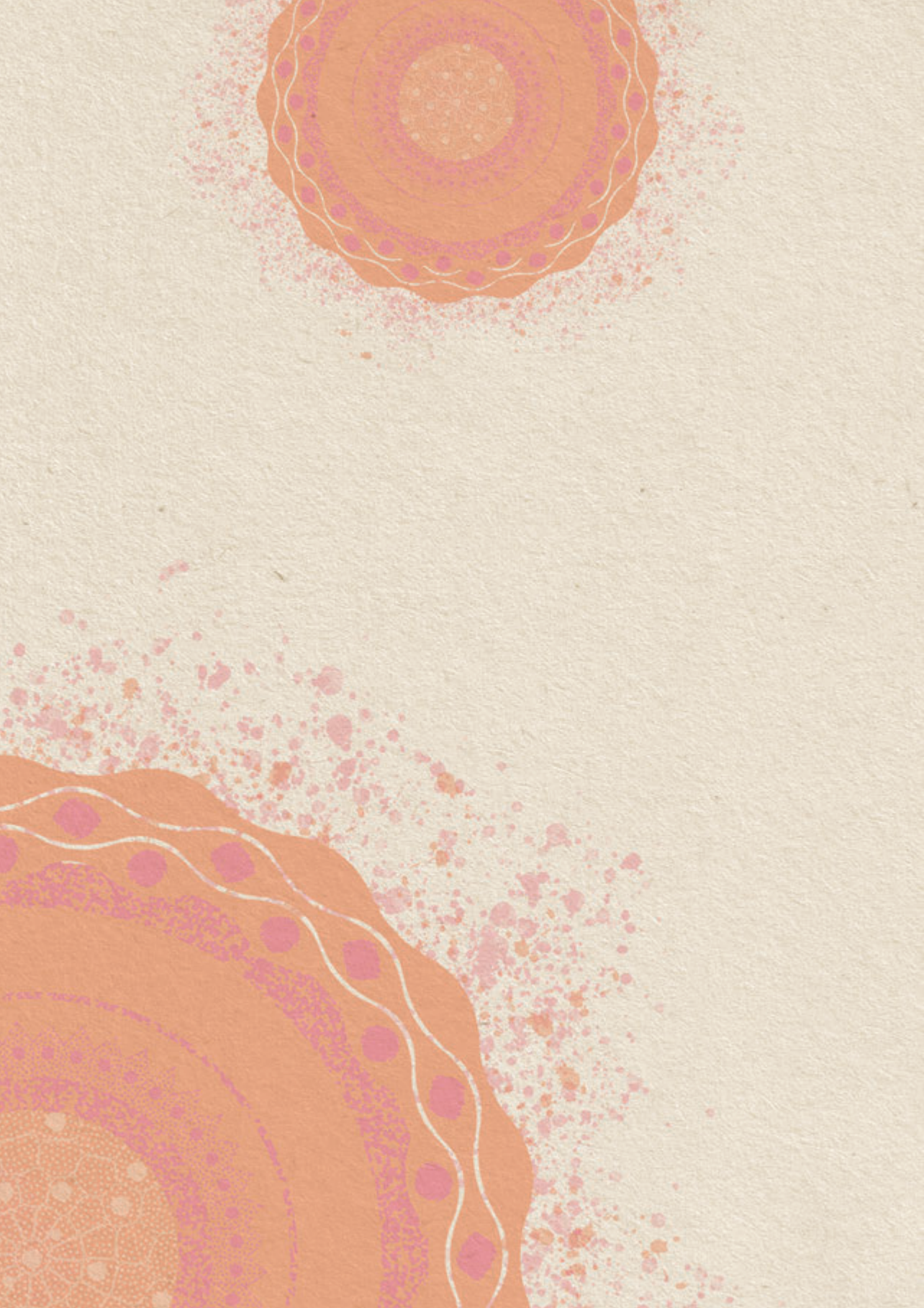


1

GENERAL INTRODUCTION

PRIMARY CUTANEOUS T-CELL LYMPHOMAS

Primary cutaneous lymphomas represent a heterogeneous group of non-Hodgkin lymphomas (NHL) presenting in the skin without evidence of extracutaneous disease at diagnosis. After the gastro-intestinal tract lymphomas, primary cutaneous lymphomas are the second most common group of extra-nodal NHL with an estimated annual incidence of 1:100.000 individuals.[1] Primary cutaneous lymphomas often differ in clinical behavior, prognosis and biological features when compared to morphologically similar lymphomas arising in lymph nodes, and therefore require different types of treatment.[2] For this reason they have been included as separate entities in recent classification systems for non-Hodgkin lymphomas, such as the World Health Organization - European Organization for Research and Treatment of Cancer (WHO-EORTC) classification for cutaneous lymphomas and the WHO classification of lymphoid neoplasms in 2016.[3,4] Within these classifications two main groups of primary cutaneous lymphomas can be distinguished: primary cutaneous T-cell lymphomas (CTCL) accounting for 75% of the cases in the Western world, and primary cutaneous B-cell lymphomas (CBCL) that account for the remaining 25%.[3] (**Table 1**)

Mycosis fungoides (MF) is the most common type of CTCL. Folliculotropic mycosis fungoides (FMF) is recognized as a rare but distinct variant of MF in the WHO-EORTC classification due to its distinctive clinicopathologic and prognostic features. The studies in this thesis are focused on this folliculotropic variant of MF and will describe further insights into clinical staging, disease course, prognostic parameters and treatment of FMF patients. This introductory chapter starts with a brief overview on the characteristics of the classical type of MF, followed by a more extensive outline on the distinctive features of FMF.

Table 1. 2018 update of the WHO-EORTC classification for cutaneous lymphomas and relative frequencies.[3]

WHO-EORTC Classification 2018	Frequency (%)
Cutaneous T-cell lymphoma (CTCL)	
MF	39
MF variants:	
Folliculotropic MF	5
Pagetoid reticulosis	<1
Granulomatous slack skin	<1
Sézary syndrome	2
Adult T-cell leukemia/lymphoma	<1
Primary cutaneous CD30+ lymphoproliferative disorders	
C-ALCL	8
Lyp	12
Subcutaneous panniculitis-like T-cell lymphoma	1
Extranodal NK/T-cell lymphoma, nasal type	<1
Chronic active EBV infection	<1
Primary cutaneous peripheral T-cell lymphoma, rare subtypes	
Primary cutaneous γ/δ T-cell lymphoma	<1
CD8+ AECTCL (provisional)	<1
Primary cutaneous CD4+ small/medium T-cell lymfoproliferative disorder (provisional)	6
Primary cutaneous acral CD8+ T-cell lymphoma (provisional)	<1
Primary cutaneous peripheral T-cell lymphoma, NOS	2
Cutaneous B-cell lymphoma (CBCL)	
Primary cutaneous marginal zone lymphoma	9
Primary cutaneous follicle center lymphoma	12
Primary cutaneous diffuse large B-cell lymphoma, legtype	4
EBV+ mucocutaneous ulcer (provisional)	<1
Intravascular large B-cell lymphoma	<1

CD8+ AECTCL: primary cutaneous aggressive epidermotropic CD8+ cytotoxic T-cell lymphoma, C- ALCL: primary cutaneous anaplastic large cell lymphoma, EBV: Epstein-Barrvirus, Lyp: lymphomatoid papulosis, MF: mycosis fungoides, NOS: not otherwise specified.

MYCOSIS FUNGOIDES

Clinical features

MF accounts for almost 50% of all primary cutaneous lymphomas and is clinically characterized by a slow evolution from patches to plaques to eventually tumors. A small minority of patients may develop extracutaneous dissemination.[2] MF typically affects older adults and occurs more frequently in males than in females. Skin lesions are preferentially localized on the buttocks and other non-sunexposed areas (**Figure 1 A-C**).

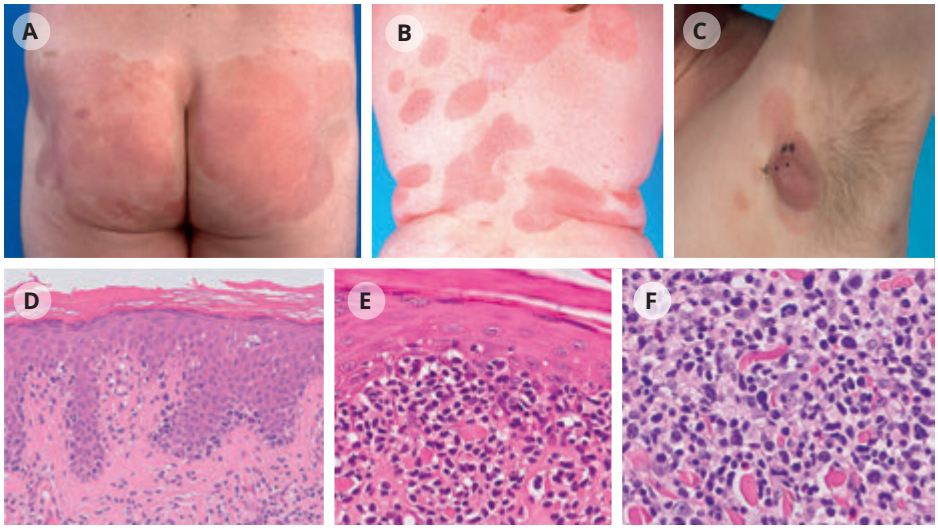


Figure 1. Examples of clinical manifestations of classic MF with limited patches (A), widespread patches, plaques (B) and a skin tumor (C) and their concordant histological pictures. Histopathologic pictures of patch- and plaque-stage MF (D and E) show sparse infiltration of atypical hyperchromatic and cerebriform T-cells into the epidermis (epidermotropism) (D) and an increased infiltrate with Pautrier microabcesses (E). Picture of tumor-stage MF (F) shows a diffuse infiltrate containing large cerebriform and blast cells.

Histology and phenotype

Patches and plaques in MF are histologically characterized by a band-like or lichenoid infiltrate in the papillary dermis consisting of atypical small- to medium-sized T-cells with convoluted (cerebriform) nuclei that infiltrate the epidermis (epidermotropism).[2] A highly characteristic feature are intra-epidermal collections of cerebriform T-cells (Pautrier microabscesses), which is observed in only a minority of cases.[5] In

tumor-stage MF, the dermal infiltrate becomes more diffuse with an increase in the proportion of tumor cells (**Figure 1 D-F**). Infiltrates may contain variable numbers of small, medium-sized, to large cerebriform cells and blast cells with prominent nuclei, and epidermotropism may disappear. The atypical MF cells usually exhibit a CD4+CD8- phenotype, but CD4-CD8+, CD4-CD8- or CD4+CD8+ are sometimes found and loss of pan-T-cell antigens such as CD2, CD3, CD5 and CD7 is a common finding, particularly in the advanced stages of the disease.[6-10]

Prognosis and prognostic features

Prognosis and risk of disease progression in MF patients are closely correlated with clinical stage.[11] Staging occurs via the clinical staging system for patients with MF/SS, based on the tumor-node-metastasis-blood (TNMB) classification. This staging system classifies both type and extent of skin lesions, and the presence and degree of lymph node, visceral and blood involvement (**Table 2: TNMB classification** and **Table 3: Clinical staging in MF/SS**).[12] Patients with patches and/or plaques (stage IA–IIA) are considered to have early-stage disease, while patients with tumors, erythroderma or nodal, blood and/or visceral involvement (stage IIB-IV) have advanced-stage disease. Patients with early-stage disease commonly run an indolent disease course and survival of MF stage IA is even comparable with age-, race- and sex-matched controls.[13-15] The prognosis becomes less favorable in patients developing MF stage IIB (tumor stage), stage III (erythroderma) or stage IV (extracutaneous disease), which have been associated with a 10-year disease-specific survival of 42%, 45% and 20%, respectively.[11] Patients usually die of systemic involvement or infections. Apart from clinical stage, independent prognostic factors associated with reduced survival in MF are advanced age, male sex, elevated lactate dehydrogenase (LDH), large cell transformation and the folliculotropic variant of MF.[11,15-21] As the folliculotropic variant of MF is the main topic of this thesis, the next paragraphs will further discuss the distinctive clinicopathologic features, diagnosis, staging, treatment and prognosis of folliculotropic mycosis fungoides.

Table 2. TNMB classification of mycosis fungoides and Sézary syndrome[12]

T (skin)	
T_1	Limited patch/ plaque (< 10% of total skin surface)
T_2	Generalized patch/ plaque (≥ 10% of total skin surface)
T_3	One or more tumors (≥ 1 cm diameter)
T_4	Erythroderma (≥ 80% of total skin surface)
N (lymph node)	
N_0	No clinically enlarged lymph nodes
N_1	Clinically enlarged lymph nodes, histologically uninvolved
N_2	Clinically enlarged lymph nodes, histologically involved (nodal architecture uneffaced)
N_3	Clinically enlarged lymph nodes, histologically involved (nodal architecture (partially) effaced)
M (viscera)	
M_0	No visceral involvement
M_1	Visceral involvement
B (blood)	
B_0	No circulating atypical (Sézary) cells (or < 5% of lymphocytes)
B_1	Low blood tumor burden (≥ 5% of lymphocytes are atypical (Sézary) cells, but does not meet criteria B2)
B_2	High blood tumor burden (positive clone and either ≥1000/μL Sézary cells or CD4/CD8 ratio>10 or CD4+CD7- cells≥ 40% or CD4+CD26- cells ≥ 30%)

Table 3. Clinical staging system for mycosis fungoides and Sézary syndrome[12]

Early-stage disease				
IA	T_1	N_0	M_0	B_{0-1}
IB	T_2	N_0	M_0	B_{0-1}
IIA	T_{1-2}	N_{1-2}	M_0	B_{0-1}
Advanced-stage disease				
IIB	T_3	N_{0-2}	M_0	B_{0-1}
III	T_4	N_{0-2}	M_0	B_{0-1}
IVA_1	T_{1-4}	N_{0-2}	M_0	B_2
IVA_2	T_{1-4}	N_3	M_0	B_{0-2}
IVB	T_{1-4}	N_{0-3}	M_1	B_{0-2}

FOLLICULOTROPIC MYCOSIS FUNGOIDES

In large series, folliculotropic mycosis fungoides makes up approximately 10% of all MF patients.[2] In 2002, van Doorn et al. studied the differences between classic MF and FMF

and found that patients with FMF differ both clinically, histologically and prognostically. Folliculotropic MF is defined by the presence of folliculotropic (infiltration of hair follicles) instead of epidermotropic infiltrates and the preferential involvement of skin lesions in the head- and neck area. FMF is generally more resistant to therapy and associated with a worse prognosis when compared to classic MF.[22] These observations were confirmed by several other studies and in recent (cutaneous) lymphoma classifications FMF has therefore been included as a distinct variant of MF.[2,23,24]

Clinical features

Clinically, FMF patients may present with (grouped) follicular papules, acneiform lesions, infiltrated plaques and/or tumors, which preferentially involve and are often most pronounced in the head- and neck area.[22-30] Patients may show keratosis pilaris-like lesions, mainly on extremities and trunk.[30-32] Skin lesions are often associated with local alopecia. The presence of infiltrated plaques in the eyebrow region with concurrent alopecia is a common and a highly characteristic feature (**Figure 2 A-G**). In rare cases FMF may present with a solitary skin lesion or with erythroderma.[33-35] Patients are often diagnosed with secondary bacterial infections. Complaints of (extensive) pruritus are common which may be difficult to manage therapeutically, whereas pruritus is usually absent in the classical type of MF.[22] **See BOX 1 – Pruritus in CTCL patients.**

Histology

Histologically, FMF is characterized by the presence of perifollicular to confluent infiltrates with variable infiltration of the follicular epithelium (folliculotropism) by small, medium-sized or sometimes large cerebriform T-cells with hyperchromatic nuclei (**Figure 2 H-J**).[2] Infiltration of the follicular epithelium may be accompanied by infiltration of the eccrine sweat glands (syringotropism). Concurrent infiltration of the epidermis (epidermotropism), characteristic of early stage classic MF, is uncommon.[22,44] Many cases show mucinous degeneration of the follicular epithelium (follicular mucinosis), which can be visualized by Alcian blue or colloidal iron staining.

Formerly in literature, these cases had been designated as MF-associated follicular mucinosis. This term has been abandoned when van Doorn et al. found no differences in clinical presentation or clinical behavior between FMF cases with or without associated follicular mucinosis. Periadnexal infiltrates contain apart from neoplastic

T-cells, a variable number of small reactive T-cells, histiocytes and sometimes clusters of B-cells. There is often a considerable admixture with eosinophils and, in particular in cases with secondary bacterial infection, plasma cells.[22,44] Histology of FMF patients presenting with early, non-infiltrated lesions such as patches containing follicle- based papules, keratosis pilaris-like lesions or acneiform lesions, usually contain sparse neoplastic infiltrates, that may be limited to a thin shell around the hair follicle.[30,31,45] With increasing infiltration of the skin lesions such as tumor-stage, the periadnexal infiltrates become more confluent or completely diffuse and may contain increasing numbers of large cerebriform cells or blast cells and hair follicles may get (partially) destructed. Large cell transformation, defined by the presence of more than 25% of blast cells or the presence of clusters of blast cells, has been reported in more than 20% of FMF cases[23,46,47] and is more commonly found than in classic MF.[48,49] Similar to classic MF, most cases of FMF exhibit a CD4+CD8- T-cell phenotype. Admixed tumor blast cells are often CD30-positive.

BOX 1. Pruritus in CTCL patients

Pruritus or 'itching' is the uncomfortable feeling that causes the desire to scratch. The exact mechanism of pruritus in both general skin disease and in CTCL patients specifically are not fully understood. Pruritus may be present in up to 88% of CTCL patients and occurs most frequently in advanced stages of disease.[36] In severe cases pruritus impacts patients' quality of life while it may be challenging to manage symptoms therapeutically.[37] Various mediators, neuropeptides and interleukins have been investigated such as histamines, substance P, IL4, IL5, IL10, IL13 and IL31, but their exact role in the pathogenesis has not fully been elucidated.[38] Conventional antipruritic agents, such as antihistamines, gamma-aminobutyric acid analogs and antidepressants have not or only been partially effective in CTCL patients.[39] In search for novel therapies, several recent studies focused on the role of interleukin 31 (IL31), since a IL31 receptor antagonist (nemolizumab) has appeared on the market. IL31 is a cytokine that is predominantly produced by activated Th2 cells.[40,41] It signals through a heterodimeric receptor that is composed of IL31A receptor and Oncostatin M receptor (OSMR).[40] This receptor is present on keratinocytes, eosinophils and afferent neurons of the dorsal root ganglia.[40] In lymphocytes and mast cells interleukin 4 induces upregulation of IL31. In eosinophils, staphylococcus toxin, interleukins 3, 4, 13 and IL31 itself upregulate IL31.[42] Expression of IL31 may be also triggered by staphylococcus aureus superantigens and colonization by staphylococcus aureus may often occur in CTCL patients, including FMF patients.[43] The role of IL31 in CTCL patients is one of the topics that will be further addressed in **chapter 7** of this thesis.

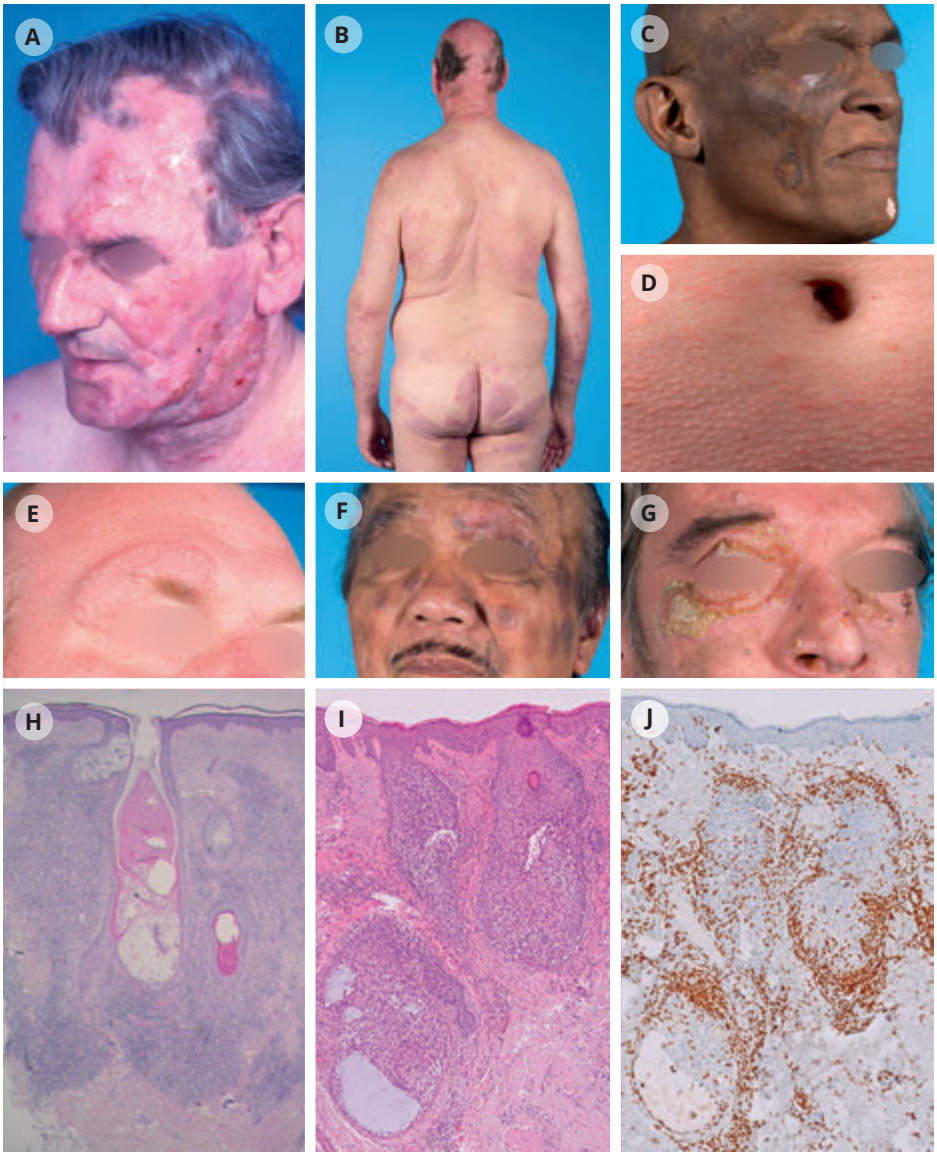


Figure 2. Clinical manifestations and histological pictures of FMF. A: pronounced tumorous skin lesions in head/neck area, B: marked alopecia on the scalp and widespread patches and plaques on the body, C: acneiform/cystic lesions on the cheek, D: characteristic keratosis pilaris-like skin lesions on the abdomen, E: facial plaque with lateral eyebrow hair loss, F: multiple facial plaques with concurrent eyebrow involvement, G: secondary bacterial infection of facial FMF lesions, H: histological picture of prominent perifollicular infiltrates with sparing of the epidermis, I: histological picture of sparse perifollicular infiltrates and mucinosis follicularis; CD3 staining shows distinct folliculotropism (J).

Staging

In classic MF the TNMB classification correlates well with survival, as previously stated (**Table 2**).[11] However, the clinical significance of this staging system for patients with FMF has been questioned. While it has been reported that patients with follicular papules, acneiform lesions or keratosis pilaris-like lesions may have early-stage disease (stage IA-IIA)[30,31,50] and patients presenting with tumors, erythroderma or extracutaneous disease have advanced FMF (stage IIB-IV), it is uncertain if patients presenting with plaques should be classified as early-stage FMF or advanced-stage FMF. Because of the deep perifollicular localization of the dermal infiltrates, it has been suggested that such patients should always be considered to have tumor-stage disease and should be treated accordingly.[22] As criteria to differentiate early- and advanced FMF are not clear, the first study in **chapter 2** will address this issue and evaluates clinical and histological characteristics of a large cohort of FMF patients in order to define criteria for early- and advanced-stage disease in FMF.

Treatment

Reports on treatment results in FMF are very scarce and controlled studies have never been performed. Management of FMF patients follows a similar therapeutic approach as used in patients with classic MF which is aimed at long-term disease control while limiting toxicity.

In general, early aggressive chemotherapy is not desirable in (F)MF patients as it is associated with considerable side effects and does not improve survival nor induces long-term remissions. Also other available treatments rarely induce durable remissions and therefore a stepwise, stage-adapted therapeutic approach is recommended.[51,52] However, as FMF has been associated with more resistance to those therapies, used in classic MF, it has been suggested that patients with FMF should be treated more aggressively, according to tumor-stage disease.[22,23] In this thesis, treatment results in a large cohort of FMF patients will be evaluated, aimed to formulate treatment recommendations in FMF. The results are presented in **chapter 3.**

Prognosis and prognostic factors

Many studies reported that patients with FMF have a worse prognosis as compared to patients with classic MF.[22-24,47] Also in large retrospective cohort studies FMF was found

to be an independent risk factor for reduced survival.[11,16,53] Independent risk factors in classic MF have been well identified, whereas prognostic factors in FMF are currently unknown and these have been the subject of further study throughout this thesis. One of the methods that has recently been used and studied for its prognostic significance in CTCL is high-throughput sequencing of the T-cell receptor (HTS-TCR). **See BOX 2 – High-throughput sequencing of the T-cell receptor in CTCL patients.**

BOX 2. High-throughput sequencing of the T-cell receptor in CTCL patients

T-cell receptors (TCRs) are translationally expressed on mature T-cells as heterodimer receptors. These heterodimer receptors are built from a combination of either α and β chains or γ and δ chains. In peripheral blood, a majority of T-cells possesses αβ TCRs, while a smaller fraction (1–5%) consists of γδ TCRs.[54] TCR formation occurs during lymphocyte development by the combination of variable (V), diversity (D), and joining (J) gene segments and deletion or insertion of nucleotides at the junctions of those segments (C, Constant gene segments). This randomized process allows for the unique T-cell receptor complimentary determining region 3 (CDR3) nucleotide sequences that enables recognition and tracking of a specific T-cell clone (**Figure 3**)**.** Four gene complexes are responsible for the variety of expressed TCRs and rearrange sequentially in a highly ordered manner, starting with *TRD*, followed by *TRG*, *TRB*, and finally *TRA*.

High-throughput sequencing (HTS) of the T-cell receptor (HTS-TCR) is a technique that allows identification and quantification of every distinct T-cell clone present within a biological sample.[55] HTS-TCR technology is based on the specific sequencing of the CDR3 region on one of the two chains of the TCR heterodimer by bias controlled multiplexed polymerase chain reaction (PCR). It is possible to identify and quantify the CDR3 segments and the V(D)J genes based on previously described sequences that can be accessed within data banks. HTS may thus give insight into the diversity of the TCR repertoire in different tissues under non-pathologic and pathologic conditions and the relative frequency of each individual clone within the full T-cell repertoire can be studied (**Figure 4**).[55] This technique has been studied in CTCL patients and shown to have both diagnostic and prognostic value. In MF, HTS of the TCR β and γ alleles was found more sensitive and specific than TCRγ PCR in detecting the pathogenic expanded T-cell clone and HTS-TCR was also found to accurately predict disease progression in early stage MF patients.[56,57] These findings prompted us to further study this HTS-TCR technique in FMF patients and results are presented in **chapter 5**.

BOX 2. *(continued)*

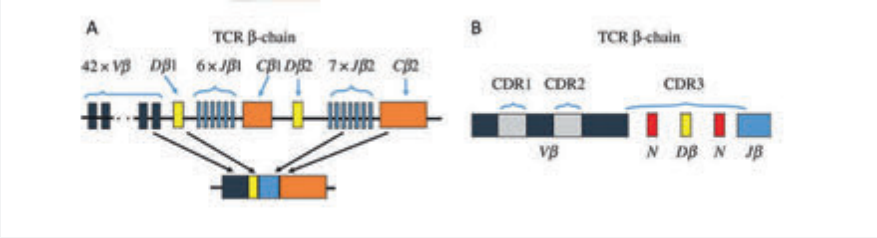


Figure 3. T-cell receptor gene rearrangement. A: Variable (V), joining (J) and constant regions (C) constitute the TCR β-chain, with additional diversity (D) regions (yellow). Segments from each region are recombined, with additional nucleotide additions, to generate each rearranged TCR. These processes generate substantial T-cell diversity. B: Hypervariable complementarity-determining regions (CDR1-CDR3) of the β-chain. CDR1 and CDR2 regions are encoded on the V region, while the most variable CDR3 region straddles the V(D)J junction.

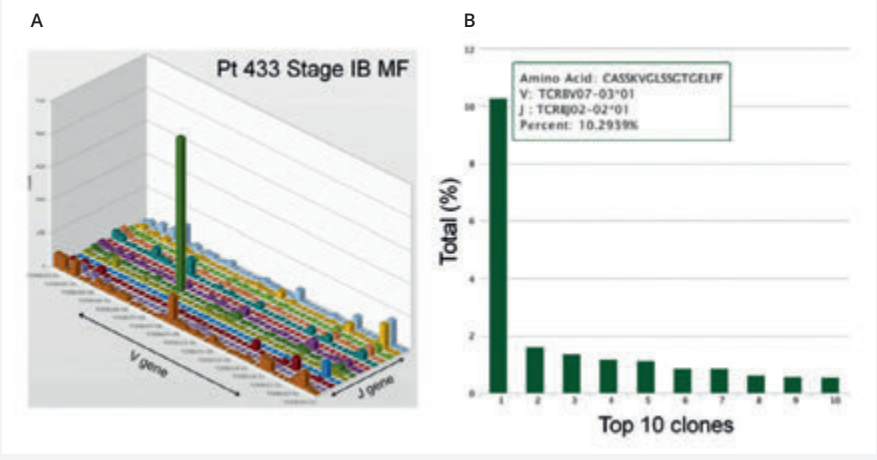


Figure 4. HTS-TCR identifies expanded populations of clonal malignant T-cells and V vs. J gene usages of T-cells from a lesional skin sample (example in A). The green peak includes the clonal malignant T-cell population, as well as other benign T-cells that share the same V and J gene usage. The individual T-cell clone sequence is shown with detailed information on the CDR3 amino acid sequence and V and J gene usage (B). The nine most frequent benign infiltrating T-cell sequences are also shown. In this example, the malignant T-cell clone makes up 10.3% of the total T-cell population in lesional skin.
Adapted from Kirsch et al, *Sci Transl Med.* 2015 October 7; 7(308). Reprinted with permission from AAAS.

AIMS AND OUTLINE OF THIS THESIS

The studies presented in this thesis were aimed to address questions regarding clinical staging, disease course, treatment and prognosis in folliculotropic mycosis fungoides.

Chapter 2 studies the clinicopathologic characteristics and disease course in a cohort of 203 FMF patients in order to identify criteria that could be used to differentiate between FMF cases with an indolent and an aggressive disease course. This study resulted in the identification of early-stage and advanced-stage FMF subgroups, implicating that these may require a different therapeutic approach. As a result, **Chapter 3** evaluates treatment results in early- and advanced-stage FMF and formulates treatment recommendations for different FMF subgroups.

Results from chapter 2 reinforced the impression that FMF patients presenting with plaques represent a prognostically heterogeneous group and that histological criteria may be helpful to differentiate indolent from aggressive cases. This patient group is further studied in chapters 4 and 5. **Chapter 4** evaluates detailed histopathologic features of FMF patients presenting with plaques and provides prognostic factors. **Chapter 5** analyses the prognostic value of T-cell receptor β gene sequencing in FMF patients presenting with plaques.

Chapter 6 describes the clinicopathologic characteristics, prognosis and treatment of a rare subgroup of FMF patients: those cases that present with a solitary skin lesion at time of diagnosis. **Chapter 7** analyses the role of interleukin 31 in pruritus and disease stage in several types of CTCL, including FMF, classic MF and Sézary syndrome.

Chapter 8 summarizes and discusses the findings described in the preceding chapters.

REFERENCES

1. Groves FD, Linet MS, Travis LB, Devesa SS. Cancer surveillance series: non-Hodgkin's lymphoma incidence by histologic subtype in the United States from 1978 through 1995. *Journal of the National Cancer Institute.* 2000;92(15):1240-1251.
2. Willemze R, Jaffe ES, Burg G, et al. WHO-EORTC classification for cutaneous lymphomas. *Blood.* 2005;105(10):3768-3785.
3. Willemze R, Cerroni L, Kempf W, et al. The 2018 update of the WHO-EORTC classification for primary cutaneous lymphomas. *Blood.* 2019;133(16):1703-1714.
4. Swerdlow SH, Campo E, Pileri SA, et al. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. *Blood.* 2016;127(20):2375-2390.
5. Nickoloff BJ. Light-microscopic assessment of 100 patients with patch/plaque-stage mycosis fungoides. *The American Journal of dermatopathology.* 1988;10(6):469-477.
6. Berti E, Tomasini D, Vermeer MH, Meijer CJ, Alessi E, Willemze R. Primary cutaneous CD8- positive epidermotropic cytotoxic T cell lymphomas. A distinct clinicopathological entity with an aggressive clinical behavior. *The American journal of pathology.* 1999;155(2):483-492.
7. Burg G, Kempf W, Cozzio A, et al. WHO/EORTC classification of cutaneous lymphomas 2005: histological and molecular aspects. *Journal of cutaneous pathology.* 2005;32(10):647-674.
8. Whittam LR, Calonje E, Orchard G, Fraser-Andrews EA, Woolford A, Russell-Jones R. CD8- positive juvenile onset mycosis fungoides: an immunohistochemical and genotypic analysis of six cases. *The British journal of dermatology.* 2000;143(6):1199-1204.
9. Diwan H, Ivan D. CD8-positive mycosis fungoides and primary cutaneous aggressive epidermotropic CD8-positive cytotoxic T-cell lymphoma. *Journal of cutaneous pathology.* 2009;36(3):390-392.
10. Hodak E, David M, Maron L, Aviram A, Kaganovsky E, Feinmesser M. CD4/CD8 double- negative epidermotropic cutaneous T-cell lymphoma: an immunohistochemical variant of mycosis fungoides. *Journal of the American Academy of Dermatology.* 2006;55(2):276-284.
11. Agar NS, Wedgeworth E, Crichton S, et al. Survival outcomes and prognostic factors in mycosis fungoides/Sezary syndrome: validation of the revised International Society for Cutaneous Lymphomas/ European Organisation for Research and Treatment of Cancer staging proposal. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology.* 2010;28(31):4730-4739.
12. Olsen E, Vonderheid E, Pimpinelli N, et al. Revisions to the staging and classification of mycosis fungoides and Sezary 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(6):1713-1722.
13. Zackheim HS, Amin S, Kashani-Sabet M, McMillan A. Prognosis in cutaneous T-cell lymphoma by skin stage: long-term survival in 489 patients. *Journal of the American Academy of Dermatology.* 1999;40(3):418-425.
14. 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(23):5830-5839.
15. Diamandidou E, Colome M, Fayad L, Duvic M, Kurzrock R. Prognostic factor analysis in mycosis fungoides/Sezary syndrome. *Journal of the American Academy of Dermatology.* 1999;40(6 Pt 1):914-924.
16. van Doorn R, Van Haselen CW, van Voorst Vader PC, et al. Mycosis fungoides: disease evolution and prognosis of 309 Dutch patients. *Archives of dermatology.* 2000;136(4):504- 510.

17. Kim YH, Liu HL, Mraz-Gernhard S, Varghese A, Hoppe RT. Long-term outcome of 525 patients with mycosis fungoides and Sezary syndrome: clinical prognostic factors and risk for disease progression. *Archives of dermatology.* 2003;139(7):857-866.
18. Talpur R, Singh L, Daulat S, et al. Long-term outcomes of 1,263 patients with mycosis fungoides and Sezary syndrome from 1982 to 2009. *Clinical cancer research : an official journal of the American Association for Cancer Research.* 2012;18(18):5051-5060.
19. Diamandidou E, Colome-Grimmer M, Fayad L, Duvic M, Kurzrock R. Transformation of mycosis fungoides/Sezary syndrome: clinical characteristics and prognosis. *Blood.* 1998;92(4):1150-1159.
20. Scarisbrick JJ, Kim YH, Whittaker SJ, et al. Prognostic factors, prognostic indices and staging in mycosis fungoides and Sezary syndrome: where are we now? *The British journal of dermatology.* 2014;170(6):1226-1236.
21. Talpur R, Sui D, Gangar P, Dabaja BS, Duvic M. Retrospective Analysis of Prognostic Factors in 187 Cases of Transformed Mycosis Fungoides. *Clinical lymphoma, myeloma & leukemia.* 2016;16(1):49-56.
22. van Doorn R, Scheffer E, Willemze R. Follicular mycosis fungoides, a distinct disease entity with or without associated follicular mucinosis: a clinicopathologic and follow-up study of 51 patients. *Archives of dermatology.* 2002;138(2):191-198.
23. Gerami P, Rosen S, Kuzel T, Boone SL, Guitart J. Folliculotropic mycosis fungoides: an aggressive variant of cutaneous T-cell lymphoma. *Archives of dermatology.* 2008;144(6):738- 746.
24. Lehman JS, Cook-Norris RH, Weed BR, et al. Folliculotropic mycosis fungoides: single-center study and systematic review. *Archives of dermatology.* 2010;146(6):607-613.
25. Klemke CD, Dippel E, Assaf C, et al. Follicular mycosis fungoides. *The British journal of dermatology.* 1999;141(1):137-140.
26. Marschalko M, Eros N, Kontar O, et al. Folliculotropic mycosis fungoides: clinicopathological analysis of 17 patients. *Journal of the European Academy of Dermatology and Venereology : JEADV.* 2015;29(5):964-972.
27. Deonizio JM, Ascef RD, Sanches JA. Folliculotropic mycosis fungoides: clinical and epidemiological evaluation in a single center in Brazil. *International journal of dermatology.* 2016;55(5):e256-261.
28. Demirkesen C, Esirgen G, Engin B, Songur A, Oguz O. The clinical features and histopathologic patterns of folliculotropic mycosis fungoides in a series of 38 cases. *Journal of cutaneous pathology.* 2015;42(1):22-31.
29. Mantaka P, Helsing P, Gjersvik P, Bassarova A, Clausen OP, Delabie J. Clinical and histopathological features of folliculotropic mycosis fungoides: a Norwegian patient series. *Acta dermato-venereologica.* 2013;93(3):325-329.
30. Muniesa C, Estrach T, Pujol RM, et al. Folliculotropic mycosis fungoides: clinicopathological features and outcome in a series of 20 cases. *Journal of the American Academy of Dermatology.* 2010;62(3):418-426.
31. Tomasini C, Kempf W, Novelli M, et al. Spiky follicular mycosis fungoides: a clinicopathologic study of 8 cases. *Journal of cutaneous pathology.* 2015;42(3):164-172.
32. Hodak E, Amitay-Laish I, Atzmony L, et al. New insights into folliculotropic mycosis fungoides (FMF): A single-center experience. *Journal of the American Academy of Dermatology.* 2016;75(2):347-355.
33. Amitay-Laish I, Feinmesser M, Ben-Amitai D, Fenig E, Sorin D, Hodak E. Unilesional folliculotropic mycosis fungoides: a unique variant of cutaneous lymphoma. *Journal of the European Academy of Dermatology and Venereology : JEADV.* 2016;30(1):25-29.
34. Kempf W, Kazakov DV, Schermesser M, et al. Unilesional follicular mycosis fungoides: report of two cases with progression to tumor stage and review of the literature. *Journal of cutaneous pathology.* 2012;39(9):853-860.

35. Marzano AV, Berti E, Lupica L, Alessi E. Unilesional follicular mycosis fungoides. *Dermatology.* 1999;199(2):174-176.

36. Vij A, Duvic M. Prevalence and severity of pruritus in cutaneous T cell lymphoma. *International journal of dermatology.* 2012;51(8):930-934.

37. Lansigan F. Navigating the treatment choices for mycosis fungoides. *Oncology (Williston Park, NY).* 2010;24(6):508, 516, 518.

38. Lewis DJ, Huang S, Duvic M. Inflammatory cytokines and peripheral mediators in the pathophysiology of pruritus in cutaneous T-cell lymphoma. *Journal of the European Academy of Dermatology and Venereology : JEADV.* 2018;32(10):1652-1656.

39. Meyer N, Paul C, Misery L. Pruritus in cutaneous T-cell lymphomas: frequent, often severe and difficult to treat. *Acta dermato-venereologica.* 2010;90(1):12-17.

40. Dillon SR, Sprecher C, Hammond A, et al. Interleukin 31, a cytokine produced by activated T cells, induces dermatitis in mice. *Nat Immunol.* 2004;5(7):752-760.

41. Cevikbas F, Wang X, Akiyama T, et al. A sensory neuron-expressed IL-31 receptor mediates T helper cell-dependent itch: Involvement of TRPV1 and TRPA1. *The Journal of allergy and clinical immunology.* 2014;133(2):448-460.

42. Saleem MD, Oussedik E, D'Amber V, Feldman SR. Interleukin-31 pathway and its role in atopic dermatitis: a systematic review. *The Journal of dermatological treatment.* 2017:1-9.

43. Talpur R, Bassett R, Duvic M. Prevalence and treatment of Staphylococcus aureus colonization in patients with mycosis fungoides and Sezary syndrome. *The British journal of dermatology.* 2008;159(1):105-112.

44. Gerami P, Guitart J. The spectrum of histopathologic and immunohistochemical findings in folliculotropic mycosis fungoides. *The American journal of surgical pathology.* 2007;31(9):1430-1438.

45. Hodak E, Amitay-Laish I, Atzmony L, et al. New insights into folliculotropic mycosis fungoides (FMF): A single-center experience. *Journal of the American Academy of Dermatology.* 2016.

46. van Santen S, Roach RE, van Doorn R, et al. Clinical Staging and Prognostic Factors in Folliculotropic Mycosis Fungoides. *JAMA dermatology.* 2016;152(9):992-1000.

47. Wieser I, Wang C, Alberti-Violetti S, et al. Clinical characteristics, risk factors and long-term outcome of 114 patients with folliculotropic mycosis fungoides. *Archives of dermatological research.* 2017;309(6):453-459.

48. Vergier B, de Muret A, Beylot-Barry M, et al. Transformation of mycosis fungoides: clinicopathological and prognostic features of 45 cases. French Study Group of Cutaneious Lymphomas. *Blood.* 2000;95(7):2212-2218.

49. Barberio E, Thomas L, Skowron F, Balme B, Dalle S. Transformed mycosis fungoides: clinicopathological features and outcome. *The British journal of dermatology.* 2007;157(2):284-289.

50. Hodak E, Amitay-Laish I, Feinmesser M, et al. Juvenile mycosis fungoides: cutaneous T-cell lymphoma with frequent follicular involvement. *Journal of the American Academy of Dermatology.* 2014;70(6):993-1001.

51. Trautinger F, Eder J, Assaf C, et al. European Organisation for Research and Treatment of Cancer consensus recommendations for the treatment of mycosis fungoides/Sezary syndrome - Update 2017. *European journal of cancer.* 2017;77:57-74.

52. Willemze R, Hodak E, Zinzani PL, Specht L, Ladetto M. Primary cutaneous lymphomas: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO.* 2013;24 Suppl 6:vi149- 154.

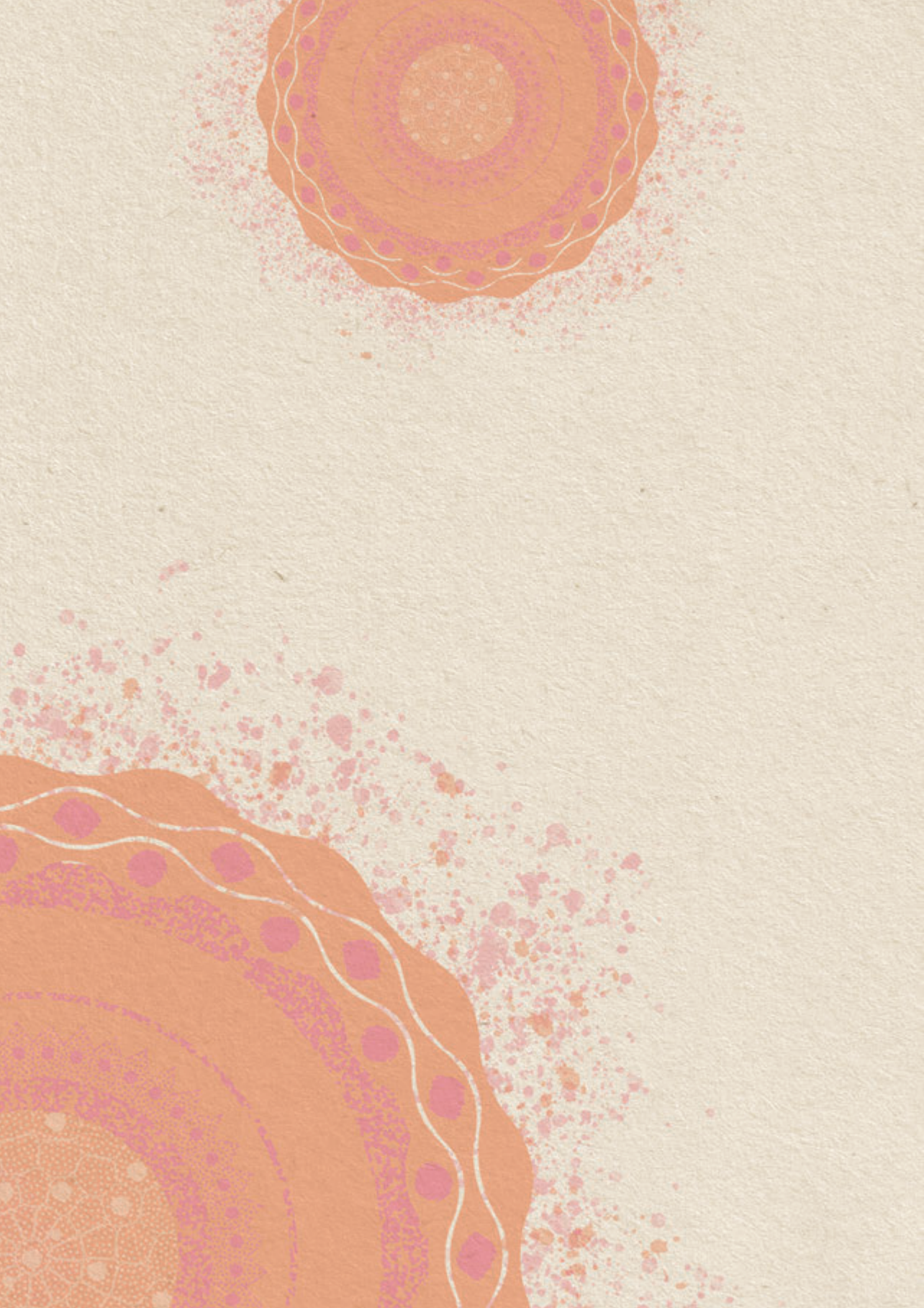
53. Benton EC, Crichton S, Talpur R, et al. A cutaneous lymphoma international prognostic index (CLIPi) for mycosis fungoides and Sezary syndrome. *European journal of cancer.* 2013;49(13):2859-2868.

54. Kaufmann SH. gamma/delta and other unconventional T lymphocytes: what do they see and what do they do? *Proc Natl Acad Sci U S A.* 1996;93(6):2272-2279.

55. Matos TR, de Rie MA, Teunissen MBM. Research Techniques Made Simple: High-Throughput Sequencing of the T-Cell Receptor. *The Journal of investigative dermatology.* 2017;137(6):e131-e138.

56. Kirsch IR, Watanabe R, O'Malley JT, et al. TCR sequencing facilitates diagnosis and identifies mature T cells as the cell of origin in CTCL. *Science translational medicine.* 2015;7(308):308ra158.

57. de Masson A, O'Malley JT, Elco CP, et al. High-throughput sequencing of the T cell receptor beta gene identifies aggressive early-stage mycosis fungoides. *Science translational medicine.* 2018;10(440).



2

CLINICAL STAGING AND PROGNOSTIC FACTORS IN FOLLICULOTROPIC MYCOSIS FUNGOIDES

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ABSTRACT

Importance: Large case series suggest that patients with folliculotropic mycosis fungoides (FMF) have a worse prognosis than patients with classic mycosis fungoides (MF). However, recent studies described a subgroup of FMF patients with a more favorable prognosis. Distinction between indolent and aggressive FMF may have important therapeutic consequences, but is hampered by the inability of the current tumor-node-metastasis-blood (TNMB) system to classify patients with FMF in a clinically meaningful way.

Objective: To differentiate between indolent and aggressive FMF, using clinico-pathological criteria and to define prognostic factors in patients with FMF.

Design: In this prospective cohort study, we followed 203 patients with FMF, included in the Dutch Cutaneous Lymphoma Registry between October 1985 and May 2014 at a tertiary referral center hosting the Dutch Cutaneous Lymphoma Registry. Overall, 220 patients with FMF had been registered, but 17 patients with incomplete follow-up data or a history of classic MF were excluded.

Main outcomes and measures: Main outcomes included clinical and histological characteristics, disease progression and survival. Prognostic factors were investigated using Cox proportional hazards regression analysis. Distinction between early plaque-stage FMF and advanced plaque-stage FMF was made by a blinded review of skin biopsies from patients presenting with plaques.

Results: In a cohort of 147 men and 56 women (median [range] age 59 [19-93] years) patients with histologically early plaque-stage FMF had a very similar overall survival (OS) rate to patients with only patches and/or follicular papules (10-year OS, 71% vs. 80%), while the survival rate of patients with histologically advanced plaque-stage FMF was almost identical to that of patients presenting with tumors (10-year OS, 25% vs. 27%). Subsequently, three clinical subgroups with significantly different survival data were distinguished: early skin-limited FMF (group A; n=84; 5- and 10-year OS: 92% / 72%); advanced skin-limited FMF (group B; n=102; 5- and 10-year OS: 55% / 28%); and FMF presenting with extracutaneous disease (group C; n=17; 5- and 10-year OS:

23% / 2%). Age at diagnosis, large cell transformation and secondary bacterial infection were independent risk factors for disease progression and/or poor survival.

Conclusion and relevance: The results of this study provide useful criteria to differentiate between indolent and aggressive FMF and confirm the existence of a subgroup of FMF with a favorable prognosis.

INTRODUCTION

In the last decade folliculotropic mycosis fungoides (FMF) has been widely recognized as a distinct variant of mycosis fungoides (MF).[1-9] Clinical and histologic features characteristic of FMF, but not or uncommonly found in classic type of MF include: 1) the histological presence of folliculotropic instead of epidermotropic neoplastic infiltrates, with or without follicular mucinosis[1,3-13]; 2) preferential localization of skin lesions in the head and neck region, with eyebrow involvement and concurrent alopecia as most characteristic feature [1,3-7,9-11,14,15]; 3) the presence of (grouped) follicular papules, alopecia, acneiform and cystic lesions [1-7,9,11,13] ; 4) the frequent occurrence of pruritus and secondary bacterial infections. [2,4-7,9] In addition, patients with FMF were shown less responsive to several first line skin-directed therapies used in classic MF, such as PUVA, and had a worse prognosis as compared to classic MF.[1,3,5-9] In our initial study 51 patients with FMF were compared with 158 patients with classic type MF, including 122 patients with plaque-stage disease (T2N0M0; stage IB) and 36 patients with tumor stage MF (T3N0M0). Survival data of patients with FMF were significantly worse than patients with plaque-stage classic MF and similar to patients with tumor stage MF, although only 14 of 51 patients had tumors or nodules at the time of diagnosis.[9] These observations were confirmed in subsequent studies and clearly indicate that the clinical staging system (TNMB system) used for MF cannot be used to classify FMF, in particular patients with plaques, in a clinically meaningful way.[3,4,16] The worse prognosis of FMF is also supported by large retrospective cohort studies of patients with MF, showing that FMF is an independent risk factor for disease progression and lower survival.[17-19] Because of its characteristic clinicopathologic features and worse prognosis, FMF was included as a distinct entity in recent cutaneous lymphoma classifications.[20,21]

However, recent studies focused attention on a subgroup of FMF patients with a favorable prognosis.[5,10,11] Distinction between FMF patients with an indolent and an aggressive clinical disease course may have important therapeutic consequences, but criteria for this indolent subgroup are still ill-defined. In the present study we reviewed the clinical and follow-up data of 203 patients with FMF, who had been followed prospectively after inclusion in the Dutch Cutaneous Lymphoma Registry. The main goal of this study was to develop criteria which could be used to differentiate between indolent and aggressive FMF. In addition, the large size of our cohort offered

the opportunity to define additional risk factors for poor outcome. Detailed treatment results and treatment recommendations will be reported separately.

PATIENTS AND METHODS

Between October 1985 and May 2014, 220 patients with FMF were included in the registry of the Dutch Cutaneous Lymphoma Group. Seventeen of 220 were excluded: eight patients because of incomplete (follow-up) data, six patients because of a history of classic epidermotropic MF for two to nine years before they developed skin tumors on the face or scalp with the histologic features of FMF, and three patients presenting with a solitary skin lesion, in whom another type of cutaneous T-cell lymphoma, in particular a primary cutaneous anaplastic large cell lymphoma could not be excluded. The final study group consisted of 203 patients. Forty-one of these 203 patients had also been included in our initial study.[9] The study was performed in accordance with the Declaration of Helsinki and the Dutch Code for Proper Secondary Use of Human Tissue, approved by the Ethics Committee of the Leiden University Medical Center.

In all cases, the diagnosis was made by an expert panel of dermatologists and pathologists at one of the regular meetings of the Dutch Cutaneous Lymphoma Group, and all cases met the diagnostic criteria of FMF as described previously.[9,20,21] The date of the first diagnostic biopsy was considered the time of diagnosis. Clinical records, clinical illustrations (available in more than 85% of patients) and follow-up data, which had been collected yearly, were evaluated. The following variables were recorded: age; sex; duration of skin lesions before diagnosis; type, preferential localization and extent of skin lesions; stage of disease according to the revised TNMB system[16]; presence of pruritus and secondary bacterial infection; presence of follicular mucinosis and presence of large cell transformation either at diagnosis or during follow-up according to established criteria[18]; type and result of initial therapy; date and type of disease progression; date of last contact or death if applicable.

Disease progression was defined by the development of clinically overt tumors in patients previously having only patch- or plaque stage disease, the development of histologically documented nodal involvement in patients with previously only skin-limited disease, the development of visceral involvement in patients with previous

skin and/or nodal disease, and death due to lymphoma. Histological lymph node involvement was assessed using the ISCL-EORTC classification system.[16]

Assessment of clinical stage

While patients with only patches, follicular papules, acneiform lesions or keratosis pilaris-like lesions clearly have early-stage disease (stage IA-IIA) and patients presenting with nodules, tumors, erythroderma and/or extracutaneous disease have advanced-stage FMF (stage IIB-IV), it is uncertain if patients presenting with plaques should be classified as early-stage FMF (stage IA-IB) or as advanced-stage FMF (stage IIB).[9] For the purpose of our study, skin biopsies from all patients presenting with plaques were reviewed in a blinded fashion. Lesions histologically showing sparse perifollicular and/or intrafollicular infiltrates containing relatively few and mainly small neoplastic T-cells were considered early plaque-stage FMF, while plaques showing more extensive confluent or diffuse infiltrates containing many often medium-sized to large tumor cells were considered to have advanced plaque-stage FMF.

Prognostic parameters

The following parameters were analyzed for their prognostic significance in FMF: sex, age at diagnosis (≤ 60 vs. >60 years), duration of skin lesions prior to diagnosis (<12months vs. 12-60months vs. >60months), extent of skin lesions (solitary vs. regional vs. generalized); large cell transformation (LCT) in the first diagnostic biopsy; presence of follicular mucinosis, pruritus, secondary bacterial infection (no vs. focal vs. extensive) and clinical stage. The term extensive secondary bacterial infection was used for patients with widespread honey-colored crusted lesions at first presentation (**Figure 1D**).

Statistical analysis

All statistical analyses were performed using the SPSS statistical software (IBM Corp). Disease-specific survival (DSS) was calculated from the date of first diagnostic biopsy until death as result of lymphoma or date of last follow-up. Overall survival (OS) was calculated from the date of diagnosis until patient's death from any cause or date of last follow-up. Progression-free survival (PFS) was calculated from the date of diagnosis to the time of disease progression or date of last follow-up. Survival curves were estimated by the method of Kaplan and Meier and comparison between curves was done by log-rank testing. Univariate analysis of parameters with possible

prognostic significance for DSS, OS or PFS was performed using Cox proportional hazards regression analysis. Factors significant at the 0.05 level in univariate analysis and age at diagnosis, regardless of statistical significance, were included in a multivariate analysis model. In this model P values below 0.05 were considered significant. To compare clinical outcome parameters among different subgroups of FMF patients a chi-square test for goodness of fit was performed for categorical variables and a Kruskal-Wallis test was performed to compare medians between subgroups. P values below 0.05 were considered significant for both tests.

RESULTS

Clinical characteristics at diagnosis

The main clinical characteristics are summarized in **Table 1**. The study included 147 males and 56 females (male to female ratio: 2.6). The median age at diagnosis was 59 years (range: 15 - 93 years). Only three of 203 patients (1.5%) were aged 18 years or younger. Most patients (86%) presented with generalized skin lesions involving multiple body regions, while nine (4%) and 20 (10%) patients had presented with solitary or localized skin lesions, respectively. Thirteen patients showed erythroderma at first presentation. This group included six patients with stage III (skin-limited disease) and seven patients with stage IV, four of whom met the criteria for peripheral blood involvement of Sézary syndrome (SS).

In 129 of 203 patients (64%) skin lesions were preferentially located and most pronounced in the head and neck area **(Figure 1)**. Infiltrated plaques in the eyebrows with concurrent alopecia were observed in more than 50% of patients, either at the time of diagnosis or during follow-up. Three patients presented with a leonine face. Only 20 of 203 (10%) patients had no skin lesions in the head and neck area at first presentation. Associated alopecia on affected skin sites was seen in about 80% of patients. Secondary bacterial infection was observed in 43 patients (21%) and was extensive in 21 of them. Almost 80% of patients complained of moderate to severe pruritus.

Four patients had a history of Hodgkin lymphoma, nine to 22 years before the diagnosis FMF was made. Three patients had a coexistent hematological disorder, including one patient with a B-cell chronic lymphocytic leukemia, one patient with a myelodysplastic syndrome and one patient with essential thrombocytosis.

Table 1. Clinical characteristics of 203 patients with follicular mycosis fungoides

Age at diagnosis, median (range) in years	59 (15-93)
Male-female ratio	2.6 (147:56)
Duration of skin lesions before diagnosis, median (range) in months	24 (1-400)
Most severe type of skin lesions at diagnosis	
Patches a/o follicular papules	67 (33%)
Plaques	58 (29%)
Tumors and nodules	55 (27%)
Erythroderma	6 (3%)
Nodal/visceral involvement	17 (8%)
Mainly head/neck involvement	129 (64%)
Alopecia	
Yes	150 (81%)
No	36 (19%)
Unknown	17
Eyebrow involvement	
At diagnosis	85 (44%)
During follow up	25 (13%)
No involvement	83 (43%)
Unknown	10
Extent of skin lesions	
Generalized	174 (86%)
Localised	20 (10%)
Solitary	9 (4%)
Pruritus	
Yes	133 (77%)
No	40 (23%)
Unknown	30
Secondary bacterial infection	
Focal	21 (10%)
Prominent	21 (10%)
Follicular mucinosis	
Present	156 (78%)
Absent	43 (22%)
Unknown	4
Large cell transformation	
Skin	33 (16%)
Extracutaneous	1 (0.5%)
Initial treatment	
Topical steroids	21 (10%)
UVB	12 (6%)
PUVA	61 (30%)
PUVA with retinoids or with IFN-a	19 (9%)
PUVA + local radiotherapy	28 (14%)
Local radiotherapy	21 (10%)
Total skin radiotherapy	20 (10%)
Systemic chemotherapy	14 (7%)
Other	7 (3%)
Complete remission on initial therapy	51 (25%)

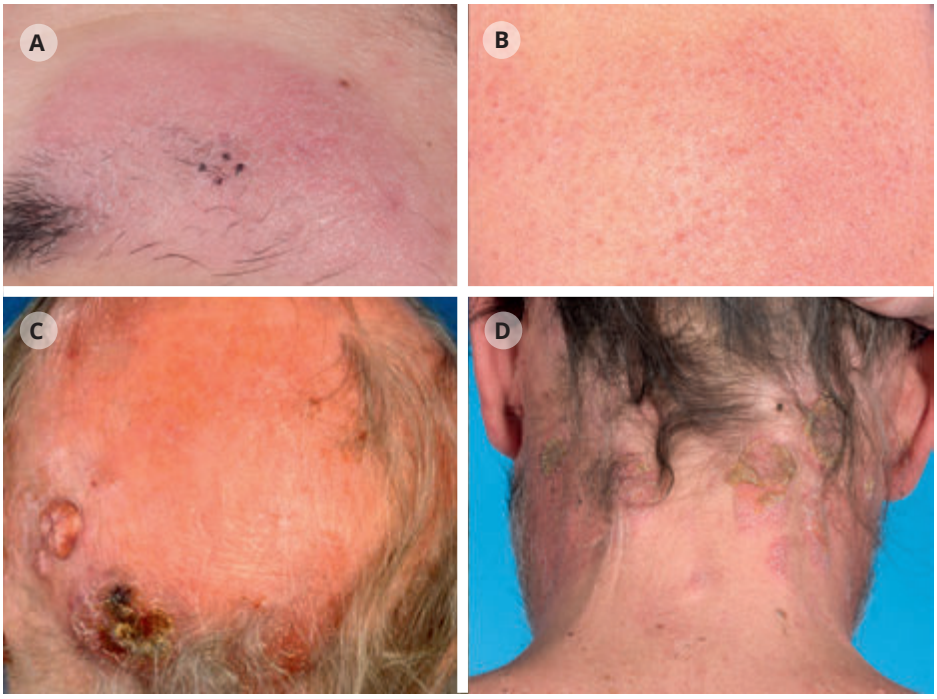


Figure 1. Clinical appearances of FMF. Solitary patch with associated alopecia in left eyebrow (A). Grouped follicular papules on the trunk (B). Alopecia and ulcerating tumors on the scalp (C); and plaques in the neck showing follicular accentuation and secondary bacterial infection (D).

Clinical stage at diagnosis

At time of diagnosis 186 patients (92%) had skin-limited disease, while seventeen patients (8%) had nodal or visceral disease at first presentation (stage IV). Among those patients with skin-limited disease 67 patients presented with only patches, follicular papules, acneiform or keratosis pilaris-like lesions (stage IA-IIA), 55 patients with nodules or tumors (stage IIB), six patients with erythroderma (stage III) and 58 patients with plaques. As noted before, it is uncertain on the basis of clinical evaluation alone, if patients presenting with plaques should be classified as stage IA-IIA or stage IIB. Based on histologic criteria (see section Patients and Methods) distinction was therefore made between patients presenting with early plaque-stage disease (n=17) and patients with advanced plaque-stage disease (n=41) **(Figure 2)**. The OS and DSS of the whole group of patients presenting with plaques (n=58) were intermediate between those of patients presenting with only follicular papules and/or patches and those of patients presenting with nodules, tumors or erythroderma. However, patients histologically

classified as early plaque-stage FMF had almost identical survival and progression data as patients presenting with only patches and follicular papules, while patients histologically classified as advanced plaque-stage FMF had a very similar course to patients presenting with tumors or nodules **(Supplementary Table 1)**. The differences in 10-year OS (80% vs. 25%; p=0.004) and 10-year DSS (100% vs. 35%; p=0.006) between those two subgroups of patients with early plaque-stage and advanced plaque-stage FMF were highly significant, which confirms the usefulness of the clinicopathologic approach used (**Figure 3A-3B**). Taken together, three clinically relevant stages were distinguished: patients with early skin-limited disease (n=84; Group A), patients with advanced skin-limited disease (n=102; Group B) and patients presenting with extracutaneous disease (n=17; Group C) (**Figure 3C-3D**).

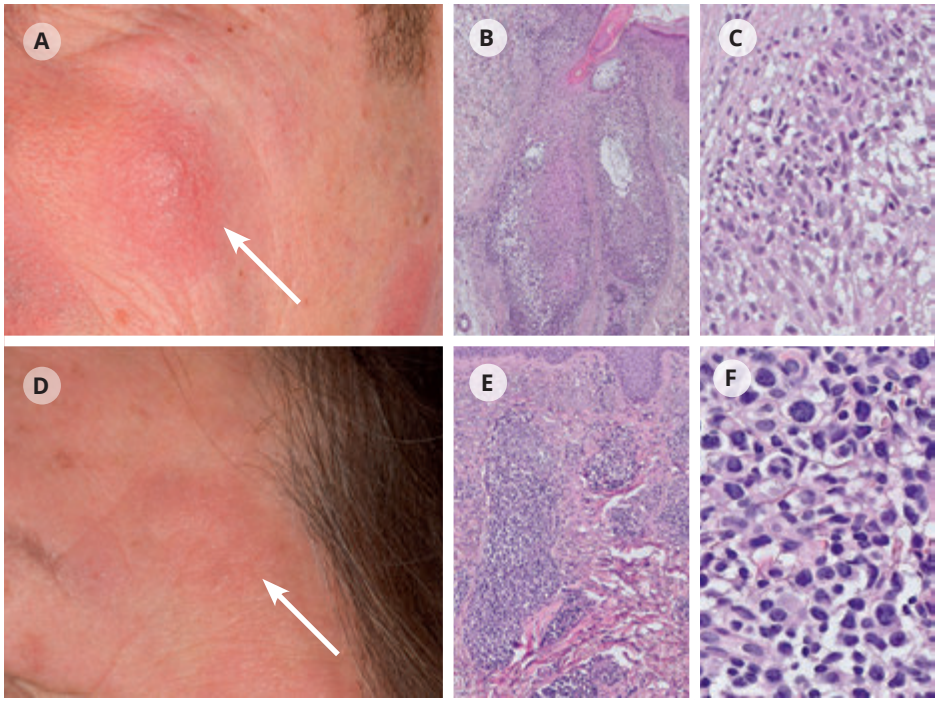


Figure 2. Clinicopathologic classification in 2 patients with FMF presenting with similar plaques on the face. Histological examination of a plaque on the left cheek of the first patient (A) shows mucin depositions (follicular mucinosis) and a sparse perifollicular and intrafollicular infiltrate (B); Detail of the infiltrate shows small neoplastic T-cells with pleomorphic nuclei (C). Histological examination of a plaque on the left temple of the second patient (D) shows dense intrafollicular infiltrates with a predominance of medium-sized to large neoplastic T-cells (E and F).

Prognostic factors

Both in univariate and multivariate analysis, age at diagnosis >60 years, clinical stage (as defined above) and the presence of extensive secondary bacterial infection were independent factors associated with reduced OS, DSS and PFS, while gender, the extent of skin lesions, the duration of skin disease prior to diagnosis, follicular mucinosis and the presence of pruritus had no effect on survival or disease progression **(Supplementary Table 2).** The presence of large cell transformation at first presentation was independently associated with reduced PFS, but not with reduced OS. The relation between LCT and DSS was borderline significant (p=0.05). Large cell transformation at first presentation was observed in 33 patients, including 21 cases in group B, 10 in group C and only two in group A. The median survival of these 33 cases was 32 months, the 5- and 10-year OS 40% and 20%, respectively, and the 5- and 10-year DSS 41% and 20%, respectively.

Extensive secondary bacterial infection was particularly found in patients with advanced FMF (19 of 21 patients) and in most cases affected skin lesions in the head and neck region (17 of 19 patients) **(Figure 1D).** The median survival of these 21 patients was only 22 months, the 5-year OS and DSS 20% and 26% respectively.

Clinical course and survival

After initial therapy, 25 of 203 (12%) patients never had a relapse and the median duration of this sustained complete remission was 68 months (range: 12-169 months); 101 patients (50%) showed continued disease without progression to a higher stage, while 77 patients (38%) showed disease progression **(Table 2)**. Altogether, 25 patients presented with (n=1) or developed visceral involvement. Visceral sites most commonly affected were the central nervous system (n=11), lungs (n=9), bone marrow (n=6) and oral or nasal mucosa (n=5), while three patients developed peripheral blood involvement. After a median follow-up of 51 months (range 3-260 months) 32 of 203 patients were in complete remission, 78 patients were alive with disease, 59 patients died of lymphoma and 34 died of unrelated disease. For those 203 patients the 5- and 10-year OS were 67% and 45%, respectively and the 5- and 10-year DSS were 75% and 60%, respectively, with significant differences between the three clinical subgroups **(Table 2; Figure 3C-3D)**. Among 84 patients with early skin-limited disease (group A) 17 patients (20%) developed skin tumors and 2 (2%) extracutaneous disease. Five (6%) patients died of

lymphoma after a median follow-up of 76 months (range: 32-198 months). The clinical presentation and histology of these five cases did not differ from other patients in group A. Interestingly, all five patients had presented with widespread follicular papules and patches, and none of them with plaques.

Table 2. Clinical course and outcome

	Total group (n=203)	Group A (n= 84)	Group B (n=102)	Group C (n=17)	P-value
Result initial treatment [a]					
Complete remission	51 (25%)	25 (30%)	25 (25%)	1 (6%)	0.22
Partial remission	104 (51%)	47 (56%)	52 (51%)	5 (29%)	0.34
Stable disease	29 (14%)	12 (14%)	14 (14%)	3 (18%)	0.75
Progression during therapy	16 (8%)	0	9 (9%)	7 (41%)	<0.05
Duration of follow-up (median (months); range)	51 (3-260)	70 (9-260)	46 (3-204)	20 (4-125)	<0.05
Clinical course after initial treatment					
Sustained complete remission [b]	25 (12%)	17 (20%)	7 (7%)	1 (6%)	<0.05
Continuous disease without progression	101 (50%)	48 (57%)	51 (50%)	2 (12%)	0.07
Disease progression	77 (38%)	19 (23%)	44 (43%)	14 (82%)	<0.05
PFS (median (months); range)	41 (2-247)	57 (4-247)	40 (2-189)	20 (4-125)	<0.05
Disease progression during follow-up					
Progressive skin lesions [c]	17 (8%)	17 (20%)	-	-	-
Nodal involvement	26 (13%)	2 (2%)	24 (24%)	-	<0.05
Visceral involvement	24 (12%)	1 (1%)	20 (20%)	3 (18%)	<0.05
Death of lymphoma	59 (29%)	5 (6%)	40 (39%)	14 (82%)	<0.05
Large cell transformation					
At diagnosis	33 (16%)	2 (2%)	21 (21%)	10 (59%)	<0.05
During follow-up	22 (11%)	4 (5%)	16 (16%)	2 (12%)	0.08
Status at last follow-up					
Alive without disease	32 (16%)	19 (23%)	12 (12%)	1 (6%)	0.08
Alive with disease	78 (38%)	50 (60%)	26 (26%)	2 (12%)	<0.05
Died of other cause	34 (17%)	10 (12%)	24 (24%)	0	<0.05
Died of FMF	59 (29%)	5 (6%)	40 (39%)	14 (82%)	<0.05
DSS (at 5 years / at 10 years)	75% / 60%	96% / 93%	65% / 40%	23% / 2%	<0.05
OS (at 5 years / at 10 years)	67% / 45%	92% / 72%	55% / 28%	23% / 2%	<0.05

Group A; early skin-limited FMF; Group B; advanced skin-limited FMF; Group C: FMF with extracutaneous disease at diagnosis. (a) Result of initial therapy of 2 patients in Group B and of 1 patient in group C was unknown. (b) Sustained complete remission: after initial complete remission no relapse was seen during follow-up. (c) Development of skin tumors in patients of group A. Patients presenting with advanced plaque stage FMF, who developed skin tumors during follow-up were not scored as progressive disease.

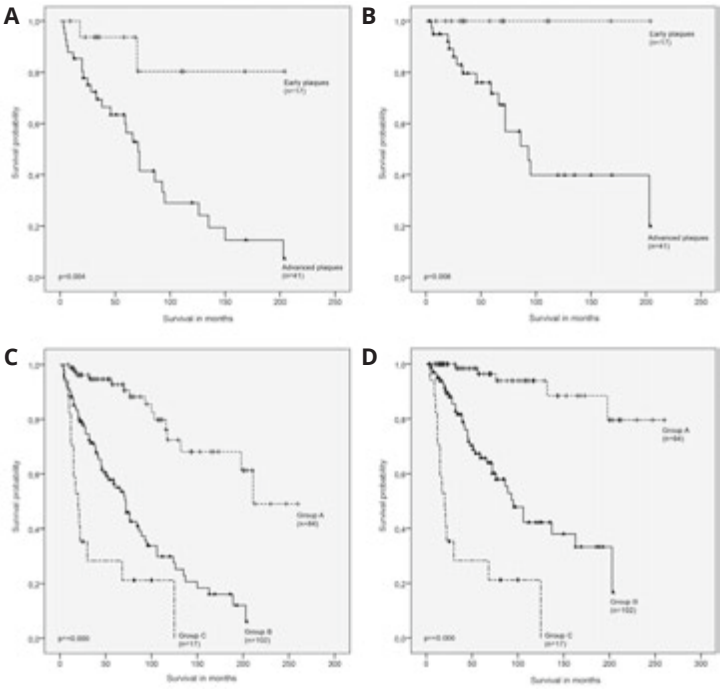


Figure 3. OS and DSS of patients with FMF. OS and DSS of patients with early and advanced plaque-stage FMF (A and B). OS and DSS of patients with early skin-limited FMF (group A), advanced skin-limited FMF (group B), and FMF presenting with extracutaneous disease (group C) (C and D). *DSS indicates disease-specific survival; FMF, folliculotropic mycosis fungoides; OS, overall survival.*

DISCUSSION

While previous studies emphasized the worse prognosis of FMF as compared to classic type MF, more recent studies focused attention on a subgroup of FMF with a favorable prognosis.[5,10,11] However, criteria to differentiate between indolent and more aggressive FMF are still ill-defined, which is mainly caused by the inability of the clinical staging system (TNMB system) to classify FMF patients presenting with plaques in a clinically meaningful way.[3,4,16] The results of the present study, in which additional histologic criteria were used to distinguish early from advanced plaque stage FMF, provide useful criteria to differentiate between indolent and aggressive FMF. Multivariate analysis showed that clinical stage, age at diagnosis, LCT and extensive secondary bacterial infection were independent risk factors for disease progression and/or poor survival.

Clinical staging in patients with FMF

In classic MF, clinical stage according to the revised TNMB criteria is the most important factor in predicting survival and risk of disease progression.[19,22] In our study FMF patients presenting with only patches and/or follicular papules (stage IA-IIA) had indeed an excellent prognosis with a 5-year OS and DSS of 92% and 95%, respectively, while patients presenting with tumors and/or nodules (stage IIB) had a 5-year OS and DSS of 50% and 59%, respectively (see **Supplementary Table 1**). The favorable prognosis of patients presenting with only patches and/or follicular papules is consistent with recent literature.[5,10,11] It is however arbitrary if FMF patients presenting with plaques should be classified as stage IA-IIA or as stage IIB.[9] Clinically identical plaques may be caused by dense neoplastic infiltrates, but also by excessive mucin depositions or an extensive inflammatory infiltrate. In the present study, distinction was therefore made between plaques histologically characterized by sparse intra- and/or perifollicular infiltrates containing relatively few and mainly small neoplastic T-cells ('early plaque-stage FMF') and plaques histologically showing more extensive confluent or diffuse infiltrates containing many often medium-sized to large tumor cells ('advanced plaque-stage FMF'). Patients histologically classified as early plaque-stage FMF had an almost equal clinical course and survival to patients presenting with only patches and/or follicular papules, while patients histologically classified as advanced plaque-stage FMF had a very similar course to patients presenting with tumors, confirming the usefulness of this clinicopathologic approach (**Supplementary Table 1**). Our results validated those of Hodak et al., presented at the 2014 meeting of the EORTC Cutaneous Lymphoma Group in Paris. Using a very similar clinicopathologic approach and very similar histologic criteria distinction could be made between early- and advanced-stage FMF (E. Hodak; personal communication). Whether patients with advanced plaque-stage disease should be classified as tumor (T3 score; stage IIB) rather than plaque-stage disease (T2 score; stage IA-IIA), as suggested by Hodak et al., is a matter of debate. The similar survival and progression rates between patients with advanced plaque-stage and tumor-stage FMF seem to justify such an upgrading. However, in the revised TNMB system, skin score is determined by clinical presentation and skin lesions histologically classified as either early or advanced plaque-stage FMF may be clinically indistinguishable (**Figure 2**).[16] Additional studies investigating the reproducibility of histology-based distinctions between early and advanced plaque-stage FMF should be awaited before further revisions of the TNMB system are made. A meeting of the EORTC Cutaneous Lymphoma

Pathology Group, in which the reproducibility of a histologic stratification is investigated by an independent panel of (dermato)pathologists is scheduled for 2017.

Prognostic factors in FMF

Previous studies have been unsuccessful to detect independent prognostic parameters, probably because of the relatively small number of patients included in these studies.[6,7] In the present study, in addition to clinical stage, age >60 years, LCT and the presence of extensive secondary bacterial infection at the time of first presentation were independent factors associated with reduced OS, DSS and/or PFS. Clinical stage of disease, age and LCT are well-known risk factors in classic MF.[19,22,23,24] The presence of secondary bacterial infection as an adverse risk factor has not been found before. It is well known that bacterial toxins acting as superantigens may stimulate the proliferation of malignant T-cells and may worsen disease in patients with a cutaneous T-cell lymphoma.[25,26] However, whether this or other mechanisms are responsible for the poor outcome in FMF patients with extensive secondary bacterial infection is unknown.

In conclusion, the results of the present study confirm the existence of a substantial subgroup of patients with FMF with a good prognosis. Apart from patients presenting with only patches and/or follicular papules, this group also contains patients with plaques histologically characterized by sparse intra- and/or perifollicular infiltrates containing relatively few and mainly small neoplastic T-cells. Distinction between patients with indolent and aggressive FMF is important, since it may have therapeutic consequences.

REFERENCES

1. Marschalko M, Eros N, Kontar O, et al. Folliculotropic mycosis fungoides: clinicopathological analysis of 17 patients. *Journal of the European Academy of Dermatology and Venereology : JEADV.* 2015;29(5):964-972.
2. Deonizio JM, Ascef RD, Sanches JA. Folliculotropic mycosis fungoides: clinical and epidemiological evaluation in a single center in Brazil. *International journal of dermatology.* 2015.
3. Demirkesen C, Esirgen G, Engin B, Songur A, Oguz O. The clinical features and histopathologic patterns of folliculotropic mycosis fungoides in a series of 38 cases. *Journal of cutaneous pathology.* 2015;42(1):22-31.
4. Mantaka P, Helsing P, Gjersvik P, Bassarova A, Clausen OP, Delabie J. Clinical and histopathological features of folliculotropic mycosis fungoides: a Norwegian patient series. *Acta dermato-venereologica.* 2013;93(3):325-329.
5. Muniesa C, Estrach T, Pujol RM, et al. Folliculotropic mycosis fungoides: clinicopathological features and outcome in a series of 20 cases. *Journal of the American Academy of Dermatology.* 2010;62(3):418-426.
6. Lehman JS, Cook-Norris RH, Weed BR, et al. Folliculotropic mycosis fungoides: single-center study and systematic review. *Archives of dermatology.* 2010;146(6):607-613.
7. Gerami P, Rosen S, Kuzel T, Boone SL, Guitart J. Folliculotropic mycosis fungoides: an aggressive variant of cutaneous T-cell lymphoma. *Archives of dermatology.* 2008;144(6):738-746.
8. Monopoli A, Annessi G, Lombardo GA, Baliva G, Girolomoni G. Purely follicular mycosis fungoides without mucinosis: report of two cases with review of the literature. *Journal of the American Academy of Dermatology.* 2003;48(3):448-452.
9. van Doorn R, Scheffer E, Willemze R. Follicular mycosis fungoides, a distinct disease entity with or without associated follicular mucinosis: a clinicopathologic and follow-up study of 51 patients. *Archives of dermatology.* 2002;138(2):191-198.
10. Tomasini C, Kempf W, Novelli M, et al. Spiky follicular mycosis fungoides: a clinicopathologic study of 8 cases. *Journal of cutaneous pathology.* 2015;42(3):164-172.
11. Hodak E, Amitay-Laish I, Feinmesser M, et al. Juvenile mycosis fungoides: cutaneous T-cell lymphoma with frequent follicular involvement. *Journal of the American Academy of Dermatology.* 2014;70(6):993-1001.
12. Gerami P, Guitart J. The spectrum of histopathologic and immunohistochemical findings in folliculotropic mycosis fungoides. *The American journal of surgical pathology.* 2007;31(9):1430-1438.
13. Hodak E, Feinmesser M, Segal T, et al. Follicular cutaneous T-cell lymphoma: a clinicopathological study of nine cases. *The British journal of dermatology.* 1999;141(2):315-322.
14. Amitay-Laish I, Feinmesser M, Ben-Amitai D, Fenig E, Sorin D, Hodak E. Unilesional folliculotropic mycosis fungoides: a unique variant of cutaneous lymphoma. *Journal of the European Academy of Dermatology and Venereology : JEADV.* 2016;30(1):25-29.
15. Kempf W, Kazakov DV, Schermesser M, et al. Unilesional follicular mycosis fungoides: report of two cases with progression to tumor stage and review of the literature. *Journal of cutaneous pathology.* 2012;39(9):853-860.
16. Olsen E, Vonderheid E, Pimpinelli N, et al. Revisions to the staging and classification of mycosis fungoides and Sezary 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(6):1713-1722.
17. Benton EC, Crichton S, Talpur R, et al. A cutaneous lymphoma international prognostic index (CLIPi) for mycosis fungoides and Sezary syndrome. *European journal of cancer.* 2013;49(13):2859-2868.

18. Benner MF, Jansen PM, Vermeer MH, Willemze R. Prognostic factors in transformed mycosis fungoides: a retrospective analysis of 100 cases. *Blood.* 2012;119(7):1643-1649.
19. Agar NS, Wedgeworth E, Crichton S, et al. Survival outcomes and prognostic factors in mycosis fungoides/Sezary syndrome: validation of the revised International Society for Cutaneous Lymphomas/ European Organisation for Research and Treatment of Cancer staging proposal. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology.* 2010;28(31):4730-4739.
20. Swerdlow SH, Campo E, Harris NL, et al. *World Health Organization Classification of Tumours of Hematopoietic and Lymphoid Tissues.* Lyon: IARC Press, 2008.
21. Willemze R, Jaffe ES, Burg G, et al. WHO-EORTC classification for cutaneous lymphomas. *Blood.* 2005;105(10):3768-3785.
22. van Doorn R, Van Haselen CW, van Voorst Vader PC, et al. Mycosis fungoides: disease evolution and prognosis of 309 Dutch patients. *Archives of dermatology.* 2000;136(4):504-510.
23. Talpur R, Singh L, Daulat S, et al. Long-term outcomes of 1,263 patients with mycosis fungoides and Sezary syndrome from 1982 to 2009. *Clinical cancer research : an official journal of the American Association for Cancer Research.* 2012;18(18):5051-5060.
24. Scarisbrick JJ, Kim YH, Whittaker SJ, et al. Prognostic factors, prognostic indices and staging in mycosis fungoides and Sezary syndrome: where are we now? *The British journal of dermatology.* 2014;170(6):1226-1236.
25. Woetmann A, Lovato P, Eriksen KW, et al. Nonmalignant T cells stimulate growth of T-cell lymphoma cells in the presence of bacterial toxins. *Blood.* 2007;109(8):3325-3332.
26. Willerslev-Olsen A, Krejsgaard T, Lindahl LM, et al. Bacterial toxins fuel disease progression in cutaneous T-cell lymphoma. *Toxins.* 2013;5(8):1402-1421.

SUPPLEMENTARY TABLES

Supplementary Table 1. Overall survival, disease specific survival and progression free survival in FMF

				OS		DSS		PFS	
Skin lesions at diagnosis	Stage	Group	No (%)	5-year	10-year	5-year	10-year	5-year	10-year
Only follicular papules a/o patches	IA-IIA	A	67 (33%)	92%	71%	95%	88%	80%	67%
Plaques Total group	IA-IIA	A-B	58 (29%)	66%	37%	79%	50%	60%	45%
Plaques (PA: early-stage [a])	NA	A	17 (8%)	83%	80%	100%	100%	78%	78%
Plaques (PA: advanced-stage [b])	NA	B	41 (20%)	56%	25%	75%	35%	52%	26%
Tumors and nodules	IIB	B	55 (27%)	50%	27%	59%	40%	57%	31%
Erythroderma [c]	III	B	6 (3%)	56%	-	68%	-	58%	-
Nodal / Visceral involvement	IV	C	17 (8%)	23%	2%	23%	2%	22%	2%

Stages according to revised ISCL/EORTC criteria.[13] OS: Overall survival, DSS: Disease specific survival, PFS: Progression free survival; NA: T-score not applicable to cases of FMF presenting with plaques.

(a) Survival of patients with early plaque stage FMF showed no significant differences compared to patients with follicular papules a/o patches (OS: p=0.89, DSS: p=0.36, PFS: p=0.99).

(b) Survival of patients with advanced plaque stage FMF showed no significant differences compared to patients with tumors and nodules (OS: p=0.99, DSS: p=0.75, PFS: p=0.87).

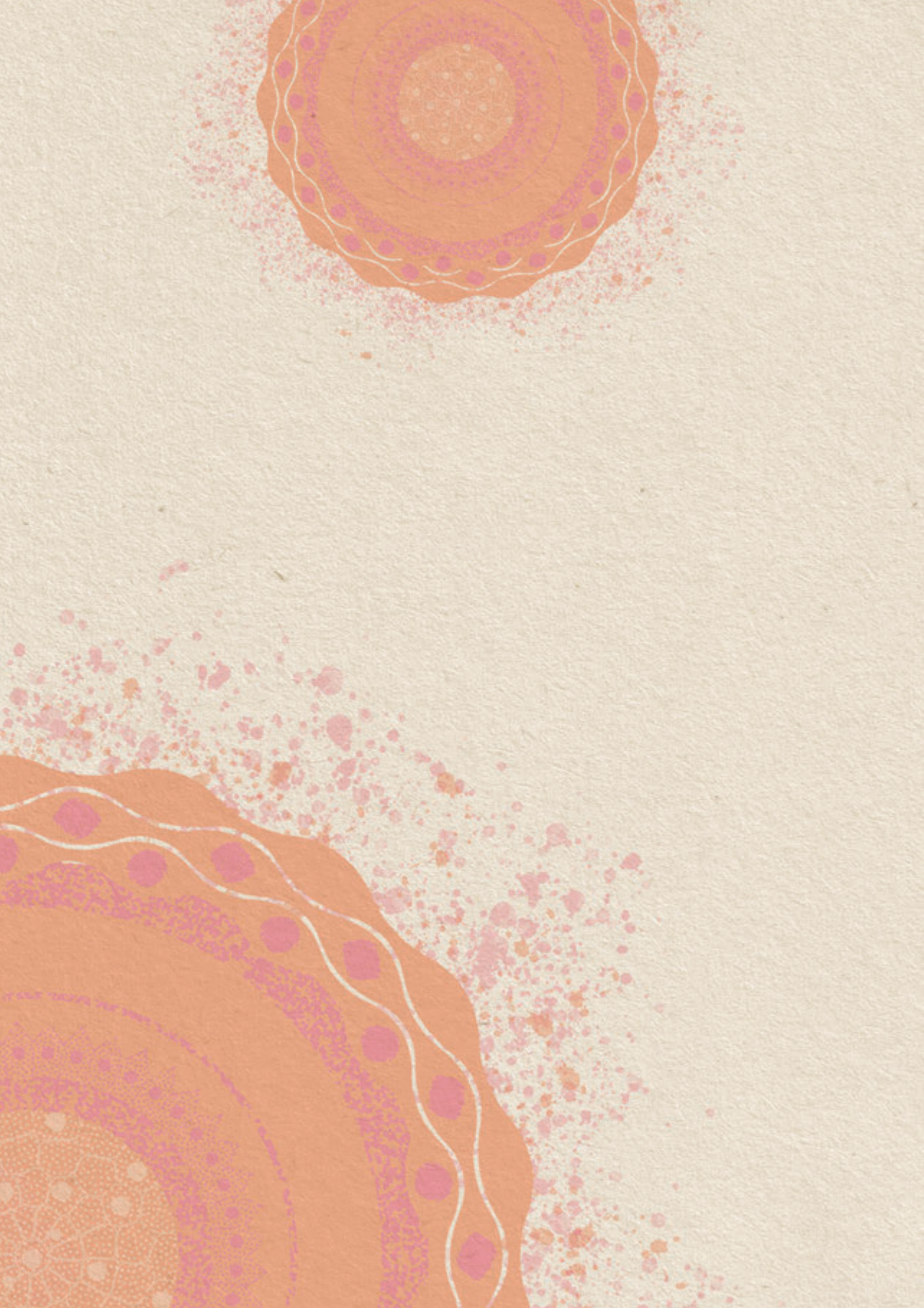
(c) Among 6 patients with skin-limited erythroderma a follow-up of 10 years was not reached.

Supplementary Table 2. Uni- and multivariate analysis of DSS, OS and PFS in 203 patients with FMF

		DSS			
	N	Univariate analysis		Multivariate analysis	
Total	**203**	**HR (95%CI)**	**P-value**	**HR (95%CI)**	**P-value**
Sex			0.60		
Women	56	1			
Men	147	0,9 (0,5-1,5)			
Age at diagnosis			0.12		0.02
≤60 years	111	1		1	
>60 years	92	1,5 (0,9-2,5)		2,0 (1,1-3,7)	
Extent of skin lesions			0.28		
Solitary	9	1			
Localised	20	2,3 (0,3-20,3)			
Generalised	174	3,8 (0,5-27,2)			
LCT at diagnosis			<0.001		0.05
No	170	1		1	
Yes	33	3,8 (2,2-6,7)		1,9 (1,0-3,6)	
Time to diagnosis			0.91		
≤12 months	59	1			
13-60 months	102	0,9 (0,5-1,6)			
> 60 months	42	1,0 (0,5-2,0)			
Mucinosis follicularis			0.28		
No	43	1			
Yes	156	0,7 (0,4-1,3)			
Pruritus			0.12		
No	40	1			
Yes	133	1,8 (0,9-3,9)			
Secondary infection			<0.001		<0.001
No	161	1		1	
Focal	21	2,1 (1,0-4,4)		1,8 (0,8-3,8)	
Prominent	21	4,8 (2,5-9,1)		4,6 (2,3-9,1)	
Clinical stage			<0.001		<0.001
Early skin-limited FMF	84	1		1	
Advanced skin-limited FMF	102	9,6 (3,8-24,7)		7,1 (2,7-18,6)	
Extracutaneous FMF	17	37,5 (13,2-107)		30,0 (9,7-92,3)	

Supplementary Table 2. *(continued)*

OS				PFS			
Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
HR (95%CI)	P-value	HR (95%CI)	P-value	HR (95%CI)	P-value	HR (95%CI)	P-value
	0.44				0.87		
1				1			
1,2 (0,7-2,0)				1,0 (0,6-1,7)			
	<0.001		<0.001		0.03		0.002
1		1		1		1	
2,9 (1,9-4,5)		3,2 (1,9-5,4)		1,6 (1,0-2,6)		2,2 (1,3-3,6)	
	0.22				0.20		
1				1			
4,9 (0,6-38,7)				3,5 (0,4-29,3)			
5,6 (0,8-40,4)				5,1 (0,7-36,7)			
	<0.001		0.07		<0.001		0.01
1		1		1		1	
2,5 (1,6-4,1)		1,8 (1,0-3,2)		3,0 (1,8-5,0)		2,1 (1,2-3,9)	
	0.51				0.74		
1				1			
1,0 (0,6-1,6)				1,2 (0,7-2,0)			
1,3 (0,8-2,3)				1,0 (0,5-1,9)			
	0.12				0.18		
1				1			
0,7 (0,4-1,1)				0,7 (0,4-1,2)			
	0.03		0.26		0.05		
1		1		1			
2,0 (1,1-3,8)		1,5 (0,8-2,9)		2,0 (1,0-3,9)			
	<0.001		<0.001		<0.001		<0.001
1		1		1		1	
2,0 (1,1-3,7)		1,7 (0,9-3,2)		1,4 (0,7-2,9)		1,2 (0,6-2,4)	
3,7 (2,1-6,3)		4,1 (2,1-8,0)		3,5 (2,0-6,2)		3,4 (1,8-6,2)	
	<0.001		<0.001		<0.001		<0.001
1		1		1		1	
5,4 (3,0-9,7)		3,3 (1,7-6,5)		2,8 (1,6-4,8)		1,9 (1,0-3,4)	
12,5 (5,9-26,7)		8,7 (3,6-21,0)		8,7 (4,3-17,9)		5,7 (2,5-13,0)	



3

RECOMMENDATIONS FOR TREATMENT IN FOLLICULOTROPIC MYCOSIS FUNGOIDES: EXPERIENCE OF THE DUTCH CUTANEOUS LYMPHOMA GROUP

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ABSTRACT

Background: Folliculotropic mycosis fungoides (FMF) is an aggressive variant of mycosis fungoides (MF) and generally less responsive to standard skin-directed therapies (SDTs). Recent studies distinguished an indolent (early-stage FMF) and a more aggressive (advanced-stage FMF) subgroup. The optimal treatment for both subgroups needs still to be defined.

Objectives: Evaluation of initial treatment results in patients with early- and advanced-stage FMF.

Methods: 203 Patients (84 early-stage, 102 advanced-stage, 17 extracutaneous FMF), included in the Dutch Cutaneous Lymphoma Registry between 1985 and 2014 were studied. Type and results of initial treatment were retrieved from the Dutch Registry. Main outcomes were complete remission (CR), sustained complete remission (SCR), partial remission (>50% improvement; PR) and overall response (OR; CR+PR).

Results: Patients with early-stage FMF were treated with non-aggressive SDTs in 67 of 84 cases, resulting in CR and OR of 28% and 83% for monotherapy topical steroids, 0% and 83% for UVB and 30% and 88% for PUVA, respectively. In patients with advanced-stage FMF these SDTs were less effective (combined CR and OR: 10% and 52%, respectively). In patients with advanced-stage FMF local radiotherapy (CR 63%; OR: 100%), total skin electron beam irradiation (CR: 59%; OR: 100%) and PUVA combined with local radiotherapy (CR: 5%; OR: 75%) were most effective.

Conclusions: The results of the present study demonstrate that not all patients with FMF should be treated aggressively. Patients with early-stage FMF may benefit very well from standard SDTs also used in early-stage classic MF and have an excellent prognosis.

INTRODUCTION

Folliculotropic mycosis fungoides (FMF) is a variant of mycosis fungoides (MF) with distinctive clinicopathologic features.[1-7] Previous studies emphasized that FMF is generally less responsive to several first-line skin-directed therapies (SDTs) and runs a more aggressive disease course, which is similar to tumor-stage classic MF and should therefore be treated accordingly.[2,3] However, more recent studies defined a subgroup of FMF patients whose FMF showed an indolent clinical behavior and an excellent prognosis. These studies indicate that not every case of FMF behaves as tumor-stage disease and that early- and advanced-stage FMF may require a different therapeutic approach. Treatment results in FMF have thus far received very little attention in the medical literature and the prevailing instructions have not been based on the most recent insights into the disease. In the present study we evaluated the results of initial treatment in the Dutch cohort of 203 FMF patients used in our previous study.[8] The main purpose of this study was to propose recommendations for optimal initial treatment in patients with early- and advanced-stage FMF.

PATIENTS AND METHODS

We studied 203 FMF patients included in the Dutch Cutaneous Lymphoma Registry between 1985 and 2014. In all cases the diagnosis of FMF and selection of initial treatment had been made by an expert panel of dermatologists and pathologists at one of the regular meetings of the Dutch Cutaneous Lymphoma Group, and all cases met the diagnostic criteria of FMF.[1] Based on the results of our previous study distinction was made between patients with early-stage skin-limited FMF (group A, n=84), advanced-stage skin-limited FMF (group B, n=102) and FMF with extracutaneous disease at first presentation (group C, n=17) (**Table 1**).[8] For each patient follow-up data, including results of initial treatment had been entered yearly in the registry. Since 1985, results of initial treatment assessed at the time of discontinuation of treatment because of (near) complete response or lack or loss of response, had been scored by means of clinical evaluation of skin lesions as: complete remission (CR; complete clearance of all skin lesions), near CR (>75% clearance of skin lesions), partial response (PR; >50% clearance of skin lesions), stable disease (SD; <25% increase to <50% clearance of skin lesions) or progressive disease (PD; ≥ 25% increase in skin lesions or progression to higher stage during treatment). Overall response (OR) rate

indicates the percentage of patients that obtained (near) CR or PR.[9] The term sustained complete remission (SCR) is used in case of no relapse during follow-up after initial CR.

Table 1 – Types of skin lesions and survival rates of three subgroups of FMF

Subgroup	Skin lesions	N	5-year OS (%)	10-year OS (%)	5-year DSS (%)	10-year DSS (%)
A	Early-stage skin-limited FMF presenting with follicular papules, follicle-based, acneiform or keratosis pilaris-like lesions and/or 'early-stage' plaques*	84	92	72	96	93
B	Advanced-stage skin-limited FMF presenting with 'advanced-stage' plaques**, tumors, nodules or erythroderma	102	55	28	65	40
C	FMF with extracutaneous localisations at first presentation (stage IVA-IVB).***	17	23	2	23	2
Total		203	67	45	75	60

N: number, OS: overall survival, DSS: disease-specific survival
** Early-stage plaques were defined as more or less elevated or infiltrated skin lesions, histologically characterized by sparse intra- or perifollicular neoplastic infiltrates containing relatively few and mainly small neoplastic T-cells.[8]*
*** Advanced-stage plaques were defined as more or less elevated or infiltrated skin lesions, histologically characterized by by extensive confluent or diffuse infiltrates containing many often medium-sized to large tumor cells. Clinically, 'early-stage plaques' and 'advanced-stage plaques' can be indistinguishable.[8]*
**** Extracutaneous FMF includes patients presenting with nodal, visceral and/or peripheral blood involvement.*

RESULTS

Type of initial treatment

The different types and results of initial treatment in the total group of FMF patients (n=203) and subgroups A (n=84), B (n=102) and C (n=17) are presented in **Table 2**.

In group A, 67 of 84 patients (80%) had primarily been treated with non-aggressive SDTs, including topical steroids, topical nitrogen mustard, UVB therapy, PUVA therapy or had received no therapy, compared with only 31 of 102 patients (30%) in group B. Combined treatment results for non-aggressive SDTs in group A showed a CR and OR of 27% and 87%, compared to 10% and 52%, respectively, in group B (p-value < 0.01). The latter group had been treated most frequently with radiotherapy-based treatment modalities, including local radiotherapy, total skin electron beam irradiation (TSEBI) and PUVA therapy combined with local radiotherapy (53 of 102; 52%), or with PUVA therapy combined with acitretin, interferon alpha or methotrexate (13 of 102; 13%). In group C, nine of 17 patients (53%) had been treated with chemotherapy.
The results of the different initial treatment modalities are described in more detail below.

Table 2 – Type and results of initial treatment in subgroups and total group of FMF patients

Group A (n=84)								
			CR	PR	OR	SD	PD	SCR
Type of therapy	n	%	%	%	%	%	%	%
Topical steroids	**18**	**21**	28	56	83	17	0	22
UVB	**6**	**7**	0	83	83	17	0	0
PUVA	**40**	**48**	30	58	88	13	0	18
PUVA+ retinoids or IFNa	**5**	**6**	0	60	60	40	0	0
PUVA+ local RT	**5**	**6**	60	40	100	0	0	40
Local RT alone	**4**	**5**	75	25	100	0	0	75
TSEB	**1**	**1**	0	100	100	0	0	0
Systemic chemotherapy	**1**	**1**	0	100	100	0	0	0
Miscellaneous*	**4**	**5**	25	50	75	25	0	25
Total group A	**84**	**100**	**29**	**57**	**86**	**14**	**0**	**20**
Group B (n=102)								
			CR	**PR**	**OR**	**SD**	**PD**	**SCR**
Type of therapy	n	%	%	%	%	%	%	%
Topical steroids	**4**	**4**	0	25	25	75	0	0
UVB	**5**	**5**	0	20	20	80	0	0
PUVA	**20**	**20**	10	50	60	25	15	5
PUVA+ retinoids or IFNa **	**13**	**13**	0	67	67	17	17	0
PUVA+ local RT	**20**	**20**	5	70	75	5	20	0
Local RT alone	**16**	**16**	63	37	100	0	0	19
TSEB	**17**	**17**	59	41	100	0	0	18
Systemic chemotherapy	**5**	**5**	20	40	60	20	20	0
Miscellaneous*	**2**	**2**	50	50	100	0	0	0
Total group B	**102**	**100**	**25**	**50**	**74**	**16**	**10**	**7**
Group C (n=17)								
			CR	**PR**	**OR**	**SD**	**PD**	**SCR**
Type of therapy	n	%	%	%	%	%	%	%
Topical steroids	**-**	**-**	-	-	-	-	-	-
UVB	**-**	**-**	-	-	-	-	-	-
PUVA	**2**	**12**	0	0	0	50	50	0
PUVA+ retinoids or IFNa	**-**	**-**	-	-	-	-	-	-
PUVA+ local RT	**2**	**12**	0	50	50	0	50	0
Local RT alone	**1**	**6**	0	100	100	0	0	0
TSEB***	**2**	**12**	0	100	100	0	0	0
Systemic chemotherapy	**9**	**53**	11	22	33	22	44	11
Miscellaneous*	**1**	**6**	0	0	0	0	100	0
Total group C	**17**	**100**	**6**	**31**	**38**	**19**	**44**	**6**
Total group (n=203)								
			CR	**PR**	**OR**	**SD**	**PD**	**SCR**
Type of therapy	n	%	%	%	%	%	%	%
Topical steroids	**22**	**11**	23	50	73	27	0	18
UVB	**11**	**5**	0	55	55	45	0	0
PUVA	**62**	**31**	23	53	76	18	6	13
PUVA+ retinoids or IFNa **	**18**	**9**	0	65	65	24	12	0
PUVA+ local RT	**27**	**13**	15	52	78	4	19	7
Local RT alone	**21**	**10**	62	38	100	0	0	29
TSEB***	**20**	**10**	53	47	100	0	0	15
Systemic chemotherapy	**15**	**7**	13	33	47	20	27	7
Miscellaneous*	**7**	**3**	29	43	71	14	14	14
Total group	**203**	**100**	**25**	**51**	**76**	**15**	**8**	**12**

*Group A: Early-stage skin-limited FMF, Group B: Advanced-stage skin-limited FMF, Group C: Extracutaneous FMF, n: number, UVB: ultraviolet B therapy, PUVA: psoralen + ultraviolet A therapy, RT: radiotherapy, TSEB: total skin electron beam therapy, CR: complete remission, PR: partial remission (>50% improvement), OR: overall response (CR+PR), SD: stable disease, PD on tx: progressive disease during initial therapy. * Miscellaneous therapies included topical nitrogen mustard (two cases in group A), methotrexate (one case in group A) or no therapy (other four cases). ** 1 missing value for therapy response *** 1 missing value for therapy response*

TREATMENT RESULTS PER TREATMENT MODALITY

Topical steroids (n=22)

Monotherapy with highly potent topical steroids had been used in 22 patients, mainly patients with early-stage FMF (18 of 22 patients). It was most effective in group A patients with limited follicle-based patches (T1-score; **Figure 1**) resulting in CR in 5 of 6 patients, in four of them without relapse during a median FU period of 26 months (range 17-63). Patients presenting with plaques, including 12 in group A and 4 in group B, showed PR in 9 of 12 (75%) and 1 of 4 (25%) cases respectively, but did not achieve CR.

UVB therapy (n=11)

Narrow-band UVB (nbUVB) therapy had been used as initial treatment in 11 patients (group A: 6 cases, group B: 5 cases), in particular in patients with widespread skin lesions localized outside the head and neck area (T2-score). None of the patients achieved CR, but PR was found in 5 of 6 cases of group A and in 1 of 5 of group B. PR occurred particularly in patients with widespread follicle-based patches, while patients with plaques, both in group A and group B, did generally not respond to this treatment.

PUVA therapy (n=62)

PUVA monotherapy was the most frequently used initial treatment modality in our cohort of FMF patients. In patients in group A CR was achieved in 12 of 40 cases (30%); seven of them did not show a relapse during a median FU of 116 months (range 12-144). Another 23 of 40 patients (57%) attained PR, including 14 patients with near CR. In contrast to treatment with potent topical steroids or nbUVB, no significant differences in results were observed between patients with follicle-based patches (n=25) and patients with plaques (n=15) (**Figure 2**), neither between patients with T1 and T2 scores. PUVA treatment appeared less effective in patients in group B, which included 20 patients with generalized plaques, in five of them with concurrent small tumorous lesions. In this group CR was observed in 2 of 20 patients (10%) and PR in another ten patients, including six patients with a near CR, resulting in an OR rate of 60%.

PUVA therapy combined with oral retinoids (n=13), interferon alpha (n=4) or methotrexate (n=1) (n=18)

This combined treatment modality had been used in 13 patients in group B presenting with plaques, or tumors or erythroderma (2 of 13 patients). In addition, a combination of PUVA and acitretin (RePUVA) was given to five patients in group A, all presenting with widespread follicular papules. None of the patients achieved CR. PR was attained in 11 of 18 cases (61%), including seven patients with near CR and one patient of which response to therapy was unknown. No significant differences in treatment results were observed between patients in group A and group B, neither between patients treated with PUVA and acitretin or PUVA combined with IFN alpha.

PUVA therapy combined with local RT (n=27)

In patients with widespread patches and plaques, PUVA therapy had often been combined with low-dose local radiotherapy for isolated tumorous plaques or tumors (**Figure 3A and 3B**). Initially, skin lesions had been treated with a dose of 20 Gy, but since 2002 low-dose RT (2x4 Gy) was preferrerd.[10] In 7 of 27 patients with more extensive plaques and tumors either acitretin (three cases) or interferon alpha (four cases) had been added as well. In general, the combination of PUVA and local RT was the most frequent treatment modality for FMF patients in group B, and resulted in CR in 1 of 20 patients (5%), near CR in 6 of 20 patients (30%) and PR in 8 of 20 (40%) cases. In group A, local RT following PUVA treatment for persistent plaques resulted in (sustained) CR in 3 of 5 cases and near CR in the other 2 patients.

Local radiation therapy (local RT) (n=21)

Local RT had been used in patients that had either presented with solitary or few localized plaques (**Figure 4**), tumors or nodules either without further skin lesions (n=11) or with concurrent follicle-based patches that were treated with topical steroids (n=10). In the first category CR was seen in all eleven cases, in four of them without relapse during a median FU period of 92 months (range 54-163). In the second category, two patients achieved sustained CR and the other eight patients a PR.

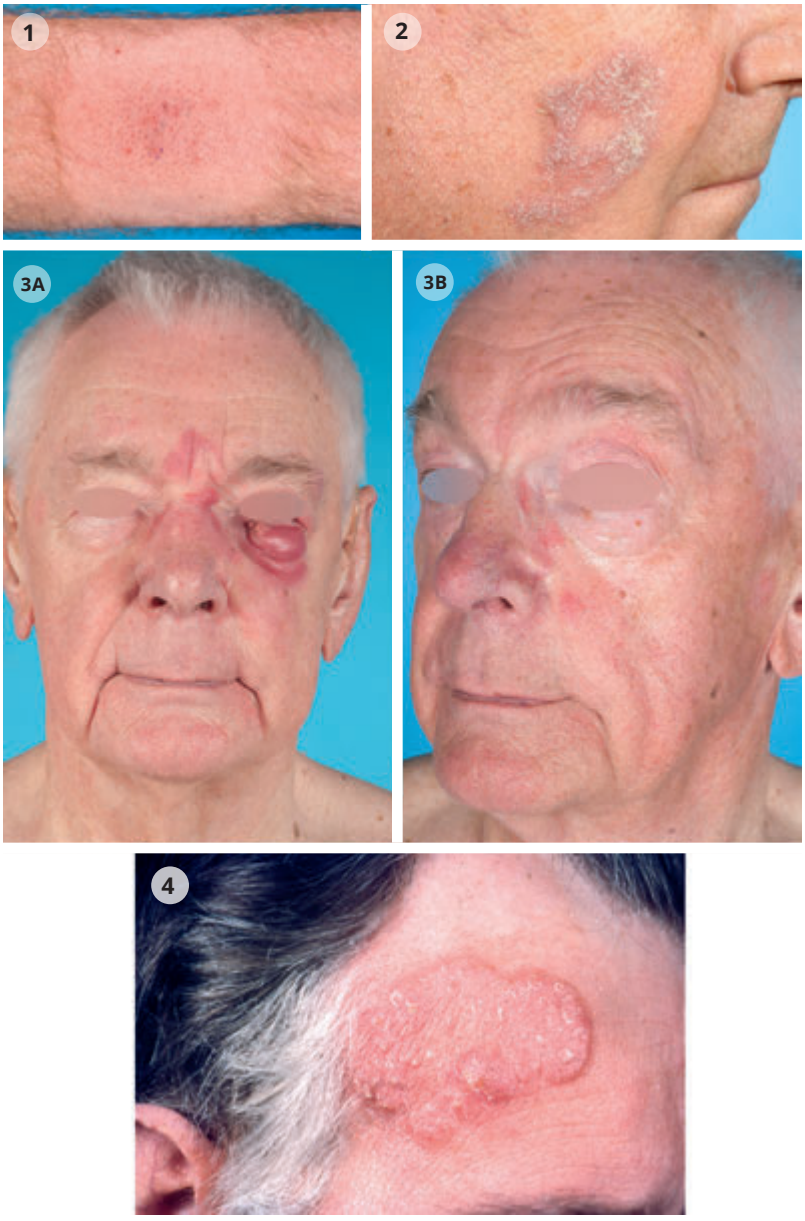


Figure 1. Detail of follicle-based patch on lower right arm with marked local alopecia.
Figure 2. Detail of a plaque (histological sparse peri-follicular neoplastic infiltrate) on the right cheek in a patient with early-stage FMF.
Figure 3. Patient with advanced-stage FMF with a subocular tumor treated with local radiotherapy (A) and result of treatment at 6 months follow-up (B).
Figure 4. Patient with advanced-stage FMF with a solitary plaque (histological diffuse neoplastic infiltrate) on the right fronto-temporal area.

Total skin electron beam irradiation (TSEBI) (n=20)

TSEBI with a standard dose of 35 Gy had mainly been used in patients presenting with widespread plaques, tumors or nodules . Results for TSEBI were excellent, resulting in CR in 10 of 20 (50%), near CR in 7 of 20 (35%) and PR in 2 of 20 (10%) and in one patient the result was unknown. Three patients had a sustained CR for 38, 57 and 169 months, but in most patients response to treatment was short lived.

Chemotherapy (n=15)

Eleven patients received CHOP(-like) courses as initial treatment: seven patients with stage IVA-IVB (group C), three patients with extensive plaques and tumors (group B) and one patient with early-stage FMF (group A), because of a concurrent clonally unrelated systemic anaplastic large cell lymphoma. Results showed sustained CR in one patient, PR in four patients, while six patients did not respond or had progressive disease. Seven of eleven patients died of lymphoma after a median follow-up of 21 months (range 7-68). The other four patients had been treated with prednisone, in two of them combined with chlorambucil. One patient with erythroderma (stage III) achieved sustained CR for more than eight years. The other three patients, who received this treatment as palliation continued to have progressive disease and died 12 to 15 months after diagnosis.

DISCUSSION

The present study showed that patients with early-stage FMF can benefit very well from non-aggressive SDTs and that not all patients with FMF should be treated similarly to patients with tumor-stage classic MF, as suggested previously.[2,3] Based on the results of this study and few available reports in literature, recommendations for effective initial treatment for different stages and clinical manifestations of FMF were formulated and consensus was reached after discussions at the regular quarterly meeting of the Dutch Cutaneous Lymphoma Group in 2015 and 2016 (see **Figure 5**). Treatment recommendations for FMF patients with extracutaneous disease (group C) have never been different from those for classic MF patients with extracutaneous disease[11] and will therefore not be further discussed.

Treatment of early-stage skin-limited FMF (group A)

Clinically, these patients present with localized or more extensive follicular papules or follicle-based patches often associated with hair loss, keratosis pilaris-like and acneiform lesions or plaques with histologically sparse peri- and intrafollicular infiltrates. This group has an excellent prognosis with a 5-year OS of 92-94%.[8,12] We have noted that patients with only limited follicle-based patches (T1) respond very well to monotherapy with potent topical steroids with CR and OR rates of 83% and 100%, respectively. Some of these patients even achieved durable SCR and may be considered cured. However, it has not been found effective in patients with plaques. In case of more widespread skin lesions nbUVB may produce PR in patients with only follicle-based patches, in particular localized outside the head/neck area, but it has again been found ineffective in patients with plaques. PUVA treatment proves much more effective, both in patients with patches and patients with plaques with CR and OR rates of 30% and 88%, respectively and sustained CR in 18% of cases. PUVA proved effective irrespective of localization and degree of extension (T1 or T2) of skin lesions. Because of the preferential localization of FMF in the head and neck area and the presumed superior efficacy we normally advise oral rather than bath PUVA. However, one study reported high efficacy of bath PUVA in 14 early-stage FMF cases with superficial or keratosis pilaris-like skin lesions that were mainly localized outside the face, resulting in CR and OR rates of 71% and 100%, respectively.[13] Taken together, non-aggressive SDTs had been used as initial treatment in 80% of early-stage FMF patients and were sufficient in most cases with a CR and OR rates of 27% and 87%, respectively. In case of residual lesions after SDTs low-dose radiotherapy (2 x 4 Gy) proves highly effective.[10] In rare cases presenting with a solitary skin lesion local radiotherapy (20 Gy) had resulted in CR in all cases.

Treatment of advanced-stage skin-limited FMF (group B)

These patients present with infiltrated plaques, histologically characterized by extensive confluent or diffuse infiltrates containing many often medium-sized to large T-cells, tumors, nodules or in rare cases erythroderma and have a less favorable prognosis with a 5-year OS of 55%, respectively.[8] Treatment with potent topical steroids or nbUVB had been ineffective in this group. PUVA monotherapy was found to be less effective than in group A, but the OR rate was still 60%. In patients with plaques this treatment can therefore be attempted first. When treatment fails or when patients present with very thick plaques or tumors, several options may be considered: (i) PUVA therapy combined

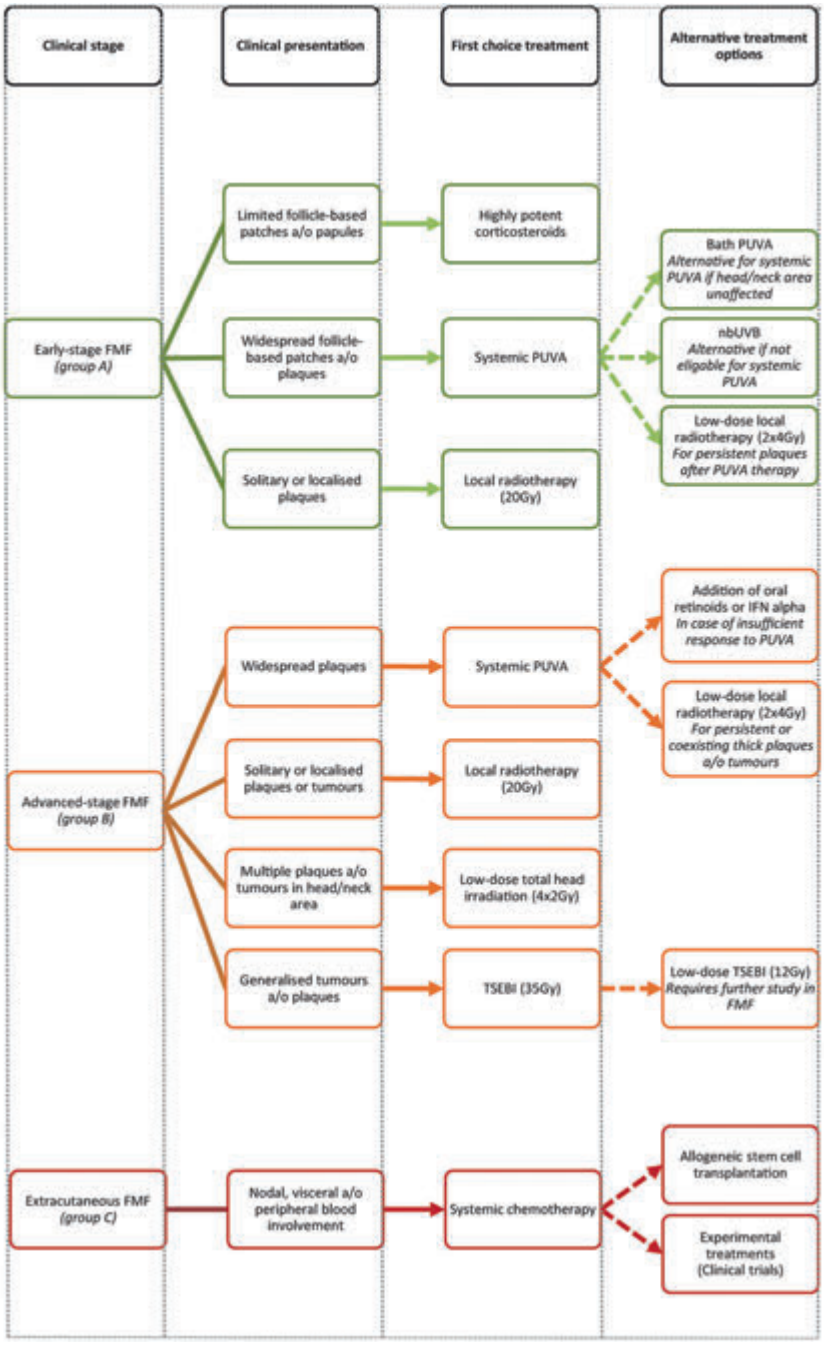


Figure 5. Algorithm with recommendations for initial treatment in patients with FMF**.**
FMF: Folliculotropic Mycosis Fungoides; a/o: and/or; PUVA: psoralen plus ultraviolet A; nbUVB: narrow-band UVB; Gy: Gray; IFN alpha: Interferon alpha; TSEBI: Total skin electron beam irradiation.

with local RT for most infiltrated lesions; (ii) TSEBI; (iii) PUVA therapy combined with IFNa or retinoids. In patients with widespread skin lesions we often prefer PUVA with additional low-dose radiotherapy for (persistent) thick plaques or tumors. This approach is very patient- and department-friendly (only two irradiations), has no side effects other than temporary hair loss in some patients, and is highly effective.[10] As patients with FMF may typically present with skin lesions on the head, total head irradiation with the same technique as used for TSEBI, but with a dose of 4 x 2 Gy and shielding of non-facial skin when appropriate, was found a very useful approach for extensive and infiltrated lesions. Concurrent less infiltrated skin lesions on trunk and extremities can be treated with PUVA. For patients presenting with a solitary or few localized plaques or tumors local radiotherapy is highly effective, may give durable CR and is the preferred mode of treatment.[2,14,15]

In patients presenting with widespread thick plaques and/or tumors TSEBI with a standard dose of 35 Gy proved an effective initial treatment with high CR and OR rates. In three of 19 cases durable complete remissions were observed, but in most patients response to treatment was short lived. Repeated use of TSEBI with a dose of 35 Gy is limited due to cumulative toxicity. Recent studies reported favorable responses of low-dose TSEBI (10-12 Gy) in classic MF, although the CR rates are lower than with conventional TSEBI (>30 Gy). An advantage of low-dose TSEBI, which is intended as palliative treatment, is that it can be used multiple times.[16,17] However, efficacy of low-dose TSEBI in FMF needs further study.

PUVA combined with retinoids or IFNa has been suggested as first-line therapy in patients with early-stage FMF with OR rates of 61% and 50%, respectively.[3] The results of the present study, in which these combinations were particularly used in patients with advanced-stage skin-limited FMF (group B) showed an OR rate of 65%, but CR was not achieved. After a good initial response disease can often be controlled effectively by continued treatment with retinoids or IFNa, without further PUVA therapy. While several studies describe efficacy of bexarotene monotherapy in patients with FMF, results on bexarotene as initial treatment have not been reported.[5,18]

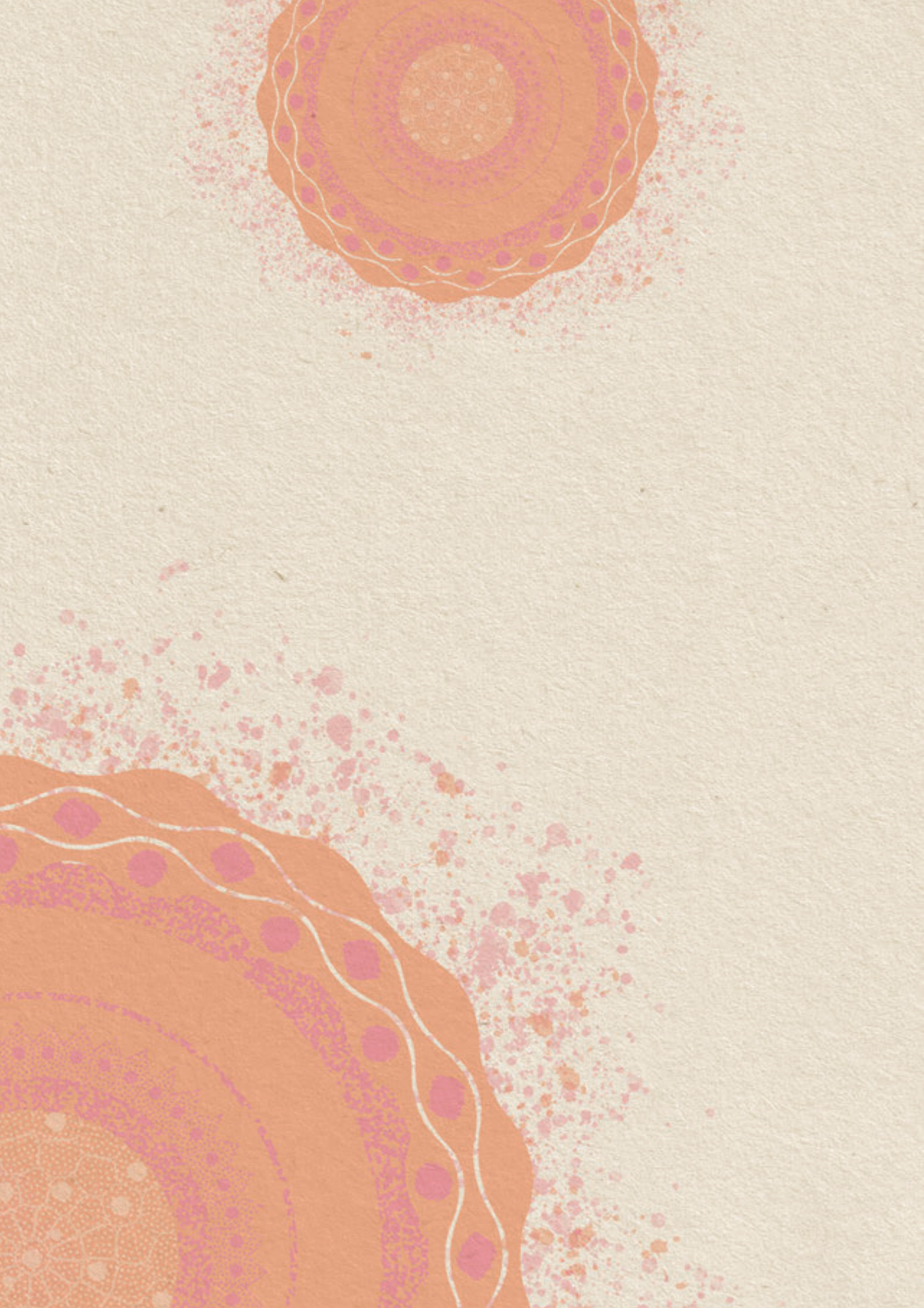
Our cohort contained six patients presenting with erythrodermic FMF (stage III). They had been treated variously with PUVA combined with IFNa (two patients) or acitretin

(one patient), TSEBI, a combination of prednisone and chlorambucil and nbUVB. The OR rate was 67% and only one patient treated with prednisone and chlorambucil achieved a CR for more than eight years. Other options that can be considered are methotrexate and extracorporeal photopheresis with or without IFNa.[11] However, our small series and the lack of published reports precludes any conclusion on the optimal treatment of patients with stage III FMF.

In conclusion, the present study demonstrates that not all patients with FMF require aggressive initial treatment. Patients with early-stage FMF have an indolent disease course and may benefit very well from non-aggressive SDTs.

REFERENCES

1. Willemze R, Jaffe ES, Burg G, et al. WHO-EORTC classification for cutaneous lymphomas. *Blood.* 2005;105(10):3768-3785.
2. van Doorn R, Scheffer E, Willemze R. Follicular mycosis fungoides, a distinct disease entity with or without associated follicular mucinosis: a clinicopathologic and follow-up study of 51 patients. *Arch Dermatol.* 2002;138(2):191-198.
3. Gerami P, Rosen S, Kuzel T, Boone SL, Guitart J. Folliculotropic mycosis fungoides: an aggressive variant of cutaneous T-cell lymphoma. *Arch Dermatol.* 2008;144(6):738-746.
4. Lehman JS, Cook-Norris RH, Weed BR, et al. Folliculotropic mycosis fungoides: single-center study and systematic review. *Arch Dermatol.* 2010;146(6):607-613.
5. Muniesa C, Estrach T, Pujol RM, et al. Folliculotropic mycosis fungoides: clinicopathological features and outcome in a series of 20 cases. *J Am Acad Dermatol.* 2010;62(3):418-426.
6. Demirkesen C, Esirgen G, Engin B, Songur A, Oguz O. The clinical features and histopathologic patterns of folliculotropic mycosis fungoides in a series of 38 cases. *J Cutan Pathol.* 2015;42(1):22-31.
7. Marschalko M, Eros N, Kontar O, et al. Folliculotropic mycosis fungoides: clinicopathological analysis of 17 patients. *J Eur Acad Dermatol Venereol.* 2015;29(5):964-972.
8. van Santen S, Roach RE, van Doorn R, et al. Clinical Staging and Prognostic Factors in Folliculotropic Mycosis Fungoides. *JAMA Dermatol.* 2016.
9. Olsen EA, Whittaker S, Kim YH, et al. Clinical end points and response criteria in mycosis fungoides and Sezary syndrome: a consensus statement of the International Society for Cutaneous Lymphomas, the United States Cutaneous Lymphoma Consortium, and the Cutaneous Lymphoma Task Force of the European Organisation for Research and Treatment of Cancer. *J Clin Oncol.* 2011;29(18):2598-2607.
10. Neelis KJ, Schimmel EC, Vermeer MH, Senff NJ, Willemze R, Noordijk EM. Low-dose palliative radiotherapy for cutaneous B- and T-cell lymphomas. *Int J Radiat Oncol Biol Phys.* 2009;74(1):154-158.
11. Trautinger F, Knobler R, Willemze R, et al. EORTC consensus recommendations for the treatment of mycosis fungoides/Sezary syndrome. *Eur J Cancer.* 2006;42(8):1014-1030.
12. Hodak E, Amitay-Laish I, Atzmony L, et al. New insights into folliculotropic mycosis fungoides (FMF): A single-center experience. *J Am Acad Dermatol.* 2016.
13. Pavlotsky F, Hodak E, Ben Amitay D, Barzilai A. Role of bath psoralen plus ultraviolet A in early-stage mycosis fungoides. *J Am Acad Dermatol.* 2014;71(3):536-541.
14. Kempf W, Kazakov DV, Schermesser M, et al. Unilesional follicular mycosis fungoides: report of two cases with progression to tumor stage and review of the literature. *J Cutan Pathol.* 2012;39(9):853-860.
15. Amitay-Laish I, Feinmesser M, Ben-Amitai D, Fenig E, Sorin D, Hodak E. Unilesional folliculotropic mycosis fungoides: a unique variant of cutaneous lymphoma. *J Eur Acad Dermatol Venereol.* 2016;30(1):25-29.
16. Hoppe RT, Harrison C, Tavallaee M, et al. Low-dose total skin electron beam therapy as an effective modality to reduce disease burden in patients with mycosis fungoides: results of a pooled analysis from 3 phase-II clinical trials. *J Am Acad Dermatol.* 2015;72(2):286-292.
17. Kamstrup MR, Gniadecki R, Iversen L, et al. Low-dose (10-Gy) total skin electron beam therapy for cutaneous T-cell lymphoma: an open clinical study and pooled data analysis. *Int J Radiat Oncol Biol Phys.* 2015;92(1):138-143.
18. Drugeon C, Charlat I, Boulinguez S, Viraben R. [Bexarotene therapy in folliculotropic cutaneous T-cell lymphoma]. *Ann Dermatol Venereol.* 2007;134(8-9):639-643.



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PLAQUE-STAGE FOLLICULOTROPIC MYCOSIS FUNGOIDES: HISTOPATHOLOGIC FEATURES AND PROGNOSTIC FACTORS IN A SERIES OF 40 PATIENTS

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ABSTRACT

Background: Folliculotropic mycosis fungoides (FMF) is a distinct variant of mycosis fungoides. Recent studies recognized indolent and aggressive subgroups of FMF, but there is controversy how patients presenting with plaques should be classified. The present study describes the histopathologic features of 40 FMF patients presenting with plaques at diagnosis. The aim of the study was to identify risk factors for disease progression and poor outcome in this group.

Methods: Clinical, histopathological, and immunophenotypical data from 40 patients with plaque-stage FMF were reviewed and analyzed for risk factors for disease progression and survival.

Results: After a median follow-up of 80 months, disease progression occurred in 20 of 40 patients. Percentage of atypical cells, cell size, percentage of Ki-67+ cells and co-existent interfollicular epidermotropism, but not the extent of perifollicular infiltrates, were associated with disease progression and reduced survival, while extensive follicular mucinosis was associated with increased survival.

Conclusions: This study underlines that FMF patients presenting with plaques represent a heterogeneous group and that a subgroup of these patients may have an indolent clinical course. It further shows that histological examination is a valuable tool to differentiate between indolent and aggressive disease.

INTRODUCTION

Folliculotropic mycosis fungoides (FMF) is recognized as a distinct variant of mycosis fungoides (MF). FMF is histopathologically characterized by the presence of folliculotropic infiltrates consisting of atypical T-cells, that often spare the epidermis.[1-3] Clinically, skin lesions are preferentially located in the head and neck region and are frequently accompanied by local alopecia and may include (grouped) follicular papules, acneiform and cystic lesions, plaques and tumors and in some patients keratosis pilaris-like skin lesions, which are located preferentially on the extremities or trunk.[1,2,4-13] Previous studies emphasized that FMF patients have a worse prognosis when compared to classic MF patients and should be treated more aggressively.[1,2] However, more recent studies reported that not all patients with FMF have an unfavorable prognosis and suggested that distinction should be made between an indolent (early stage) and an aggressive group (advanced stage) of FMF.[5,7,11] Patients presenting with only follicle-based patches, acneiform, or keratosis pilaris-like lesions have early-stage disease (stages IA-IIA), run an indolent clinical course and have an excellent prognosis, while patients presenting with nodules, tumors, erythroderma, and/or extracutaneous disease have advanced FMF (stages IIB-IV) and usually show a much more aggressive clinical course.[5] It is however uncertain how patients presenting with plaques should be categorized. In a study of Hodak et al, all patients with infiltrated plaques were upstaged and considered to have tumor-stage disease (stage IIB).[7] In contrast, studies of our own group showed that a subset of patients presenting with plaques may have a favorable clinical course. Using histopathological criteria, distinction was made between cases with early plaque-stage FMF and advanced plaque-stage FMF. It was found that patients with early plaque-stage FMF had the same indolent clinical behavior as patients with patch-stage FMF, whereas patients with advanced plaque-stage FMF had the same clinical behavior and prognosis as patients with tumor-stage FMF.[5] Histopathologic classification as either early or advanced plaque-stage disease was based on a rough estimate of the extent and cellular composition of the perifollicular infiltrates in a single hematoxylin and eosin (H&E)-stained section, but predefined criteria were lacking.[5] In the present study, clinical, histopathological and immunophenotypical characteristics of 40 patients with plaque-stage FMF at diagnosis were evaluated. The aim of this study was to describe histopathological characteristics of plaque-stage FMF and assess parameters associated with disease progression and survival.

PATIENTS AND METHODS

The database of the Dutch Cutaneous Lymphoma Group was searched for patients with FMF, who met the following criteria: the clinical presence of plaques without concurrent tumors or extracutaneous disease at the time of diagnosis, availability of clinical illustrations, and availability of relevant immunostainings or tissue blocks to perform additional stains. Plaques were defined clinically as palpable, mostly elevated skin lesions with a diameter > 1 cm.[14]

In order to study the risk of disease progression or death due to lymphoma, only patients with a follow-up period of at least 60 months or death before that time were selected. The total study group included 40 patients with plaque-stage FMF. In all cases, the diagnosis of FMF met the criteria of the WHO-EORTC classification and was confirmed by an expert panel of dermatologists and pathologists of the Dutch Cutaneous Lymphoma Group.[15] Clinical and follow-up data were retrieved from the Dutch Cutaneous Lymphoma Registry and from medical records. The date of first diagnostic biopsy was considered the time of diagnosis. Disease progression was defined as the development of skin tumors (stage IIB) or extracutaneous disease (stage IV) or death due to lymphoma. The study was performed in accordance with the Declaration of Helsinki and in accordance with our in-house Biobank protocol, as approved by our Medical Ethics Committee.

Histopathologic parameters

Histopathologic sections from the initial diagnostic skin biopsy including routinely stained H&E sections and a panel of immunostains were reviewed by three of the authors (SvS, PJ, and RW) blinded to the clinical and follow-up data. The following parameters were evaluated: extent of neoplastic infiltrate (sparse (1) or (2) prominent perifollicular and perivascular infiltrates confined to the perifollicular area; (3) confluent perifollicular and interfollicular infiltrates; (4) completely diffuse infiltrates), the percentage of atypical cells in the infiltrate (<10%; 11%-25%; >25%), size of the neoplastic T-cells (predominantly small/medium or predominantly medium/large), the percentage of blast cells (<10%; 11%-25%; >25%), degree of folliculotropism (mild, moderate, or extensive), presence or absence of interfollicular epidermotropism, presence or absence of syringotropism, presence of follicular mucinosis (no or minimal vs. prominent) and presence or absence

of eosinophils and neutrophils (no or few vs. prominent). Immunohistochemical sections, routinely stained for CD2, CD3, CD4, CD5, CD8, CD20, CD30, CD68, Ki-67, and cytotoxic proteins (TIA-1, granzyme B) were reviewed to determine the phenotype of neoplastic cells and the extent and composition of admixed inflammatory infiltrate. The presence of Ki-67+ cells was scored as more or less than 10%.

Statistical analysis

All statistical analyses were performed using the SPSS statistical software (IBM SPSS Statistics 23). To determine statistically significant clinical or histopathological differences between patients with disease progression (P-FMF) and patients without disease progression (NP- FMF) independent samples t tests and χ^2 tests were used. P-values <0.05 were considered statistically significant. Disease-specific survival (DSS) was calculated from the date of diagnostic biopsy until death as result of lymphoma or date of last follow-up. Overall survival (OS) was calculated from the date of diagnosis until patient's death from any cause or date of last follow-up. Progression-free survival (PFS) was calculated from the date of diagnosis until time of disease progression or death. Survival curves were calculated using Kaplan-Meier analyses and log-rank tests were used for comparison between survival curves. Using a Cox proportional hazards model, univariate analysis of age, and all histopathologic parameters was performed. Factors significant in univariate analysis were included in a multivariate analysis model. In both models, P-values below 0.05 were considered significant.

RESULTS

Clinical characteristics

The study included 29 males and 11 females (ratio: 2.6) with a median age at diagnosis of 56 years (range: 19-82 years). Distinction was made between patients with disease progression (P-FMF) and patients without disease progression for at least 5 years after diagnosis (NP-FMF). In the P-FMF group, the median time from diagnosis to disease progression was 30 months (range, 3-136 months) and occurred within 5 years after diagnosis in 17 of 20 patients and after 61, 66 and 136 months in the other three patients. The main clinical characteristics of these two groups are presented in **Table 1**.

The median age at diagnosis of patients in the P-FMF group was higher than in the NP-FMF group (62 vs. 51 years, respectively, $P = 0.004$). No significant differences between the P-FMF group and the NP-FMF group were found in the extent of skin lesions, the predominant involvement of the head and neck region, the presence of pruritus, and the initial type of treatment. The number of patients achieving complete remission upon initial treatment was higher in the NP-FMF group than in the P-FMF group (60% vs. 15%, respectively, $P = 0.003$), but overall response rates were comparable (75% vs. 65%, respectively). Follow-up data of the NP-FMF group showed sustained complete remission in eight of 20 patients, while 12 patients had relapsing skin disease without progression beyond plaque-stage disease. In the P-FMF group, five of 20 patients developed only skin tumors (stage IIB), whereas 15 patients developed extracutaneous disease with or without skin tumors (stage IV). After a median follow-up of 59 months, four patients in this P-FMF group were alive with ongoing disease, 15 patients died of lymphoma and one died of unrelated disease. The 5-year DSS and OS in the P-FMF group were both 45%, compared to 100% and 90%, respectively, in the NP-FMF group.

Histopathology

The main histopathological findings in these 40 patients with plaque-stage FMF are summarized in **Table 2**. In 35 of 40 cases, biopsies were obtained from skin lesions in the head and neck area. In two cases of the NP-FMF group and three cases of the P-FMF group, skin lesions on the back (four cases) or arm (one case) were biopsied. Twenty cases showed sparse (n= 4) or prominent (n= 14) infil trates confined to the perifollicular area (**Figures 1 and 2**). Confluent perifollicular and perivascular infiltrates or completely diffuse infiltrates were observed in the other 22 cases and were more common in the P-FMF group (**Figures 3 and 4**). Infiltration of the follicular epithelium by atypical lymphocytes was mild to moderate in 23 cases and extensive in 17 cases, including five of 20 (25%) in the NP-FMF group and 12 of 20 (60%) in the P-FMF group.

Table 1. Clinical characteristics of 40 patients with plaque-stage folliculotropic mycosis fungoides

	Total group (n = 40)	NP-FMF (n = 20)	P-FMF (n = 20)	*P*-value
Median age at diagnosis (range; months)	56 (19-82)	51 (19-68)	62 (29-82)	<0.01
Male-female (ratio)	29-11 (2.6)	15-5 (3.0)	14-6 (2.3)	0.72
Median time from first skin lesion to diagnosis (months)	24 (3-300)	10 (2-360)	36 (3-300)	0.19
Extent of skin lesions				0.11
Solitary	2 (5%)	2 (10%)	0	
Localized	2 (5%)	2 (10%)	0	
Generalized	36 (90%)	16 (80%)	20 (100%)	
Predominant head/neck Involvement	32 (80%)	16 (80%)	16 (80%)	1.0
Pruritus				0.34
Yes	30 (75%)	13 (65%)	17 (85%)	
No	8 (20%)	5 (25%)	3 (15%)	
Unknown	2 (5%)	2 (10%)	—	
Type of initial treatment				0.61
Topical steroids	4 (10%)	3 (15%)	1 (5%)	
Narrow-band UVB	2 (5%)	0 (0%)	2 (10%)	
PUVA	16 (40%)	9 (45%)	7 (35%)	
PUVA + local radiotherapy	6 (15%)	2 (10%)	4 (20%)	
Local radiotherapy	6 (15%)	3 (15%)	3 (15%)	
Total skin radiotherapy	6 (15%)	3 (15%)	3(15%)	
Result initial treatment				0.03
Complete remission	15 (37%)	12 (60%)	3 (15%)	
Partial remission	13 (32%)	3 (15%)	10 (50%)	
Stable disease	10 (25%)	4 (20%)	6 (30%)	
Progressive disease	2 (5%)	1 (5%)	1 (5%)	
Median duration follow-up (range) (months)	80 (6-320)	104 (38-320)	59 (6-241)	0.05
Status at last follow-up				<0.01
Alive without disease	7 (17%)	7 (35%)	0 (0%)	
Alive with ongoing disease	14 (35%)	10 (50%)	4 (20%)	
Died of lymphoma	15 (37%)	0 (0%)	15 (75%)	
Died of unrelated disease	4 (10%)	3 (15%)	1 (5%)	
Disease-specific survival at 5/10 years	72%/57%	100%/100%	45%/24%	<0.01
Overall survival at 5/10 years	68%/54%	90%/90%	45%/24%	<0.01

Abbreviations: NP-FMF: non-progressive folliculotropic mycosis fungoides; P-FMF, progressive folliculotropic mycosis fungoides; PUVA: psoralen plus ultraviolet A therapy; UVB: ultraviolet B therapy.

Table 2. Histopathologic characteristics of 40 patients with plaque-stage folliculotropic mycosis fungoides

	Total group (n = 40)	NP-FMF (n = 20)	P-FMF (n = 20)	*P*-value
Location of skin biopsy				0.63
Head and neck area	35 (88%)	18 (90%)	17 (85%)	
Trunk or extremities	5 (12%)	2 (10%)	3 (15%)	
Extent of dermal infiltrate				0.52
1. sparse perifollicular infiltrate	4 (10%)	3 (15%)	1 (5%)	
2. prominent perifollicular infiltrate	14 (35%)	8 (40%)	6 (30%)	
3. confluent peri−/intrafollicular infiltrates	18 (45%)	7 (35%)	11 (55%)	
4. completely diffuse infiltrate	4 (10%)	2 (10%)	2 (10%)	
Folliculotropism				0.05
Mild infiltration	6 (15%)	5 (25%)	1 (5%)	
Moderate infiltration	17 (42%)	10 (50%)	7 (35%)	
Extensive infiltration	17 (42%)	5 (25%)	12 (60%)	
Follicular mucinosis				0.01
No or focal spots	20 (50%)	6 (30%)	14 (70%)	
Moderate to extensive	20 (50%)	14 (70%)	6 (30%)	
Interfollicular epidermotropism[a]				0.01
Absent	26 (68%)	16 (88%)	10 (50%)	
Present	12 (32%)	2 (12%)	10 (50%)	
Syringotropism				0.68
Absent	33 (83%)	17 (85%)	16 (80%)	
Present	7 (17%)	3 (15%)	4 (20%)	
Percentage atypical cells[b]				0.02
<10%	7 (17%)	5 (25%)	2 (10%)	
10%-25%	12 (30%)	9 (45%)	3 (15%)	
>25%	21 (53%)	6 (30%)	15 (75%)	
Size of atypical cells				<0.01
Predominantly small-medium	33 (83%)	20 (100%)	13 (65%)	
Predominantly medium-large	7 (17%)	0 (0%)	7 (35%)	
Percentage of blast cells[c]				0.15
Absent or few <10%	34 (85%)	19 (95%)	15 (75%)	
10%–25%	3 (7%)	1 (5%)	2 (10%)	
>25% (blastic transformation)	3 (7%)	0 (0%)	3 (15%)	
Percentage of CD30+ cells				0.21
<10%	33 (83%)	18 (90%)	15 (75%)	
>10%	7 (17%)	2 (10%)	5 (25%)	
Ki-67				<0.01
<10%	29 (73%)	19 (95%)	10 (50%)	
>10%	11 (28%)	1 (5%)	10 (50%)	

Table 2. *(continued)*

	Total group (n = 40)	NP-FMF (n = 20)	P-FMF (n = 20)	*P*-value
Reactive CD8+ cells[d]				0.42
Absent or few <10%	16 (42%)	6 (32%)	10 (53%)	
Moderate 10%–25%	19 (50%)	11 (58%)	8 (42%)	
Many >25%	3 (8%)	2 (11%)	1 (5%)	
Eosinophils				0.29
Absent or few	29 (72%)	13 (65%)	16 (80%)	
Prominent	11 (28%)	7 (35%)	4 (20%)	

Abbreviations: NP-FMF, non-progressive folliculotropic mycosis fungoides; P-FMF, progressive folliculotropic mycosis fungoides.

[a]In two cases evaluation not possible because of ulceration.[b]Percentage of atypical cells >25% was used as a prognostic factor for statistical analysis. [c]Percentage of blast cells >10% was used as a prognostic factor for statistical analysis. [d]Two cases with CD8+ tumor cells were excluded.

Follicular mucinosis was prominent in 14 of 20 cases (70%) in the NP-FMF group, compared to six of 20 (30%) P-FMF cases ($P = 0.01$), but was absent or only focally present in the other 20 cases. Coexistent infiltration of the interfollicular epidermis by atypical T-cells was observed in 12 of 38 evaluable cases (31%), and was much more common in the P-FMF group than in the NP-FMF group (50% vs 11%, respectively $P = 0.01$)). Epidermotropism was mild in six cases and prominent in another six cases, but was always less pronounced than coexistent folliculotropism. Syringotropism was seen in seven of 40 cases, without any difference between the two subgroups (**Figure 5**).

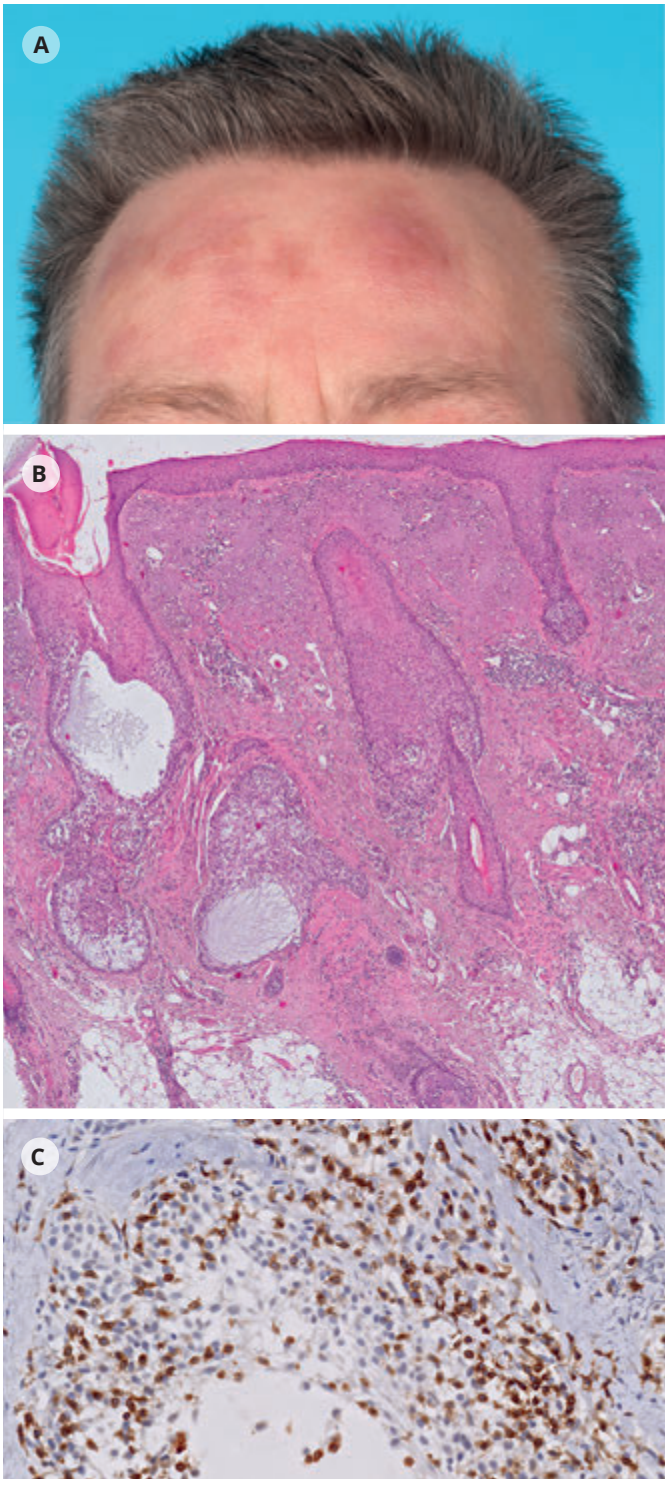


Figure 1. Plaques on a forehead (A). Histopathologic examination shows sparse intrafollicular and perifollicular infiltrates and extensive follicular mucinosis, which may explain the induration of the skin lesions (B, hematoxylin and eosin [H&E], ×40). Folliculotropic infiltrate shows positive staining for CD3 (C, original magnification, ×400)

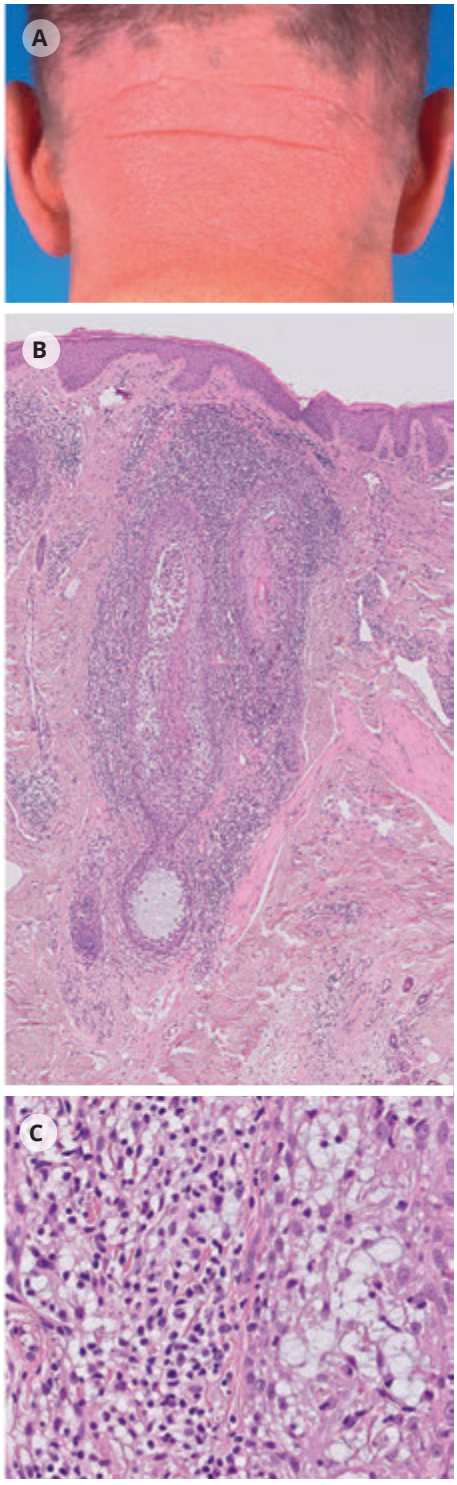


Figure 2. Plaque in the neck with follicular accentuation (A); Prominent perifollicular infiltrates (B, hematoxylin and eosin [H&E], x40); Detail of the infiltrate shows infiltration by small atypical T-cells (C, H&E, ×400)

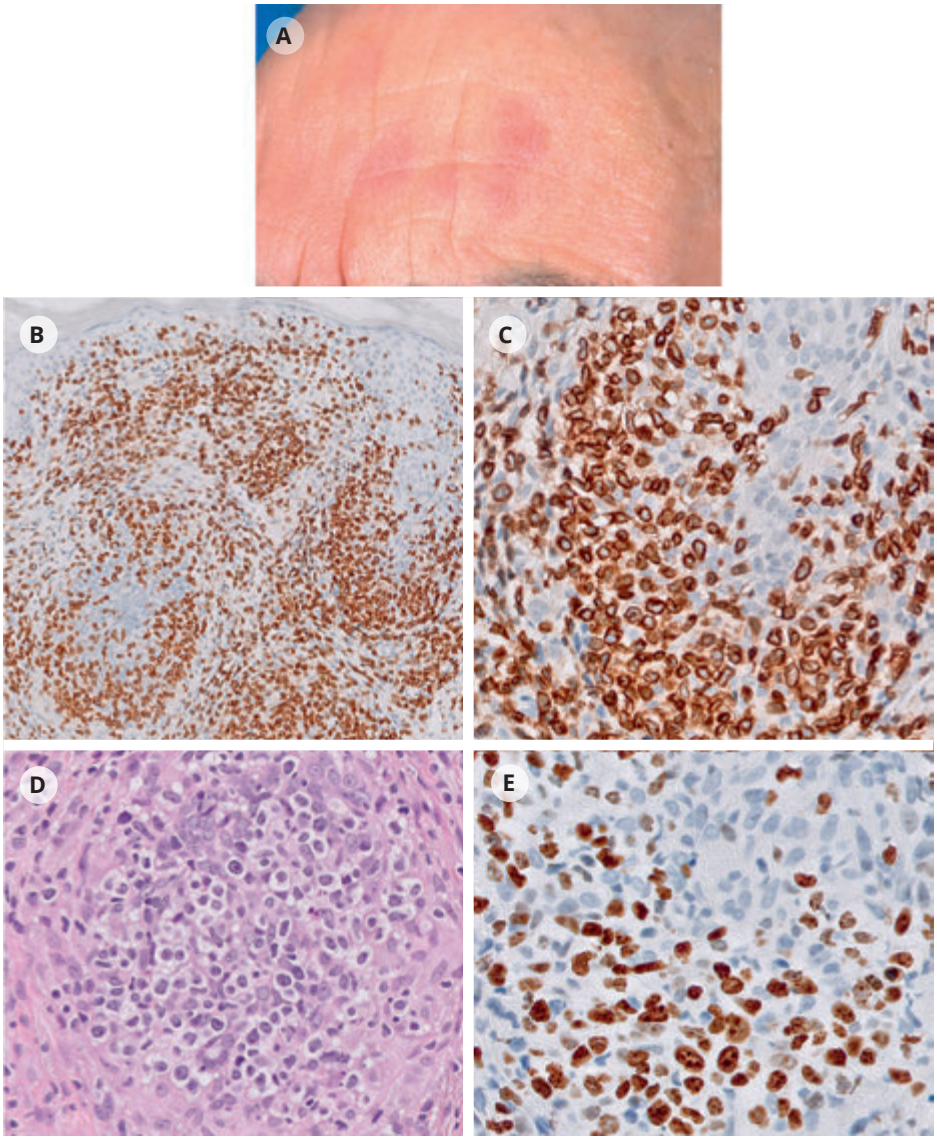


Figure 3. Plaques on a forehead (A). CD3 staining shows confluent perifollicular and perivascular infiltrates throughout the dermis (B, original magnification, ×40). Detail of infiltrate showing infiltration of follicular epithelium by large atypical lymphocytes (C, hematoxylin and eosin [H&E], ×400). Atypical T-cells express CD3 (D, original magnification, ×400) and Ki-67 staining shows a high proliferation rate (E, original magnification, ×400)

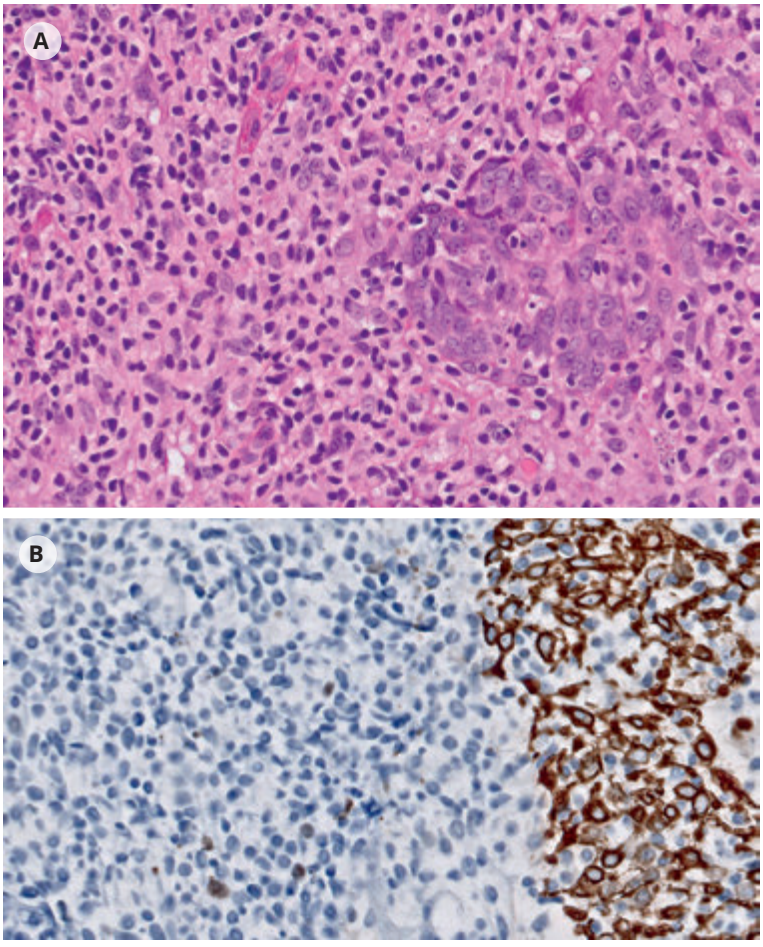


Figure 4. Detail of diffuse dermal infiltrate showing a dense infiltrate of small atypical T-cells infiltrating follicular epithelium (A, hematoxylin and eosin [H&E], × 400); Keratin staining highlights remnants of follicular structures (B, original magnification, ×400)

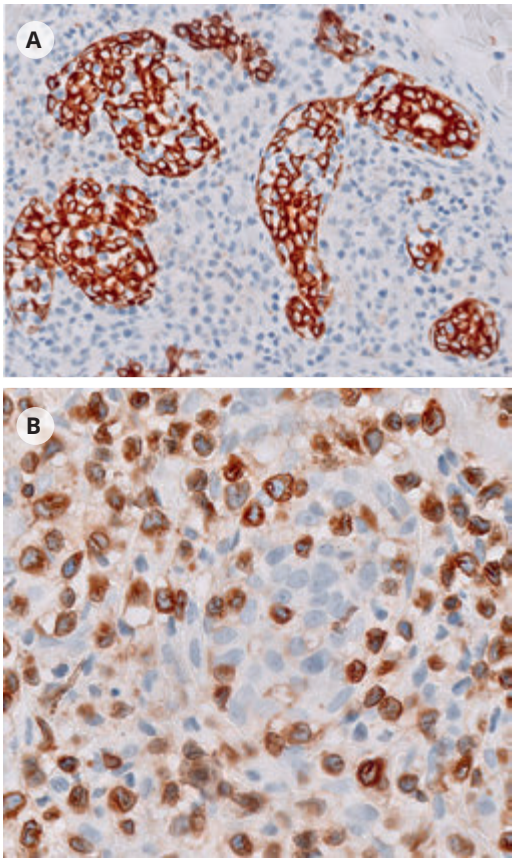


Figure 5. Keratin staining shows infiltration of large atypical lymphocytes into sweat glands (syringotropism) (A, original magnification, ×400); Expression of CD3 by syringotropic T-cells (B, original magnification, ×400)

Percentages of atypical cells, including cells with hyperchromatic and cerebriform nuclei and blast cells, in the dermal infiltrates were less than 10% in seven cases, 10% to 25% in 12 cases and more than 25% in 21 cases. Percentages above 25% were more frequent in the P-FMF than in the NP-FMF group (75% vs 30%, respectively; *P* = 0.02). In 33 of 40 cases (83%), these atypical cells were predominantly small to medium-sized (**Figures 2C and 4A**). In the other seven cases, all seven belonging to the P-FMF group, they were predominantly medium-sized to large (**Figure 3C**). Blast cells were either few or absent in 34 of 40 cases (85%). In six cases, they made up more than 10% of the T-cell infiltrate, but only three cases had percentages more than 25% and were considered to show large cell transformation.[16] The neoplastic cells had a CD3+, CD4+, CD8- T-cell phenotype in 38 cases and a CD3+, CD4-, CD8+ T-cell phenotype in two cases. Expression of CD30 by more than 10% of the neoplastic T-cells was observed in two of 20 cases (10%) in the

NP-FMF group and five of 20 cases (25%) in the P-FMF group. Percentages of more than 10% Ki-67 positive cells were detected in 11 of 40 cases and were much more common in the P-FMF group than in the NP-FMF group (50% and 5%, respectively $P = <0.01$) (**Figure 3E**). Dermal infiltrates contained variable numbers of admixed inflammatory cells, but comparison of the two subgroups of plaque-stage FMF showed no significant differences. Percentages of admixed CD8+ T-cells varied between 10% to more than 25% in 20 cases with a CD4+ T-cell phenotype, while percentages lower than 10% were found in 18 cases. Within the follicular epithelium CD8+ T-cells were generally few or absent. CD79a showed clusters of small B-cells in 26 of 40 cases, with formation of reactive germinal centers in two cases. Considerable numbers of eosinophils were observed in 11 of 40 cases, (clusters of) plasma cells in 11 cases, while neutrophils were generally absent. CD68 staining revealed considerable numbers of histiocytes in most cases, while variable numbers of multinucleated giant cells were observed in 12 of 40 cases.

Prognostic factors

Factors that significantly correlated with both disease progression and reduced survival included the presence of more than 25% atypical cells in dermal infiltrates, the size of neoplastic T-cells, the presence of coexistent interfollicular epidermotropism and presence of more than 10% Ki-67 positive cells. Presence of more than 10% blast cells was associated increased disease progression. In contrast, presence of prominent follicular mucinosis was associated with reduced disease progression and increased survival (**Table 3**). The extent of perifollicular infiltrates, which had been used in our previous study as one of the parameters to differentiate between early and advanced plaque stage disease[5], had no effect on prognosis and disease progression. Other factors that did not correlate with disease progression or survival were extent of folliculotropism, presence or absence of syringotropism, presence or absence of many eosinophils and percentages of admixed reactive CD8+ T-cells, CD30+ T-cells, CD79a+ B-cells, plasma cells, and CD68+ histiocytes. In multivariate analysis, size of neoplastic T-cells was independently associated with reduced PFS, whereas the presence of >10% Ki-67 positive cells was associated with reduced survival.

Table 3. Univariate and multivariate analysis of relevant histopathologic features in plaque-stage folliculotropic mycosis fungoides

		DSS			
		Univariate analysis		Multivariate analysis	
	40	HR (95%CI)	*P*-value	HR (95%CI)	*P*-value
Age at diagnosis			**0.004**		**0.11**
≤60 years	26	1			
>60 years	14	4.7 (1.6-13.4)			
Extent of infiltrate			0.79		-
Minimal perifollicular	4				
Prominent perifollicular	14				
Extensive perifollicular/confluent	18				
Completely diffuse	4				
Folliculotropism			0.50		-
Mild	6				
Moderate	17				
Extensive	17				
Follicular mucinosis			**0.04**		0.61
No or focal spots	20	1			
Moderate to extensive (lakes)	20	0.30 (0.10-0.94)			
Interfollicular epidermotropism			**0.003**		0.33
Absent	26	1			
Present	12	4.9 (1.7-13.8)			
Percentage atypical cells			**0.005**		0.28
<25%	19	1			
>25%	21	8.6 (1.9-38.3)			
Cell size			**0.008**		0.23
Small-medium	33	1			
Medium-large	7	4.4 (1.5-13.0)			
Percentage of blast cells			0.38		-
<10%	34				
>10%	6				
Percentage of Ki-67+ cells			**<0.001**		**0.02**
<10%	29	1		1	
>10%	11	7.7 (2.7-22.3)		5.3 (1.3-21.2)	

Note: Bold values were considered significant, as p<0.05 was considered significant.
Abbreviations: CI, confidence interval; DSS, disease-specific survival; HR: hazard ratio; N: number; OS, overall survival; PFS: progression-free survival.

Table 3. *(continued)*

OS				PFS			
Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
HR (95%CI)	*P*-value	HR (95%CI)	*P*-value	HR (95%CI)	*P*-value	HR (95%CI)	*P*-value
	0.001		**0.04**		**0.001**		0.10
1 5.2 (2.0-13.4)				1 5.8 (2.1-15.8)			
	0.69		-		0.51		-
	0.12		-		**0.04**		-
					Ref 0.36 0.06		
	0.009		0.96		**0.02**		0.75
1 0.25 (0.9-0.71)				1 0.31 (0.12-0.81)			
	0.007		0.56		**0.005**		0.34
1 3.5 (1.4-8.7)				1 3.6 (1.5-8.8)			
	0.005		0.48		**0.006**		0.60
1 5.0 (1.6-15.2)				1 4.2 (1.5-11.7)			
	0.006		0.37		**<0.001**		**0.03**
1 4.0 (1.5-10.7)				1 9.5 (3.2-27.6)		1 4.3 (1.1-16.3)	
	0.08		-		**0.01**		0.95
				1 3.7 (1.3-11.2)			
	<0.001		**0.03**		**<0.001**		0.27
1 7.3 (2.7-19.7)		1 4.2 (1.2-14.7)		1 5.3 (2.1-13.0)			

DISCUSSION

The present study describes the histopathologic features of 40 FMF plaques biopsied at diagnosis. The aim of this study was to identify risk factors for disease progression and poor outcome in this group. After a median follow-up of 80 months, disease progression occurred in 20 of 40 patients. Histopathologic evaluation revealed that the presence of more than 25% atypical cells in dermal infiltrates, size of neoplastic cells, the presence of more than 10% blast cells, presence of interfollicular epidermotropism, and presence of more than 10% Ki-67 positive cells were associated with decreased DSS, OS and/or PFS. Prominent follicular mucinosis was associated with increased survival and reduced risk of disease progression.

In a recent study of Hodak et al, extent and depth of peri-follicular infiltrates were significantly greater in advanced-stage FMF than in early-stage FMF. In addition, eosinophils and plasma cells in dermal infiltrates as well as pruritus were significantly more common in advanced-stage FMF.[7] However, in that study early-stage FMF included follicle-based patches and acneiform and keratosis pilaris-like lesions that mainly involved trunk and extremities, while advanced-stage FMF did not only include tumors, but also infiltrated plaques that preferentially involved the head and neck region. It should be noted that our study included only plaques and that in 80% of cases biopsies had been taken from the most infiltrated lesions in the head and neck area. This might explain why extent and depth of perifollicular infiltrates as well as the other abovementioned risk factors were not different between the two groups in the present study.

The observation that co-existent interfollicular epidermotropism is a risk factor for disease progression and reduced survival was unexpected and is at present unexplained. In 11 of 12 cases, with co-existent epidermotropism skin biopsies were obtained from characteristic FMF lesions in the face and none of these patients had concurrent skin lesions typical of classic MF.

Previous studies in classic MF showed that low numbers of admixed CD8+ T-cells are associated with disease progression and an inferior prognosis.[17-19] In the present study, such an association was not found. Notably, the number of reactive CD8+ T-cells

infiltrating into the follicular epithelium was very low, similar to the few or absent reactive CD8+ T-cells in the epidermis of early-stage classic MF.

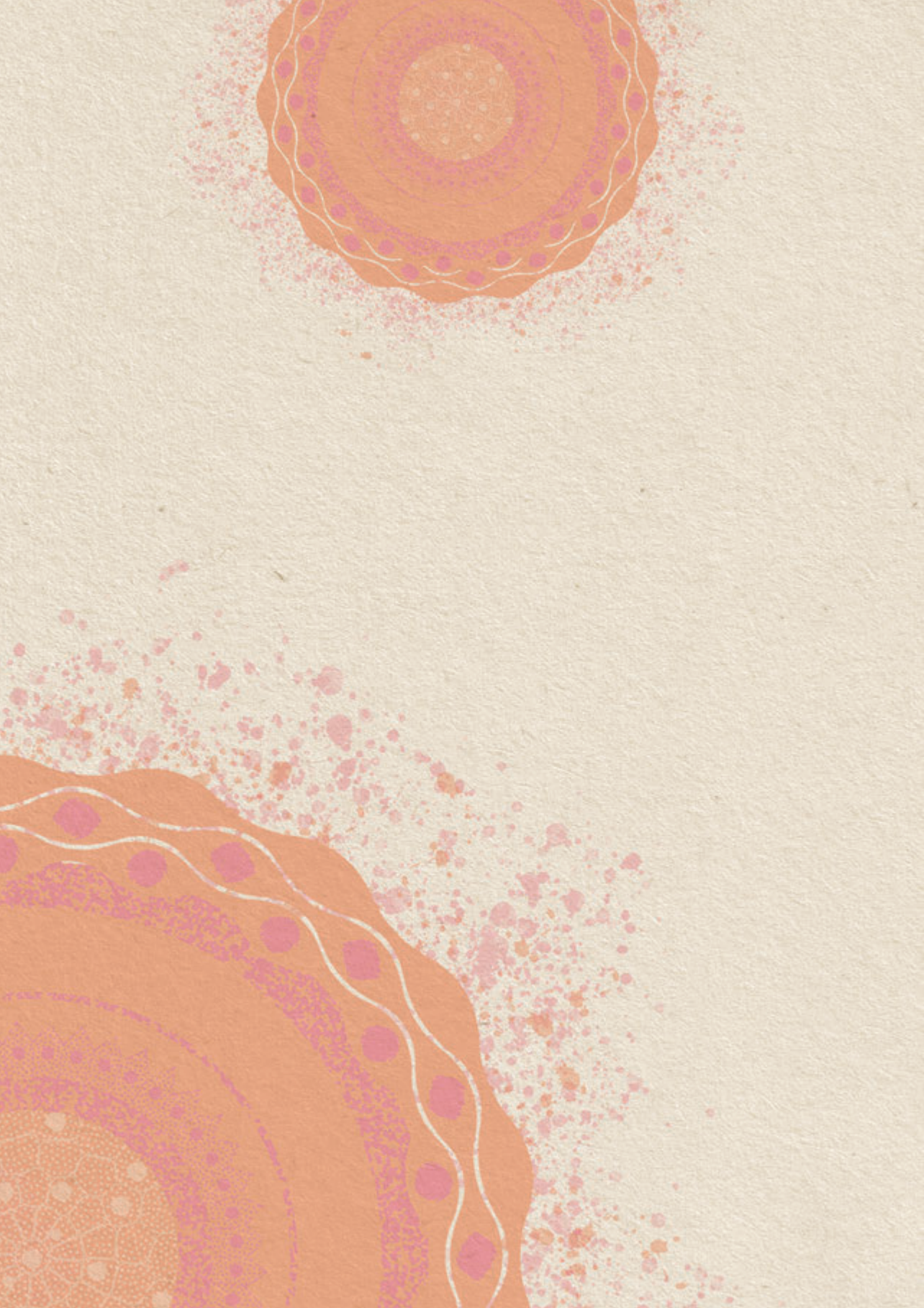
Furthermore, we found that age at diagnosis and lack of complete response upon initial treatment were clinical prognostic factors associated with disease progression in plaque-stage FMF. Age is a well-reported prognostic factor in both classic MF and FMF, while complete response to initial treatment has been reported as a favorable prognostic factor in classic MF.[5,6,20-23]

In conclusion, the results of the present study show that FMF patients presenting with plaques represent a heterogeneous group and that a subgroup of these patients may have an indolent clinical course. Our results argue against the suggestion that all cases of FMF with plaques should be considered to have tumor-stage disease. They further show that histopathological examination is a valuable tool to differentiate between indolent and aggressive disease.

REFERENCES

1. van Doorn R, Scheffer E, Willemze R. Follicular mycosis fungoides, a distinct disease entity with or without associated follicular mucinosis: a clinicopathologic and follow-up study of 51 patients. *Arch Dermatol*. 2002;138(2):191-198.
2. Gerami P, Rosen S, Kuzel T, Boone SL, Guitart J. Folliculotropic mycosis fungoides: an aggressive variant of cutaneous T-cell lymphoma. *Arch Dermatol*. 2008;144(6):738-746.
3. Gerami P, Guitart J. The spectrum of histopathologic and immunohistochemical findings in folliculotropic mycosis fungoides. *Am J Surg Pathol*. 2007;31(9):1430-1438.
4. Lehman JS, Cook-Norris RH, Weed BR, et al. Folliculotropic mycosis fungoides: single-center study and systematic review. *Arch Dermatol*. 2010;146(6):607-613.
5. van Santen S, Roach RE, van Doorn R, et al. Clinical staging and prognostic factors in folliculotropic mycosis fungoides. J*AMA Dermatol*. 2016;152(9):992-1000.
6. Wieser I, Wang C, Alberti-Violetti S, et al. Clinical characteristics, risk factors and long-term outcome of 114 patients with folliculotropic mycosis fungoides. *Arch Dermatol Res*. 2017;309(6):453-459.
7. Hodak E, Amitay-Laish I, Atzmony L, et al. New insights into folliculotropic mycosis fungoides (FMF): a single-center experience. *J Am Acad Dermatol*. 2016;75(2):347-355.
8. Marschalko M, Eros N, Kontar O, et al. Folliculotropic mycosis fungoides: clinicopathological analysis of 17 patients. *J Eur Acad Dermatol Venereol*. 2015;29(5):964-972.
9. Deonizio JM, Ascef RD, Sanches JA. Folliculotropic mycosis fungoides: clinical and epidemiological evaluation in a single center in Brazil. *Int J Dermatol*. 2015;55(5):e256-e261.
10. Demirkesen C, Esirgen G, Engin B, Songur A, Oguz O. The clinical features and histopathologic patterns of folliculotropic mycosis fungoides in a series of 38 cases. *J Cutan Pathol*. 2015;42(1): 22-31.
11. Muniesa C, Estrach T, Pujol RM, et al. Folliculotropic mycosis fungoides: clinicopathological features and outcome in a series of 20 cases. *J Am Acad Dermatol*. 2010;62(3):418-426.
12. Mantaka P, Helsing P, Gjersvik P, Bassarova A, Clausen OP, Delabie J. Clinical and histopathological features of folliculotropic mycosis fungoides: a Norwegian patient series. *Acta Derm Venereol*. 2013;93 (3):325-329.
13. Tomasini C, Kempf W, Novelli M, et al. Spiky follicular mycosis fungoides: a clinicopathologic study of 8 cases. *J Cutan Pathol*. 2015; 42(3):164-172.
14. Nast A, Griffiths CE, Hay R, Sterry W, Bolognia JL. The 2016 international league of dermatological societies' revised glossary for the description of cutaneous lesions. *Br J Dermatol*. 2016;174(6):1351- 1358.
15. Willemze R, Cerroni L, Kempf W, et al. The 2018 update of the WHO-EORTC classification for primary cutaneous lymphomas. *Blood*. 2019;133(16):1703-1714.
16. Salhany KE, Cousar JB, Greer JP, Casey TT, Fields JP, Collins RD. Transformation of cutaneous T cell lymphoma to large cell lymphoma. A clinicopathologic and immunologic study. *Am J Pathol.* 1988;132(2): 265-277.
17. Vermeer MH, van Doorn R, Dukers D, Bekkenk MW, Meijer CJ, Willemze R. 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(23): 4322-4329.
18. Hoppe RT, Medeiros LJ, Warnke RA, Wood GS. CD8-positive tumor-infiltrating lymphocytes influence the long-term survival of patients with mycosis fungoides. *J Am Acad Dermatol*. 1995;32(3): 448-453.
19. Goteri G, Filosa A, Mannello B, et al. Density of neoplastic lymphoid infiltrate, CD8+ T cells, and CD1a+ dendritic cells in mycosis fungoides. *J Clin Pathol*. 2003;56(6):453-458.

20. Agar NS, Wedgeworth E, Crichton S, et al. Survival outcomes and prognostic factors in mycosis fungoides/Sezary 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(31):4730- 4739.
21. Benton EC, Crichton S, Talpur R, et al. A cutaneous lymphoma inter- national prognostic index (CLIPi) for mycosis fungoides and Sezary syndrome. *Eur J Cancer*. 2013;49(13):2859-2868.
22. Talpur R, Singh L, Daulat S, et al. Long-term outcomes of 1,263 patients with mycosis fungoides and Sezary syndrome from 1982 to 2009. *Clin Cancer Res*. 2012;18(18):5051-5060.
23. 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(4):504-510



5

TUMOR CLONE FREQUENCY CALCULATION USING HIGH THROUGHPUT SEQUENCING OF THE T-CELL RECEPTOR β GENE IN FOLLICULOTROPIC MYCOSIS FUNGOIDES PATIENTS

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Cutaneous T-cell lymphomas (CTCL) are a heterogenous group of lymphoproliferative disorders defined by the clonal expansion of malignant T-lymphocytes in the skin. Classic mycosis fungoides (MF) is the most common type accounting for 60% of CTCLs and more than seventy percent of these patients present with early-stage disease (≤IIA) corresponding with an indolent disease course.[1,2] These patients are characterized by infiltration of malignant T-lymphocytes in the epidermal epithelium (epidermotropism) and clinically by flat erythematous, scaly patches or more elevated plaques typically localized on sun shielded areas.[3] The folliculotropic variant of mycosis fungoides (FMF) is diagnosed in approximately 10% of MF patients and is characterized by infiltration of malignant T-cells into the hair follicle epithelium instead of epidermal epithelium.[1] FMF carries distinct clinical features and a subset of FMF patients have a more aggressive clinical course as compared to classic MF.[4] Risk of disease progression within 5 years is reported in 8-21% of early-stage classic MF patients[2,5] and has been reported in approximately 30-40% of FMF patients.[2,4] Early identification of this subset of patients at risk for progressive and potentially fatal disease in both classic MF and FMF is of utmost importance, as it can facilitate accurate allocation of patients towards successful allogeneic stem cell transplantation and potential curative disease before their disease becomes treatment refractory. In the search for helpful parameters to early identify (F)MF patients at risk for disease progression, recent studies have successfully used high-throughput DNA sequencing of the T-cell receptor beta gene (HTS-TRB) to calculate the tumor clone frequency (TCF) from a lesional skin biopsy. This TCF represents the fraction of the most prevalent, identical T-cell receptor B gene (TRB) productive CDR3 sequences, relative to the total amount of TRB productive sequence reads. Ideally, clonally expanded tumor cells represent the highest TCF relative to polyclonal benign infiltrated T-cells.[6,7] Previous studies demonstrated that TCF >25% was a strong and independent prognostic factor for both progression-free survival (PFS) and overall survival (OS) in early-stage classic MF patients.[6] Here, we investigated whether the TCF calculated by HTS-TRB from FMF lesional skin could also identify patients with an adverse prognosis in FMF. Therefore, DNA was extracted from 46 residual paraffin embedded and frozen skin biopsies, taken at diagnosis from lesional skin from 41 FMF patients, that had clinically presented with plaques. The date of diagnosis was also the starting point for survival analysis. HTS-TRB had been performed using the Adaptive Biotechniologies, USA, platform that was also used to study TCF in classic MF.[6] Our study group included 24 FMF cases with disease progression, defined as evolution towards a higher TNMB stage, including 16 cases that had died from

disease during a median follow up duration of 68 months (range 6-320 months). TCF from 41 FMF cases ranged widely from 0.95-72.01% with a median of 11.00% (**Figure 1**). Median TCF in FMF patients with a non-progressive and a progressive disease course was 9.38% vs. 14.19%, respectively (p=0.15). In order to study the association between TCF and risk of disease progression or (lymphoma-related) death , a cox proportional hazards analysis was performed for TCF as a continuous variable. This resulted in non-statistical significant hazard ratios (HR) with 95% confidence intervals (CI) of 1.01 (0.99-1.03 ; p=0.39) ; 1.01 (0.99-1.04; p=0.27) and 1.02 (1.00-1.04; p=0.12) for PFS, OS and disease-specific survival (DSS), respectively. Then, log rank tests for specific TCF cutoff values, ranging from 5-50 percent, calculated with 5 percent incremental steps, were conducted to examine whether these cutoff values were associated with poor survival. Non-statistical significant trends in Kaplan Meier curves were observed with TCF cutoff values 15%, 20% and 25% for DSS and OS. In contrast, in classic MF a relative continuous relation between TCF and HRs for PFS and OS has been reported with the strongest association at TCF >25%.[6] In our FMF cohort a TCF >25% was found in 12/41 patients, of which 8/12 patients (67%) developed disease progression and 7/12 patients (58%) eventually died due to lymphoma (median follow-up in this group: 60 months (range 6-179). A TCF ≤25% was observed in 29/41 patients,

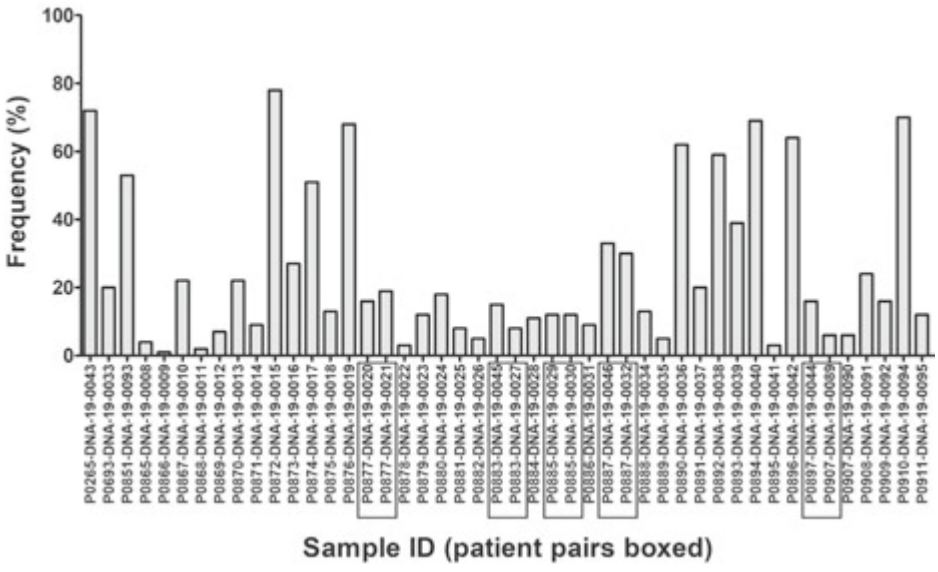
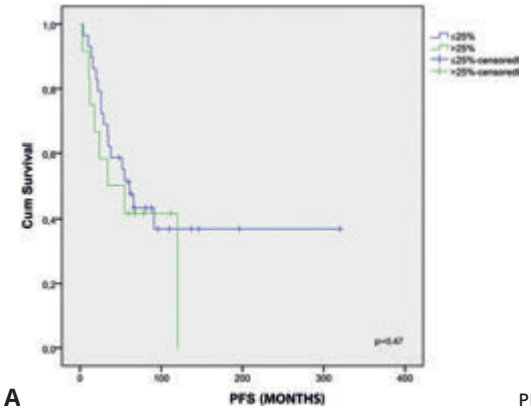
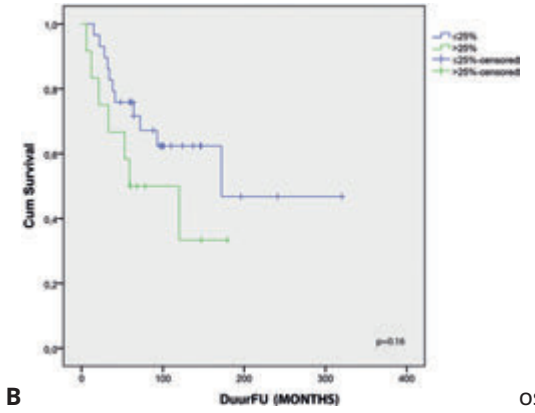


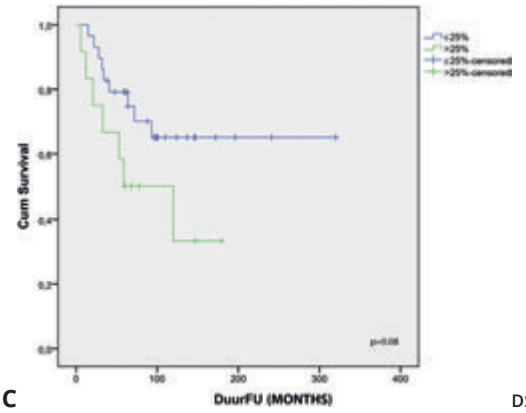
Figure 1. Tumor clone frequencies (TCF) in percentages in 46 lesional folliculotropic mycosis fungoides (FMF) skin samples after high throughput sequencing of T-cell receptor beta gene (HTS-TRB).



A PFS, p=0.47



B OS, p=0.16



C DSS, p=0.08

Figure 2. Survival analyses in patients with plaque-stage FMF with a tumor clone frequency (TCF) ≤25% or >25%. Survival plots using Kaplan Meier analysis for patients with a tumor clone frequency (TCF) of ≤25% or >25%. Kaplan Meier curves in panel A show no difference in progression-free survival (PFS) between these two groups. Panels B and C show a statistically non-significant trend for overall survival (OS) and disease-specific survival (DSS), respectively. P-values between groups ≤25% or >25% were calculated using log-rank analyses.

of which disease progression and lymphoma related death was seen in 16/29 (55%) and 9/29 (31%) patients, respectively (median follow-up in this group: 88 months (range 15-320)). Calculation of Kaplan Meier curves followed by log rank analyses studying two subgroups with TCF ≤25% or >25% resulted in p-values of 0.47, 0.16 and 0.08 for PFS, OS and DSS respectively (**Figure 2**).

This lack of correlation was further analyzed using detailed histopathological parameters that were previously analyzed for their prognostic function in a separate publication on a similar cohort.[8] Both proliferation rate and the size of neoplastic cells were found to be independent factors associated with adverse prognosis[8] and these factors, available for the majority of patients in the current cohort, were able to distinguish cases with a significantly different survival (**Supplementary table 2**).

In conclusion, we found that in 41 cases with plaque-stage FMF TCF did not correlate significantly with survival. It should be noted that in previous studies on classic MF not only patients with plaques but also with patches were included.[6] We focused on FMF patients with plaques as previous studies have shown that this group is heterogeneous in terms of prognosis and that the use of histological criteria enabled a distinction between patients with an indolent and aggressive disease course.[4] Based on the results of the present study there is no additional value for TCF in the identification of plaque-stage FMF patients with an adverse prognosis. However, it cannot be excluded that non-statistical significant trends would reach significance in a larger study cohort. Whether TCF calculation from HTS-TRB analysis is attributive in early FMF should therefore be further tested by larger studies and on different clinical presentations of early-stage FMF. Our results may further underscore that MF and FMF are distinct CTCL variants and that the aggressiveness of a tumor is not only reflected by tumor clone size. Further studies should focus not only on TCF but also on critical genetic alterations and cellular activation pathways that are involved in disease progression of FMF.

CONFLICTS OF INTEREST

TSK and RAC received a grant from the National Institutes of Health (NIH) and National Cancer Institute (NCI) for T-cell receptor sequencing (Grant: R01 CA203721).
All other co-authors state no conflict of interest.

REFERENCES

1. Willemze R, Cerroni L, Kempf W, et al. The 2018 update of the WHO-EORTC classification for primary cutaneous lymphomas. *Blood.* 2019;133(16):1703-1714.
2. Agar NS, Wedgeworth E, Crichton S, et al. Survival outcomes and prognostic factors in mycosis fungoides/Sezary 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(31):4730-4739.
3. Willemze R, Jaffe ES, Burg G, et al. WHO-EORTC classification for cutaneous lymphomas. *Blood.* 2005;105(10):3768-3785.
4. van Santen S, Roach RE, van Doorn R, et al. Clinical Staging and Prognostic Factors in Folliculotropic Mycosis Fungoides. *JAMA Dermatol.* 2016.
5. Talpur R, Singh L, Daulat S, et al. Long-term outcomes of 1,263 patients with mycosis fungoides and Sezary syndrome from 1982 to 2009. *Clin Cancer Res.* 2012;18(18):5051-5060.
6. de Masson A, O'Malley JT, Elco CP, et al. High-throughput sequencing of the T cell receptor beta gene identifies aggressive early-stage mycosis fungoides. *Sci Transl Med.* 2018;10(440).
7. Matos TR, de Rie MA, Teunissen MBM. Research Techniques Made Simple: High-Throughput Sequencing of the T-Cell Receptor. *J Invest Dermatol.* 2017;137(6):e131-e138.
8. van Santen S, Jansen PM, Quint KD, Vermeer MH, Willemze R. Plaque stage folliculotropic mycosis fungoides: histopathologic features and prognostic factors in a series of 40 patients. *J Cutan Pathol.* 2020;47(3):241-250.

SUPPLEMENTARY TABLES

Supplementary Table 1. Patient characteristics, histopathological features and TCF values

		Patient characteristics and disease course					Histopathological features [c]			HTS
Sample no.	Biopsy location	Age (years)	Gender (M/F)	FU (months)	Progression? [a]	Status at last FU	Proliferation rate [b]	Cell size	Blasts (%)	**TCF (%)**
1	Forehead	66	M	172	**P+**	D0	Low	medium-large	>25	**7,89**
2	Temple	69	M	6	**P+**	**D+**	High	medium-large	<10	**42,19**
3	Temple	59	F	33	**P+**	**D+**	High	medium-large	>25	**26,40**
4	Temple	57	M	28	**P+**	**D+**	High	medium-large	<10	**8,89**
5	Eyebrow	77	M	15	**P+**	**D+**	High	medium-large	10-25	**8,87**
6	Face	55	M	120	**P+**	**D+**	High	small-medium	<10	**46,65**
7	Eyebrow	82	F	53	**P+**	**D+**	High	small-medium	<10	**45,61**
8	Neck	51	F	59	**P+**	**D+**	High	small-medium	10-25	**28,48**
9	Periocular	62	F	41	**P+**	**D+**	High	small-medium	<10	**24,51**
10	Neck	51	F	59	**P+**	**D+**	High	small-medium	10-25	**23,56**
11	Cheek	63	M	32	**P+**	**D+**	High	small-medium	10-25	**16,24**
12	Supraorbital	65	M	64	**P+**	**D+**	Low	small-medium	<10	**23,60**
13	Forehead	61	M	93	**P+**	**D+**	Low	small-medium	<10	**11,00**
14	Supraorbital	71	M	22	**P+**	**D+**	Low	small-medium	<10	**7,45**
15	Scalp	69	F	34	**P+**	**D+**	Low	small-medium	<10	**7,34**
16	Supraorbital	71	M	22	**P+**	**D+**	Low	small-medium	<10	**4,65**
17	Back	49	M	72	**P+**	**D+**	Low	small-medium	<10	**2,04**
18	Back	57	F	12	**P+**	**D+**	NA	NA	NA	**61,05**
19	Occipital	52	F	21	**P+**	**D+**	NA	NA	NA	**49,16**
20	Lower arm	49	F	124	**P+**	A+	High	medium-large	>25	**9,33**
21	Shoulder	29	M	147	**P+**	A+	Low	small-medium	<10	**47,27**
22	Forehead	63	M	241	**P+**	A+	Low	small-medium	<10	**19,24**
23	Scapule	66	M	64	**P+**	A+	Low	small-medium	<10	**12,13**
24	Neck	39	M	147	**P+**	A+	Low	small-medium	<10	**5,35**
25	Face	60	M	101	**P+**	A+	Low	small-medium	<10	**3,70**
26	Eyebrow	56	M	60	**P+**	A0	NA	NA	NA	**10,00**
27	Scalp	68	M	38	P0	D0	Low	small-medium	<10	**3,90**
28	Scalp	68	M	38	P0	D0	Low	small-medium	<10	**3,17**
29	Cheek	58	M	137	P0	A+	Low	small-medium	<10	**15,29**
30	Shoulder	52	F	48	P0	A+	Low	small-medium	<10	**10,47**
31	Forehead	52	F	48	P0	A+	Low	small-medium	<10	**6,56**
32	Cheek	58	M	320	P0	A+	Low	small-medium	<10	**6,30**
33	Supraorbital	53	F	96	P0	A+	Low	small-medium	<10	**5,81**

Supplementary Table 1. *(continued)*

		Patient characteristics and disease course					Histopathological features [c]			HTS
Sample no.	Biopsy location	Age (years)	Gender (M/F)	FU (months)	Progression? [a]	Status at last FU	Proliferation rate [b]	Cell size	Blasts (%)	**TCF (%)**
34	Shoulder	66	M	23	P0	A+	Low	small-medium	<10	**5,26**
35	Temple	21	M	98	P0	A+	Low	small-medium	<10	**4,35**
36	Flank	59	M	179	P0	A+	NA	NA	NA	**72,01**
37	Wrist	54	M	78	P0	A+	NA	NA	NA	**43,04**
38	Eyebrow	69	M	60	P0	A+	NA	NA	NA	**32,26**
39	Trunk	40	M	100	P0	A+	NA	NA	NA	**21,43**
40	Eyebrow	29	F	88	P0	A0	Low	small-medium	<10	**16,06**
41	Eyebrow	19	M	59	P0	A0	Low	small-medium	<10	**9,38**
42	Eyebrow	25	F	63	P0	A0	Low	small-medium	<10	**4,26**
43	Forehead	29	F	196	P0	A0	Low	small-medium	<10	**2,16**
44	Occipital	55	M	146	P0	A0	Low	small-medium	<10	**1,41**
45	Cheek	50	M	110	P0	A0	Low	small-medium	<10	**0,95**
46	Flank	68	M	68	P0	A0	NA	NA	NA	**41,74**

Abbreviations: A+ : alive with disease, A0: alive without disease, D+ : death due to lymphoma, D0: death due to non-lymphoma related cause, FU: follow-up, F: female, HTS: high throughput sequencing, M: male, NA: not available, No.: number, P+: progressive disease course, TCF: tumor clone frequency percentage

[a] Disease progression was defined as the development of skin tumors (stage IIB) or extracutaneous disease (stage IV) or death due to lymphoma (D+).

[b] Proliferation rate was scored 'low' (<10% Ki67+ neoplastic cells) or 'high' (>10% Ki67+ neoplastic cells).

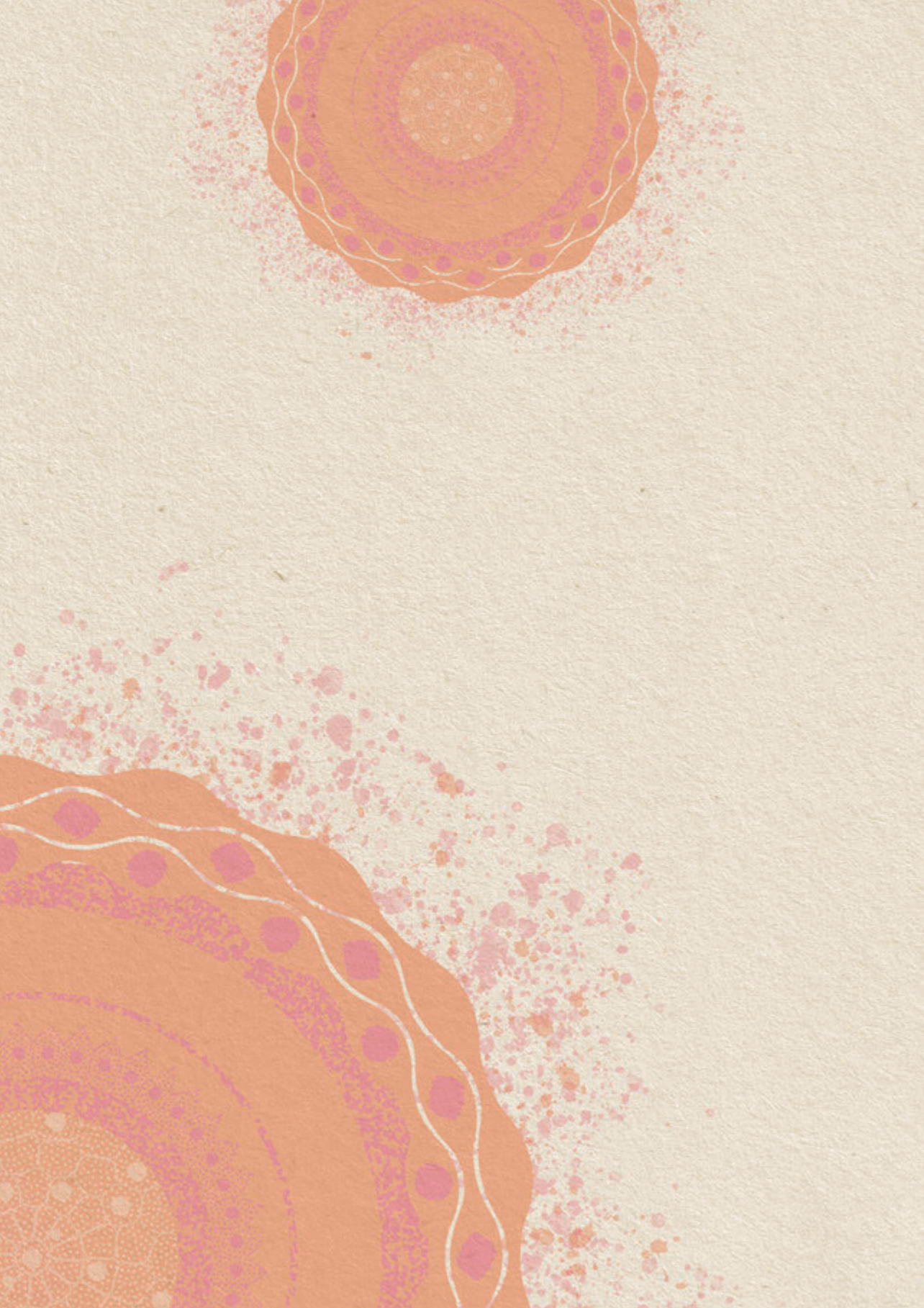
[c] Histopathologic features were scored on biopsies taken at diagnosis from (the most pronounced) plaque.

Supplementary Table 2. Kaplan Meier and log rank analyses of previously reported prognostic histopathologic factors in the current FMF patient cohort.[1]

	N	5year overall survival	5year disease specific survival	5year progression-free survival
Proliferation rate <10% vs. >10%	23 vs. 10	87% vs. 20% (p<0.001)	91% vs. 20% (p<0.001)	87% vs. 20% (p<0.001)
Cell size small-medium vs. medium-large	27 vs. 6	74% vs. 33% (p=0.02)	77% vs. 33% (p=0.06)	74% vs. 33% (p=0.04)

FMF: folliculotropic mycosis fungoides; KM: Kaplan Meier; N: number of patients; proliferation rate was scored as <10% Ki67+ neoplastic cells or >10% Ki67+ neoplastic cells at diagnostic biopsy of a plaque.

[1] van Santen et al; Plaque stage folliculotropic mycosis fungoides: histopathologic features and prognostic factors in a series of 40 patients. Journal of Cutaneous Pathology., 2021.



6

FOLLICULOTROPIC MYCOSIS FUNGOIDES PRESENTING WITH A SOLITARY LESION: CLINICOPATHOLOGICAL FEATURES AND LONG-TERM FOLLOW-UP DATA IN A SERIES OF 9 CASES

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ABSTRACT

Background: Folliculotropic mycosis fungoides (FMF) is a distinct variant of mycosis fungoides, which in rare cases may present with a solitary lesion. Reported cases describe an excellent prognosis, but follow-up was generally short. Herein, clinicopathologic characteristics, long-term follow up data of 9 patients with solitary FMF are presented and differential diagnosis is discussed.

Methods: From a cohort of 203 patients with FMF, nine cases with solitary FMF were selected. Clinical data and histological sections obtained at diagnosis and during follow-up were reviewed.

Results: Skin lesions, in all patients located on the head, went into complete remission after treatment with radiotherapy (6 cases) or topical steroids (1 case) or regressed spontaneously (2 cases). After a median follow-up of 89 months (range 51-203 months), 5 patients were still in complete remission, 2 patients had developed multiple skin relapses, while 2 patients had progressed to extracutaneous and fatal disease. Histologically, all patients showed marked folliculotropism, associated with syringotropism (4 cases) and/or follicular mucinosis (5 cases). Large cell transformation was observed at first presentation (2 cases) and during follow-up (3 cases).

Conclusions: Long-term follow-up data indicate that patients with solitary FMF do not always have an indolent clinical course and therefore require long-term follow-up.

INTRODUCTION

Folliculotropic mycosis fungoides (FMF) is a rare but distinct variant of mycosis fungoides (MF), histologically characterized by the presence of folliculotropic infiltrates, often with sparing of the epidermis, and clinically by the preferential involvement of the head and neck region.[1] Clinical manifestations include the presence of (grouped) follicular papules, plaques and tumors, and in some patients acneiform or keratosis pilaris-like skin lesions.[2-4] Infiltrated plaques or tumors in the eyebrow region are a highly characteristic feature and often skin lesions are associated with alopecia. Very uncommonly FMF may present with a solitary skin lesion, with only 28 cases documented in literature to date.[5-14] There is however no consensus about the definition of this condition. In most reports, solitary or unilesional FMF is defined by a solitary skin lesion with histopathological features of FMF.[6-10,12,14] However, in other studies unilesional FMF is defined by skin lesions in a single skin area covering <5% of body surface, which may contain multiple papules or nodules.[5,11,13] Patients with a solitary skin lesion (19 of 28 cases) presented most frequently with an (infiltrated) plaque or tumor (14 of 19 cases)[6-10,13,14], which were primarily localized in the head- and neck area (17of 19 cases). In contrast, patients with multiple papules within a small single skin area presented mainly with lesions on the limbs and trunk (7 of 9 cases) and only two cases with facial or scalp involvement.[11,13] Irrespective of the clinical presentation, initial treatment consisted most frequently of local radiotherapy[5,6,8-11,13,] but also excision[7,10,11,13], psoralen with ultraviolet A therapy with or without retinoids[10,11], topical steroids[8] and in one case even multiagent chemotherapy was used[7] , resulting in complete remissions in most cases. Most reports emphasize the favorable course of patients with solitary or unilesional FMF. Disease progression was reported in only three cases, including two patients showing progression from either a patch or a plaque to tumor stage disease preceding treatment with local radiotherapy[9] , and one patient presenting with a solitary papule in the right eyebrow, who developed relapsing and progressive plaque-like lesions histologically showing large cell transformation.[7] Dissemination to extracutaneous sites or lymphoma-related deaths have not been reported thus far. However, in most reports duration of follow-up was relatively short.

Herein, we present another nine patients with solitary FMF. In contrast to previous studies, more than half of our patients showed large cell transformation, either at

presentation or during follow-up, and two of nine patients developed extracutaneous and ultimately fatal disease. Differentiation between FMF and other types of cutaneous T-cell lymphoma may be challenging, in particular in patients presenting with a solitary tumor. The aim of our report is to describe the clinicopathologic features and differential diagnosis of FMF patients presenting with a solitary skin lesion, and to emphasize the necessity of long-term follow-up.

PATIENTS AND METHODS

From a prospective study cohort of 203 patients with FMF that were included in the Dutch Cutaneous Lymphoma Registry between 1985 and 2014, nine cases (4%) that had presented with a solitary skin lesion were selected for this study. Clinical and follow-up data were retrieved from the Dutch Cutaneous Lymphoma Registry and from medical records. The study was performed in accordance with the Declaration of Helsinki and the Dutch Code for Proper Secondary Use of Human Tissue, approved by the ethics committee of the Leiden University Medical Center. In all cases the diagnosis of FMF met the criteria of the WHO-EORTC classification and was confirmed by an expert panel of dermatologists and pathologists of the Dutch Cutaneous Lymphoma Group.[1] One of these patients (Table 1; case 3) has been reported previously, at that time still designated as alopecia mucinosa, despite the presence of highly atypical peri- and intrafollicular infiltrates.[15] Histologic sections from the initial biopsy and in total 19 biopsies obtained from four patients during follow-up were reviewed. The following histological criteria were examined: localization and cellular composition of the malignant infiltrate, presence of large cell transformation defined by the presence of more than 25% or large clusters of blast cells, extent of folliculotropism (+ minimal, ++ pronounced, +++ follicular destruction), syringotropism (- absent, + present) and epidermotropism (- absent, + present), the presence of follicular mucinosis (- absent, ± focal, +pronounced). Immunohistochemical sections, routinely stained for CD2, CD3, CD4, CD5, CD7, CD8, CD20, CD30, CD68, Ki-67 and cytotoxic proteins (TIA-1, granzyme B) were reviewed to determine the phenotype of the neoplastic cells and the presence of marker loss by these cells.

RESULTS

Clinical findings

The clinical characteristics of the nine patients are presented in **Table 1**. All nine patients (8 males, 1 female) had presented with a solitary skin lesion, localized in the head- and neck area. Median age at diagnosis was 57 years (range 19-68 years) and median time to diagnosis was 3 months (range 1-24 months). Two patients had presented with a typical eyebrow patch or plaque with associated alopecia. The other patients presented with an indurated plaque or tumorous lesion. Pruritus was reported in only one patient. Treatment with local radiotherapy (6 cases) or topical steroids (1 case) resulted in complete remission in all of them. The other two patients did not receive active treatment, because of complete spontaneous resolution of their skin lesion after biopsy (cases no. 5 and 8). Five of nine patients did not develop other skin lesions during a median follow-up of 59 months (range, 51-191 months). Two patients (cases 7 and 8) developed only multiple solitary skin relapses at distant sites, which were all successfully treated with low-dose radiotherapy (2 x 4 Gy), excision or topical steroids. The two remaining patients developed progressive skin lesions, five and 78 months after diagnosis, and ultimately developed extracutaneous disease with involvement of lymph nodes, bone marrow and visceral organs (see Table 1). Multiagent chemotherapy was unsuccessful and both patients died of their disease, 89 and 203 months after diagnosis, respectively. After a median follow-up of 89 months (range 51-203 months), six patients were alive without evidence of disease, one patient was alive with relapsing skin disease and two patients had died of lymphoma.

Histological findings

Histological characteristics of the initial diagnostic skin biopsies and a selection of follow-up biopsies are presented in **Table 2**. Initial biopsies of four cases (nos. 1-4) typically showed perifollicular to diffuse infiltrates with pronounced infiltration of the follicular epithelium by small to medium-sized pleomorphic T-cells with cerebriform nuclei and associated follicular mucinosis (**Figures 1A-F**). The other five cases (nos. 5-9) showed diffuse folliculotropic infiltrates and focal follicular mucinosis in one of them.

Table 1 – Clinical characteristics of nine patients with unilesional folliculotropic mycosis fungoides

Case no.	Sex/Age (years)	Clinical presentation	Initial treatment	Result	Course	Relapse (months)	Treatments relapse (result)	Status last follow-up (months)
1	M/25	Patch left eyebrow with alopecia	RT	CR	SCR	-	-	Ao, 55
2	M/19	Plaque left eyebrow with alopecia	Topical steroids	CR	SCR	-	-	Ao, 59
3	M/33	Plaque above left eyebrow	RT	CR	PD	plaque right eyebrow (78) fol-lowed by progres-sive skin lesions on face and trunk systemic disease (nodal and bone marrow) (178)	IL steroids, PUVA, topical nitrogen mustard, TSEBI, local RT (PD) Systemic chemo-therapy (PD)	D+, 203
4	F/59	Infiltrated plaque right forehead	RT	CR	SCR	-	-	Ao, 191
5	M/59	Firm papule chin	Spontaneous remission	CR	SCR	-	-	Ao, 51
6	M/50	Tumor scalp with alopecia	RT	CR	SCR	-	-	Ao, 65
7	M/61	Tumor glabella	RT	CR	RD	papule left eyebrow (39) papule left eyebrow (49)	Excision (CR) Topical steroids (CR)	Ao, 109
8	M/68	Tumor upper lip	Spontaneous remission	CR	RD	tumor glabella (29) follicle-based patches shoulder and leg (85) plaque lower lip(122) plaque on nose (134)	Low-dose RT (CR) Topical steroids (CR) Low-dose RT (CR) Low-dose RT (CR)	A+, 134
9	M/57	Tumor on tip of the nose	RT	CR	PD	plaque left side nose (5) followed by progressive skin lesions on face and trunk systemic disease (lung, nodal, CNS) (80)	PUVA+ interferon alpha, Low-dose RT (PD) Systemic chemotherapy (PD)	D+, 89

M: male, F: female, CR: complete remission, SCR: sustained complete remission, RD: relapsing disease (same stage), PD: progressive disease (to stage IV), D+: death of lymphoma, Ao: Alive without evidence of disease, A+: alive with disease; RT: radiotherapy, IL : intralesional CNS: central nervous system, TSEBI: total skin electron beam irradiation.

Table 2 - Histological characteristics of nine patients with solitary folliculotropic mycosis fungoides

No.	Clinical presentation (years after diagnosis)	Localisation of infiltrate	FT	ST	ET	Follicular mucinosis	Predominant morphology tumor cells	Immuno-phenotype	Blast cells (%)	LCT
1	Patch left eyebrow	Periadnexal	++	-	-	+	Small pleomorphic cells	CD4+	-	-
2	Flat plaque left eyebrow	Periadnexal	++	-	-	+	Small pleomorphic cells	CD4+	+	-
3	Plaque above left eyebrow	Periadnexal to diffuse	++	-	-	+	Small/medium-sized pleomorphic cells	CD4+	+	-
3FU	Plaque nose (14 yrs)	Diffuse	++	-	-	+	Small to large pleomorphic cells and (partially) CD30+ blast cells	CD4-	++	-
3FU	Plaque nose (16 yrs)	Diffuse	++	+	-	+	Small to large pleomorphic cells and (partially) CD30+ blast cells	CD4-	+++	+
4	Infiltrated plaque right forehead	Periadnexal to diffuse	++	-	-	+	Small/medium-sized pleomorphic and scattered CD30+ blast cells	CD4+	++	-
5	Papule chin	Diffuse	++	+	-	-	Small/medium-sized pleomorphic cells	CD4+	-	-
6	Tumor scalp	Diffuse	+++	+	-	-	Small/medium-sized pleomorphic cells and CD30+ blast cells	CD4+	+++	+
7	Tumor glabella	Diffuse	++	+	-	-	Small/medium-sized pleomorphic and scattered CD30+ blast cells	CD4+	+	-
7FU	Papule left eyebrow (4 yrs)	Periadnexal	++	-	-	-	Medium-sized pleomorphic and clusters of CD30+ blast cells	CD4+	++	+
8	Tumor upper lip	Diffuse	++	-	-	±	Small/medium-sized pleomorphic	CD4+	-	-
8FU	Tumor glabella (2 yrs)	Diffuse	++	-	-	-	Small/medium-sized pleomorphic and CD30+ blast cells	CD4+	+++	+
9	Tumor nose	Diffuse	+++	+	+	-	Small to large pleomorphic and CD30- blast cells	CD4-CD8-	+++	+
9FU	Tumors back and wrist (5 yrs)	Periadnexal	++	+	-	-	Small/medium-sized pleomorphic cells and scattered CD30- blast cells	CD4-CD8-	+	-

No.: number; FU: follow up biopsy; FT: folliculotropism + minimal, ++ pronounced, +++ follicular destruction; ST: syringotropism - absent, + present ; ET: epidermotropism - absent, + present, Follicular mucinosis: - absent, ± focal, + pronounced, Blast cells: - <5% + 5-10% ++ 11-25% +++ >25% LCT: large cell transformation

Infiltration of the eccrine sweat glands (syringotropism) was observed in four cases and pronounced epidermotropism with formation of Pautrier's microabscesses in one case (Table 2). The perifollicular infiltrates consisted of small to medium-sized pleomorphic T-cells and blast cells, admixed with histiocytes, small lymphocytes, eosinophils, which were abundant in four cases (nos. 2, 3, 5 and 8) and plasma cells. In three cases (nos. 4, 6 and 7) large clusters of small B-cells were observed. The proportion of blast cells varied between less than 5% to at most 15% in seven cases. Two cases met the criteria of large cell transformation with percentages of blast cells exceeding 25% (no. 9) or even 50% (no. 6). Apart from case 9, blast cells were predominantly CD30-positive. In one case (no.6) more than 80% of the neoplastic T-cells expressed CD30, more than half of them large blast cells. Because of the marked folliculotropism and syringotropism, nicely visualized by additional staining with a monoclonal antibody against keratin, as well as the presence of many small to medium-sized pleomorphic T-cells with cerebriform nuclei, both in the follicular epithelium and in the perifollicular infiltrates, the diagnosis transformed FMF rather than primary cutaneous anaplastic large cell lymphoma (C-ALCL) was preferred (**Figures 2A-E**). In another case (case 9), because of the density of the dermal infiltrates, mainly consisting of small to medium-sized pleomorphic T-cells with cerebriform nuclei, epithelial structures could hardly be recognized, and differentiation between FMF and a diagnosis of peripheral T-cell lymphoma, not otherwise specified (PTCL, NOS) was challenging. Also in this case a diagnosis of FMF was preferred because of the typical morphology of the neoplastic cells (cerebriform nuclei) and the presence of marked folliculotropism and syringotropism. Follow-up biopsies in this patient showed perifollicular infiltrates with pronounced follicular infiltration by small to medium-sized pleomorphic T-cells consistent with a diagnosis of FMF. In three cases (nos. 3, 7 and 8) skin lesions obtained during follow-up showed a marked increase in the number of blast cells (either CD30-positive or CD30-negative) compared to the initial biopsies and met the criteria of large cell transformation (see Table 2). Immunohistochemical examination demonstrated a CD3+CD4+CD8- phenotype with variable loss of pan-T-cell antigens in eight of nine cases. One case (no. 9) showed a CD3+CD4-CD8- phenotype, both in the initial and follow-up biopsies. An aberrant phenotype was also found in the two cases in which the skin lesions showed complete spontaneous resolution after biopsy. In case 5 the neoplastic CD4+ T-cells showed complete loss of CD7 and expressed cytotoxic proteins (TIA-1 and granzyme B), while case 8 showed (partial) loss of CD2 and CD5.

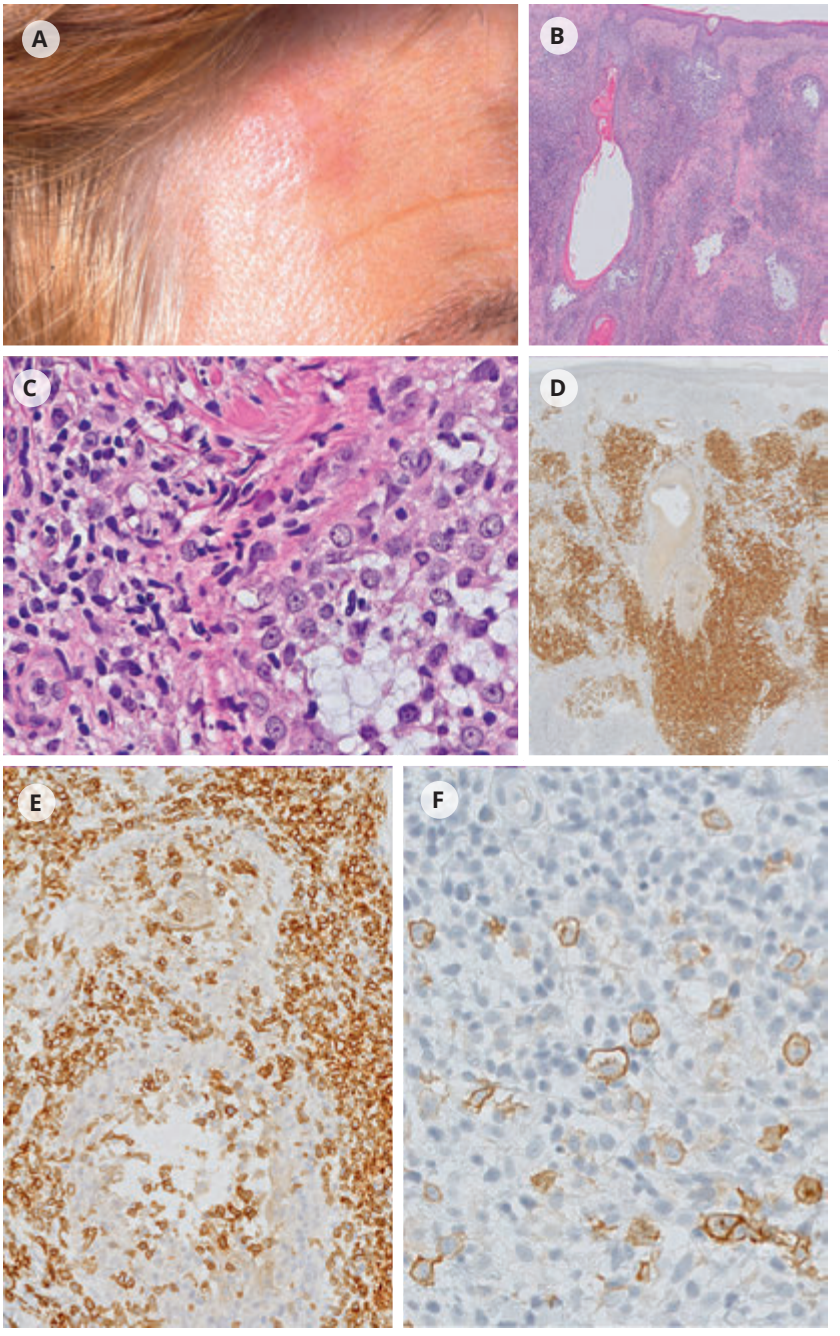


Figure 1. Case 4: Erythematous infiltrated plaque on the right forehead (A). Histology shows periadnexal to diffuse infiltrates with sparing of the epidermis and follicular mucinosis (B) and infiltration of the follicular epithelium by hyperchromatic small pleomorphic lymphocytes (C) that stained positive for CD3 (D and E). Perifollicular infiltrates show scattered CD30 positive blast cells (F).

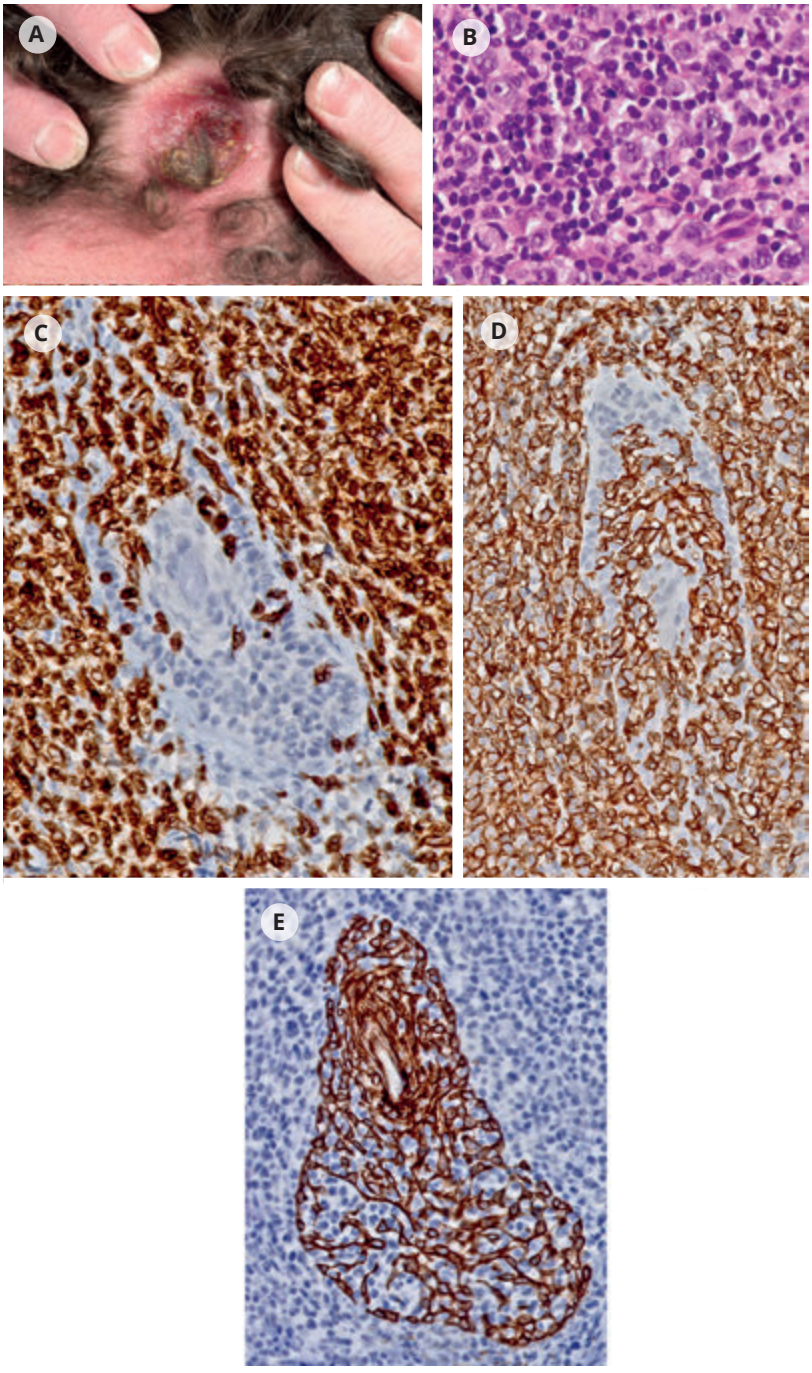


Figure 2. Case 6: Crusted tumor in the neck within an area of alopecia (A). Dermal infiltrate shows a mixture of small/medium-sized pleomorphic cells and blast cells (B). Folliculotropic infiltrates show a positive staining for CD3 (C) and CD30 (D). Folliculotropism is nicely visualized by a keratin staining (E).

DISCUSSION

The present study describes the clinicopathologic features and long-term follow-up data of nine patients with FMF presenting with a solitary skin lesion. This study included only patients presenting with one solitary lesion, who had been followed for a median of 89 months (range 4-17 years). All patients had presented with a skin lesion in the head- and neck area and achieved complete remission after initial treatment, which included local radiotherapy in six of nine cases. Interestingly, in two patients the skin lesions showed complete spontaneous resolution after biopsy (**Figures 3A-D**). Five cases achieved a long-lasting remission for several years after initial treatment. However, in contrast to previous studies, two of nine patients developed systemic disease and died of systemic lymphoma, 7.5 and 17 years after diagnosis. In addition, large cell transformation, either at presentation (case 6 and 9) or during follow-up (cases 3, 7 and 8) was observed in five of nine cases. These observations demonstrate that not all patients presenting with solitary FMF run an uneventful clinical course and indicate that long-term follow-up is warranted. Histologically, the diagnosis of solitary FMF was rather straightforward in seven of nine patients. Clinically, these patients presented with a solitary patch, plaque or tumor in the head- and neck area with the characteristic histological features of FMF, including perifollicular to diffuse folliculotropic (and syringotropic) infiltrates, that mainly consisted of atypical CD3+, CD4+, CD8- small to medium-sized cerebriform lymphocytes , variable numbers of mostly CD30+ blast cells and associated follicular mucinosis (4 of 7 cases). Since cases of advanced FMF often contain variable numbers of CD30+ blast cells, differentiation between FMF and C-ALCL may sometimes be difficult, in particular in FMF patients presenting with a solitary lesion and/or cases with a diffuse proliferation of CD30+ blast cells, as observed in case 6 of the present series. In this patient the diagnosis transformed FMF was based on the presence of marked folliculotropism and syringotropism as well as the presence of many small to medium-sized pleomorphic T-cells with cerebriform nuclei, both in the follicular epithelium and in between the blast cells in the perifollicular infiltrates.[16] The combination of folliculotropism and syringotropism has been described in previous reports on FMF, and is also referred to as adnexotropic MF.[17,18] In cases with diffuse dermal infiltrates with partial or even complete destruction of the epithelial structures differentiation between FMF and other types of CTCL may be extremely difficult or even impossible. In our previous study on 203 patients with FMF [19] a few patients with a diffuse proliferation of CD30+ blast cells and no discernible epithelial structures had initially been misclassified as C-ALCL.

The correct diagnosis could only be made after development of new skin lesions with the characteristic clinicopathologic features of FMF. In such cases staining with monoclonal antibodies against keratin may be useful to visualize infiltration of residual hair follicles and sweat glands by neoplastic T-cells and facilitate a definite diagnosis of FMF. In patients presenting with a solitary lesion histologically showing an atypical T-cell infiltrate, which disappears spontaneously after biopsy, as observed in two of our patients (cases 5 and 8), a diagnosis of cutaneous T-cell pseudolymphoma should be considered. However, in both cases the presence of a diffuse infiltrate of small to medium-sized pleomorphic T-cells, prominent folliculotropism and an aberrant CD4+ T-cell phenotype supported a diagnosis of FMF and argued against a diagnosis of T-cell pseudolymphoma.

In conclusion, the long-term follow-up data of our patients presenting with solitary FMF indicate that these patients do not always have an indolent clinical course, but may develop progressive cutaneous and extracutaneous disease, and therefore require long-term follow-up.

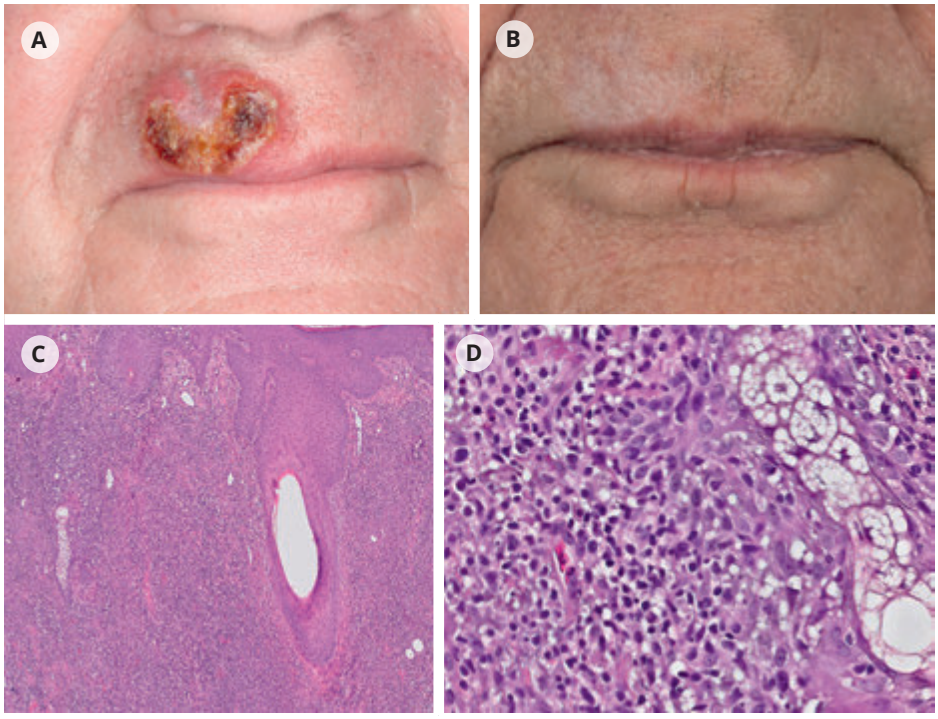
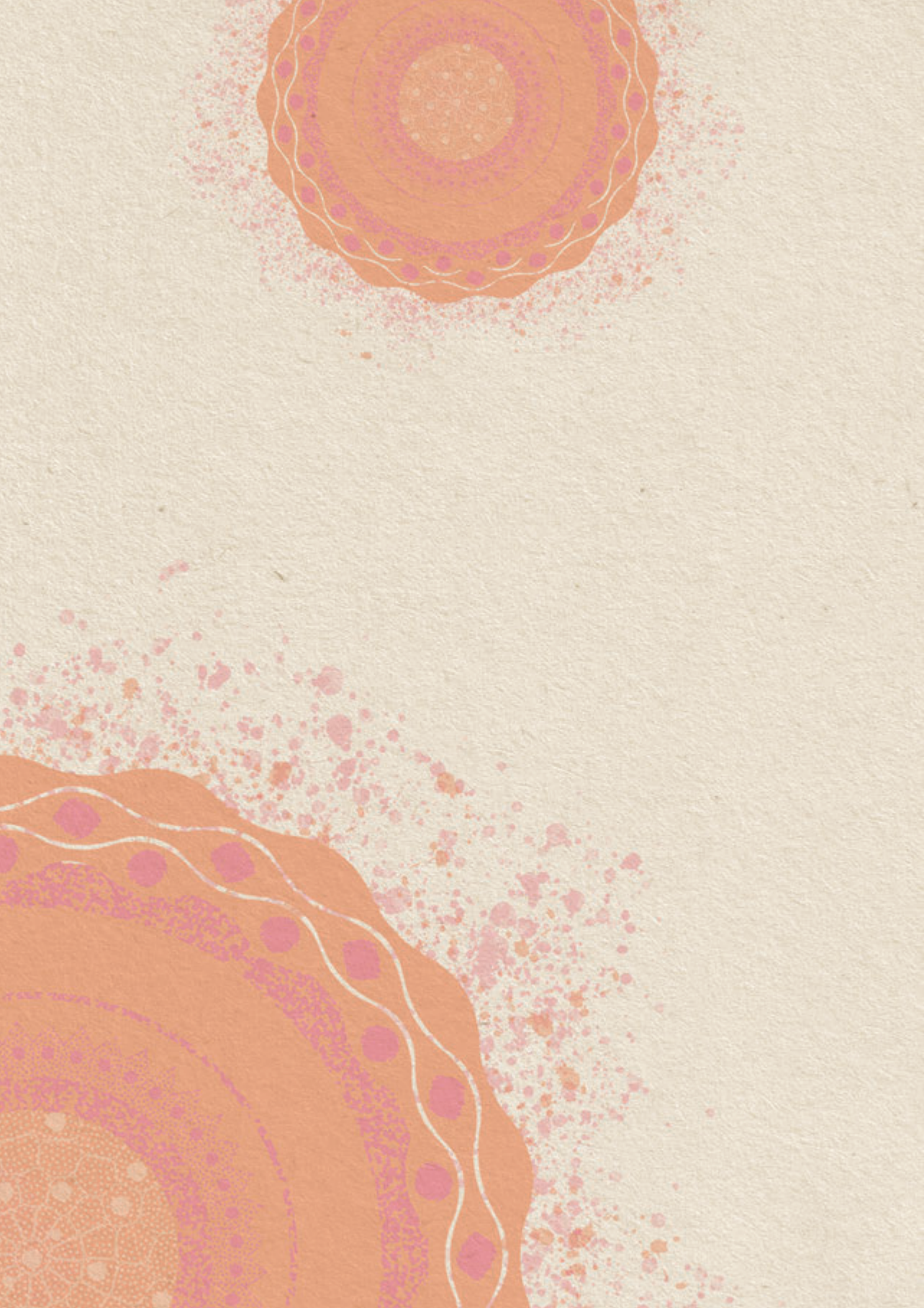


Figure 3. Case 8: Crusted tumor on the upper lip (A) that resolved spontaneously after skin biopsy (B). Histology shows a diffuse dermal infiltrate (C) mainly composed of small/medium sized pleomorphic cells with marked folliculotropism (D).

REFERENCES

1. Willemze R, Jaffe ES, Burg G, et al. WHO-EORTC classification for cutaneous lymphomas. *Blood.* 2005;105(10):3768-3785.
2. van Doorn R, Scheffer E, Willemze R. Follicular mycosis fungoides, a distinct disease entity with or without associated follicular mucinosis: a clinicopathologic and follow-up study of 51 patients. *Archives of dermatology.* 2002;138(2):191-198.
3. Gerami P, Rosen S, Kuzel T, Boone SL, Guitart J. Folliculotropic mycosis fungoides: an aggressive variant of cutaneous T-cell lymphoma. *Archives of dermatology.* 2008;144(6):738-746.
4. Lehman JS, Cook-Norris RH, Weed BR, et al. Folliculotropic mycosis fungoides: single-center study and systematic review. *Archives of dermatology.* 2010;146(6):607-613.
5. Marzano AV, Berti E, Lupica L, Alessi E. Unilesional follicular mycosis fungoides. *Dermatology.* 1999;199(2):174-176.
6. Hsiao PF, Hsiao CH, Tsai TF, Jee SH. Unilesional folliculotropic/syringotropic cutaneous T-cell lymphoma presenting as an indurated plaque on the nape. *International journal of dermatology.* 2006;45(10):1268-1270.
7. Setyadi HG, Nash JW, Duvic M. The solitary lymphomatous papule, nodule, or tumor. *Journal of the American Academy of Dermatology.* 2007;57(6):1072-1083.
8. Ally MS, Pawade J, Tanaka M, et al. Solitary mycosis fungoides: a distinct clinicopathologic entity with a good prognosis: a series of 15 cases and literature review. *Journal of the American Academy of Dermatology.* 2012;67(4):736-744.
9. Kempf W, Kazakov DV, Schermesser M, et al. Unilesional follicular mycosis fungoides: report of two cases with progression to tumor stage and review of the literature. *Journal of cutaneous pathology.* 2012;39(9):853-860.
10. Pileri A, Agostinelli C, Grandi V, et al. Unilesional folliculotropic mycosis fungoides: a true rarity in the clinico-pathologic spectrum? *Giornale italiano di dermatologia e venereologia : organo ufficiale, Societa italiana di dermatologia e sifilografia.* 2014;149(6):639-642.
11. Amitay-Laish I, Feinmesser M, Ben-Amitai D, Fenig E, Sorin D, Hodak E. Unilesional folliculotropic mycosis fungoides: a unique variant of cutaneous lymphoma. *Journal of the European Academy of Dermatology and Venereology : JEADV.* 2016;30(1):25-29.
12. Cerroni L, Fink-Puches R, Back B, Kerl H. Follicular mucinosis: a critical reappraisal of clinicopathologic features and association with mycosis fungoides and Sezary syndrome. *Archives of dermatology.* 2002;138(2):182-189.
13. Muniesa C, Estrach T, Pujol RM, et al. Folliculotropic mycosis fungoides: clinicopathological features and outcome in a series of 20 cases. *Journal of the American Academy of Dermatology.* 2010;62(3):418-426.
14. Baykal C, Atci T, Sari SO, Ekinci AP, Buyukbabani N. Underrecognized clinical features of folliculotropic mycosis fungoides: a large clinical series. *Journal der Deutschen Dermatologischen Gesellschaft = Journal of the German Society of Dermatology : JDDG.* 2017.
15. Sentis HJ, Willemze R, Scheffer E. Alopecia mucinosa progressing into mycosis fungoides. A long-term follow-up study of two patients. *The American Journal of dermatopathology.* 1988;10(6):478-486.
16. Benner MF, Jansen PM, Vermeer MH, Willemze R. Prognostic factors in transformed mycosis fungoides: a retrospective analysis of 100 cases. *Blood.* 2012;119(7):1643-1649.
17. Garcovich A, Garcovich S, Massi G. An unusual variant of granulomatous adnexotropic cutaneous T-cell lymphoma. *The British journal of dermatology.* 2003;148(2):363-365.
18. Dubin DB, Hurowitz JC, Brettler D, et al. Adnexotropic T-cell lymphoma presenting with generalized anhidrosis, progressive alopecia, pruritus, and Sjogren's syndrome. *Journal of the American Academy of Dermatology.* 1998;38(3):493-497.
19. van Santen S, Roach RE, van Doorn R, et al. Clinical Staging and Prognostic Factors in Folliculotropic Mycosis Fungoides. *JAMA dermatology.* 2016.



7

SERUM AND CUTANEOUS TRANSCRIPTIONAL EXPRESSION LEVELS OF IL31 ARE MINIMAL IN CUTANEOUS T-CELL LYMPHOMA VARIANTS

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ABSTRACT

Aim: Recent studies suggested a role for IL31 in the pathogenesis of pruritus and disease severity in patients with cutaneous T-cell lymphomas (CTCL). However, discrepant results were reported for IL31 serum levels, transcriptional expression levels or immunohistochemistry studies and its relation to pruritus intensity and/or disease severity in CTCL. Most studies did not distinguish between different CTCL variants. We investigated IL31 serum levels in different subtypes of CTCL, including Mycosis Fungoides (MF) (typically not pruritic), Folliculotropic Mycosis Fungoides (FMF) and Sézary syndrome (SS) (both often pruritic).

Methods: From 54 CTCL patients (17 SS, 21 FMF and 16 classic MF) serum samples were analyzed with a high sensitivity V-PLEX immunoassay for IL31. The study group included 35/54 (65%) patients with complaints of pruritus. Thirty-five patients had advanced stage disease (≥ stage IIB). A visual analog scale score (VAS score) for pruritus was available in 29 CTCL patients (7 SS, 9 FMF and 13 classic MF) and in other cases complaints of pruritus were retrieved medical records. qPCR analyses for *IL31* expression were performed in lesional skin biopsies from 8 CTCL patients. Serum samples from 4 healthy individuals without pruritus and from 5 atopic dermatitis (AD) patients with severe pruritus were included as controls.

Results: In 11/54 (20%) of CTCL patients low serum levels of IL31 were detected (mean 0.48pg/mL, range 0.20-1.39pg/mL) including 6/17 (35%) SS patients (mean 0.57pg/mL) and 5/21 (24%) FMF patients (mean 0.33 pg/mL). All 11 patients with detectable levels of IL31 reported complaints of moderate to severe pruritus and 9/11 patients presented with advanced stage disease (≥IIB). qPCR analyses resulted in lowly expressed *IL31* expression levels in 4 of 8 patients; these patients all suffered from pruritus and advanced stage disease.

Conclusions: Translational and transcriptional expression levels of IL31 were very low or undetectable in CTCL patients. Detectable low IL31 serum levels were exclusively observed in SS and FMF patients and not in patients with classic MF. However, these marginal IL31 levels in a small proportion of CTCL patients do not support an essential role for IL31 in CTCL patients.

INTRODUCTION

Cutaneous T-cell lymphomas (CTCL) are a heterogeneous group of malignant non-Hodgkin lymphomas of skin-homing T-lymphocytes, of which Mycosis fungoides (MF) and Sézary syndrome (SS) are the most commonly investigated variants.[1] Pruritus is a common sign in CTCL patients which can be a therapeutically challenging to manage.[2] The underlying mechanisms causing pruritus in these patients are currently poorly understood. Recent studies found pruritic skin inflammation in transgenic mice overexpressing IL31 and demonstrated significantly elevated serum and mRNA IL31 levels in atopic dermatitis (AD) and prurigo nodularis suggesting a role for IL31 in the pathogenesis of pruritic skin diseases.[3-7] Although most studies predominantly focused on patients with atopic dermatitis (AD), a few studies investigated IL31 in CTCL patients as well.[8-12] IL31 is a cytokine that is predominantly produced by activated T-helper 2 (Th2) cells and CLA+ CD45RO+ effector memory T-cells and signals through a heterodimeric receptor composed of IL31 receptor A (IL31RA) and oncostatin M receptor (OSMR).[3] Recent findings with IL31RA antigen therapy showed a reduction of pruritus in AD patients,[13] indicating that IL31 might be of similarly therapeutic interest in CTCL patients. However, previous studies focusing on IL31 in CTCL patients have reported variable results regarding the correlation between IL31 levels and pruritus intensity,[9,10,12] disease severity [8,11] or both parameters [8]. However, these studies typically did not distinguish between different CTCL variants. Because Sézary syndrome (SS) and the folliculotropic variant of Mycosis Fungoides (FMF) are CTCL variants that are characterized by (intense) pruritus while classic Mycosis Fungoides (MF) is typically not, we hypothesized that differences in pruritic and non-pruritic variants of CTCL might explain discrepancies reported in literature and that pruritic CTCL patients may be associated with higher levels of IL31. The aim of the current study was to investigate translational IL31 levels in serum and cutaneous *IL31* transcriptional expression in pruritic and non-pruritic CTCL variants and to study the relation of IL31 with regard to pruritus and clinical disease stage.

MATERIAL AND METHODS

Patients, samples and controls

From 54 CTCL patients (16 classic MF, 21 FMF and 17 SS) a total of 68 serum samples (16 classic MF, 25 FMF, 27 SS) were included in the study (**Table 1 and 2**). In all cases the diagnosis had been established by an expert panel of dermatologists and pathologists, highly experienced in CTCL and in accordance with the WHO-EORTC classification.[1] Staging at time of sample collection was performed according to the Tumor-Node-Metastasis (TNM) classification for MF and SS[14] and resulted in 19 early-stage (stage I-IIA) and 35 advanced-stage (stage IIB-IV) CTCL patients. From ten patients, two or three consecutive serum samples had been included in this study. These samples had been obtained at different time points during a patients' disease course while patients remained to have either early- or advanced-stage disease. Information whether a patient did or did not suffer from pruritus at time of sampling had been retrieved from medical records or from a visual analog scale score (VAS score) for pruritus which had been available in 29 CTCL patients (13 classic MF, 9 FMF and 7 SS). Patients with an available VAS score were considered to suffer from pruritus when VAS was ≥3.3 (VAS ≥3.3 -6.6 moderate pruritus; VAS ≥6.6 severe pruritus). Patients with VAS scores <3.3 were considered to have minimal pruritus and were not counted as patients suffering from pruritus. Serum samples had been stored at –80°C until use. Thirty-one of 68 serum samples had been collected over a 5 month-period prior to IL31 analysis and the remaining 37 derived from our biobank and had been stored for a median duration of 6 years (range 1-16 years) until IL31 analysis.

Serum of 4 healthy individuals and 5 AD patients with severe pruritus (VAS scores ranging from 6.7-8.7) were included as controls for IL31 determination. The study was performed in accordance with our in-house biobank protocol (B20.046), approved by the institutional review board committee of Leiden University Medical Center, the Netherlands and in accordance with the principles of the Declaration of Helsinki. Patients' written informed consent was obtained prior to collection of blood serum.

IL31 immunoassay

IL31 concentrations in serum samples from different CTCL patient groups and controls were examined using a sensitive V-PLEX multi-spot assay (Th17 panel 1 (human), K15085D, MSD Maryland, USA). Verification of our results was done with the IL31,

SECTOR K151XAD-1 (MSD) (IL31 single analyte assay). As a quality control, a standard curve with 8 dilutional steps was run on the same plate along with the investigated CTCL serum samples for both the multi-spot V-PLEX assay as for the verification single analyte assay (**Figure 1**).

qPCR analysis

Quantitative PCR (qPCR) to determine IL31 mRNA expression was performed in 8 CTCL patients of which frozen skin biopsies (n=9, 2 biopsies originated from the same patient) were obtained at same date of serum sample collection. RNA was isolated using the RNeasy Mini Kit (Qiagen, Hilden, Germany), which included on-column DNase digestion. cDNA synthesis was performed using the iScript cDNA Synthesis Kit (Bio-Rad, Veenendaal, The Netherlands) and qPCR was performed with SYBR Green Super mix (Bio-Rad). *IL31* expression was examined using qPCR on a CFX384 PCR detection system (Bio-Rad) by measuring relative gene expression against stably expressed reference genes *ERCC3* and *TMEM87A*[15] using intron-spanning primers (**Supplementary Table 1**) according to the delta-delta Cq method.[16]

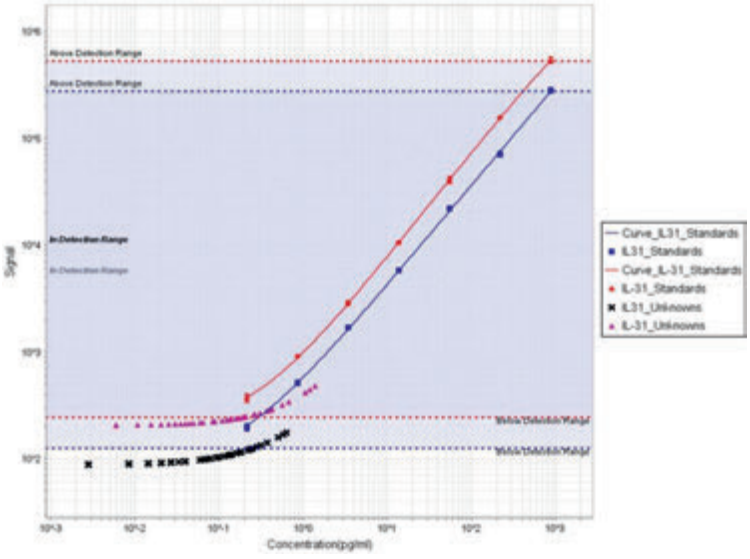

Figure 1 Standard curves for IL31 V-PLEX multispot analysis (Th17 panel) and single analyte analysis. Standard curves for both the original experiment and the verification experiment demonstrate similar observations and reveal that a minority of CTCL serum samples exceed the IL31 detection threshold.

RESULTS

Serum levels

Results are displayed in **Table 1** (summarized) and **Table 2**. In 11/54 (20%) CTCL patients serum levels of IL31 could be detected (mean 0.48pg/mL, range 0.20-1.39pg/mL). These included 6/17 (35%) SS patients (mean 0.57pg/mL, range 0.20-1.39pg/mL) and 5/21 (24%) FMF patients (mean 0.33 pg/mL, range 0.20-0.43pg/mL); IL31 could not be detected in classic MF. Nine of 11 patients with detectable levels of IL31 presented with advanced stage disease (≥IIB) and reported complaints of (moderate to severe) pruritus. Still, in 69% of patients that had reported (moderate to severe) pruritus IL31 could not be detected. Information on pruritus in our 11 detectable cases had been available from medical records in 10 cases and in only 1 case a VAS score was available (score 5.9). As a consequence, correlation analysis between VAS scores and IL31 concentrations could not be performed. In three patients with consecutive serum samples at various time points, IL31 could be detected repeatedly in low amounts (in 2 of 3 included samples). In the other patients with consecutive serum samples, IL31 could not be detected. Serum levels of IL31 could be detected in two out of five patients with AD (1.06 and 1.21 pg/mL with VAS scores of 6.8 and 6.7 respectively) and were not detectable in four healthy controls. Since IL31 is one of the eight measured cytokines in the utilized Th17 panel (including IL17A, IL21, IL22, IL23, IL27, IL31, and MIP3α) we have been able to compare cytokine levels relative to IL31. We observed that among the total set of measurable cytokines, IL31 was ubiquitously lowly expressed or undetectable. Positive as well as negative results from both patient and control samples were confirmed using a high-sensitivity single analyte IL31 assay.

Table 1. Patient characteristics and IL31 levels[a]

Diagnosis	No. Patients	Stage I-IIA	Stage IIB-IVB	Pruritus	No pruritus	Available VAS score	No. patients with detectable IL31 (mean, pg/mL)
SS	17	-	17	16	1	7	6 (0.57)
FMF	21	9	12 [b]	16	5	9	5 (0.33)
MF	16	10	6 [c]	3	13	13	0 (-)
Total	**54**	**19**	**35**	**35**	**19**	**29**	**11 (0.48)**

SS Sézary syndrome, FMF folliculotropic mycosis fungoides, MF classic mycosis fungoides, No. number, I-IIA early stage of disease according to the International Society of Cutaneous Lymphomas (ISCL),[14] IIB-IVB advanced stage of disease according to ISCL, VAS visual analogue scale (for pruritus)

*[a] See detailed information on this study cohort in **Table 2**.*

[b] 10 out of 12 patients with advanced stage FMF reported moderate to severe pruritus.

[c] 1 out of 6 patients with advanced stage MF reported moderate to severe pruritus.

Table 2. Detailed characteristics of included CTCL patients and controls, IL31 serum detectability and relation with pruritus

No.	CTCL subtype	E / A	TNMB stage	Pruritus?	Pruritus degree	Pruritus information	IL31 Serum concentration (pg/mL)	IL31 Serum concentration (pg/mL) in consecutive samples *(time between sampling in months)*	qPCR data?
1	SS	A	IV	+	Severe	MR	1.39	0.31(13), ND(55)[a]	n/p
2	SS	A	IV	+	Severe	MR	1.07	-	LOW
3	SS	A	IV	+	Severe	MR	0.68	0.37(31), ND(43)[a]	ND
4	SS	A	IV	+	Severe	MR	0.57	-	n/p
5	FMF	E	IB	+	Moderate	MR	0.43	-	n/p
6	FMF	A	III	+	Severe	MR	0.41	ND(133)	LOW
7	FMF	A	III	+	Moderate	MR	0.40	-	n/p
8	SS	A	IV	+	Severe	MR	0.27	-	n/p
9	FMF	E	IA	+	Moderate	VAS (5.9)	0.21	-	n/p
10	FMF	A	IIB	+	Moderate	MR	0.20	-	LOW
11	SS	A	IV	+	Severe	MR	0.20	0.26(55), ND(25)[a]	ND
12	MF	E	IB	+	Severe	VAS (9.2)	ND	-	n/p
13	SS	A	IV	+	Severe	VAS (7.4)	ND	-	n/p
14	FMF	E	IB	+	Severe	VAS (7.0)	ND	ND(1)	n/p
15	SS	A	IV	+	Severe	VAS (6.8)	ND		n/p
16	SS	A	IV	+	Moderate	VAS (5.4)	ND	-	n/p
17	MF	A	IIB	+	Moderate	VAS (4.9)	ND	-	n/p
18	MF	E	IA	+	Moderate	VAS (4.9)	ND	-	n/p
19	FMF	E	IB	+	Moderate	VAS (4.5)	ND	-	n/p
20	SS	A	IV	+	Moderate	VAS (4.0)	ND	ND(15)	n/p
21	FMF	A	IIB	+	Moderate	VAS (3.8)	ND	-	n/p
22	FMF	A	IIB	+	Moderate	VAS (3.3)	ND	ND(3), ND(55)	n/p
23	MF	E	IA	-	Low	VAS (2.0)	ND	-	n/p
24	MF	E	IB	-	Low	VAS (1.7)	ND	-	n/p
25	SS	A	IV	-	Low	VAS (1.5)	ND	-	n/p
26	MF	E	IA	-	Low	VAS (0.6)	ND	-	n/p
27	FMF	E	IA	-	Low	VAS (0.5)	ND	-	n/p
28	FMF	E	IA	-	Low	VAS (0.3)	ND	-	n/p
29	MF	E	IA	-	Low	VAS (0.1)	ND	-	n/p
30	MF	E	IB	-	Low	VAS (0.1)	ND	-	n/p
31	FMF	A	IIB	-	No	VAS (0.0)	ND	-	n/p
32	FMF	E	IA	-	No	VAS (0.0)	ND	-	n/p
33	MF	E	IB	-	No	VAS (0.0)	ND	-	n/p

Table 2. *(continued)*

No	CTCL subtype	E / A	TNMB stage	Pruritus?	Pruritus degree	Pruritus information	IL31 Serum concentration (pg/mL)	IL31 Serum concentration (pg/mL) in consecutive samples *(time between sampling in months)*	qPCR data?
34	MF	E	IA	-	No	VAS (0.0)	ND	-	n/p
35	MF	E	IB	-	No	VAS (0.0)	ND	-	n/p
36	MF	A	IIB	-	No	VAS (0.0)	ND	-	n/p
37	MF	A	IIB	-	No	VAS (0.0)	ND	-	n/p
38	SS	A	IV	+	Severe	MR	ND	-	n/p
39	SS	A	IV	+	Severe	MR	ND	-	n/p
40	FMF	A	IIB	+	Severe	MR	ND	-	ND
41	FMF	A	IV	+	Severe	MR	ND	-	n/p
42	SS	A	IV	+	Moderate	MR	ND	ND(14)[b]	n/p
43	SS	A	IV	+	Moderate	MR	ND	ND(20)[c]	n/p
44	SS	A	IV	+	Moderate	MR	ND	ND(33)[d]	n/p
45	SS	A	IV	+	Moderate	MR	ND	-	n/p
46	FMF	E	IA	+	Moderate	MR	ND	-	n/p
47	FMF	E	IIA	+	Moderate	MR	ND	-	n/p
48	FMF	A	IIB	+	Moderate	MR	ND	-	LOW
49	FMF	A	IV	+	Moderate	MR	ND	-	n/p
50	FMF	A	IV	+	Moderate	MR	ND	-	n/p
51	FMF	A	IIB	-	No	MR	ND	-	n/p
52	MF	A	IV	-	No	MR	ND	-	n/p
53	MF	A	IIB	-	No (Painful skin)	MR	ND	-	LOW (2x)
54	MF	A	IIB	-	No	MR	ND	-	n/p
C	AD	N.A.	-	+	Severe	VAS (6.8)	1.06	-	-
C	AD	N.A.	-	+	Severe	VAS (6.7)	1.21	-	-
C	AD	N.A.	-	+	Severe	VAS (8.0)	ND	-	-
C	AD	N.A.	-	+	Severe	VAS (8.6)	ND	-	-
C	AD	N.A.	-	+	Severe	VAS (8.7)	ND	-	-
C	Healthy	N.A.	-	-	-	-	ND	-	-
C	Healthy	N.A.	-	-	-	-	ND	-	-
C	Healthy	N.A.	-	-	-	-	ND	-	-
C	Healthy	N.A.	-	-	-	-	ND	-	-

Table 2. *(continued)*
A: advanced-stage disease (according to TNMB staging system stages IIB-IV are considered advanced-stage disease); AD: atopic dermatitis; CTCL: cutaneous T-cell lymphoma; C: control sample; E: early-stage disease (stages IA-IIA are considered early-stage disease); FMF: folliculotropic mycosis fungoides; mo: months; LOW: lowly expressed (Cq>31); MF: mycosis fungoides, MR: (retrieved from) medical records; N.A. not applicable; ND: not detected; No.: patient number; n/p: not performed; qPCR quantitative polymerase chain reaction; SS: Sézary syndrome; TNMB: tumor node metastasis blood staging system; VAS: visual analogue scale (VAS <3,3 was considered low; VAS 3,3-6,6 was considered moderate, VAS >6,6 was considered severe).
[a] In these patients IL31 could be detected in one of two follow-up samples, while these patients continued to suffer from a similar degree of pruritus.
[b] At follow up sampling, this SS patient had no complaints of pruritus (VAS score of 0.0).
[c] At follow up sampling, this SS patient suffered from minimal complaints of pruritus (VAS score of 2.0).
[d] At follow up sampling, this SS patient had no complaints of pruritus (retrieved from medical record).

qPCR

In 4 of 8 patients *IL31* could be detected in very low, not in quantitatively measurable levels (Cq>31) (**Supplementary Figure 1**). These four patients suffered from pruritus (one case described a painful skin) and advanced stage disease and in two patients very low IL31 serum levels were detected.

DISCUSSION

Pruritus is a common distressing symptom in CTCL patients that may be challenging to manage therapeutically.[17] Over the past years, IL31 has been reported to play a central role in the pathogenesis of pruritus[3,4] and an IL31 receptor antagonist is being developed as a novel therapeutic strategy for the reduction of pruritus in AD patients.[13]

The aim of the current study was to investigate the role of IL31 in CTCL patients. This study was the first investigating the role of IL31 among the following three (major) CTCL subtypes: classic MF, FMF and SS. It is known that pruritus in CTCL is more common in FMF and SS compared to classic MF and is more prevalent among patients with advanced stage disease.[2,18] Therefore, at the start of our study, we hypothesized that serum samples from the more pruritic variants of CTCL (FMF and SS) and from more advanced stages of disease express differentially higher IL31 serum levels. The results of our study revealed very low IL31 serum levels in 11 CTCL patients but undetectable levels in the remaining 43 CTCL patients. As a matter of fact, IL31 was exclusively found in serum from FMF and SS patients, was not detectable in patients with classic MF and concerned advanced stages of disease in 82% of cases. It can therefore not be excluded

that marginal concentrations might play a role in at least a minority of CTCL patients suffering from pruritus or advanced stage disease. However, in 69% of all patients that reported moderate to severe pruritus in our cohort, IL31 could not be detected, questioning an important role for IL31 related pruritus in our CTCL patients.

In literature, studies focusing on IL31 in serum of CTCL patients have shown diverse findings. Our results are in line with the study of Möbs et al, that reported equally low IL31 serum values and only few samples exceeded the threshold for unequivocal quantification.[12] Several other studies reported increased IL31 serum concentrations in CTCL[8,9,11] , but in all these studies the concentration of IL31 was low when compared to values reported in AD patients.[5,6,19] Likewise, in the study of Singer et al. not all CTCL patients demonstrated detectable IL31 serum values while only leukemic stages of disease were investigated.[9] Being aware of the previously published low IL31 concentrations in CTCL, for the current study we selected a highly sensitive immunoassay (V-PLEX multi-spot and single analyte) characterized by a large dynamic range. In addition, the serum diluents used in these immunoassays have been optimized to eliminate possible interferences from heterophilic antibodies, a known source of non-specific signals in ELISA based assays.[20]

With respect to disease severity, our results found almost all detectable IL31 serum levels and transcriptional levels in advanced stages of disease, which is in line with the study of Ohmatsu et al. that found a correlation between IL31 concentrations and CTCL disease severity.[8] However, it should be noted that it is difficult to determine whether our observation is biased by the relative small proportion of positive samples; as other cohorts with few positive samples found no relation with disease stage.[12]

As we found that in the large majority of CTCL patients with moderate to severe pruritus IL31 could not be detected in serum or skin, this might suggest that other mediators are more important in the pathogenesis of pruritus in CTCL, as has been proposed before.[11]. Although some studies found a relation between the amount of IL31 and pruritus severity in immunohistochemistry studies[10], other studies reported that cutaneous IL31 in human subjects may not exert a direct pruritic effect in the skin, thus questioning a direct relation between cutaneous IL31 and pruritic symptoms.[21] In line with our findings in serum, we found that transcriptional expression for *IL31* in skin was not

quantifiable further suggesting that alternative mechanisms are more important in the pathogenesis of pruritus in CTCL.

CONCLUSIONS

In conclusion, using a highly sensitive V-PLEX IL31 immunoassay on well characterized patient groups, we demonstrated very low IL31 serum levels in a minority of patients with pruritic CTCL variants (FMF and SS) and no detectable levels in classic MF patients. Expression of IL31 mRNA in pruritic CTCL skin was similarly low and could not be detected in quantitative measurable levels, by means of qPCR. We cannot exclude that marginal concentrations might play a role in at least a minority of CTCL patients suffering from pruritus or advanced stage disease. However, our observations do not support a prominent role for IL31 in the pathogenesis of pruritus and disease activity in CTCL patients and further studies elucidating crucial mechanisms in CTCL pruritus are highly warranted.

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REFERENCES

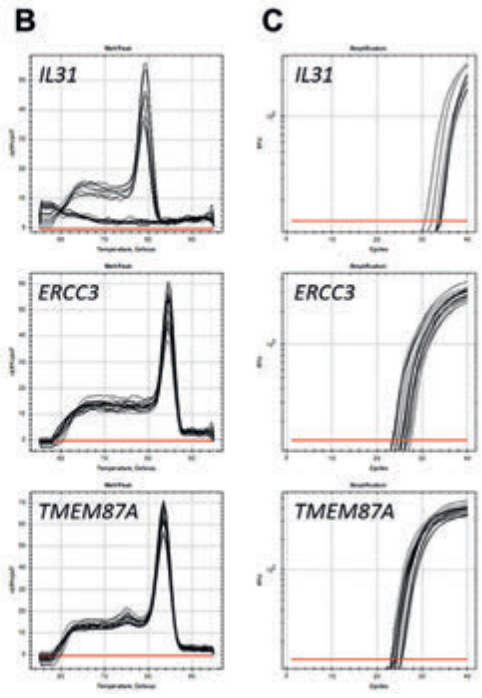
1. Willemze R, Cerroni L, Kempf W, et al. The 2018 update of the WHO-EORTC classification for primary cutaneous lymphomas. *Blood.* 2019;133(16):1703-1714.
2. Vij A, Duvic M. Prevalence and severity of pruritus in cutaneous T cell lymphoma. *Int J Dermatol.* 2012;51(8):930-934.
3. Dillon SR, Sprecher C, Hammond A, et al. Interleukin 31, a cytokine produced by activated T cells, induces dermatitis in mice. *Nat Immunol.* 2004;5(7):752-760.
4. Sonkoly E, Muller A, Lauerma AI, et al. IL-31: a new link between T cells and pruritus in atopic skin inflammation. *J Allergy Clin Immunol.* 2006;117(2):411-417.
5. Szegedi K, Kremer AE, Kezic S, et al. Increased frequencies of IL-31-producing T cells are found in chronic atopic dermatitis skin. *Exp Dermatol.* 2012;21(6):431-436.
6. Raap U, Wichmann K, Bruder M, et al. Correlation of IL-31 serum levels with severity of atopic dermatitis. *J Allergy Clin Immunol.* 2008;122(2):421-423.
7. Saleem MD, Oussedik E, D'Amber V, Feldman SR. Interleukin-31 pathway and its role in atopic dermatitis: a systematic review. *J Dermatolog Treat.* 2017:1-9.
8. Ohmatsu H, Sugaya M, Suga H, et al. Serum IL-31 levels are increased in patients with cutaneous T-cell lymphoma. *Acta Derm Venereol.* 2012;92(3):282-283.
9. Singer EM, Shin DB, Nattkemper LA, et al. IL-31 is produced by the malignant T-cell population in cutaneous T-Cell lymphoma and correlates with CTCL pruritus. *J Invest Dermatol.* 2013;133(12):2783-2785.
10. Nattkemper LA, Martinez-Escala ME, Gelman AB, et al. Cutaneous T-cell Lymphoma and Pruritus: The Expression of IL-31 and its Receptors in the Skin. *Acta Derm Venereol.* 2016;96(7):894-898.
11. Malek M, Glen J, Rebala K, et al. Il-31 does not correlate to pruritus related to early stage cutaneous T-cell lymphomas but is involved in pathogenesis of the disease. *Acta Derm Venereol.* 2015;95(3):283-288.
12. Mobs M, Gryzik S, Haidar A, Humme D, Beyer M, Vandersee S. Analysis of the IL-31 pathway in Mycosis fungoides and Sezary syndrome. *Arch Dermatol Res.* 2015;307(6):479-485.
13. Ruzicka T, Hanifin JM, Furue M, et al. Anti-Interleukin-31 Receptor A Antibody for Atopic Dermatitis. *N Engl J Med.* 2017;376(9):826-835.
14. Olsen E, Vonderheid E, Pimpinelli N, et al. Revisions to the staging and classification of mycosis fungoides and Sezary 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(6):1713-1722.
15. van Kester MS, Borg MK, Zoutman WH, et al. A meta-analysis of gene expression data identifies a molecular signature characteristic for tumor-stage mycosis fungoides. *J Invest Dermatol.* 2012;132(8):2050-2059.
16. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods.* 2001;25(4):402-408.
17. Lansigan F. Navigating the treatment choices for mycosis fungoides. *Oncology (Williston Park).* 2010;24(6):508, 516, 518.
18. van Doorn R, Scheffer E, Willemze R. Follicular mycosis fungoides, a distinct disease entity with or without associated follicular mucinosis: a clinicopathologic and follow-up study of 51 patients. *Arch Dermatol.* 2002;138(2):191-198.

19. Kim S, Kim HJ, Yang HS, Kim E, Huh IS, Yang JM. IL-31 Serum Protein and Tissue mRNA Levels in Patients with Atopic Dermatitis. *Ann Dermatol.* 2011;23(4):468-473.
20. Martins TB, Pasi BM, Litwin CM, Hill HR. Heterophile antibody interference in a multiplexed fluorescent microsphere immunoassay for quantitation of cytokines in human serum. *Clin Diagn Lab Immunol.* 2004;11(2):325-329.
21. Hawro T, Saluja R, Weller K, Altrichter S, Metz M, Maurer M. Interleukin-31 does not induce immediate itch in atopic dermatitis patients and healthy controls after skin challenge. *Allergy.* 2014;69(1):113-117.

SUPPLEMENTARY MATERIAL

A

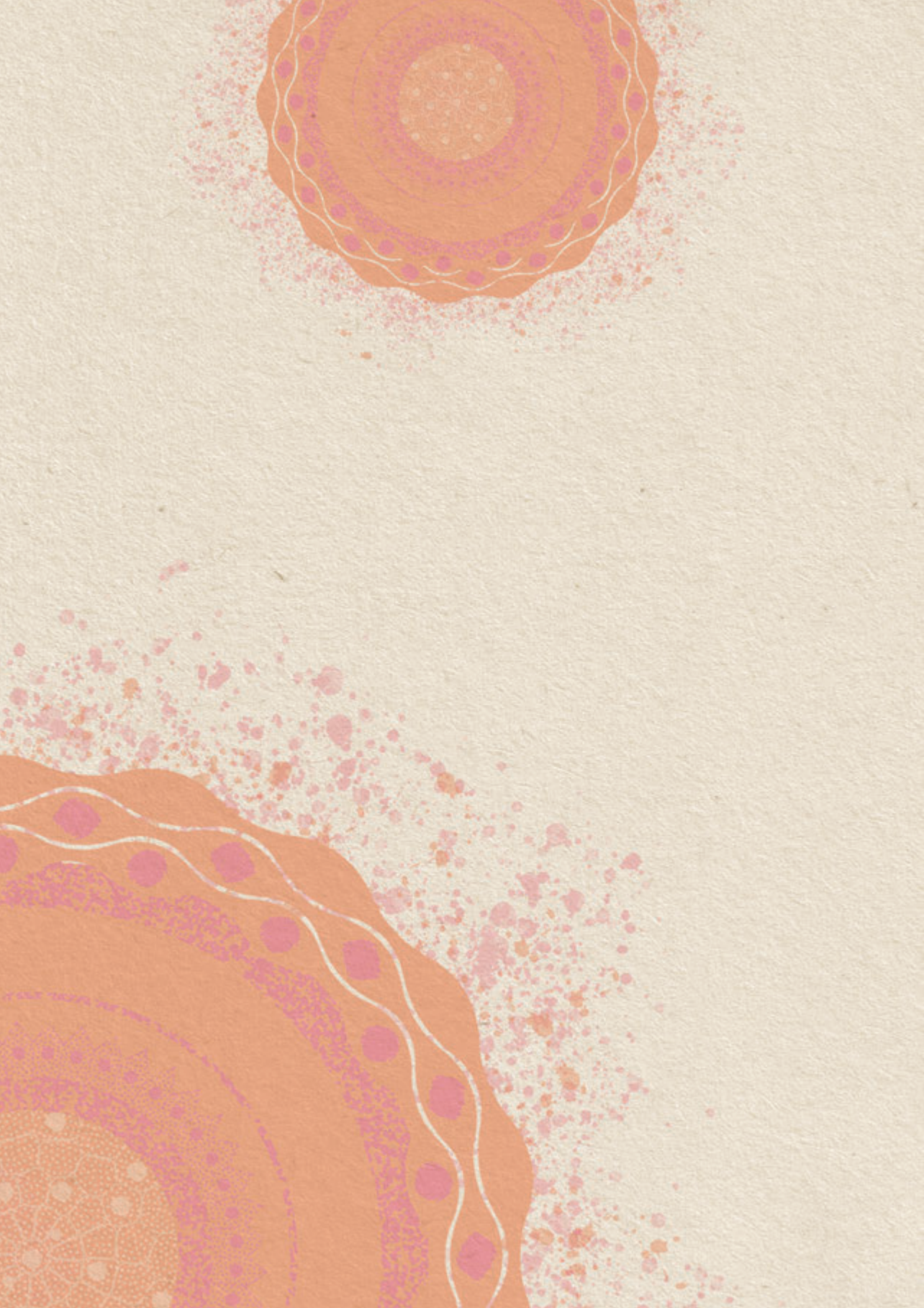
Origin	*IL31* (Mean Cq)	*ERCC3* (Mean Cq)	*TMEM87A* (Mean Cq)
FMF	31.4	24.5	23.8
SS	*not detectable*	25.5	24.1
SS	*not detectable*	25.5	24.1
MF	33.8	23.7	23.2
MF	34.1	25.5	24.0
SS	33.6	27.3	25.5
FMF	*not detectable*	26.2	25.0
FMF	34.0	23.6	23.2
FMF	*not detectable*	26.7	25.3



Supplementary Figure 1 *IL31* qPCR results from 9 CTCL skin biopsies. (A) Mean cycle quantification (Cq) values for *IL31* and reference genes *ERCC3* and *TMEM87A* as performed in duplicate. (B) Post-PCR assessment of amplification specificity by melting curve analysis. (C) Log-transformed amplification plots. *NB: results from patient with MF belong to the same patient (see also patient 53 from Table 2).*
FMF: folliculotropic mycosis fungoides, SS: Sézary syndrome, MF: classic mycosis fungoides

Supplementary Table 1. Primer sequences for qPCR

Gene	Forward primer (5´-3´)	Reverse primer (5´-3´)
IL31	CCCTCTCGAAGATGCTTTTG	CGTGTAATTCTGGGACACGA
ERCC3	ATATCCAAGGTAGGTGACACTTCG	TTGTACTCTTCTGCAACCATCCC
TMEM87A	CATCTGGACAACCATGAAGTTCAG	AGGATCATGGAGAACAGCAAGC



8

SUMMARY AND DISCUSSION

The studies presented in this thesis were focused on folliculotropic mycosis fungoides (FMF), a primary cutaneous T-cell lymphoma which in recent lymphoma classifications is recognized as a distinct variant of mycosis fungoides (MF).[1] It is characterized by the presence of perifollicular infiltrates with variable infiltration of neoplastic T-cells into the hair follicles (folliculotropism), the preferential localization of skin lesions in the head- and neck area and a worse prognosis as compared to classic MF.[2,3] Studies in this thesis aimed to address questions regarding clinical staging, prognostic parameters and treatment in FMF. The main issues addressed concerned:

1 The development of criteria to identify subgroups of FMF patients with an indolent and more aggressive clinical disease course.
2 The lack of additional prognostic parameters in FMF patients.
3 The need for specific treatment recommendations in different FMF subgroups.
4 The role of the cytokine interleukin-31 (IL-31) in (severe) pruritic cutaneous T-cell lymphoma (CTCL) variants, including FMF.

In this final chapter, the results of these studies are summarized and discussed based on recent literature. The chapter will end with concluding remarks and future perspectives.

IDENTIFICATION OF INDOLENT AND AGGRESSIVE FMF SUBGROUPS

In 2002, van Doorn et al. studied the difference between classic MF and FMF and found that FMF patients carry a worse prognosis as compared to classic MF patients.[2] This observation was confirmed by subsequent studies and also supported by large retrospective cohort studies that showed that the folliculotropic variant of MF was an independent risk factor for disease progression and impaired survival.[3-6] Because of this impaired survival and also because of the deeper perifollicular localization of neoplastic infiltrates, FMF patients were invariably considered to have tumor-stage disease, regardless of the clinical appearance of skin lesions.[2] Yet over time, several reports emerged that described subgroups of FMF patients with very indolent clinical disease courses.[7-10] As a consequence, we aimed to identify criteria that could be used to differentiate between FMF cases with an indolent and aggressive clinical behavior. Therefore, we studied the clinical and follow-up data of a cohort of 203 FMF patients and the results of this study are presented in **Chapter 2**. FMF patients presenting with follicle-

based patches, follicular papules, keratosis pilaris-like and/or acneiform (comedones, milia or cystic) skin lesions followed an indolent clinical course with a 5- and 10-year disease specific survival (DSS) of 95% and 88%, respectively (**Table 1**). The clinical presentations in this group were similar to those of indolent FMF cases previously described in smaller series.[7-11] In contrast, FMF patients that presented with tumors and nodules without concurrent extracutaneous disease at diagnosis followed a much more aggressive clinical course with 5- and 10-year DSS rates of 59% and 40%, respectively (**Table 1**). The prognosis of 58 FMF patients presenting with plaques at diagnosis was intermediate between the above mentioned indolent (early-stage) and aggressive (advanced-stage) subgroups and had 5- and 10-year DSS rates of 79% and 50%, respectively.

Table 1. Prognosis of subgroups of Folliculotropic Mycosis Fungoides (FMF)

Skin lesions at diagnosis	*N*	5-year DSS (%)	10-year DSS (%)		FMF subgroup	5-year DSS(%)	10-year DSS (%)
Follicular papules, patches, keratosis pilaris-like lesions, acneiform lesions	*67*	95	88	→	**Early-stage skin-limited FMF**	96	93
Early plaques[1]	*17*	100	100				
Advanced plaques[2]	*41*	75	35	→	**Advanced-stage skin-limited FMF**	65	40
Tumors and nodules	*55*	59	40				
Erythroderma	*6*	68	NR				
Nodal / Visceral involvement	*17*	23	2	→	**Extracutaneous FMF**	23	2

DSS: disease specific survival according to van Santen et al.[12] ; FMF: folliculotropic mycosis fungoides; N: number of patients, NR: not reached.

[1] Early plaques were defined as more or less elevated or infiltrated skin lesions, histologically characterized by sparse peri/intrafollicular infiltrates containing mainly small- to medium sized cerebriform/pleomorphic cells.

[2] Advanced plaques were defined as more or less elevated or infiltrated skin lesions, histologically characterized by by dense neoplastic infiltrates containing mainly medium-sized to large cerebriform/pleomorphic cells.

There is ongoing discussion if FMF patients with plaques should be classified as early stage disease as in classic MF (stage IA-IIA) or as advanced stage disease (stage IIB). Further analysis of our group of patients presenting with plaques showed considerable heterogeneity in prognosis. By reviewing the skin biopsies at diagnosis of our 58 plaque-stage FMF patients in a blinded fashion, it was shown that histopathologic criteria were useful to make a distinction between indolent and aggressive plaque-stage cases. Plaque-stage FMF that was histologically characterized by sparse intra- or perifollicular neoplastic infiltrates containing relatively few and mainly small neoplastic T-cells (early plaque-stage FMF) had excellent survival rates with a 10-year DSS of 100%, very similar to the indolent

course of patients with above mentioned early-stage FMF. On the contrary, plaque-stage FMF patients histologically showing more extensive confluent or diffuse infiltrates containing many often medium-sized to large tumor cells (advanced plaque-stage FMF) had a very similar course to patients presenting with tumors with a 10-year DSS of 35% (**Table 1**). Altogether, the results of our study presented in **chapter 2** resulted in the definition of three clinically relevant subgroups of FMF: patients with early-stage skin-limited FMF, patients with advanced-stage skin-limited FMF and FMF patients presenting with concurrent cutaneous and extracutaneous disease (**Table 1**). Clinical differences between early- and advanced-stage skin-limited FMF are further summarized in **Table 2**.

Table 2. Differences between early and advanced stage (skin-limited) folliculotropic mycosis fungoides (FMF)

	Early stage FMF	Advanced stage FMF
Clinical presentations	Follicular papules Follicle-based patches Acneiform lesions Keratosis pilaris-like lesions Early plaques[1]	Advanced plaques[2] Tumors Nodules Erythroderma
Mainly head / neck involvement	51%	78%
Eyebrow involvement[3]	34%	50%
Pruritus	69%	80%
Secondary bacterial infection	Rare	Common
Histopathology	Sparse intra- and perifollicular infiltrates with relatively few mainly small to medium-sized neoplastic T-cells	Confluent perifollicular tot diffuse dermal infiltrates containing many medium-sized tot large neoplastic T-cells and often blast cells
Large cell transformation	Rare	Common (20-30%)
Treatment	Topical steroids PUVA Local radiotherapy	PUVA PUVA + local radiotherapy PUVA + interferon or retinoids TSEBI
Disease specific survival 5/10yr	96% / 93%	65% / 40%
Overall survival 5/10yr	92% / 72%	55% / 28%

[1] Early plaques were defined as more or less elevated or infiltrated skin lesions, histologically characterized by sparse peri/intrafollicular infiltrates containing mainly small- to medium sized cerebriform/pleomorphic cells.
[2] Advanced plaques were defined as more or less elevated or infiltrated skin lesions, histologically characterized by by dense neoplastic infiltrates containing mainly medium-sized to large cerebriform/pleomorphic cells.
[3] Eyebrow involvement present at diagnosis.
FMF: Folliculotropic Mycosis Fungoides, PUVA: Psoralen Ultraviolet A treatment, TSEBI: Total Skin Electron Beam Irradiation; yr: year.

In a study by Hodak et al. significant survival differences were also found between early and advanced subgroups of FMF.[11] However, in that study patients presenting with plaques were categorized primarily on clinical criteria, e.g. the presentation with flat plaques was considered early-stage disease, while infiltrated plaques were upstaged to tumor-stage disease.[11] However, results from chapter 2 do not support an approach to upstage all infiltrated plaques to tumor-stage disease, as a subset of plaques in our cohort carried a favorable prognosis and these plaques were clinically indistinguishable from those in patients with advanced plaque-stage FMF. It should also be stressed that in the current TNMB system stage is determined by clinical presentation and additional histological criteria are not taken into account. Further studies on the impact of additional histologic criteria on the staging of plaque-stage FMF, and perhaps also plaque-stage classic MF, are needed.At present, a large international study is being performed to investigate the reproducibility of diverse clinical and histological criteria to stage FMF patients. These results should be awaited before further definitive adjustments in current staging systems are made.

PROGNOSTIC PARAMETERS IN FMF

Clinical and histological prognostic parameters

Prognostic parameters are relatively well-studied in classic MF, but are almost lacking in FMF. Therefore in **chapter 2**, we also investigated various clinical and histological parameters for their prognostic significance in our cohort of 203 FMF patients. Clinical stage, age (older than 60 years), large cell transformation at diagnosis and the presence of extensive secondary bacterial infection at the time of first clinical presentation appeared independent factors associated with reduced OS, DSS, and/or PFS. Analogous to classic MF, clinical stage of disease, age and large cell transformation are well-known risk factors in classic MF and were also reported in one other FMF cohort.[6,13-16]. However, the presence of secondary bacterial infection as an adverse risk factor has not been described before. It is known that bacterial toxins acting as superantigens may stimulate the proliferation of malignant T-cells and that antibiotic or eradication treatment have been associated with clinical improvement in CTCL patients.[17-19] It warrants further study whether this or other mechanisms are responsible for poor outcome in FMF patients with extensive secondary bacterial infection and if treatment regimens focusing on preventing or lowering bacterial load could be beneficial in FMF patients.

Histological prognostic parameters in FMF patients presenting with plaques

As it became apparent from the results of chapter 2 that histological criteria appeared helpful in the distinction between patients with early- and advanced plaque-stage FMF, a detailed study on histopathologic characteristics and parameters associated with survival in plaque-stage FMF was performed in **chapter 4**. In order to study the risk for disease progression or death due to lymphoma, 20 patients with a progressive disease course and 20 patients without disease progression during at least 60 months were compared. Histopathologic evaluation revealed that the presence of >25% atypical cells in the infiltrate, size of neoplastic T-cells, presence of >10% blast cells, presence of >10% Ki-67 positive cells and prominent interfollicular epidermotropism were associated with disease progression and/or decreased survival, while extensive follicular mucinosis turned out to be associated with increased survival. The extent of the perifollicular infiltrates, which had been used in our study described in chapter 2 as one of the parameters to differentiate between early and advanced plaque stage FMF, had no effect on prognosis or disease progression. In contrast, in a study of Hodak et al. the extent or density of neoplastic infiltrate was described to be an independent prognostic factor, but this study had included also tumors and (follicle-based) patches, instead of only plaques.[11]

Prognostic molecular parameters in FMF patients

As described in the introductory chapter of this thesis, high throughput sequencing (HTS) of the T-cell receptor (TCR) is a relatively new technique that allows an accurate identification of the unique CDR3 region of a TCR. As a consequence, this technique enables the sensitive quantification of every distinct T-cell clone present within CTCL infiltrates and allows the study of the relative frequency of each individual clone (tumor clone frequency) within the full T-cell repertoire of an infiltrate.[20,21] In a study on 141 cases with early stage classic MF it was found that tumor clone frequency (TCF) greater than 25% in lesional skin was associated with reduced progression-free survival with a hazard ratio (HR) of 4.9. **Chapter 5** describes the results of this technique in a population of 41 FMF patients presenting with plaques, including 24 cases with and 17 without disease progression. TCF varied widely from 0.95-72.01% (median 11.00%) and was ≥25% in 12/41 cases. We did not find significant associations between different TCF cut off values and PFS, OS or DSS while HRs were calculated for TCFs variying from 5-50%, each calculation with a 5% interval. Therefore, TCF calculation has no value in early risk stratification of FMF patients with plaque-stage disease.

Difference with classic MF may be explained by the fact that both patch- and plaque-stage had been included. Furthermore, striking variations in usage of Vbeta reportoires were found between (predominantly classic MF) cases reported by de Masson et al. and the differences regarding Vbeta usage in FMF patients will be topic for further investigation. Use of different Vbeta repertoires may further support the notion that classic MF and FMF harbor some biologic and pathogenic differences in terms of antigen stimulation that could drive the onset or evolution of the disease.

MANAGEMENT AND TREATMENT OPTIONS IN FMF

Controlled studies on treatment of FMF patients are completely lacking and even reports on treatment results in FMF are scarce. In addition, the above mentioned recognition of indolent and aggressive subgroups in FMF (early skin-limited, advanced skin-limited and extracutaneous) implies that these subgroups require a different therapeutic approach.

As a result, the study in **chapter 3** investigated treatment responses of different treatment modalities in 203 FMF patients. This chapter describes that a stepwise, stage-adapted therapeutic approach, similar to classic MF, can be followed in FMF patients. Furthermore, beneficial outcomes for certain FMF subgroups resulted in the proposal of specific treatment recommendations (see chapter 3 for algorithm).

Management in early-stage skin-limited FMF

Early-stage skin-limited FMF patients with limited follicle-based patches respond very well to monotherapy with potent topical steroids. In case of more widespread skin lesions, oral PUVA treatment was found very effective, both in cases with follicle-based patches and in patients with plaques. In patients with follicle-based patches who are not eligible for oral PUVA, nbUVB or bath PUVA may be an alternative, in particular for lesions located outside the head and neck area. However, in patients with plaques nbUVB and bath PUVA are generally not effective. In cases with residual lesions after skin-directed therapies (SDTs), low-dose local radiotherapy proves highly effective. Treatment with topical nitrogen mustard has also been used effectively in early-stage (F)MF for more than 50 years, however this treatment is no longer available and effectiveness of variants of this treatment, such as chlormethine gel, are now available and are further investigated.[22]

Management in advanced-stage skin-limited FMF

In advanced (skin-limited) FMF patients, monotherapy with topical steroids and nbUVB are ineffective. In patients with infiltrated plaques, PUVA can be attempted, although CR may not be achieved. In this group, radiotherapy-based treatment modalities have been found most effective. In patients with widespread skin lesions, combined treatment with PUVA and concomitant low-dose radiotherapy for (persistent) thick plaques or tumors is often performed and found very effective. For patients presenting with a few localized plaques or tumors local radiotherapy yields excellent responses, may give durable CR and is the preferred mode of treatment. In patients presenting with widespread thick plaques and/or tumors, total skin electron beam irradiation (TSEBI) with a standard dose of 35 Gy proved effective with high CR and OR rates and durable responses in some patients. However, in most patients, response to TSEBI is short lived. In addition, reports on durability of responses and dosage regimens are variable.[16,23] Advantages of lower dosage TSEBI regimens include lower toxicity and the opportunity of multiple retreatments. Recent studies report nearly similar effectivity of low-dose TSEBI (10-12Gy) regimens in classic MF and additionally investigate the effect of low-dose TSEBI combined with adjuvant (systemic) treatments.[24,25] Clinical trials on low-dose TSEBI regimens combined with adjuvant brentuximab (https://clinicaltrials.gov/ct2/show/NCT02822586) and adjuvant interleukin 12 (https://clinicaltrials.gov/ct2/show/NCT02542124) are currently ongoing. Whether these regimens are also effective in patients with FMF warrants further study and stratification of treatment results for FMF patients is essential.

While the combination of PUVA therapy with IFNa or retinoids is frequently described in advanced (skin-limited) disease, convincing evidence that this combined therapy reaches higher response rates is currently lacking and controlled studies in both MF and FMF should be a target for further research.

Management in extracutaneous FMF

In FMF patients with concurrent extracutaneous as well as refractory advanced skin-limited disease single-agent chemotherapy, such as gemcitabine or liposomal doxorubicin should be considered before aggressive multi-agent (CHOP-like) chemotherapy regimens are used. As an alternative, in CD30-positve cases, brentuximab can be used and particularly in younger patients an allogeneic stem cell transplantation

should be considered. Allogeneic stem cell transplantations using reduced-intensity conditioning regimens have shown promising results and may be the only therapy with curative potential.[26] However, a low tumor burden at the time of transplantation is considered critically important[27] and may in patients with extensive skin lesions be achieved by the combination of TSEBI as a debulking agent prior to transplantation.[26] Still, the urgent need for well-designed, multicenter trials is especially apparent for these patients.

Management and follow-up of solitary FMF

Chapter 6 describes the clinicopathologic features, long-term follow-up data and challenges in diagnosing the uncommon clinical presentation of a solitary FMF lesion. The results of a case series of 9 FMF patients with a solitary lesion show that this peculiar clinical presentation may run a heterogeneous disease course. The results presented in chapter 2 and chapter 6 show that local radiotherapy may give durable CR in solitary FMF. It is therefore considered the preferred mode of treatment, which is in accordance with other studies performed in both solitary classic MF and solitary FMF.[28-30] Previous studies emphasized the indolent clinical behavior of patients with solitary FMF. Development of extracutaneous disease or lymphoma-related deaths have never been reported, although follow-up periods were rather short in these studies.[28,29,31] However, chapter 6 reports that progressive disease may start years after a CR on initial treatment and that two of nine patients developed extracutaneous disease and died 89 and 203 months after diagnosis, indicating that long-term follow-up is required also in patients with solitary FMF.

IL31 as a valuable target in the management of pruritus in FMF?

Pruritus is a frequent distressing symptom in FMF and other CTCL patients, is often more severe in advanced stages of disease and can be very difficult to manage therapeutically.[32,33] Moderate to severe pruritus has been reported in 68-80% of FMF cases.[3,16,34] The underlying mechanisms causing pruritus in FMF and CTCL patients are currently poorly understood. Recent literature has focused on interleukin 31 (IL31) as a possible beneficial target for improving pruritus in CTCL patients, following successful studies with IL31 targeted therapy improving pruritus in atopic dermitis patients.[35] Several studies have described increased IL31 serum or transcriptional levels in the pathogenesis of pruritus and/or disease severity of CTCL, while other groups have

reported undetectable values.[36-40] Therefore, we investigated IL31 levels in serum and cutaneous *IL31* transcriptional expression in pruritic and non-pruritic CTCL variants and the relation between IL31 and clinical disease stage. The results of this study are described in **chapter 7** and reveal low and undetectable values of IL31 in 31% and 69% of our CTCL patients that suffered from pruritus, respectively. Our results are in line with the IL31 study of Möbs et al, that reported equally low IL31 serum values and only few samples exceeded the threshold for quantification.[40] Several other studies stressed increased IL31 serum concentrations in CTCL[36,37,39] , but in all these studies the concentration of IL31 was relatively low when compared to values reported in AD patients.[41-43] Our results do not support an essential role for IL31 in the pathogenesis of pruritus in CTCL patients and stress the importance to further elucidate crucial mechanisms in CTCL pruritus, other than IL31.

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

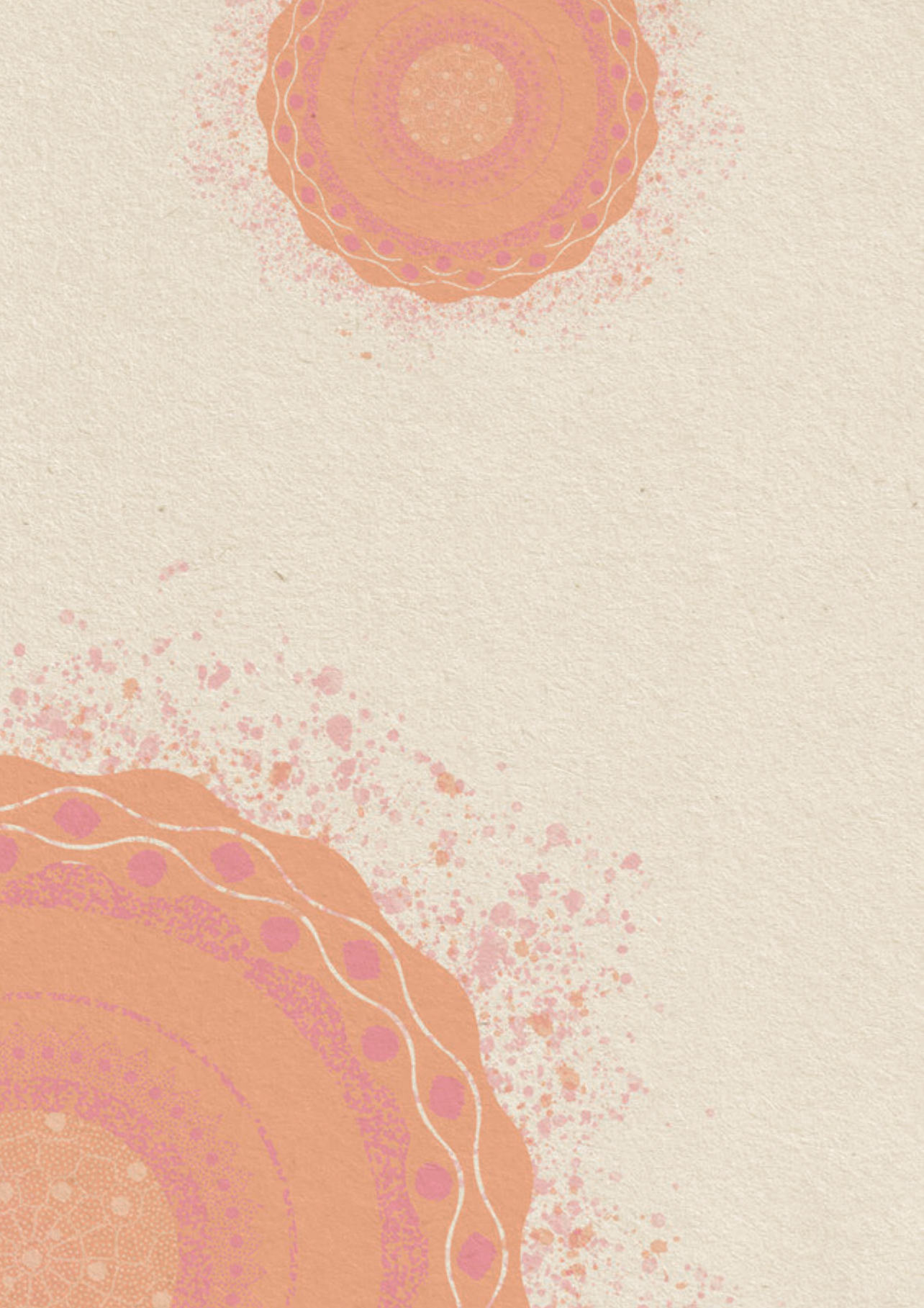
The different studies in this thesis show that there are important prognostic subgroups among FMF patients, which can be better recognized through a good clinical assessment of their skin and critical appraisal of their histopathology. Contrasting the previous notion that all FMF patients carry an aggressive clinical course, we found that a subgroup of FMF patients run an indolent disease course and may respond to relatively safe skin-directed therapies. As a result, the studies in this thesis aid in a better risk stratification of FMF patients and in the search for appropriate treatment. Still, therapeutical improvement is much desired for FMF patients. Further insights in FMF's specific biology, including molecular and genetic events driving the disease may yield the achievement of new treatment targets. Furthermore, as genetic profiling and gene sequencing are gaining ground for improved prognostic characterization and treatment selection, these approaches may be similarly interesting for further study in FMF patients. In addition, current and future studies investigating (targeted) therapies in large prospective (multi-center) MF cohorts, should specifically stratify their results for FMF patients in order to expand our knowledge on treatment outcomes in this specific MF variant.

REFERENCES

1. Willemze R, Jaffe ES, Burg G, et al. WHO-EORTC classification for cutaneous lymphomas. *Blood.* 2005;105(10):3768-3785.
2. van Doorn R, Scheffer E, Willemze R. Follicular mycosis fungoides, a distinct disease entity with or without associated follicular mucinosis: a clinicopathologic and follow-up study of 51 patients. *Arch Dermatol.* 2002;138(2):191-198.
3. Gerami P, Rosen S, Kuzel T, Boone SL, Guitart J. Folliculotropic mycosis fungoides: an aggressive variant of cutaneous T-cell lymphoma. *Arch Dermatol.* 2008;144(6):738-746.
4. Benton EC, Crichton S, Talpur R, et al. A cutaneous lymphoma international prognostic index (CLIPi) for mycosis fungoides and Sezary syndrome. *Eur J Cancer.* 2013;49(13):2859-2868.
5. Benner MF, Jansen PM, Vermeer MH, Willemze R. Prognostic factors in transformed mycosis fungoides: a retrospective analysis of 100 cases. *Blood.* 2012;119(7):1643-1649.
6. Agar NS, Wedgeworth E, Crichton S, et al. Survival outcomes and prognostic factors in mycosis fungoides/Sezary 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(31):4730-4739.
7. Bakar O, Seckin D, Demirkesen C, Baykal C, Buyukbabani N. Two Clinically Unusual Cases of Folliculotropic Mycosis Fungoides: One with and the Other without Syringotropism. *Ann Dermatol.* 2014;26(3):385-391.
8. Muniesa C, Estrach T, Pujol RM, et al. Folliculotropic mycosis fungoides: clinicopathological features and outcome in a series of 20 cases. *J Am Acad Dermatol.* 2010;62(3):418-426.
9. Tomasini C, Kempf W, Novelli M, et al. Spiky follicular mycosis fungoides: a clinicopathologic study of 8 cases. *J Cutan Pathol.* 2015;42(3):164-172.
10. Hodak E, Amitay-Laish I, Feinmesser M, et al. Juvenile mycosis fungoides: cutaneous T-cell lymphoma with frequent follicular involvement. *J Am Acad Dermatol.* 2014;70(6):993-1001.
11. Hodak E, Amitay-Laish I, Atzmony L, et al. New insights into folliculotropic mycosis fungoides (FMF): A single-center experience. *J Am Acad Dermatol.* 2016.
12. van Santen S, Roach RE, van Doorn R, et al. Clinical Staging and Prognostic Factors in Folliculotropic Mycosis Fungoides. *JAMA Dermatol.* 2016;152(9):992-1000.
13. 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(4):504-510.
14. Talpur R, Singh L, Daulat S, et al. Long-term outcomes of 1,263 patients with mycosis fungoides and Sezary syndrome from 1982 to 2009. *Clin Cancer Res.* 2012;18(18):5051-5060.
15. Scarisbrick JJ, Prince HM, Vermeer MH, et al. Cutaneous Lymphoma International Consortium Study of Outcome in Advanced Stages of Mycosis Fungoides and Sezary Syndrome: Effect of Specific Prognostic Markers on Survival and Development of a Prognostic Model. *J Clin Oncol.* 2015;33(32):3766-3773.
16. Wieser I, Wang C, Alberti-Violetti S, et al. Clinical characteristics, risk factors and long-term outcome of 114 patients with folliculotropic mycosis fungoides. *Arch Dermatol Res.* 2017.
17. Woetmann A, Lovato P, Eriksen KW, et al. Nonmalignant T cells stimulate growth of T-cell lymphoma cells in the presence of bacterial toxins. *Blood.* 2007;109(8):3325-3332.
18. Willerslev-Olsen A, Krejsgaard T, Lindahl LM, et al. Bacterial toxins fuel disease progression in cutaneous T-cell lymphoma. *Toxins (Basel).* 2013;5(8):1402-1421.

19. Talpur R, Bassett R, Duvic M. Prevalence and treatment of Staphylococcus aureus colonization in patients with mycosis fungoides and Sezary syndrome. *Br J Dermatol.* 2008;159(1):105-112.
20. de Masson A, O'Malley JT, Elco CP, et al. High-throughput sequencing of the T cell receptor beta gene identifies aggressive early-stage mycosis fungoides. *Sci Transl Med.* 2018;10(440).
21. Matos TR, de Rie MA, Teunissen MBM. Research Techniques Made Simple: High-Throughput Sequencing of the T-Cell Receptor. *J Invest Dermatol.* 2017;137(6):e131-e138.
22. Denis D, Beneton N, Laribi K, Maillard H. Management of mycosis fungoides-type cutaneous T-cell lymphoma (MF-CTCL): focus on chlormethine gel. *Cancer Manag Res.* 2019;11:2241-2251.
23. van Santen S, van Doorn R, Neelis KJ, et al. Recommendations for treatment in Folliculotropic Mycosis Fungoides: Report of the Dutch Cutaneous Lymphoma Group. *Br J Dermatol.* 2017.
24. Rivers CI, Singh AK. Total Skin Electron Beam Therapy for Mycosis Fungoides Revisited With Adjuvant Systemic Therapy. *Clin Lymphoma Myeloma Leuk.* 2019;19(2):83-88.
25. Elsayad K, Susek KH, Eich HT. Total Skin Electron Beam Therapy as Part of Multimodal Treatment Strategies for Primary Cutaneous T-Cell Lymphoma. *Oncol Res Treat.* 2017;40(5):244-252.
26. Virmani P, Zain J, Rosen ST, Myskowski PL, Querfeld C. Hematopoietic Stem Cell Transplant for Mycosis Fungoides and Sezary Syndrome. *Dermatol Clin.* 2015;33(4):807-818.
27. Trautinger F, Eder J, Assaf C, et al. European Organisation for Research and Treatment of Cancer consensus recommendations for the treatment of mycosis fungoides/Sezary syndrome - Update 2017. *Eur J Cancer.* 2017;77:57-74.
28. Ally MS, Pawade J, Tanaka M, et al. Solitary mycosis fungoides: a distinct clinicopathologic entity with a good prognosis: a series of 15 cases and literature review. *J Am Acad Dermatol.* 2012;67(4):736-744.
29. Kempf W, Kazakov DV, Schermesser M, et al. Unilesional follicular mycosis fungoides: report of two cases with progression to tumor stage and review of the literature. *J Cutan Pathol.* 2012;39(9):853-860.
30. Hsiao PF, Hsiao CH, Tsai TF, Jee SH. Unilesional folliculotropic/syringotropic cutaneous T-cell lymphoma presenting as an indurated plaque on the nape. *Int J Dermatol.* 2006;45(10):1268-1270.
31. Amitay-Laish I, Feinmesser M, Ben-Amitai D, Fenig E, Sorin D, Hodak E. Unilesional folliculotropic mycosis fungoides: a unique variant of cutaneous lymphoma. *J Eur Acad Dermatol Venereol.* 2016;30(1):25-29.
32. Vij A, Duvic M. Prevalence and severity of pruritus in cutaneous T cell lymphoma. *Int J Dermatol.* 2012;51(8):930-934.
33. Ahern K, Gilmore ES, Poligone B. Pruritus in cutaneous T-cell lymphoma: a review. *J Am Acad Dermatol.* 2012;67(4):760-768.
34. van Santen S, Roach RE, van Doorn R, et al. Clinical Staging and Prognostic Factors in Folliculotropic Mycosis Fungoides. *JAMA Dermatol.* 2016.
35. Ruzicka T, Hanifin JM, Furue M, et al. Anti-Interleukin-31 Receptor A Antibody for Atopic Dermatitis. *N Engl J Med.* 2017;376(9):826-835.
36. Ohmatsu H, Sugaya M, Suga H, et al. Serum IL-31 levels are increased in patients with cutaneous T-cell lymphoma. *Acta Derm Venereol.* 2012;92(3):282-283.
37. Singer EM, Shin DB, Nattkemper LA, et al. IL-31 is produced by the malignant T-cell population in cutaneous T-Cell lymphoma and correlates with CTCL pruritus. *J Invest Dermatol.* 2013;133(12):2783-2785.
38. Nattkemper LA, Martinez-Escala ME, Gelman AB, et al. Cutaneous T-cell Lymphoma and Pruritus: The Expression of IL-31 and its Receptors in the Skin. *Acta Derm Venereol.* 2016;96(7):894-898.
39. Malek M, Glen J, Rebala K, et al. Il-31 does not correlate to pruritus related to early stage cutaneous T-cell lymphomas but is involved in pathogenesis of the disease. *Acta Derm Venereol.* 2015;95(3):283-288.

40. Mobs M, Gryzik S, Haidar A, Humme D, Beyer M, Vandersee S. Analysis of the IL-31 pathway in Mycosis fungoides and Sezary syndrome. *Arch Dermatol Res.* 2015;307(6):479-485.
41. Szegedi K, Kremer AE, Kezic S, et al. Increased frequencies of IL-31-producing T cells are found in chronic atopic dermatitis skin. *Exp Dermatol.* 2012;21(6):431-436.
42. Raap U, Wichmann K, Bruder M, et al. Correlation of IL-31 serum levels with severity of atopic dermatitis. *J Allergy Clin Immunol.* 2008;122(2):421-423.
43. Kim S, Kim HJ, Yang HS, Kim E, Huh IS, Yang JM. IL-31 Serum Protein and Tissue mRNA Levels in Patients with Atopic Dermatitis. *Ann Dermatol.* 2011;23(4):468-473.



9

NEDERLANDSE SAMENVATTING

LIST OF ABBREVIATIONS

LIST OF PUBLICATIONS

CURRICULUM VITAE

NAWOORD

NEDERLANDSE SAMENVATTING

Primair cutane lymfomen zijn non-Hodgkin lymfomen die zich in de huid presenteren, zonder dat er op het moment van diagnose aanwijzingen zijn voor lokalisaties elders in het lichaam. Er zijn twee belangrijke hoofdgroepen te onderscheiden: de primair cutane T-cellymfomen (CTCL), die in ongeveer 75% van de gevallen voorkomen en de primair cutane B-cellymfomen (CBCL), die verantwoordelijk zijn voor de overige 25% .

Mycosis fungoides (MF) is het meest voorkomende CTCL type. Folliculotrope mycosis fungoides (FMF) wordt erkend als een zeldzame maar specifieke variant van MF vanwege zijn kenmerkende klinische, histopathologische en prognostische kenmerken en beslaat tot ongeveer 10% van alle gestelde MF diagnoses. In tegenstelling tot de klassieke vorm van MF, waarbij neoplastische T-cellen de opperhuid (epidermis) infiltreren (epidermotropie), wordt FMF gekenmerkt door de aanwezigheid van neoplastische T-cellen in en rond de haarfollikels (folliculotropie). Voorkeurslokalisaties van de huidafwijkingen bij FMF zijn het hoofd- en halsgebied, hetgeen veelal gespaard blijft bij de klassieke vorm van MF. In 2002 bestudeerden van Doorn et al. het verschil tussen klassieke MF en FMF en stelden vast dat FMF patiënten een slechtere prognose hebben in vergelijking met patiënten met een klassieke vorm van MF. Deze observatie werd bevestigd door latere studies en ook ondersteund door grote retrospectieve cohortstudies die aantoonden dat de folliculotrope variant van MF een onafhankelijke risicofactor voor ziekteprogressie en verminderde overleving is. Vanwege deze verminderde overleving en ook vanwege de diepere perifolliculaire lokalisatie van neoplastische infiltraten, werd aangenomen dat FMF patiënten moesten worden beschouwd als 'tumorstadium' van de ziekte, ongeacht het klinische uiterlijk van de huidlaesies, en derhalve een agressiever behandelschema nodig hadden. Toch verschenen er in de loop van de tijd verschillende studies in de literatuur die subgroepen van FMF patiënten met een zeer indolent klinisch ziekteverloop beschreven en een goede reactie op de niet-agressieve eerstelijns behandelingen, zoals die voor het vroege stadium van klassieke MF worden voorgesteld. Als gevolg daarvan was het doel om in dit proefschrift verschillende zaken in kaart brengen: 1) het ziekteverloop van een groot cohort FMF patiënten, 2) prognostische criteria die een onderscheid kunnen maken tussen FMF patiënten met een indolent en met een snel progressief beloop 3) de behandelresultaten en behandelaanbevelingen voor specifieke FMF subgroepen.

ZIEKTEBELOOP FMF PATIËNTEN

In **hoofdstuk 2** zijn de klinische en follow-up gegevens van een cohort van 203 FMF patiënten bestudeerd. Het bleek dat FMF patiënten met patches met folliculaire tekening, folliculaire papels, keratosis pilaris-achtige en / of acneïforme huidlaesies (comedonen, milia of cysten) een indolent klinisch verloop volgden met een 5- en 10-jaars ziektespecifieke overleving van respectievelijk 95% en 88%. Deze groep werd derhalve geclassificeerd als 'vroeg-stadium FMF'. De klinische presentaties in deze groep waren vergelijkbaar met die van patiënten met een indolente vorm van FMF die eerder in kleinere series waren beschreven. Daarentegen volgden FMF patiënten met tumoren en noduli bij diagnose zonder gelijktijdige systemische of lymfeklierbetrokkenheid een veel agressiever klinisch beloop met een 5- en 10-jaars ziektespecifieke overleving van respectievelijk 59% en 40% en deze patiënten konden worden geclassificeerd als 'gevorderd stadium' van de ziekte. Deze percentages betroffen bij FMF patiënten met plaques bij diagnose respectievelijk 79% en 50% en deze cijfers liggen daarmee prognostisch tussen het bovengenoemde 'vroege stadium' en 'gevorderde stadium' in.

Aangezien er discussie bestond in de literatuur of FMF patiënten met plaques moeten worden geclassificeerd als vroeg stadium, zoals bij klassieke MF (stadium IA-IIA), of als gevorderd stadium (stadium IIB), werd verdere analyse verricht op deze subgroep. FMF patiënten met plaques bij diagnose bleken een aanzienlijke heterogeniteit te vertonen in prognose. Door de huidbiopten bij diagnose te bekijken van deze groep patiënten (geblindeerd voor de klinische gegevens), werd aangetoond dat histopathologische criteria nuttig zijn om onderscheid te maken tussen patiënten die een indolent of meer agressief ziektebeloop zullen volgen. Plaque-stadium FMF met daarbij de histologische kenmerken van spaarzame intra- of perifolliculaire neoplastische infiltraten met relatief weinig en voornamelijk kleine neoplastische T-cellen (vroeg plaquestadium FMF) hadden uitstekende overlevingspercentages met een 10-jaars ziektespecifieke overleving van 100%, zeer vergelijkbaar met het gunstige beloop van FMF patiënten met de bovengenoemde vroege fase FMF. Daarentegen, plaque-stadium FMF patiënten die histologisch meer uitgebreide of diffuse infiltraten vertoonden ofwel veel middelgrote tot grote tumorcellen bevatten (gevorderd plaquestadium FMF), hadden een vergelijkbaar beloop als patiënten die zich presenteren met tumoren en noduli met een 10-jarige ziektespecifieke overleving van 35%. Al met al resulteerden de resultaten

van deze studie in hoofdstuk 2 in de definitie van drie klinisch en prognostisch relevante subgroepen van FMF: patiënten met 1) FMF in een vroeg stadium, waartoe ook de groep met 'vroeg plaque-stadium FMF' werd ingedeeld 2) FMF in een gevorderd stadium, waartoe ook de groep met 'gevorderd plaque-stadium' werd ingedeeld en 3) FMF-patiënten met gelijktijdige cutane en extracutane ziekte.

ONDERZOEK NAAR PROGNOSTISCHE CRITERIA IN FMF PATIENTEN

Omdat uit de resultaten van hoofdstuk 2 duidelijk werd dat histologische criteria behulpzaam zijn bij het onderscheid tussen plaquestadium patiënten met vroege en gevorderde FMF, werd in **hoofdstuk 4** een gedetailleerd onderzoek uitgevoerd naar histopathologische kenmerken en parameters geassocieerd met de kans op ziekteprogressie en overleving bij deze groep FMF patiënten. Om het risico op ziekteprogressie of overlijden als gevolg van lymfoom te bestuderen, werden patiënten met een progressief ziekteverloop en patiënten zonder ziekteprogressie (gedurende ten minste 60 maanden na diagnose) met elkaar vergeleken. Histopathologische evaluatie onthulde dat de volgende factoren geassocieerd waren met een verhoogde kans op ziekteprogressie en/of verminderde overleving: de aanwezigheid van >25% atypische cellen in het infiltraat, de grootte van neoplastische T-cellen, de aanwezigheid van >10% blastaire tumorcellen, de aanwezigheid van >10% Ki-67-positieve cellen (een marker voor de delingsactiviteit van de tumorcellen) en opvallende mate van interfolliculaire epidermotropie. Daarnaast werd aangetoond dat de aanwezigheid van uitgebreide folliculaire mucinose juist geassocieerd is met een verhoogde overlevingskans. De omvang van de neoplastische infiltraten, die in onze studie in hoofdstuk 2 was gebruikt als een van de criteria om onderscheid te maken tussen vroeg en gevorderd plaquestadium FMF, had geen effect op de prognose of ziekteprogressie.

Hoofdstuk 5 onderzoekt de vraag of het gebruik van high throughput sequencing (HTS) van de T-celreceptor (TCR) prognostische betekenis kan hebben voor FMF patiënten die zich presenteren met plaques bij diagnose. Dit betreft een nieuwe moleculaire techniek die nauwkeurig elke afzonderlijke T-celkloon in een biologisch sample kan identificeren. Ook kan de relatieve frequentie van elke individuele kloon binnen het volledige T-celrepertoire van een infiltraat worden bepaald (tumorkloonfrequentie). In tegenstelling tot wat eerder is aangetoond voor klassieke MF, werden er in de studie

in hoofdstuk 5 geen evidente significante associaties tussen tumorkloonfrequentie percentages en prognose aangetoond voor deze FMF subgroep.

BEHANDELRESULTATEN EN -AANBEVELINGEN

De herkenning van een indolente (vroeg stadium) en meer agressief verlopende vorm van FMF (gevorderd stadium) impliceert dat deze prognostisch verschillende patiëntsubgroepen een andere therapeutische benadering vereisen. Als gevolg hiervan onderzocht de studie in **hoofdstuk 3** de behandelresultaten van verschillende behandelingsmodaliteiten bij diverse subgroepen FMF patiënten.

De resultaten in dit hoofdstuk tonen dat patiënten met de indolente vorm van FMF goed reageren op behandeling met niet-agressieve, op de huid gerichte therapieën. Zeer beperkte vlakke patches met folliculaire papels verkrijgen goede resultaten op de behandeling met klasse IV topicale steroïden. Voor patiënten met meer uitgebreide afwijkingen bleek orale PUVA-therapie zeer effectief. Daarentegen was UVB-therapie minder effectief en kon geen complete remissie van de huidafwijkingen induceren. Bij patiënten met een agressieve vorm van FMF bleken bovengenoemde behandelingen niet meer afdoende, alhoewel orale PUVA bij patiënten met plaques geprobeerd kan worden. Bij voorkeur wordt orale PUVA-therapie bij deze patiënten gecombineerd met lage dosis radiotherapie (2 x 4 Gy) voor dikkere plaques of tumoren, die onvoldoende op PUVA reageren. Als alternatief kan orale PUVA gecombineerd worden met acitretin of interferon alfa. Patiënten met zeer uitgebreide plaques en/of tumoren komen in aanmerking voor totale huidbestraling met elektronen, deze behandeling bleek zeer effectief, echter is het effect in de meerderheid van de gevallen kortdurend. Chemotherapie worden vaak vanwege betere en minder toxische alternatieven niet verkozen bij patiënten met uitgebreide huidafwijkingen, maar is wel geïndiceerd voor patiënten met systemische betrokkenheid van hun cutane lymfoom. Daarnaast is er toenemende aandacht voor de toepassing van targeted therapies, waarbij brentuximab een belangrijke rol speelt. Studieresultaten van dergelijke behandelingen dienen separate uitkomsten voor klassieke MF en FMF te rapporteren.

In **hoofdstuk 6** is een bijzondere subgroep FMF patiënten beschreven die zich bij diagnose presenteert met slechts één huidafwijking (solitaire FMF). De meest effectieve

therapie in deze patiëntgroep is lokale radiotherapie, waarbij gekozen wordt voor een dosis van 20Gy om daarmee langdurige remissie of volledige curatie te induceren. Eerdere studies hebben het indolente ziektebeloop benadrukt van patiënten met solitaire FMF en het ontstaan van extracutane ziekte of overlijden als gevolg van de ziekte zijn nooit gerapporteerd. De in hoofdstuk 6 beschreven studie vermeldt echter bij twee van de negen beschreven cases ontwikkeling naar extracutane ziekte en overlijden. Ook werd gevonden dat progressieve ziekte nog jaren na een complete respons op de initiële behandeling kan ontstaan, hetgeen aangeeft dat langdurige follow-up nodig is voor deze bijzondere patiëntengroep.

Tenslotte is in **hoofdstuk 7** gekeken naar de rol van IL31 in de meest voorkomende type cutane T cel lymfomen, waaronder FMF. Dit cytokine speelt een centrale rol in de pathogenese van jeuk van atopische dermatitis patiënten. Jeuk is ook in de meerderheid van de FMF patiënten een belangrijk en hinderlijk symptoom, echter is de rol van IL31 in cutane T cel lymfomen nog onvoldoende opgehelderd. Verschillende onderzoeken hebben verhoogde transcriptieniveaus en/of serumconcentraties van IL31 in CTCL beschreven, terwijl andere groepen niet-detecteerbare waarden rapporteerden. Daarom hebben we IL31-niveaus in serum en de cutane IL31-transcriptie-expressie onderzocht bij verschillende CTCL-varianten waaronder FMF. De resultaten van deze studie tonen zeer lage of niet-detecteerbare waarden van IL31 bij respectievelijk 31% en 69% van de CTCL-patiënten met jeuk. Deze uitkomsten ondersteunen geen cruciale rol voor IL31 in de pathogenese van jeuk bij CTCL-patiënten en benadrukken het belang om andere mechanismen van jeuk bij CTCL patiënten, inclusief FMF patiënten, verder op te helderen.

LIST OF ABBREVIATIONS

AD	Atopic dermatitis
C-ALCL	Primary cutaneous anaplastic large cell lymphoma
CBCL	Primary cutaneous B cell lymphomas
CDR3	Complimentary determining region 3
CHOP	Cyclophosphamide Hydroxydaunorubicin Oncovin Prednisone
CI	Confidence interval
Cq	Cycle quantification
CR	Complete remission
CTCL	Primary cutaneous T cell lymphomas
DSS	disease specific survival
EORTC	European Organization for Research and Treatment of Cancer
FMF	Folliculotropic mycosis fungoides
HR	Hazard ratio
HTS-TCR	High-throughput sequencing of the T cell receptor
HTS-TRB	High-throughput DNA sequencing of the T cell receptor beta gene
IL31	Interleukin 31
IL31RA	Interleukin 31 receptor A
ISCL	International Society for Cutaneous Lymphomas
LCT	Large cell transformation
LDH	Lactate dehydrogenase
MF	(Classic) mycosis fungoides
NbUVB	Narrow-band ultraviolet B therapy
NCI	National Cancer Institute
NHL	non-Hodgkin lymphomas
NIH	National Institutes of Health
NP-FMF	Non-progressive folliculotropic mycosis fungoides
OR	Overall response
OS	Overall survival
OSMR	Oncostatin M receptor
PCR	Polymerase chain reaction
PD	Progressive disease
P-FMF	Progressive folliculotropic mycosis fungoides

PFS	Progression free survival
PR	Partial remission
PUVA	Psoralen with ultraviolet A therapy
RePUVA	Retinoid and psoralen with ultraviolet A therapy
RT	Radiotherapy
SCR	Sustained complete remission
SDT	Skin directed therapy
SS	Sézary syndrome
TCF	Tumor clone frequency
TCR	T cell receptor
TNMB	Tumor-node-metastasis-blood
TRB	T cell receptor beta gene
TSEBI	Total skin electron beam irradiation
VAS	Visual analogue scale
WHO	World Health Organisation

LIST OF PUBLICATIONS

van Santen, S., W.H. Zoutman, A. de Masson, K.D. Quint, R. Willemze, N. Gerard, J.E. Teague, T.S. Kupper, R.A. Clark, C.P. Tensen, M.H. Vermeer. Tumor clone frequency calculation using high throughput sequencing of the T-cell receptor β gene in folliculotropic mycosis fungoides patients. Manuscript accepted for publication in Journal of Investigative Dermatology.

van Santen, S., J.J. Out, W.H. Zoutman, K.D. Quint, R. Willemze, M.H. Vermeer, C.P. Tensen. Serum and cutaneous transcriptional expression levels of IL31 are minimal in cutaneous T-cell lymphoma variants. Biochemistry and biophysics reports (2021) 4;26:101007

van Santen, S., P.M. Jansen, K.D. Quint, M.H. Vermeer, R. Willemze. Plaque-stage folliculotropic mycosis fungoides: histopathologic features and prognostic factors in a series of 40 patients. Journal of Cutaneous Pathology (2020) 45(2): 122-128.

van Santen, S., P.M. Jansen, M.H. Vermeer, R. Willemze. Folliculotropic mycosis fungoides presenting with a solitary lesion: Clinicopathological features and long-term follow-up data in a series of 9 cases. Journal of Cutaneous Pathology (2018) 45(2): 122-128.

van Santen, S., M.H. Vermeer, R. Willemze. Classification and recommended treatment options for folliculotropic mycosis fungoides. Expert Opinion on Orphan Drugs (2017) 6:1, 35-45.

van Santen, S., R. van Doorn, K.J. Neelis, L.A. Daniëls, B. Horvath, M.S. Bruijn, C.J.G. Sanders, M.M. van Rossum, E.R.M. de Haas, J.C.J.M. Veraart, M.W. Bekkenk, M.H. Vermeer, R. Willemze. Recommendations for treatment in folliculotropic mycosis fungoides: report of the Dutch Cutaneous Lymphoma Group. British Journal of Dermatology (2017) 177(1): 223-228.

van Santen, S., R. van Doorn, M.H. Vermeer, R. Willemze. Diagnostiek en behandeling van folliculotrope mycosis fungoides. Nederlands Tijdschrift voor Dermatologie en Venereologie (2016) 26 (11): 677-81.

van Santen, S., R.E.J. Roach, R. van Doorn, B. Horvath, M.S. Bruijn, C.J.G. Sanders, J.C. de Pooter, M.M. van Rossum, E.R.M. de Haas, J.C.J.M. Veraart, M.W. Bekkenk, M.H. Vermeer, R. Willemze. Clinical Staging and Prognostic Factors in Folliculotropic Mycosis Fungoides. JAMA Dermatology (2016) 152(9): 992-1000.

van Santen, S., M.R.T.M. Thissen, N.A. Kukutsch. Melanomen bij kinderen in Nederland. Nederlands Tijdschrift voor Geneeskunde (2015) 159: A9425.

CURRICULUM VITAE

Suzanne van Santen werd geboren op 28 maart 1987 te Strijen. In 2005 behaalde zij cum laude haar eindexamen aan het gymnasium van het Oude Hoven college te Gorinchem en begon in datzelfde jaar haar studie geneeskunde in Maastricht. Haar belangstelling voor de dermatologie werd aangewakkerd gedurende haar coschappen. Dit resulteerde in een keuzestage bij de afdeling dermatologie in Maastricht Universitair Medisch Centrum (MUMC) onder leiding van dermatoloog dr. Kelleners en in een wetenschapsstage betrekking hebbende op de cutane immunologie bij de afdeling immunologie en allergologie in het MUMC onder leiding van allergoloog dr. Nieuwhof. Na een vormingsjaar als basisarts ANIOS interne geneeskunde in het Laurentius ziekenhuis te Roermond maakte zij de stap naar een carrière in de dermatologie en werkte als ANIOS dermatologie in het Catharina Ziekenhuis te Eindhoven onder supervisie van prof. Steijlen. Zowel de klinische als wetenschappelijke kanten van de dermatologie, in het bijzonder het immuunsysteem van de huid, bleven Suzanne boeien en deze interesses kwamen samen in 2015. In dat jaar startte zij onder leiding van prof. Willemze en prof. Vermeer een gecombineerd traject met zowel promotieonderzoek en patiëntenzorg op het gebied van de cutane lymfomen als de opleiding tot dermatoloog aan het Leids Universitair Medisch Centrum (LUMC). Het huidige proefschrift is het resultaat van haar promotieonderzoek. Per 1 januari 2019 is zij gestart met haar specialisatieopleiding tot dermatoloog in het LUMC welke zij momenteel voortzet. Suzanne woont met haar gezin in Den Haag.

NAWOORD

We may dream alone or strive alone, but true success always needs and is best celebrated with the help and support of other people.

(adapted from Farrah Gray, 1984)

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