Flow cytometry for the assessment of blood tumour burden in cutaneous T-cell lymphoma: towards a standardized approach

Maarten H. Vermeer,1 Helene Moins-Teisserenc,2,3 Martine Bagot,4,5 Pietro Quaglino6 and Sean Whittaker7

1Dermatology Department, Leiden University Medical Center, Leiden, the Netherlands
2Université de Paris, Institut de Recherche Saint-Louis, INSERM UMR1160, Paris, France
3Hematology Laboratory and 4Department of Dermatology, AP-HP, Hôpital Saint-Louis, Paris, France
5Université de Paris, Institut de Recherche Saint-Louis, INSERM UMR5976, Onco-Dermatology and Therapies, Paris, France
6Dermatological Clinic, Department of Medical Sciences, University of Turin Medical School, Turin, Italy
7St. John’s Institute of Dermatology, School of Basic and Medical Biosciences, King’s College London, Guy’s Hospital, London, SE1 9RT, UK

Corresponding author
Maarten Vermeer.
Email: m.h.vermeer@lumc.nl

Accepted for publication
9 February 2022

Funding sources
This work was supported by Kyowa Kirin, Inc.

Conflicts of interest
M.H.V. has received research grants from Kyowa Kirin and Takeda, and has also received consultancy grants from Kyowa Kirin. H.M.-T. is a consultant for Innate Pharma and has received research grants from Kyowa Kirin. H.M.-T. has been an advisor and speaker for Helsinn-Recordati, Innate Pharma, Kyowa Kirin, Takeda and Galderma. P.Q. received honoraria for his participation in advisory boards and speaker fees from Therakos, 4SC, Kyowa Kirin, Takeda, Celgene and Helsinn. S.W. declares no conflicts of interest.

Ethics statement
Ethics approval was not required for this review.

DOI 10.1111/bjd.21053

Abstract

Mycosis fungoides (MF) and Sézary syndrome (SS) are the best-studied subtypes of cutaneous T-cell lymphoma, a rare non-Hodgkin lymphoma that primarily presents in the skin but can also involve blood, lymph nodes and viscera. The role of blood involvement in the assessment and staging of MF and SS has evolved in recent years from being classed as simply ‘present’ or ‘absent’, with no impact on staging, to full analysis of abnormal peripheral blood T cells using flow cytometry (FC) to detect and quantify aberrant T-cell phenotypes and polymerase chain reaction (PCR) to characterize T-cell receptor gene rearrangements. These sensitive peripheral blood assessments are replacing manual Sézary cell counts and have become an important part of clinical workup in MF and SS, providing the potential for more accurate prognosis and appropriate management. However, although international recommendations now include guidelines for FC analysis of peripheral blood markers for staging purposes, many clinics only perform these analyses in patients with advanced-stage lymphoma, if at all, and there is still a need for standardized use of validated markers. Standardization of a single effective multiparameter FC panel would allow for accurate identification and quantification of blood tumour burden for diagnosis, staging, assessment of therapeutic response, and monitoring of disease progression at all stages of disease. Once defined, validation of an MF/SS biomarker FC panel will enable uptake into clinical settings along with associated standardization of protocols and reagents. This review discusses the evolution of the role of FC in evaluating blood involvement in MF and SS, considers recently published international guidelines and identifies evidence gaps for future research that will allow for standardization of FC in MF and SS.

Introduction

Cutaneous T-cell lymphomas (CTCL) are a heterogeneous group of non-Hodgkin T-cell lymphomas that primarily present in the skin but can also progress to involve blood, lymph nodes and viscera. The best-studied CTCL subtypes are mycosis fungoides (MF) and Sézary syndrome (SS), together accounting for around two-thirds of all CTCL cases.1,2 Disease-specific staging in MF and SS is the major predictor of prognosis.3–5 MF is generally considered to be indolent with a variable type and extent of skin disease, and includes a subset of patients presenting with or developing extracutaneous disease.6 Previously thought to originate from mature tissue-resident T cells expressing skin-homing markers, multifocal skin presentation and failure of skin-directed treatments to
achieve cure or long-term remissions, represent two aspects of the disease that disagree with this model of pathogenesis. Recent evidence suggests that neoplastic T-cell clones are present in the peripheral blood of patients with MF, even in the early stages of the disease, and haematogenous spread of neoplastic cells may consecutively seed skin lesions.\(^7\)–\(^9\)

Early-stage MF (stage IA, IB, IIA) often has little impact on life expectancy; estimated median survival for stage IA disease is 35–5 years.\(^1\)\(^,\)\(^4\)\(^,\)\(^5\)\(^,\)\(^10\)\(^,\)\(^11\) However, up to 34% of patients with early-stage disease progress to advanced-stage disease (stage IIB to IVB); overall survival is estimated at 4–7 years for stage IIB compared with 15–8 years for stage IIA.\(^1\)\(^,\)\(^4\)

SS is a rare and aggressive CTCL subtype characterized by overt blood involvement (leukaemic cell count ≥ 1000 per mm\(^3\)) at diagnosis, erythroderma and generalized lymphanenopathy, and is associated with a median survival of around 3 years.\(^1\)\(^,\)\(^2\)\(^,\)\(^4\)

In 2007, blood classification was added to the tumour–node–metastasis (TNM) classification of MF/SS by the International Society for Cutaneous Lymphomas (ISCL) and the European Organisation for Research and Treatment of Cancer (EORTC), based on recognition of blood involvement as an independent negative prognostic factor, leading to the TNM-blood (TNMB) classification system.\(^12\)\(^–\)\(^16\) In these recommendations, blood assessment was based on both morphology (manual Széary cell counting) and flow cytometry (FC). It was acknowledged that FC offered a more objective tool for identifying and quantifying neoplastic lymphocytes in blood\(^1\)\(^,\)\(^2\) compared with manual cell counts, which are notoriously subjective.

In recent years, FC usage has expanded in this indication and has been demonstrated to be of value for disease staging, prognostic evaluation, guiding treatment approach, and therapeutic response assessment. However, there is still a need for standardization of FC methodologies to provide objective, consistent and quantifiable measures of blood tumour burden in MF/SS.\(^1\)\(^,\)\(^17\) This review discusses the evolution of FC in evaluating blood involvement in MF/SS, considers recently published international guidelines, and identifies evidence gaps for future research that will allow for FC standardization in MF/SS.

**History of the development of flow cytometry in cutaneous T-cell lymphomas**

Before the TNMB classification, blood involvement was considered to be either ‘absent’ (B\(_0\)) or ‘present’ (B\(_1\)) and was not included in clinical staging (Table 1).\(^18\) In 2007, the ISCL/EORTC revised MF/SS staging and classification guidelines defined the following three stages of blood involvement: B\(_0\), absence of significant blood involvement; B\(_1\), low blood tumour burden and B\(_2\), high blood tumour burden (Table 1).\(^12\) In recognition of the fact that proportional measures can vary depending on overall leucocyte count, the use of absolute counts was suggested in a 2011 ISCL/United States Cutaneous Lymphoma Consortium (USCLC)/EORTC update,\(^19\) with a subsequent update published in 2021.\(^20\) These definitions are still used in relevant MF/SS treatment guidelines, including those of the EORTC, the European Society for Medical Oncology (ESMO), and the National Comprehensive Cancer Network in the USA.\(^21\)\(^–\)\(^23\) The 2018 ESMO guidelines recommend peripheral blood FC for all MF stages but state that the test may be more appropriate in patients with suspected SS, while the EORTC recommend FC for measuring blood involvement at all MF stages and in SS, as accurate blood class assignment is necessary for overall disease staging and consequent management.\(^17\)\(^,\)\(^22\)

The ISCL/EORTC criteria also provided subcategories for B\(_0\)/B\(_1\) disease to account for the absence or presence of a T-cell clone identified by analysis of the T-cell receptor (TCR) gene (Table 1). Polymerase chain reaction (PCR) has replaced Southern blot analysis as the ‘gold standard’ for clonality testing\(^24\) and the development of standardized BIOMED-2/ EuroClonality multiplex PCR protocols has greatly supported T-cell clonality assessment and diagnosis of lymphoid malignancies.\(^25\)\(^,\)\(^26\) TCR gene rearrangement in peripheral blood T cells can occur early in MF and has prognostic significance if it is the same as that found in the skin.\(^1\)\(^,\)\(^12\)\(^,\)\(^15\) Based on these criteria, diagnosis of SS requires B\(_2\) blood involvement with a T-cell clone in peripheral blood identical to that found in the skin.\(^1\)\(^,\)\(^12\)\(^,\)\(^19\)\(^,\)\(^23\) B\(_2\)-level blood involvement can also occur rarely in patients with MF who have nonerythrodermic disease.\(^1\)\(^,\)\(^27\)

Historically, detection of Széary cells has been performed using light-microscopic analysis, a time-consuming and subjective technique with high rates of interobserver variability.\(^12\)\(^,\)\(^18\)\(^,\)\(^29\) This has now largely been replaced by FC analysis, which offers immunophenotyping of T-cell subsets and assessment of clonality.\(^17\)\(^,\)\(^28\)\(^,\)\(^29\) TCR gene sequencing in conjunction with FC can increase blood classification accuracy, particularly for patients with low tumour burden.\(^1\)

**Current guidelines for peripheral blood staging and assessment of blood response**

There was previously no objective definition of blood class using FC, and centres adopted different definitions in their publications. The potential use of FC to measure numbers of CD4\(^+\) CD26\(^–\) and/or CD4\(^+\) CD7\(^–\) T-cell populations to quantify blood involvement was discussed in the 2011 ISCL/USCLC/EORTC consensus statement as a reasonable and quantifiable method for use in clinical trials.\(^19\) An EORTC Cutaneous Lymphoma Task Force Committee later published recommendations for blood classification and blood response criteria to bridge the transition from manual Széary cell counting to a more standardized use of FC.\(^17\) FC analysis was recommended to count absolute numbers of abnormal (CD4\(^+\) CD26\(^–\) and/or CD4\(^+\) CD7\(^–\)) T cells, owing to the potential for proportional skewing based on overall lymphocyte counts,\(^30\) and recommended FC as the method to measure blood involvement in all stages of MF and SS.\(^17\)

The most recent guidelines update (ISCL/USCLC/EORTC) published in 2021, defines blood classifications B\(_0\), B\(_1\) and B\(_2\)
Table 1  Evolution of blood involvement classification for mycosis fungoides and Sézary syndrome

| Classification                      | B0: No significant Sézary cell presence (< 5%) |
|------------------------------------|------------------------------------------------|
| TNM classification established1    | • B0: Lack of significant blood involvement, indicated by ≤ 5% Sézary cells |
|                                    | • B1: Low blood tumour burden, indicated by > 5% Sézary cells, but < 1000 per µL and/or absence of T-cell clone |
|                                    | • B1a: T-cell clone absent |
|                                    | • B1b: T-cell clone present |
|                                    | • B2: High blood tumour burden, indicated by ≥ 1000 Sézary cells per µL with T-cell clone present |
|                                    | • Alternatively, Sézary cell count may be substituted by CD4+ or CD3+ cells with CD4/CD8 ratio of ≥ 10 |
|                                    | or expanded CD4+ cells with abnormal immunophenotype including CD7 or CD26 loss (either ≥ 40% CD4+ CD7− or ≥ 30% CD4+ CD26−) |
| TNM classification revised to TNMB12| • Flow cytometry used to measure absolute CD4+ CD7− or CD4+ CD26− counts |
|                                    | • B0: Absolute count is < 250 per µL |
|                                    | • B1: Absolute count is from 250 per µL to < 1000 per µL |
|                                    | • B2: Absolute count is ≥ 1000 per µL with T-cell clone present |
| EORTC-CLTF recommendation,17 based upon parameters first presented by ISCL-USCLC-EORTC19| • Flow cytometry used to measure absolute CD4+ CD7− or CD4+ CD26− counts or another aberrant lymphocyte population that has been identified by flow cytometry. Absolute counts should be determined by the percentage of aberrant lymphocytes identified by flow cytometry, multiplied by the total lymphocyte count of a complete blood count. Alternatively, the percentage of aberrant CD45+ leukocytes multiplied by the white blood cell count may be used |
| ISCL-USCLC-EORTC modified staging20| • B0: Absolute count is < 250 per µL |
|                                    | • B1a: T-cell clone absent or equivocal |
|                                    | • B1b: T-cell clone present and identical to skin |
|                                    | • B2a: T-cell clone absent or equivocal |
|                                    | • B2b: T-cell clone present and identical to skin |
|                                    | • B3: Blood involvement cannot be quantified according to agreed guidelines |
|                                    | • B3a: T-cell clone absent or equivocal |
|                                    | • B3b: T-cell clone present and identical to skin |

CLTF, Cutaneous Lymphoma Task Force; EORTC, European Organisation for Research and Treatment of Cancer; ISCL, International Society for Cutaneous Lymphoma; TNM, tumour–node–metastasis; TNMB, tumour–node–metastasis–blood; USCLC, United States Cutaneous Lymphoma Consortium.

by FC using an absolute count of CD4+ CD26−, CD4+ CD7− or other aberrant lymphocyte population identified by FC, with an additional Bc classification where blood involvement cannot be quantified according to agreed guidelines (Table 1).20 T-cell counts are based on flow gating using markers to select cells of interest. However, gating strategies vary and, therefore, a set of basic gating principles has been proposed.31

This recommendation for FC evaluation of the immunophenotype of abnormal T cells in blood complements the analysis of skin biopsy samples in which immunohistochemical analysis of CD2, CD3, CD4, CD5, CD7, CD8, CD20 and CD30 expression and molecular analysis of TCR gene rearrangements are recommended.23

Despite international recommendations, FC analysis and tracking of blood involvement is often performed only in patients with advanced-stage disease.20,21 Therefore, we present our consensus recommendations for cases where use of FC in MF/SS is especially appropriate, in centres where it may not be practical or possible to assess and track blood tumour burden for all patients (Table 2).

Biomarkers used for flow cytometry analysis of mycosis fungoides and Sézary syndrome peripheral blood involvement

Although the EORTC definitions are of value and utilized in clinical practice, there is still need for a widely used
Recent efforts have focused on further identifying the most useful cell-surface markers to immunophenotypically characterize Sézary cells and distinguish them from non-neoplastic T cells. The most common abnormalities in T cells of MF/SS are the loss of CD7 or CD16 followed by reduced or absent CD2 and/or CD3. Identification of higher numbers of T cells with abnormal expression of CD3, CD7, CD26 or TCR variants (TCRαβ, TCRγδ) has been correlated with more advanced stages of MF and shown to predict disease progression. Recently, the six-biomarker panel of CD3, CD4, CD7, CD8, CD26 and CD45 has been recommended as the minimum combination of antibodies to be included in a single six-colour FC assay for MF/SS.

Flow cytometry use: consensus recommendations

| Clinical flags for the use of flow cytometry | How often during follow-up? |
|--------------------------------------------|-----------------------------|
| Patient with advanced-stage disease (stage IIB and above) | Every 3 months in those patients with abnormal flow cytometry at baseline |
| Intractable pruritus | In the case of disease/stage progression |
| Generalized patches and/or plaques (T2A/T2B) | Upon development of any clinical flags presented in the left-hand column |
| Erythroderma | |
| Lymphocytosis on WBC | |
| High serum LDH | |
| Lack of response to treatment | |

LDH, lactate dehydrogenase; WBC, white blood cell count.

standardized and validated set of markers. Recent efforts have focused on further identifying the most useful cell-surface markers to immunophenotypically characterize Sézary cells and distinguish them from non-neoplastic T cells.

Table 2: Consensus recommendations for minimum use of flow cytometry for mycosis fungoides and Sézary syndrome when not performed at all stages.

The most common abnormalities in T cells of MF/SS are the loss of CD7 or CD16 followed by reduced or absent CD2 and/or CD3. Identification of higher numbers of T cells with abnormal expression of CD3, CD7, CD26 or TCR variants (TCRαβ, TCRγδ) has been correlated with more advanced stages of MF and shown to predict disease progression. Recently, the six-biomarker panel of CD3, CD4, CD7, CD8, CD26 and CD45 has been recommended as the minimum combination of antibodies to be included in a single six-colour FC assay for MF/SS.

The presence of cell populations with low (‘dim’) expression of certain markers on FC has been included as part of the diagnostic criteria for other haematological malignancies. SS has been commonly reported to include a ‘dim’ CD3, CD4 or CD2 population. It has been suggested that the presence of ‘dim’ T-cell marker expression, coupled with CD26 and/or TCR-Vβ analysis, may represent a good tool for tumour burden assessment. Good resolution of a ‘dim’ population is reliant on fluorochrome choice, particularly in multicoulour combinations.

While the CD3+ CD4+ CD7+ and/or CD26+ immunophenotype is characteristic of MF/SS, it is not specific, as intrapatient heterogeneity of MF/SS cell markers has been observed. Healthy CD4+ T cells in patients with SS often display aberrant phenotypes, not only with regard to CD7 and CD26, but also in relation to the usual markers of naive/memory cells and exhaustion, similar to those seen in Sézary cells.

Additional potentially useful markers for FC are also under evaluation, although no consensus has been reached. It is suggested that additional antigens should be combined with CD3, CD4, CD, and CD26, at the very least (Figure 1).
Recent studies have investigated the expression of killer-cell immunoglobulin-like receptors (KIRs) (e.g. CD158a, CD158b and CD158k) and adhesion molecules (CD164); however, results have generally been inconclusive. The identification of KIR3DL2/CD158k on malignant cells in SS and advanced MF has greatly helped the detailed study of the malignant clone. Since it was first described by Bagot et al. in 2001, the reliability of this marker for detecting Sézary cells in blood and skin has been demonstrated by several teams. In a recent study, the presence of KIR3DL2+ Sézary cells ≥ 200 μL−1 correlated with an SS diagnosis at a sensitivity of 88.6% and specificity of 96.3%; this contrasts with Boonk et al. who found a similar specificity of 95%, but a sensitivity of only 33%. However, the difference in sensitivity may be related to the use of fresh vs. frozen samples in the Roelens and Boonk studies, respectively. Tracking Sézary cells with KIR3DL2/CD158k, which remains constant over time, allows discrimination from benign cells that may have the same aberrant phenotypes with defects in nonspecific marker expression. Indeed, the decrease of such normal T cells expressing CD4+ CD7− or CD4+ CD26− may not indicate neoplastic cell clearance and, conversely, the presence of these cells may underestimate the rate of blood response. Since 2014, KIR3DL2/CD158k analysis has formed part of the routine care of patients with erythroderma at Hospital Saint-Louis in Paris, and, together with Bordeaux and Clermont-Ferrand centres, the Moin-Tessierenc team provides harmonized FC protocols to all French laboratories and standardized guidelines for analysis. Further international validation of this marker is required to confirm its usefulness for diagnostics, prognostics and evaluation of therapies.

As T-cell antigenic variations are not always specific to MF/SS, evaluation of TCR gene rearrangements is also recommended to confirm clonality in diagnosis and monitoring, and to correlate with other molecular findings from skin or node biopsy analyses. This is critical, as peripheral blood nonpathogenic T-clones can occur in healthy individuals, especially with increasing age, but these clones are not identical to those in skin or node biopsies. Identification of an identical T-cell clone in the skin and in the blood has been shown to be associated with a poor prognosis, even in patients with a B3 blood classification.

The use of FC panels of TCR-Vβ-specific antibodies to screen for Vβ domain expression in T-cell proliferations was originally proposed two decades ago, based on the assessment of large series of antibodies in normal and malignant T cells. This method has shown efficacy for both initial blood staging and for assessment of therapeutic response, and can be used to confirm that the features of the T-cell clone in blood are similar to those of the skin biopsy T cells. However, this TCR-Vβ-specific antibody panel does not identify all different TCR-Vβ chain variables, and the expression of different TCR-Vβ varies in healthy individuals, making interpretation of small clones challenging.

The future of flow cytometry in mycosis fungoides/Sézary syndrome

While progress has been made towards development of a standardized biomarker set to identify and track disease in MF/SS, further improvements are still required and protocols must be disseminated to encourage universal adoption of a single effective and validated standard. Standardization of a single effective multiparameter FC panel will allow for accurate identification and quantification of blood tumour burden for diagnosis, staging and assessment of therapeutic response,
including the development of escape variants and monitoring of disease progression. Blood staging is not only relevant to initial diagnosis, but also as an indicator of response to therapy. Thus, FC needs to be evaluated as a criterion for determining blood response, and as a method for both detection of resistant clones to therapy and minimal residual disease assessment. Moreover, FC can be useful to identify the expression of specific cell markers to be recognized by monoclonal antibodies for treatment as targeted therapies. Current potential therapeutic targets and relevant therapies include CD30 (brentuximab vedotin, licensed in CD30+ CTCL), CCR4 (mogamulizumab), PD-1 (nivolumab and pembrolizumab), CD158k/KIR3DL2 (lacutamab), CD47 (TTI-621) and CD52 (alemtuzumab).

One example of a successful standardization method is the EuroFlow programme, established to develop standardized protocols for diagnostic use and monitoring treatment response by FC in various lymphomas and leukaemias. This programme is being used to design a marker set for MF/SS, with the six-biomarker antibody set and TCR clonality markers as a starting point. For example, CD3, CD4, CD8 and CD45 would be included as the backbone markers, and CD7 and CD26 would be included to identify MF/SS cells. Other markers could then be tested in combination with this backbone set to identify and validate the most clinically useful final set to be used in conjunction with TCR clonality testing. Prospective multicentre studies and collaborations between EORTC and EuroFlow are under way to develop rapid, accurate, standardized protocols and validated cut-off values for the assessment of blood involvement in CTCL.

Accurate quantification of blood involvement could also help to ascertain whether it would be clinically relevant to identify a further cut-off, higher than B2, to characterize patients with a very high blood burden, potentially with a worse prognosis, who therefore would be candidates for a more aggressive therapeutic strategy.

There are a number of barriers to overcome in the use of FC for the assessment of blood tumour burden in CTCL. Firstly, markers may overlap with normal or reactive T-cell populations. Secondly, clonality evaluation using TCR-Vβ antibodies covers only about 70% of the TCR repertoire, is ineffective for those T cells that have lost TCR expression, and is expensive to perform. Finally, standardized protocols, including the development of standard operating procedures for reagents, procedures and analysis, must be adhered to and technicians should be trained in the use of appropriate reagents.

Recent FC studies using EuroFlow-based panels to analyse blood samples from 24 patients with SS demonstrated extensive inter- and intrapatient phenotypic heterogeneity with changes over time, which could be tracked by next-generation sequencing of TCRs to identify a patient’s tumour-specific ‘barcode’. Multicentre follow-up studies are currently being performed to develop a new standard for improved blood staging and disease monitoring.

Conclusions

This review focuses on the relevance of adequate blood monitoring in patients with MF/SS during the disease course in order to better tailor treatment strategies and define the clinical behaviour of the disease. Recent advances support the use of FC to quantify blood tumour burden, using absolute counts of abnormal T cells, but there is a need to test the clinical implications of this in prospective studies. Moreover, an adequate blood tumour burden definition could help in identifying the prognostic and/or predictive role played by blood involvement in early disease, and in understanding whether patients with a very high blood tumour burden could be classified in a separate stage. For this objective, and to better characterize the clonal evolution and potential heterogeneity of circulating T-cell subclones, a better definition of the circulating clonal cells is needed, specifically in terms of FC markers that would be able to identify neoplastic cells.

Acknowledgments

The authors would like to thank Emma Butterworth of Excerpta Medica for medical writing assistance, which was funded by Kyowa Kirin, Inc.

References

1 Vermeer MH, Nicolay JP, Scarisbrick JJ, Zinzani PL. The importance of assessing blood tumour burden in cutaneous T-cell lymphoma. Br J Dermatol 2021; 185:19–25.
2 Willemze R, Cerroni L, Kempf W et al. The 2018 update of the WHO-EORTC classification for primary cutaneous lymphomas. Blood 2019; 133:1703–14.
3 Horwitz SM, Olsen EA, Ducic M et al. Review of the treatment of mycosis fungoides and Sézary syndrome: a stage-based approach. J Natl Compr Canc Netw 2008; 6:436–42.
4 Agar NS, Wedgeworth E, Crichton S et al. Survival outcomes and prognostic factors in mycosis fungoides/Sézary syndrome: validation of the revised International Society for Cutaneous Lymphomas/European Organisation for Research and Treatment of Cancer staging proposal. J Clin Oncol 2010; 28:4730–9.
5 Maguire A, Puelles J, Raboisson P et al. Early-stage mycosis fungoides: epidemiology and prognosis. Acta Derm Venereol 2020; 100: adv0013.
6 Kim YH, Bagot M, Pinter-Brown L et al. Mogamulizumab versus vorinostat in previously treated cutaneous T-cell lymphoma (MAVORIC): an international, open-label, randomised, controlled phase 3 trial. Lancet Oncol 2018; 19:1192–204.
7 Iyer A, Hennessey D, O’Keefe S et al. Skin colonization by circulating neoplastic clones in cutaneous T-cell lymphoma. Blood 2019; 134:1517–27.
8 Xiao MZX, Hennessey D, Iyer A et al. Transcriptomic changes during stage progression of mycosis fungoides. Br J Dermatol 2022; 186:520–31.
9 Dobos G, Assaf C. Transcriptomic changes during stage progression of mycosis fungoides: from translational analyses to their potential clinical implications. Br J Dermatol 2022; 186:387–8.
10 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; 118:5830–9.
11 Scarisbrick JJ, Quaglini P, Prince HM et al. The PROCLIP international registry of early-stage mycosis fungoides identifies substantial diagnostic delay in most patients. Br J Dermatol 2019; 181:350–7.
12 Olsen E, Vonderheid E, Pimpinelli N et al. Revisions to the staging and classification of mycosis fungoides and Sézary syndrome: a proposal of the International Society for Cutaneous Lymphomas (ISCL) and the cutaneous lymphoma task force of the European Organisation of Research and Treatment of Cancer (EORTC). Blood 2007; 110:1713–22.
13 Kim YH, Liu HL, Mraz-Gernhardt S et al. Long-term outcome of 525 patients with mycosis fungoides and Sézary syndrome: clinical prognostic factors and risk for disease progression. Arch Dermatol 2003; 139:857–66.
14 Toro JR, Stoll HL Jr, Stomper PC, Oseroft AR. Prognostic factors and evaluation of mycosis fungoides and Sézary syndrome. J Am Acad Dermatol 1997; 37:58–67.
15 Fraser-Andrews EA, Woolford AJ, Russell-Jones R et al. Detection of a peripheral blood T cell clone is an independent prognostic marker in mycosis fungoides. J Invest Dermatol 2000; 114:17–21.
16 Scarisbrick JJ, Whittaker S, Evans AV et al. Prognostic significance of tumor burden in the blood of patients with erythrodermic primary cutaneous T-cell lymphoma. Blood 2001; 97:624–30.
17 Scarisbrick JJ, Hodak E, Bagot M et al. Blood classification and blood response criteria in mycosis fungoides and Sézary syndrome using flow cytometry: recommendations from the EORTC cutaneous lymphoma task force. Eur J Cancer 2018; 93:47–56.
18 Bunn PA Jr, Lamberg SI. Report of the Committee on Staging and Classification of Cutaneous T-Cell Lymphomas. Cancer Treat Rep 1979; 63:725–8.
19 Olsen EA, Whittaker S, Kim YH et al. Clinical end points and response criteria in mycosis fungoides and Sézary 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; 29:5958–607.
20 Olsen EA, Whittaker S, Willemze R et al. Primary cutaneous lymphoma: recommendations for clinical trial design and staging update from the ISCL, USCLC, and EORTC. Blood 2021; https://doi.org/10.1182/blood.2021020507.
21 Trautinger F, Eder J, Assaf C et al. European Organisation for Research and Treatment of Cancer consensus recommendations for the treatment of mycosis fungoides/Sézary syndrome – update 2017. Eur J Cancer 2017; 77:57–74.
22 Willemze R, Hodak E, Zinzani PL et al. Primary cutaneous lymphomas: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2018; 29 (Suppl. 4):iv30–40.
23 National Comprehensive Cancer Network Guidelines. Primary cutaneous lymphomas, version 2.2021. Available at: https://www.nccn.org/guidelines/guidelines-detail?category=1&id=1491 (last accessed 26 March 2021).
24 Langerak AW, Groenen PJ, Brüggemann M et al. EuroClonality/BIOMED-2 guidelines for interpretation and reporting of Ig/TCR clonality testing in suspected lymphoproliferations. Leukemia 2012; 26:2159–71.
25 van Dongen JJ, Langerak AW, Brüggemann M et al. Design and standardization of PCR primers and protocols for detection of clonal immunoglobulin and T-cell receptor gene recombinations in suspect lymphoproliferations: report of the BIOMED-2 Concerted Action BMH4-CT98-3936. Leukemia 2003; 17:2257–317.
26 Brüggemann M, White H, Gaulard P et al. Powerful strategy for polymerase chain reaction-based clonality assessment in T-cell malignancies Report of the BIOMED-2 Concerted Action BMH4 CT98-3936. Leukemia 2007; 21:215–21.
27 Talpur R, Singh L, Daulat S et al. Long-term outcomes of 1,263 patients with mycosis fungoides and Sézary syndrome from 1982 to 2009. Clin Cancer Res 2012; 18:5051–60.
28 Morice WG, Katzmann JA, Pittelkow MR et al. A comparison of morphologic features, flow cytometry, TCR-Vbeta analysis, and TCR-PCR in qualitative and quantitative assessment of peripheral blood involvement by Sézary syndrome. Am J Clin Pathol 2006; 125:364–74.
29 Guitart J. Sézary syndrome and mycosis fungoides flow cytometric evaluation: the clinicians’ perspective. Cytometry B Clin Cytom 2021; 100:129–31.
30 Scarisbrick JJ. Staging of mycosis fungoides and Sézary syndrome: time for an update? EM Hemoth 2018; 6:92–100.
31 Horna P, Wang S, Wolniak KL et al. Flow cytometric evaluation of peripheral blood for suspected Sézary syndrome or mycosis fungoides: international guidelines for assay characteristics. Cytometry B Clin Cytom 2021; 100:142–53.
32 Boonk SE, Zoutman WH, Marie-Cardine A et al. Evaluation of immunophenotypic and molecular biomarkers for Sézary syndrome using standard operating procedures: a multicenter study of 59 patients. J Invest Dermatol 2016; 136:1364–72.
33 Hristov AC, Vonderheid EC, Borowitz MJ. Simplified flow cytometric assessment in mycosis fungoides and Sézary syndrome. Am J Clin Pathol 2011; 136:944–53.
34 Najid S, Tensen CP, van der Sluijs-Gelling AJ et al. Improved Sézary cell detection and novel insights into immunophenotypic and molecular heterogeneity in Sézary syndrome. Blood 2021; 138:2539–54.
35 Yazici S, Bülşüş Başkan E, Budak F et al. Flow cytometric analysis of T, B, and NK cells antigens in patients with mycosis fungoides. J Immunol Res 2015; 2015:856340.
36 Illingsworth A, Johansson U, Huang S et al. International guidelines for the flow cytometric evaluation of peripheral blood for suspected Sézary syndrome or mycosis fungoides: assay development/optimization, validation, and ongoing quality monitors. Cytometry B Clin Cytom 2021; 100:156–82.
37 Bernengo MG, Novelli M, Quaglini P et al. The relevance of the CD4+ CD26- subset in the identification of circulating Sézary cells. Br J Dermatol 2001; 144:125–35.
38 Kelemen K, Guitart J, Kuzel TM et al. The usefulness of CD26 in flow cytometric analysis of peripheral blood in Sézary syndrome. Am J Clin Pathol 2008; 129:146–56.
39 Roelens M, de Masson A, Ram-Wolff C et al. Revisiting the initial diagnosis and blood staging of mycosis fungoides and Sézary syndrome with the KIR3DL2 marker. Br J Dermatol 2020; 182:1415–22.
40 Rawstron AC, Kreuzer KA, Soosapilla A et al. Reproducible diagnosis of chronic lymphocytic leukemia by flow cytometry: an European Research Initiative on CLL (ERIC) & European Society for Clinical Cell Analysis (ESCCA) Harmonisation project. Cytometry B Clin Cytom 2018; 94:121–8.
41 Novelli M, Fava P, Sarda C et al. Blood flow cytometry in Sézary syndrome: new insights on prognostic relevance and immunophenotypic changes during follow-up. Am J Clin Pathol 2015; 143:57–69.
42 van Dongen JJ, Lhermitte L, Böttcher S et al. EuroFlow antibody panels for standardized n-dimensional flow cytometric
immunophenotyping of normal, reactive and malignant leukocytes. Leukemia 2012; 12:1908–75.
43 Jones CL, Ferreira S, McKenzie RC et al. Regulation of T-plastin expression by promoter hypomethylation in primary cutaneous T-cell lymphoma. J Invest Dermatol 2012; 132:2042–9.
44 Pullitzer MR, Horna P, Almeida J. Sézary syndrome and mycosis fungoides: An overview, including the role of immunophenotyping. Cytometry B Clin Cytom 2021; 100:132–8.
45 Roelens M, Delord M, Ram-Wolff C et al. Circulating and skin-derived Sézary cells: clonal but with phenotypic plasticity. Blood 2017; 130:1468–71.
46 Roelens M, de Masson A, Ram-Wolff C et al. Letter to the editor with regard to the article entitled “Sézary syndrome and mycosis fungoides: An overview, including the role of immunophenotyping”. Cytometry B Clin Cytom 2021; 100:139–40.
47 Campbell JJ, Clark RA, Watanabe R, Kupper TS. Sézary syndrome and mycosis fungoides arise from distinct T-cell subsets: a biologic rationale for their distinct clinical behaviors. Blood 2010; 116:767–71.
48 Ferenczi K, Fulbright RC, Pinkus J et al. Increased CCR4 expression in cutaneous T cell lymphoma. J Invest Dermatol 2002; 119:1405–10.
49 Huet D, Bagot M, Loyaux D et al. SC5 mAb represents a unique tool for the detection of extracellular vimentin as a specific marker of Sézary cells. J Immunol 2006; 176:652–9.
50 Johnson LD, Banerjee S, Kruglov BE et al. Targeting CD47 in Sézary syndrome with SIRPα. Blood Adv 2019; 3:1145–53.
51 Picker LJ, Michie SA, Rott LS, Butcher EC. A unique phenotype of naive T cells in contact dermatitis is due to differential tissue usage in the leukemic phase of cutaneous T cell lymphoma. J Exp Med 2005; 204:651–61.
52 Sakamoto M, Miyagaki T, Kamijo H et al. CD147-cyclophilin A interactions promote proliferation and survival of cutaneous T-cell lymphoma. Int J Mol Sci 2021; 22:7889.
53 Samimi S, Benoit B, Evans K et al. Increased programmed death-1 expression on CD4+ T cells in cutaneous T-cell lymphoma: implications for immune suppression. Arch Dermatol 2010; 146:1382–8.
54 Sterry W, Bruhn S, Kienue N et al. Dominance of memory over naïve T cells in contact dermatitis is due to differential tissue immigration. Br J Dermatol 1990; 123:59–64.
55 Vonderheid EC, Boselli CM, Conroy M et al. Evidence for restricted Vβ usage in the leukemic phase of cutaneous T-cell lymphoma. J Invest Dermatol 2005; 124:651–61.
56 Willemze R, van Vlodt WA, Hermans J et al. Diagnostic criteria in Sézary’s syndrome: a multiparameter study of peripheral blood lymphocytes in 32 patients with erythroderma. J Invest Dermatol 1983; 81:392–7.
57 Wood GS, Hong SB, Sasaki DT et al. Les-8/CD7 antigen expression by CD3+ T cells: comparative analysis of skin and blood in mycosis fungoides/Sézary syndrome relative to normal blood values. J Am Acad Dermatol 1990; 22:602–7.
58 Lima M, Almeida J, dos Anjos Teixeira M et al. Utility of flow cytometry immunophenotyping and DNA ploidy studies for diagnosis and characterization of blood involvement in CD4+ Sézary’s syndrome. Hematologica 2003; 88:874–87.
59 Jiang L, Yuan CM, Hubachek J et al. Variable CD52 expression in mature T cell and NK cell malignancies: implications for alemtuzumab therapy. Br J Haematol 2009; 145:173–9.