BJD
British Journal of Dermatology

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 Quaglino[6] and Sean Whittaker[7]

[1]Dermatology Department, Leiden University Medical Center, Leiden, the Netherlands
[2]Université de Paris, Institut de Recherche Saint-Louis, INSERM UMR1160, Paris, France
[3]Haematology Laboratory and [4]Departement of Dermatology, AP-HP, Hôpital Saint-Louis, Paris, France
[5]Université de Paris, Institut de Recherche Saint-Louis, INSERM UMRS976, Onco-Dermatology and Therapies, Paris, France
[6]Dermatological Clinic, Department of Medical Sciences, University of Turin Medical School, Turin, Italy
[7]St. John's Institute of Dermatology, School of Basic and Medical Biosciences, King's College London, Guy's Hospital, London, SE1 9RT, UK

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.

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. M.B. 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

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.[1] 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 lymphadenopathy, 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 Sézary cell counting) and flow cytometry (FC). It was acknowledged that FC offered a more objective tool for identifying and quantifying neoplastic lymphocytes in blood[12] 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.[4,12,15] 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.[1,2,19,23] B_2-level blood involvement can also occur rarely in patients with MF who have nonerythrodermic disease.[1,27]

Historically, detection of Sézary cells has been performed using light-microscopic analysis, a time-consuming and subjective technique with high rates of interobserver variability.[12,28,29] 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 Sézary 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

TNM classification established[1]	• B0: No significant Sézary cell presence (< 5%) • B1: Sézary cells present (> 5%); record total white blood cell count, total lymphocyte counts and number of Sézary cells per 100 lymphocytes

TNM classification revised to TNMB[12]	• B_0: Lack of significant blood involvement, indicated by ≤ 5% Sézary cells
	  • B_{0a}: T-cell clone absent
	  • B_{0b}: T-cell clone present
	• B_1: Low blood tumour burden, indicated by > 5% Sézary cells, but < 1000 per μL and/or absence of T-cell clone
	  • B_{1a}: T-cell clone absent
	  • B_{1b}: T-cell clone present
	• B_2: 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−)

EORTC-CLTF recommendation,[17] based upon parameters first presented by ISCL-USCLC-EORTC[19]	• Flow cytometry used to measure absolute CD4+ CD7− or CD4+ CD26− counts • B_0: Absolute count is < 250 per μL • B_1: Absolute count is from 250 per μL to < 1000 per μL • B_2: Absolute count is ≥ 1000 per μL with T-cell clone present

ISCL-USCLC-EORTC modified staging[20]	• 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+ leucocytes multiplied by the white blood cell count may be used
	• B_0: Absolute count is < 250 per μL
	  • B_{0A}: T-cell clone absent or equivocal
	  • B_{0B}: T-cell clone present and identical to skin
	• B_1: Absolute count is from 250 per μL to < 1000 per μL
	  • B_{1A}: T-cell clone absent or equivocal
	  • B_{1B}: T-cell clone present and identical to skin
	• B_2: Absolute count is ≥ 1000 per μL
	  • B_{2A}: T-cell clone absent or equivocal
	  • B_{2B}: T-cell clone present and identical to skin
	• B_X: Blood involvement cannot be quantified according to agreed guidelines
	  • B_{XA}: T-cell clone absent or equivocal
	  • B_{XB}: 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 B_X 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.[29,30] 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

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

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) • Intractable pruritus • Generalized patches and/or plaques (T2A/T2B) • Erythroderma • Lymphocytosis on WBC • High serum LDH • Lack of response to treatment	• Every 3 months in those patients with abnormal flow cytometry at baseline • In the case of disease/stage progression • Upon development of any clinical flags presented in the left-hand column

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

standardized and validated set of markers.[17,31] 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.[31,32]

The most common abnormalities in T cells of MF/SS are the loss of CD7 or CD26 followed by reduced or absent CD2 and/or CD3.[28,33,34] 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.[33,35] 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.[31,36] CD3 is associated with the TCR and has value for gating the FC analysis to target T cells. Surface expression of CD3 has also been shown to be diminished in 40–80% of MF/SS cases with blood involvement.[31] CD4 and CD8 are T-cell markers that identify the relevant T-cell subset, and most cases involve CD4+ T cells. CD8 is included to allow for CD4+ CD8+ double-positive cells to be gated out, as these are more commonly involved in reactive conditions and complicate the phenotypic evaluation of the abnormal T-cell subset.[31] While CD8− CD4− ('double-negative') cases do exist, they are rare and only case studies/series are available. Therefore, these cases are the subject of ongoing identification, which further emphasizes the importance of standardized approaches to FC, in order to allow for meaningful interpretation of data generated from these cases. Loss of CD7 and/or CD26 is a common finding in MF/SS, identified in 50–80% and 70–100% of cases with blood involvement, respectively; however this is also often observed in reactive CD4 T-cell conditions. When CD4+ CD7− cells comprised > 40% of the CD4+ T-cell population, researchers were able to discriminate between SS and erythrodermic inflammatory dermatoses (EID) with a sensitivity of 54% and specificity of 100%.[32] The same study also examined CD4+ CD26− cells and found that when these comprised ≥ 30% of the CD4+ T-cell population, SS vs. EID could be distinguished with a sensitivity of 86% and specificity of 47%; when there were > 80% CD26− cells within the CD4+ population, the specificity was 100% and sensitivity was 66%.[32] However, when considering the percentage value of the CD4+ CD26− subset, in the original paper by Bernengo *et al.*,[37] the positive predictive value was 100% for a cut-off value of 30%; sensitivity was 97% and specificity was 100%. Both of these markers have been associated with disease stage and disease progression in MF/SS.[31,33,38]

However, the reference population (either total lymphocytes or CD4+ T cells) used for CD7− or CD26− percentages was not consistent between centres. In a prospective study that included 254 patients referred for testing for CTCL at initial diagnosis, the best specificity and sensitivity was obtained when total lymphocyte count was used as the reference subset at the thresholds indicated by EORTC/ISCL.[39] Finally, CD45 inclusion is suggested as a way to identify haematopoietic (CD45+) cells and exclude CD45− nonhaematopoietic cells from the analysis.[31]

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.[40] SS has been commonly reported to include a 'dim' CD3, CD4 or CD2 population.[33,41] 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.[41] Good resolution of a 'dim' population is reliant on fluorochrome choice, particularly in multicolour combinations.[41,42]

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.[43,44] 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 that seen in Sézary cells.[45,46] Analysis of CD4+ T cells by Roelens *et al.* found that CD4+ CD45RA− CD26− CD27+ CD28+ PD1+ T cells represented 58·5% of Sézary cells and 49·2% of healthy CD4+ T cells in a patient with SS, but only 3·8% of CD4+ T cells in a healthy donor.[46] In addition, healthy and malignant CD4+ T cells often evolve in the same way under treatment. Given these common phenotypes, the reliability of extended phenotypes using only nonspecific markers for Sézary cells may be questionable.[46]

Additional potentially useful markers for FC are also under evaluation, although no consensus has been reached.[44] It is suggested that additional antigens should be combined with CD3, CD4, CD, and CD26, at the very least (Figure 1).[31,37,47–63]

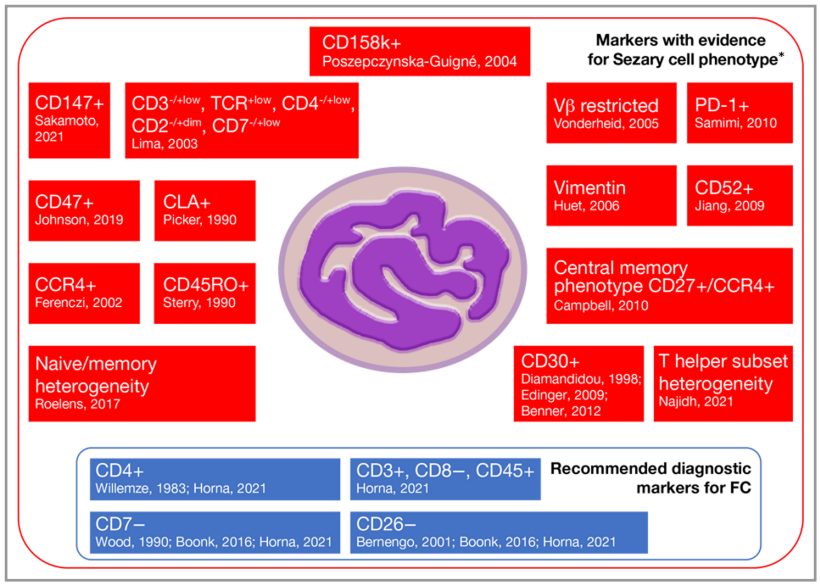

Figure 1 Markers with evidence for the Sézary cell phenotype and those recommended as diagnostic markers for FC. FC, flow cytometry; PD-1, programmed cell death-1. *Not limited to markers routinely detected by FC.

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.[44] 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,[64] the reliability of this marker for detecting Sézary cells in blood and skin has been demonstrated by several teams.[45,65,66] In a recent study,[39] 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.*[32] 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.[46] 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.[39] Since 2014, KIR3DL2/CD158k analysis has formed part of the routine care of patients with erythroderma at Hospital Saint-Louis in Paris,[46] and, together with Bordeaux and Clermont-Ferrand centres, the Moins-Teisserenc 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 B_0 blood classification.[4]

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.[67,68] 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.[17,31,69] 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[70] 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.[71] 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).[71]

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.[42,72–75] 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.[31] 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.[1,34]

Accurate quantification of blood involvement could also help to ascertain whether it would be clinically relevant to identify a further cut-off, higher than B_2, 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.[17,34,44] 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.[17,31] 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.[73]

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,[34] which could be tracked by next-generation sequencing of TCRs to identify a patient's tumour-specific 'barcode'.[76–78] 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, Duvic 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**: adv00013.
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, Quaglino P, Prince HM *et al.* The PROCLIPI 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 Sezary 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-Gernhard S *et al.* Long-term outcome of 525 patients with mycosis fungoides and Sezary syndrome: clinical prognostic factors and risk for disease progression. *Arch Dermatol* 2003; **139**:857–66.
14 Toro JR, Stoll HL Jr, Stomper PC, Oseroff 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**:117–21.
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**:2598–607.
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.2021012057.
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 BHM4 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? *EMJ Hematol* 2018; **6**:92–100.
31 Horna P, Wang SA, 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**:142–55.
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 Najidh 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 Yazıcı S, Bülbül 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 Illingworth 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, Quaglino 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; **26**: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 Pulitzer MP, 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. Sezary 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, Fuhlbrigge 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 Sezary cells. *J Immunol* 2006; **176**:652–9.
50 Johnson LDS, Banerjee S, Kruglov O *et al.* Targeting CD47 in Sézary syndrome with SIRPαFc. *Blood Adv* 2019; **3**:1145–53.
51 Picker LJ, Michie SA, Rott LS, Butcher EC. A unique phenotype of skin-associated lymphocytes in humans. Preferential expression of the HECA-452 epitope by benign and malignant T cells at cutaneous sites. *Am J Pathol* 1990; **136**:1053–68.
52 Poszepczynska-Guigné E, Schiavon V, D'Incan M *et al.* CD158k/KIR3DL2 is a new phenotypic marker of Sezary cells: relevance for the diagnosis and follow-up of Sezary syndrome. *J Invest Dermatol* 2004; **122**:820–3.
53 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.
54 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.
55 Sterry W, Bruhn S, Künne N *et al.* Dominance of memory over naive T cells in contact dermatitis is due to differential tissue immigration. *Br J Dermatol* 1990; **123**:59–64.
56 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.
57 Willemze R, van Vloten 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.
58 Wood GS, Hong SR, Sasaki DT *et al.* Leu-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.
59 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. *Haematologica* 2003; **88**:874–87.
60 Jiang L, Yuan CM, Hubacheck 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.
61 Diamandidou E, Colome-Grimmer M, Fayad L *et al.* Transformation of mycosis fungoides/Sezary syndrome: clinical characteristics and prognosis. *Blood* 1998; **92**:1150–9.
62 Edinger JT, Clark BZ, Pucevich BE *et al.* CD30 expression and proliferative fraction in nontransformed mycosis fungoides. *Am J Surg Pathol* 2009; **33**:1860–8.
63 Benner MF, Jansen PM, Vermeer MH, Willemze R. Prognostic factors in transformed mycosis fungoides: a retrospective analysis of 100 cases. *Blood* 2012; **119**:1643–9.
64 Bagot M, Moretta A, Sivori S *et al.* CD4(+) cutaneous T-cell lymphoma cells express the p140-killer cell immunoglobulin-like receptor. *Blood* 2001; **97**:1388–91.
65 Klemke CD, Brade J, Weckesser S *et al.* The diagnosis of Sézary syndrome on peripheral blood by flow cytometry requires the use of multiple markers. *Br J Dermatol* 2008; **159**:871–80.
66 Moins-Teisserenc H, Daubord M, Clave E *et al.* CD158k is a reliable marker for diagnosis of Sézary syndrome and reveals an unprecedented heterogeneity of circulating malignant cells. *J Invest Dermatol* 2015; **135**:247–57.
67 van den Beemd R, Boor PP, van Lochem EG *et al.* Flow cytometric analysis of the Vβ repertoire in healthy controls. *Cytometry* 2000; **40**:336–45.
68 Langerak AW, van Den Beemd R, Wolvers-Tettero IL *et al.* Molecular and flow cytometric analysis of the Vβ repertoire for clonality assessment in mature TCRαβ T-cell proliferations. *Blood* 2001; **98**:165–73.
69 Feng B, Jorgensen JL, Jones D *et al.* Flow cytometric detection of peripheral blood involvement by mycosis fungoides and Sézary syndrome using T-cell receptor Vβ chain antibodies and its application in blood staging. *Mod Pathol* 2010; **23**:284–95.
70 Halkes CJM, Zoutman WH, van der Fits L *et al.* Mutation in PIGA results in a CD52-negative escape variant in a Sézary syndrome patient during alemtuzumab treatment. *J Invest Dermatol* 2015; **135**:1199–202.
71 Quaglino P, Fava P, Pileri A *et al.* Phenotypical markers, molecular mutations, and immune microenvironment as targets for new treatments in patients with mycosis fungoides and/or Sézary syndrome. *J Invest Dermatol* 2021; **141**:484–95.
72 Kalina T, Flores-Montero J, van der Velden VH *et al.* EuroFlow standardization of flow cytometer instrument settings and immunophenotyping protocols. *Leukemia* 2012; **26**:1986–2010.
73 Kalina T, Brdickova N, Glier H *et al.* Frequent issues and lessons learned from EuroFlow QA. *J Immunol Methods* 2019; **475**:112520.
74 Theunissen P, Mejstrikova E, Sedek L *et al.* Standardized flow cytometry for highly sensitive MRD measurements in B-cell acute lymphoblastic leukemia. *Blood* 2017; **129**:347–57.
75 Flores-Montero J, Sanoja-Flores L, Paiva B *et al.* Next generation flow for highly sensitive and standardized detection of minimal residual disease in multiple myeloma. *Leukemia* 2017; **31**:2094–103.
76 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. *Sci Transl Med* 2015; **7**:308ra158.
77 Sufficool KE, Lockwood CM, Abel HJ *et al.* T-cell clonality assessment by next-generation sequencing improves detection sensitivity in mycosis fungoides. *J Am Acad Dermatol* 2015; **73**:228–36.e222.
78 Weng WK, Armstrong R, Arai S *et al.* Minimal residual disease monitoring with high-throughput sequencing of T cell receptors in cutaneous T cell lymphoma. *Sci Transl Med* 2013; **5**:214ra171.