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Review

Blood classification and blood response criteria in mycosis fungoides and Sézary syndrome using flow cytometry: recommendations from the EORTC cutaneous lymphoma task force

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Abstract Our current mycosis fungoides (MF) and Sézary Syndrome (SS) staging system includes blood-classification from B0-B2 for patch/plaque/tumour or erythroderma based on manual Sézary counts but results from our EORTC survey confirm these are rarely performed in patch/plaque/tumour MF, and there is a trend towards using flow cytometry to measure blood-class. Accurately assigning blood-class effects overall stage and the 'global response' used to measure treatment responses in MF/SS and hence impacts management. The EORTC Cutaneous Lymphoma Task Force Committee have reviewed the literature and held a Workshop (June 2017) to agree a definition of blood-class according to flow cytometry.

No large study comparing blood-class as defined by Sézary count with flow cytometry has been performed in MF/SS. The definition of blood-class by flow cytometry varies between publications. Low-level blood involvement occurs in patch/plaque/tumour much less than

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erythroderma ($p < 0.001$). The prognostic relevance of blood involvement (B1 or B2) in patch/plaque/tumour is not known. Studies have not shown a statistically worse difference in prognosis in erythrodermic MF patients with low-level blood involvement (IIIB) versus those without (IIIA), but Sezary patients who by definition have a leukaemic blood picture (staged IVA1 or higher) have a worse prognosis.

For consistency flow, definition for blood-class must be an objective measurement. We propose absolute counts of either CD4+CD7-or CD4+CD26-where B0<250/μL, B1 = 250/μl −<1000/μL and B2≥1000/μL plus a T-cell blood clone. Fluctuations between B0 and B1 should not be considered in the treatment response criteria until further prognostic information is known.

1. Introduction

Blood-classification was introduced into the TNM classification of mycosis fungoides (MF) and Sezary syndrome (SS) in the 2007 revision [1]. Blood-class from B0-B2 was defined using manual Sézary counts based on morphology, but these are highly subjective and have never been validated in a multicentre prospective international study. B2 was defined as ≥1000/μL Sézary cells and, in those with erythroderma and a positive blood T-cell clone identical to skin, were the diagnostic criteria for SS. B1 was defined as greater than 5% circulating Sézary cells (as a percentage of circulating lymphocytes) using a manual Sézary count (on a peripheral blood smear) to differentiate those patients with low-level blood involvement from B0; thereby defining B0 as ≤5% circulating Sézary cells [1]. Blood involvement may occur in MF and SS especially in advanced stages of disease notably erythroderma (defined as >80% of skin involved), but blood involvement may also occur in patch/plaque or tumour MF. In erythroderma, blood-class defines the patients who do not reach the criteria for SS stage (IVA1) as stage IIIA (blood-class;B0) or stage IIIB (blood-class; B1). Blood-class is similarly recorded for patch/plaque or tumour MF, but B0-1 does not change stage, whereas B2 is designated IVA1 independent of the level of skin involvement. Circulating Sezary cells have recently been shown to have a diverse phenotype from skin Sezary cells despite demonstrating clonality with skin-derived Sezary cells showing a more advanced maturation pattern with differences in cytokine/chemokine receptor expression [2]. CD4+CD7- and CD4+CD26-subsets are the most commonly used to identify the neoplastic population in MF/SS. However, partial loss of these markers may occur on benign T-lymphocytes with ageing, in inflammatory dermatoses or following therapy [1–5]. Other aberrant phenotypes may also occur but are less common.

As there is no objective definition of blood-class using flow cytometry, centres have adopted different definitions for publications. The EORTC is leading a Prospective Cutaneous Lymphoma International Prognostic Index study (PROCLIPI study) in early-stage MF which collects staging, survival and treatment data. For the TNMB stage to be comparative and consistent in future publications, an agreement for the definition of blood-class is required. Furthermore, as response in blood is considered as part of treatment response used for clinical trials in MF/SS, it is essential that there is a universal objective definition. Here, we review the literature and report on our EORTC Workshop defining blood-class in MF/SS.

2. Material and methods

We reviewed the literature of blood involvement in MF/SS using flow cytometry, through an internet search of relevant medical databases (e.g. PUBMED, MEDLINE) as well as a targeted search of relevant professional bodies and their guidelines (e.g. EORTC, ISCL (International Society for Cutaneous Lymphoma), UK-CLG (UK-Cutaneous Lymphoma Group). We performed an EORTC survey of centres detailing their current practice for measuring blood involvement by flow cytometry and reviewed preliminary PROCLIPI data.

Following discussions at the EORTC Annual General Meeting (April, 2017), an EORTC Blood Classification Workshop to review the literature and define blood-class using flow cytometry was held (June 2017).

3. Results

3.1. Workshop discussion and interpretation of current staging and response criteria

A comprehensive literature review confirms that there is no objective universal definition of blood-class in MF/SS according to flow cytometry. The adoption of CD26-cells in the assessment of blood involvement in MF/SS followed the work by Bernengo *et al.* [3] which compared the percentage of CD4+CD26-% in 103 MF/SS patients with blood-class defined by Sézary counts. Of 151 MF patients, 14 (9%) had B1 (≥5% Sézary cells

Table 1
Comparison of blood-class measurements according to flow cytometry in MF/SS.

Blood-class according to flow	B0	B0 comments	B1	B1 Comments	B2	B2 comments
Vonderheid et al 2002 [3]	Not defined by flow		Not defined by flow		A CD4/CD8 ratio of 10 or more due to an increase in CD3 or CD4 cells by flow cytometry or aberrant expression of pan T-cell markers (CD2, CD3, CD4, CD5) by flow cytometry. Deficient CD7 expression on T-cells or expanded CD4+CD7- ≥40%	
Olsen et al, 2007 [1]	Not defined by flow		Not defined by flow		TCR clone plus expanded CD4+ or CD3 population with CD4:CD8>10 or expanded CD4+ with an abnormal phenotype including loss of CD7- or CD26- (≥40% CD4+/CD7- or ≥30% CD4+/CD26-)	Definition of expanded CD3 or CD4 population not defined
Olsen et al, 2011 [4]	Absence of a clone and either fewer than 20% Sézary cells or fewer than 250 Sézary cells/μL.[Vonderheid 2003 24] Data suggest that a normal value for CD4+CD26- or CD4+CD7- cells by flow cytometry is lower than 15%. Based on an upper limit of normal value of 1600/μL for CD4 cells in the blood, an absolute count of lower than 250/μL CD4+/CD26- or CD4+CD7- cells would appear to be a normal value for these CD4 subsets and could also be used to define the absence of or normalisation of blood involvement (B0)	From text, not included in any table. Definition of B0 is given twice 1.A normal value for CD4+CD26- or CD4+CD7- cells by flow cytometry is lower than 15% and 2. An absolute count of lower than 250/μL CD4+/CD26- or CD4+CD7- cells would appear to be a normal value	250-<1000 μL	As default with B0 as <250 μL and B2 as ≥ 1000/μL	≥1000/μL cells with positive clone; one of the following can be substituted for Sézary cells: CD4/CD8 ≥10, CD4+CD7- cells ≥40% or CD4+CD26- cells ≥30%	Not specified in Table 1 but presume substitution of ≥1000/μL cells with flow measurements only defines B2 in patients with an expanded CD4 population.

(*continued on next page*)

Table 1 (*continued*)

Blood-class according to flow	B0	B0 comments	B1	B1 Comments	B2	B2 comments
Benton et al, 2014 [19]	<500 Sézary cells/μL	Assessed by flow cytometry	500-1000 Sézary cells/μL	Assessed by flow cytometry	>1000 Sézary cells/μL or 35% of lymphocytes as CD4+CD26- or CD4+CD7-	Assessed by flow cytometry
Gibson et al 2016 [25]			CD4:CD8>5, >20% CD4+/CD7- or >20% CD4+/CD26-	B1 flow definition determined without prognostic data		
Vonderheid et al 2017 [9]	<30% CD4+CD26- and <1000/μL		≥30% CD4+CD26- and <1000/μL		CD4+CD26- ≥1000/μL	
NCCN Guidelines 2017 [28]	Absence of significant blood involvement: ≤5% of peripheral blood lymphocytes or <250/mcL are atypical (Sézary) cells or < 15% CD4+/CD26—or CD4+/CD7—cells of total lymphocytes		Low blood tumour burden: >5% of peripheral blood lymphocytes are atypical (Sézary) cell or ≥ 15% CD4+CD26 − or CD4+CD7 − of total lymphocytes but do not meet the criteria of B0 or B2		High blood tumour burden: ≥1000/mcL Sézary cells (CD4+/CD26 − or CD4+/CD7 − cells by flow cytometry) or CD4/CD8 ≥ CD8 ≥ 10 or ≥40% CD4/CD7 −or ≥30% CD4+/CD26 − cells of total lymphocytes	
PROCLIPI	<250/μL CD4+CD7- or CD4+CD26- cells/μL	The absolute value of <250/μL was chosen as defined in Clinical Endpoints paper and is the approximately 15% or an expanded CD4+ level of 1600/μL. 15% has been suggested as the upper limit of normal for CD4+CD7- or CD4+CD26- cells.	250/μL - <1000/μL CD4+CD7- or CD4+CD26- cells/μL		≥1000/μL CD4+CD7- or CD4+CD26- cells/μL (or other aberrant phenotype) plus evidence of a relevant T-cell clone in blood (identical to skin clone)	≥1000/μL was the original definition for B2 using manual Sézary count and B2 was shown to have a poorer survival in advanced-stage patients compared with B0 [17]

Abbreviations: B0, absolute counts of either CD4+CD7-or CD4+CD26-are <250/μL cells; B1, CD4+CD7-or CD4+CD26-counts are between 250/μL−<1000/μL cells; B2, CD4+CD7-cells or CD4+CD26-cells or other aberrant phenotype ≥1000/μL in the presence of a relevant T-cell clone in blood; MF, mycosis fungoides; SS, Sézary syndrome.

but <1000). 13 of 14 of these patients had >30% CD4+CD26-cells and none of the patients with B0 MF or any of the 88 patients with benign inflammatory erythroderma or healthy controls had >30% CD4+CD26-cells. CD4+CD26-% was greater than 40% in all 52 SS patients [3]. A statistically significant direct relationship between CD26-percentage values and the Sézary cell count percentage was found in B1 and B2 patients ($p < 0.001$).

The number of centres performing manual Sézary counts declined as this was highly subjective and required considerable experience with flow cytometry becoming the more popular alternative for measuring blood involvement in MF/SS. The ISCL suggested that B2 may be defined by flow in patients with a T-cell clone in blood as 1) expanded CD4+/CD3+ cells with CD4/CD8 ratio of ≥10, (2) expanded CD4+ cells with abnormal immunophenotype including loss of CD7/CD26 (≥40% CD4+CD7-or ≥30% CD4+CD26-), but no definition of expanded CD4+ cells was given [4]. This led to different interpretation for measuring B2 blood-class between centres. Furthermore, no definition of B0/B1 using flow cytometry was provided. The use of CD4/CD8 ratio>10 to classify B2 is reliant on the absolute CD8 count which may be reduced by treatment resulting in a raised ratio with a normal CD4 count and the relevance of this has not been tested. We found a CD4/8 > 10 in 2–10.5% of patients with patch/plaque/tumour MF but in 52.2% with erythroderma (Table 2).

TCR clonality in blood should be recorded as a/b alongside blood-class but does not alter the overall stage or blood response [5]. For T-cell clonality to be relevant, it must be identical to the skin T-cell clone as blood clones may occur in the elderly population with unknown significance. The prognostic significance of a TCR blood clone in MF is not yet proven [6]. Agar *et al.* found T-cell clonality to be of prognostic importance in B0 patients ($p < 0.001$) but not B1. A relevant T-cell blood clone is a diagnostic criterion for Sezary. However according to our EORTC survey (Table 3), blood tests performed at centres in different stages of MF/SS found only 7/31 (23%) centres are performing TCR studies on all stages of MF/SS patients.

Clonality may be tested using PCR with biomed 2 but PCR is not quantitative. Clonality using V beta antibodies allows quantification of the T-cell clone but at present only covers around 70% of the T-cell receptor repertoire. Currently, clonality using V beta antibodies is not routinely performed and remains expensive. High throughput sequencing is a novel technique which allows near to 100% of T-cells clones to be identified and is quantitative but is only available in highly specialised centres, and costs are likely to be prohibitive as a routine diagnostic test. Yoo *et al.* [8] found an identical peripheral T-cell clone in 2/30 (6.7%) patients with T1/T2, 5/13 (38.4%) with T3 and 9/14 (64.3%) with T4 disease. Our preliminary PROCLIPI data also found an increased likelihood of a relevant T-cell clone in blood with increasing T class 1 to 3 despite the lack of association of increasing blood-class with T-classes Table 2.

The Clinical Endpoints [2011], which introduced measuring response to treatment in skin, blood, node and viscera in MF/SS for use in clinical trials, further defined B0-2 using flow cytometry [5], Table 1. However, the criteria for blood-class are open to interpretation, normal flow measurements for B0 were defined as CD4+CD26-or CD4+CD7-cells <250/μL or < 15% by flow cytometry and studies adopted different definitions for B0-2 (Table 1). Furthermore, these values are arbitrary and not backed up by prospective multicentre international studies.

Our survey of 31 EORTC sites confirmed flow cytometry to be the preferred method of tracking blood involvement (Table 3). Few centres are performing manual Sézary counts on all MF/SS patients, and 7 centres (23%) no longer perform manual counts. Flow cytometry is performed in all centres but frequently restricted to advanced stages. Only 11 sites (35%) of sites perform flow on all stages of MF/SS (Table 3). A recent publication from the United States found the number of sites performing Sézary counts was just over half (51%), however, >90% measured CD4+CD7-cells [9].

After interim analysis of PROCLIPI blood data, it was striking that blood involvement through T class 1–3 was similar (Table 2), but blood involvement in erythrodermic disease is significantly higher $p < 0.001$. We therefore divided our discussions into blood-class in patch/plaque or tumour MF and erythroderma.

3.2. Blood-class B0-B2 in patch/plaque or tumour MF patients

Most patients with early-stage MF are B0, but B1 and B2 may be seen in early-stage MF with Talpur *et al.* [10] reporting 2.0% of stage IA with B1 and 0.6% with B2 [6] using a definition of B1 (aleukaemic >500 and < 1000 cells/mL), or B2 (leukaemic >1000 cells/mL) with no significant difference in the survival between B0 and B1 in early-stage MF patients.

Previously, Harmon *et al.* [11] reported on CD4+CD7-in the blood of 31 patients with benign dermatoses, 35 patients who had CTCL and 16 normal controls. There was no statistical difference between the mean % CD4+CD7-circulating cells for the normal control (5.8%) or the benign dermatoses (9.3%; $p = 0.13$) nor between benign dermatoses and MF ($p = 0.80$). The patients with pre-SS and SS had a higher mean percentage of CD4+CD7-cells (22.4% and 35.5%, respectively) than patients with benign dermatoses ($p < 0.01$). In the retrospective study on 1422 MF patients reported by the Italian Study Group for Cutaneous Lymphoma [12], among patients with stage IVA1, only 5% had T1 and 28% T2. Recently, Vonderheid and Hou [13] reported B2 as defined by Sézary cells ≥1000/μL in 7 of 373

Table 2
Preliminary PROCLIPI data from Europe showing blood-class, nodal-class, flow cytometry, serum LDH and blood T-cell clone according to T class.

	T1a	T1b	T2a	T2b	T3	T4
No. patients	125	97	112	152	56	81
B0	121 (96.8%)	90 (92.8%)	102 (91.1%)	138 (90.8%)	52 (92.9%)	23 (28.4%)
B1	4 (3.2%)	5 (5.2%)	9 (8.0%)	10 (6.6%)	4 (7.1%)	18 (22.2%)
B2	0	2 (2.1%)	1 (0.9%)	4 (2.7%)	0	40 (49.4%)
N0	123 (98.4%)	93 (95.9%)	110 (98.2%)	129 (84.9%)	38 (67.9%)	39 (48.4%)
Nx	2 (1.6%)	1 (1.1%)	0	15 (9.9%)	10 (17.9%)	21 (26.0%)
N1-2	0	3 (3.1%)	2 (1.8%)	3 (2.0%)	5 (8.9%)	10 (12.3%)
N3	0	0	0	5 (3.3%)	3 (5.4%)	11 (13.6%)
CD4:CD8>3.5	4/52 (7.7%)	9/56 (16.1%)	14/76 (18.4%)	21/86 (24.4%)	11/37 (29.7%)	52/67 (77.6%)
CD4:CD8>10	1/52 (1.9%)	2/56 (3.6%)	2/76 (2.6%)	9/86 (10.5%)	2/37 (5.4%)	35/67 (52.2%)
CD4+CD7->15%	2/35 (5.7%)	12/36 (33.3%)	9/33 (27.3%)	18/57 (32.1%)	7/22 (31.8%)	32/36 (88.9%)
CD4+CD7->40%	0/35	2/36 (5.6%)	3/33 (9.1%)	8/57 (14.0%)	0/22	22/36 (61.1%)
CD4+CD26->15%	10/35 (28.6%)	16/36 (44.4%)	14/33 (42.4%)	31/57 (54.4%)	14/22 (63.6%)	33/36 (91.7%)
CD4+CD26->30%	1/35 (2.9%)	9/36 (25.0%)	6/33 (18.2%)	17/57 (29.8%)	6/22 (27.8%)	27/36 (75.0%)
Raised LDH	5/85 (5.9%)	13/80 (16.3%)	8/76 (10.5%)	33/114 (29.0%)	15/49 (30.6%)	52/78 (66.7%)
Identical TCR blood clone	1/20 (5.0%)	3/31 (9.7%)	5/31 (16.1%)	14/53 (26.4%)	5/24 (20.8%)	26/43 (60.4%)

Abbreviations: B0, absolute counts of either CD4+CD7-or CD4+CD26-are <250/μL cells; B1, CD4+CD7-or CD4+CD26-counts are between 250/μL–<1000/μL cells; B2, CD4+CD7-cells or CD4+CD26-cells or other aberrant phenotype ≥1000/μL in the presence of a relevant T-cell clone in blood; MF, mycosis fungoides; SS, Sézary syndrome; PROCLIPI, Prospective Cutaneous Lymphoma International Prognostic Index.

(1.88%) patients with patch/plaque or tumour MF. The CD4+CD26-percentage was ≥30% in 15/373 (4%) of patch, plaque/tumour patients (including 1.4% with patch, 7.3% with plaque and 7% with tumour disease compared with 70% in erythroderma).

Rare cases of B2 in patch/plaque MF are described in the literature, and the prognostic relevance is not known compared with patch/plaque patients with lower or no blood involvement [14–17]. A study of 6 patients fulfilling B2 without erythroderma, 1 of 2 patients with T1-stage MF had died, but there were no disease-specific deaths during a median follow-up of 9 years [16]. Conversely, a recent study from the Mayo clinic of 16 patients diagnosed between 1976 and 2010 with CTCL having B2 but without erythroderma (most commonly patches and plaques [n = 12] had a median time from identification of B2 to death of just 3.6 years; range, 0.5–8.7 years) which was similar survival to their SS patients p = 0.08 [17].

The PROCLIPI study which opened in 2015 is prospectively collecting, at diagnosis and follow-up, data on patients with MF/SS and will determine the prognostic significance of blood-class with time. Preliminary PROCLIPI data from Europe similarly identified a small percentage of patients with patch/plaque or tumour to have B1 (5.9%) and B2 (1.3%). Blood involvement was present at low levels through T class T1-T3 with no significant trend for increased blood involvement with T1-T3 class and was significantly lower than T4 (erythrodermic patients) with 22.2% B1 and 49.4% B2. Table 2 reports preliminary PROCLIPI data from Europe showing blood-class, nodal-class, flow cytometry, serum LDH and blood T-cell clone according to T-class. According to the revised staging in 2007, patch/plaque and tumour patients with B2 are staged IVA1 with implications for treatment and survival, but no previous study has prospectively studied the significance of blood-class in patch/plaque or tumour disease at diagnosis and through the course of the disease, and it is unclear how blood involvement/class fluctuates with time/disease course. Such that the quantification of blood involvement may not be important for staging or therapeutic response in patch/plaque or tumour disease.

The percentage of patients with measurable blood involvement by flow in skin class T1 through to T3 appears similar suggesting that this flow measurement may not necessarily reflect severity of MF in skin and as T-class and stage have prognostic significance then blood involvement as measured by B0-2 may not have prognostic relevance in patch/plaque or tumour disease.

Defining blood-class using absolute values of CD4+CD7-or CD4+CD26-cells, as shown under PROCLIPI in Table 1, has the advantage of allowing every patient to be defined a blood-class, but it must be appreciated that small movements in blood involvement can allow a blood-class change. At present, blood response from B1 to B0 is defined as a CR [5], and yet we have no real insight into the dynamics of blood involvement in patch/plaque and tumour patients and how this fluctuates with time, therapy or disease progression. There are exceptional cases when B2 is found in patients with patch/plaque or tumours who do not progress to SS, and we do not understand the clinical or prognostic consequences of this scenario [10,11,16,17]. Such patients will be followed up prospectively in PROCLIPI to determine any relevance.

3.3. Blood-class B0-B2 in erythrodermic MF/SS

Staging in erythroderma is determined by blood-class and the management of patients with erythroderma is determined by stage [18]. It is therefore of vital

Table 3
Showing the blood tests performed at different sites in according to stage in MF/SS patients.

Principal investigator	Site	FBC	TCR	Flow	Sézary count
Scarisbrick	Birmingham	All	All	All	Erythrodermic
Vakeva	Helsinki	All	All	All	IIIA–IVB
Estrach	Barcelona	All	IIIA–IV	IIIA–IV	IIIB–IV
Piris	Santander	IB–IV	IV	All	All
Servitige	Barcelona	IB–IV	IB–IV	IB–IV	IB–IV
Papadavid	Athens	All	If flow abnormal	IIB–IV	IIB–IV
Pujol	Barcelona	All	All	All	All
Azurdia	Liverpool	All	All	All	All
Klemke	Karlsruhe	All	IIIA–IV	IIA–IV All but CD4+26-	IIIA–IV
Beylot-Barry	Bordeaux	IIB–IV	All	IIB–IV select on IB	IIB–IV plus CD158k
Cerroni	Graz	All	Select	IIB–IV	IIB–IV
Ortiz	Madrid	IB–IV	IB–IV	IB–IV	None
Dunill	Bristol	All	IIA–IV	IIA–IV CD4+26- only	None
Busschots	Leuven	All		select	None
Berti	Milan	All	IIB–IV	IIIA–IV select IIB	IIIA–IV
Vermeer	Leiden	All	IIIA–IV	IIIA–IV limited not CD4+7- CD4+26-	IIIA–IV
Wehkamp	Kiel	All	If flow abnormal	All	All
Wobser	Würzburg	IIB–IV	IIB–IV	IIB–IV	IIB–IV
Bagot	Paris	All	IIB–IV	IIB–IV	IIB–IV
Stadler	Minden	All	IIB–IV	All	All
Trautinger	St Poelten	IIB–IV	IIB–IV	IIB–IV select on IB, IIA not CD4+CD26-	None
Marschalkó	Semmelweis	All	IB–IV	IB–IV select adv for CD4+7- CD4+26-	IIA–IV
Goldberg	Tel Aviv	All	All	All	All
Pimpinelli	Florence	IB–IV	III–IV	III–IV	None
Nicolay	Mannheim	All	IIB–IV	All	IIB–IV
Cowan	Manchester	All	None	All	None
Matin	Oxford	All	All	IV	Erythrodermic
Bates	Southhampton	All	IIIA–IV	IIIA–IV	IIIA–IV
MacKay	Glasgow	All	III above	If Sézary cells on film	III above
Hodak	Petah Tiqva	All	III above, select on earlier stages	All	All
Quaglino	Torino	All	If flow abnormal	IIB to IV	All

Abbreviations: MF, mycosis fungoides; SS, Sézary syndrome.

importance that blood-class differentiates different prognostic groups if used to determine patient treatment.

The clinical presentation of erythroderma with <1000/μL is long recognised and was termed 'Pre-Sézary' by Winkelmann in 1974 [19] and defined as a chronic, steroid unresponsive erythroderma, lymphadenopathy, lymphocytic band at dermo-epidermal junction and fewer than <1000 circulating Sézary cells/mm^3. Whilst some of these patients progress to meet the Sézary criteria, a number of patients continue a more benign clinical path [20,21].

A recent large study of 1275 advanced patients confirmed that erythrodermic CTCL patients have varying blood tumour burden such that 31% of erythrodermic patients were B0, 20% B1 and 49% B2 [22]. Throughout all advanced stages of MF/SS, there was no significant difference between survival in B0 or B1 but survival in B2 was significantly worse p = 0.021 although many of these B2 patients had extensive nodal involvement (IVA2) which is known to carry a poor prognosis. There was no significant difference in median survival or 5-year survival in IIIA, IIIB or IVA1 with median survival in IIIA 61.7 months (5-year survival = 60.2%), IIIB 58.2 months (5-year survival = 55.7%) and IVA1 55.7 months (5-year survival = 48.3%). Preliminary analysis of erythrodermic patients in the European PROCLIPI data set confirms this split with 28.4% of erythrodermic patients B0, 22.2% B1 and 49.4% B2. Talpur *et al.* [10] reported on 1263 MF/SS patients. The risk of disease progression was significantly greater for 192 B2 patients (including 184 of 188 [97.9%] erythrodermic patients and 8 of 1052 [0.8%] patch/plaque or tumour patients) as compared with risk of disease progression in patients with B0–1 in all T-classes ($p < 0.0001$). Agar *et al.* [7] reported on 1502 patients and found a worse overall survival and disease-specific survival between B2 compared with B1 (p = 0.04). The same data set was used to determine a cutaneous lymphoma prognostic index and found B1-2 (where B1 was >5% circulating Sézary cells by manual counts) was an independent prognostic variable, but this was not significant using the validation set from MD Anderson [23]. Importantly the data definitions of B1 varies between these publications Table 1. There are no prognostic

studies comparing B1 as ≥5% Sézary cells on manual counts with B1 defined by flow as an absolute count of either ≥250/μL − <1000/μL CD4+CD7-or CD4+CD26-cells.

A study of 84 patients with erythrodermic CTCL stratified blood involvement into 5 subsets H0-4 and found a stepwise statistically significant increase in CTCL-specific deaths where H4 was >10,000/μL Sézary cells/[24] suggesting in erythrodermic disease that an increasing peripheral blood tumour burden may be inversely proportional to survival in MF/SS. This finding was replicated in a study of 124 erythrodermic patients from MD Anderson with median survival in H0-2 of 7.6 years compared with 2.4 years in H4 ($p = 0.01$) [25] and implies a higher blood tumour burden is a poor prognostic factor.

A recent publication from France reported on 31 erythrodermic stage patients with B0-1 (13 IIIA and 18 IIIB). Six of 18 patients with B1 developed B2 and fulfilled the criteria for SS, and none with B0, over a median follow-up of 1.6 years [21]. This agrees with the conception that some patients with B1 have 'pre-Sézary'. A large study from Novelli *et al.* [26] of flow cytometry found loss of CD26 to be the most frequent phenotypic abnormality and confirmed ≥30% as a reliable tool when differentiating from inflammatory erythroderma.

A relevant point of variation between publications is the definition and requirement for an expanded CD4 population in the definition of B2. This is mentioned but not defined in the 2007 Staging paper [1] and was based on an upper limit of normal value of 1600/μL for CD4+ cells in the Clinical Endpoints paper [5].

The prognostic relevance of erythrodermic patients with either B0 or B1 is not yet proven prospectively. In addition, the relevance may be different where historically B1 was defined as ≥5% Sézary cells compared with B1 defined by flow cytometry. From previously published data, there was no significant difference in median survival between IIIA and IIIB patients [13,22]. Patients may fluctuate between blood-class overtime and with treatment. Some patients with B1 will progress to SS but others continue with a relatively low blood tumour burden and may be referred to as erythrodermic MF. SS is defined as patients with erythroderma, ≥1000/μL circulating Sézary cells and presence of a relevant T-cell clone. Recent studies have focused on developing improved diagnostic markers for SS. A multicentre study of 59 patients with SS found loss of CD26 in ≥80% of CD4+ cells or loss of CD7 in ≥40% of CD4+ cells could differentiate SS patients from inflammatory erythroderma with high specificity [27]. Other possible markers included DNM3, TWIST1, EPHA4 and PLS3. A further recent publication suggested KIR3DL2 to be highly specific in diagnosing SS [28]. Further international studies are required to determine their value.

4. Discussion

4.1. Summary recommendations for blood-class, staging and blood response criteria

For any staging system to be clinically useful in the management of patients; it must be objective, reproducible, and increasing stage should be predictive of patients' outcome. Staging criteria in MF/SS were revised more than 10 years ago, and since this time, further information on the prognostic importance of stage has been published in several large studies [7,10,22]. Whilst we await our large-scale international prospective PROCLIPI study to determine the prognostic importance of TNMB and other biomarkers, we propose the following considerations should be applied to the current staging and clinical end-points.

1) Recommendations for defining blood class

Flow cytometry is our recommended method of measuring blood involvement in all stages of MF and SS. To ensure all patients are designated a blood-class, there is a requirement to select a cut off between classes. We propose that this is determined using absolute flow counts of CD4+CD7-or CD4+CD26-. This will allow blood-class to be comparative between centres for prospective testing.

For staging patch/plaque or tumour patients, B2 should be tracked prospectively to determine if this is of clinical significance at present these patients are assigned stage IVA1 [1].

2) Definition of Sezary syndrome

Sezary syndrome (stage IVA1 and above) should be defined as patients with erythroderma, B2 blood involvement and a circulating TCR clone identical to skin. Bone marrow involvement is not a defined criteria for SS as its' relevance is not currently understood. It is not routinely performed in centres. The prognostic relevance of eythrodermic patients with minor blood involvement (B0-1) (stage IIIA/B) is not yet known.

3) Blood response criteria

The relevance of blood-class in MF/SS is not only related to stage but also contributes to the response to therapy in clinical trials. For response criteria in blood, we suggest that for a complete response, B2 should improve to B0 as B2 to B1 may not be clinically relevant. Improvement from B1 to B0 should not meet CR as the significance of this is not yet known. A PR should be considered as a 50% reduction in blood tumour burden only in those with B2 because the relevance of B0/B1 is not known. Fluctuations between B0 and B1 should not be included as treatment response as movement between B0 and B1 does not alter management in erythroderma

Table 4
Recommendations for blood response criteria for MF/SS.

Response	Definition	Change from end-points 2011
Complete response (CR)	• B0, B1 no CR possible • B2 to B0	B1 to B0 no longer considered CR
Partial response (PR)	• B0, B1 no PR possible • B2 as 50% reduction in absolute count	No change
Stable disease (SD)	• B0,B1, B2 fails to attain CR, PR or PD	No change
Progressive disease (PD)	• B0 or B1 to B2 with an increase in absolute counts of ≥50% • B2 with an increase in absolute counts of ≥50% • Loss of response with an increase in absolute counts ≥1000/μL and ≥50% from nadir	Remove the need for at least 5000 neoplastic cells/μL for PD
Relapse	• Increase in absolute counts of ≥1000/μL in those with CR	Relapse not possible in B0-1 as no CR in these patients

Abbreviations: Absolute count, absolute flow counts using CD4+CD7-, CD4+CD26-or other aberrant phenotype; B0, absolute counts of either CD4+CD7-or CD4+CD26-are <250/μL cells; B1, CD4+CD7-or CD4+CD26-counts are between 250/μL–<1000/μL cells; B2, CD4+CD7-cells or CD4+CD26-cells or other aberrant phenotype ≥1000/μL in the presence of a relevant T-cell clone in blood.

or patch/plaque or tumour MF patients and may not be clinically relevant, and low-level blood involvement with CD4+7- or CD4+26-cells are also seen in benign dermatoses. Hence, we propose B1 to B0 should not be recorded as a CR for any patients and B1-B0-B1 should not be considered a relapse Table 4. We also propose the elevated number of neoplastic cells for progressive disease (PD) as >5000/μL should be lowered to ≥1000/μL plus a ≥50% increase from baseline or nadir for 'loss of response' Table 4. A possible 500% increase in blood tumour burden to 5000/μL before PD is reached was used from the CLL definition [5,29] but is deemed too high and not in line with response criteria for PD in skin (>25%), lymph nodes (>50%) or viscera (>50%). It is important to recognise within the clinical trial setting that blood response criteria needs to be evaluated in relation to skin responses as part of the global response assessment [5]. Future studies might provide improved tools for identifying and quantifying tumour cells. Combined with longitudinal clinical studies, this might determine better cut off values for blood-class.

4.2. Recommendations

- Either CD4+CD7-or CD4+CD26-may be used for measuring B-class where B0<250/μL (250 SI units), B1 = 250/μl–<1000/μL (250–1000 SI units) and B2≥1000/μL (1000 SI units) plus a relevant T-cell blood clone.
- Movement between B0 and B1 should not be considered in the treatment response criteria
- In B2, an increase in absolute counts of ≥50% should be considered PD without the current requirement for ≥5000/μL
- The significance of B2 in patch/plaque/tumour MF is unknown, and patients should be tracked

5. Conclusion

Consistency in flow definition for blood-class is critical for evaluation of patient outcome and must be an objective measurement. Our current knowledge of the implications of 'minor' blood involvement (B0-1) is rudimentary and should not be used as a primary indicator to change patients' treatments.

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Conflict of interest statement

None.

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