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The biomarker landscape in mycosis fungoides and Sézary syndrome

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Abstract

The practice of pre-emptive individualized medicine is predicated on the discovery, development and application of biomarkers in specific clinical settings. Mycosis fungoides and Sézary syndrome are the two most common type of cutaneous T-cell lymphoma, yet diagnosis, prognosis and disease monitoring remain a challenge. In this review, we discuss the current state of biomarker discovery in mycosis fungoides and Sézary syndrome, highlighting the most promising molecules in different compartments. Further, we emphasize the need for continued multicentre efforts to validate available and new biomarkers and to develop prospective combinatorial panels of already discovered molecules.

Keywords

cell surface marker; cutaneous T-cell lymphoma; gene expression; screening; secretory protein

1 INTRODUCTION

In the era of personalized medicine, the study of malignancy-related biomarkers has led to advances in early detection of various cancers, improved prognostication and monitoring of disease burden.

Cutaneous T-cell lymphomas (CTCL) are a heterogeneous group of extranodal lymphomas with mycosis fungoides (MF) the most common variant, and Sézary syndrome (SzS) an aggressive, leukaemic variant.[1] Differential diagnosis of early-stage MF is a clinically challenging task as many patients present with non-specific eczematous patches. With histological examination, a 40% false-negative rate and a 44% false-positive rate have been estimated in the diagnosis of early disease.[2,3] Furthermore, biopsy interpretation of early MF is frequently subjective, with studies demonstrating poor reproducibility of diagnoses rendered by different pathologists or even the same pathologist.[4–6] Even molecular

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CONFLICT OF INTERESTS

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techniques have may have low sensitivity and specificity in early stages. One such technique, clonal rearrangements of the T-cell receptor (TCR) gamma gene by polymerase chain reaction (PCR) are positive only in 74% of early MF biopsies.[7] Further, sensitivity of a given marker has been shown to vary with technique used and the density of tumor cells in a sample.[8] Early-stage MF is generally indolent with little effect on overall life expectancy; however, rapid progression and extracutaneous spread of malignant T cells occur in a subset of patients.[1,9] Prediction of which MF/SzS patients will progress is difficult, although recent collaborative efforts have been undertaken to better prognosticate outcomes.[10]

Taken together, the challenges of diagnosis and prognosis underscore the need for reliable markers for MF/SzS. In this review, we provide an overview of diagnostic and prognostic biomarkers in MF/SzS and discuss their potential clinical applicability. Current ongoing collaborative efforts will enable us to overcome the above-mentioned limits and determine combinations of biomarkers with the most clinical relevance.

2 FUNCTIONS OF BIOMARKERS IN MF/SZS

Ideal characteristics of molecular biomarkers vary depending on their intended clinical application.[11] We propose four functions biomarkers can perform in MF/SzS:

1. Biomarkers used for screening purposes must be inexpensive, easily and robustly measurable using minimally invasive routine laboratory techniques, and should discriminate between malignant and benign processes. Early-stage MF may be confused with benign inflammatory conditions such as psoriasis, eczema or other dermatoses (e.g. lymphomatoid drug eruptions, pityriasis rubra pilaris, prodromal bullous dermatoses).[12] Considering the low prevalence of MF and the high prevalence of these benign dermatoses, a screening strategy must achieve high specificity and sensitivity of more than 75% to avoid an unacceptable level of false-positive results. Such markers should also be used prior to potentially harmful therapy. Misdiagnosis of MF can lead to a choice of treatment harmful to patients; for example, cyclosporine and TNF-α inhibitors cause rapid lymphoma progression in patients who are treated for presumed psoriasis, but in fact have undiagnosed MF.[12]

2. Diagnostic biomarkers should accurately identify malignant T cells in peripheral blood and in tissue including skin, lymph nodes and bone marrow. They should assess tumor burden and differentiate MF/SzS from other diseases. Diagnostic markers should also allow for subtyping of cutaneous lymphoma, as some variants need to be precisely diagnosed in order to provide appropriate therapeutic guidance. Specific diagnostic markers may require more elaborate, time-consuming and complex methods compared with screening markers and are only applied to cases with a high level of suspicion for MF/SzS. Such biomarkers should have very high levels of sensitivity and specificity of more than 95%.

3. Prognostic Biomarkers, which predict biological behaviour, should accurately differentiate aggressive from indolent disease. Current staging of MF/SzS uses TNMB classification with disease presentation in the skin (T), lymph nodes (N),

viscera (M) and blood (B) stratifying patients into early stage (IA to IIA) and advanced stage (IIB to IVB).[13] Advanced stage at the time of presentation correlates with poor prognosis, although outcomes within a particular TNMB stage fall into a wide range. For example, stage IVA/B disease which signifies either blood, nodal or visceral involvement has an overall 5-year survival rate of 0%–40%.[14] An improvement of prognostication within a given TNMB stages may be achieved with a combination of biomarkers.

4. Biomarkers of disease activity must reflect changing tumor dynamics, meaning the marker should quantitatively or qualitatively change before clinically significant progression or remission.[11] These markers are used for detecting occult recurrences in patients who are in clinical remission and for monitoring response to therapy.

Some existing and emerging markers fall into more than one of these four categories. To more closely examine specific putative markers and biomarker panels, we will review serum-based, cell surface, genetic and epigenetic markers (Figure 1).

3 SERUM MARKERS AND SECRETORY PROTEINS

A number of serum markers have been studied in MF/SzS, and while these molecules are non-specific, many are already routinely tested via simple, cheap serum studies. Elevated serum lactate dehydrogenase (LDH) has been associated with advanced stage[15] and aggressive disease in MF/SzS patients.[16–19] Elevated beta-2 microglobulin (β2M)[18] and specific IgE levels towards environmental or food allergens have each been associated with poor prognosis in SzS patients; although unlike LDH, data are limited and they are not widely used.[20] Due to their low specificity, these markers are unlikely to have screening or diagnostic applications, but may be useful in prognostic panels or measures of disease activity.

Secretory proteins including interleukins, growth factors, interferons and necrosis factors are measured in a blood draw with an enzyme-linked immunosorbant assay (ELISA) or in a tissue section using immunohistochemistry (IHC). Elevated soluble IL-2 receptor was upregulated in the serum of SzS patients compared with cutaneous B-cell lymphoma and T-cell inflammatory diseases but did not correlate with disease outcome in CTCL patients.[21,22] Elevated IL-13 was identified in the skin of MF/SzS patients and AD patients but not normal skin or psoriasis, and levels of IL-13 increased with increasing MF/SzS stage.[23] Elevated IL-31 has been identified in MF/SzS with conflicting data on whether higher levels are associated with pruritus.[24–26] Thymus and activation-regulated chemokine (TARC/CCL17) is a chemoattractant elevated in MF compared with psoriasis and healthy controls, with higher levels in tumor-stage disease compared with early stage.[27] Additionally, CCR4, one of the receptors for TARC, is expressed on epidermotropic cells in patch, plaque and tumor-stage MF.[27] Technological advances in cytokine measurement have produced addressable bead or chip-based assays for quantitation of up 100 serum proteins in a single sample. A panel of upregulated TNFR1, TNFR2 and IL-12 tested using a Luminex multiplex assessment of serum proteins in MF/SzS reliably distinguished cases from normal controls with 88% sensitivity and 98% specificity.[22] Application of these

technologies could allow for testing of a multiple-marker panel with a single blood draw to screen patients with eczematous and psoriasiform dermatoses as well as to diagnose and prognosticate MF/ SzS patients.

Multiplex immunoassays have another potential application in a promising biomarker class: cancer-specific autoantibodies.[28] Prior to clinical signs and symptoms of cancer, the immune system senses tumor cells and produces autoantibodies to tumor-associated antigens. In solid organ cancers, these autoantibodies have been detected years before clinical manifestations of disease, making them excellent early screening tools.[29,30] Interestingly, several chaperons proteins (HSP60, HSP71 and HSPA5) were identified as antigens for cutaneous lymphoma by proteome serology.[31] Further exploration of the role these autoantibodies may play in detecting MF/SzS is warranted.

4 LEUCOCYTE POPULATION CHANGES

A complete blood count is a non-specific test with useful prognostic implications. Elevated white blood cell (WBC) count, absolute lymphocyte count and absolute eosinophilia have been associated with increased disease progression and disease-specific death in MF/SzS patients.[10,32] Severe cytopenias can also be a sign of marrow involvement and an indication for bone marrow biopsy. More specific than total WBCs, changes in the T-cell population can correlate with disease activity. Low numbers of CD8+ T cells (<600/mL) have been associated with a worse prognosis in MF/SzS patients.[33–35] T-cell size can also be prognostic as large-cell transformation (LCT) to ≥4 times a normal lymphocyte portends a worse prognosis.[36,37] Atypical lymphocytes are larger with irregular nuclear contour, identified by high scatter on flow cytometry.[38] LCT of disease in MF/SzS patients is associated with overall reduction in life expectancy to 37 months compared to 163 months in untransformed disease.[36] As LCT is temporally intertwined with disease progression, it may be more clinically relevant to consider predictive markers of pending LCT to identify patients at risk before their disease progresses. Elevated β2M, LDH and CD25 have been identified as possible predictors of transformation.[36,39] Additionally, cells that demonstrate large-cell transformation may be CD30+ or CD30−. In a study of patients with large-cell transformation of mycosis fungoides, CD30 positivity was a predictor of improved survival.[40]

5 CELL SURFACE MARKERS

Significant efforts have been made to identify changes in cell surface markers specific to circulating Sézary cells or infiltrating cells in MF, summarized in Table 1. Flow cytometry on peripheral blood mononuclear cells (PBMCs) and IHC on formalin-fixed paraffin-embedded skin biopsies are used to characterize surface marker expression. Importantly, multicolour flow cytometry allows for the simultaneous measurement of eleven or more markers on a single cell.[41] Double immunoenzyme staining and multispectral immunofluorescence imaging allow for more than one marker to be visualized at a time in histopathological analysis.[42] Thus, as new markers continue to be identified, additional parameters can easily be added to these existing approaches.

New technology has recently been utilized for TCR analysis. TCR-Vβ analysis either by flow cytometry or IHC in frozen tissue has historically been used to identify clonal populations in circulating T cells or lymphocytic infiltrates, allowing for differentiation between MF/SzS and inflammatory conditions.[13,43,44] Further, patients with a single gene rearrangement clone detected in multiple concurrent biopsy samples at the time of diagnosis were more likely to have progressive disease than those with multiple TCR clones.[45] TCR-Vβ analysis using these methods does not include all TCR-Vβ families, so a clone may be suspected but not identified. TCR gene rearrangements have also been identified with TCR PCR analysis. Recent introduction of high-throughput TCR sequencing of CDR3 has improved the identification of malignant T cells. Next-generation sequencing is on its way to offer full spectrum clonal analysis of α-, β-, γ-and δ-submits. This methodology provides a full spectrum of clones in the sample making it easy to follow a particular clone for identification of minimal residual disease, including malignant cells in lymph nodes missed by traditional histopathological analysis.[46,47] The utility of TCR analysis as a stand-alone biomarker of MF/SzS is limited as other T-cell lymphoproliferative conditions and cutaneous lymphoid dyscrasias also demonstrate clonal rearrangement of TCR genes;[48,49] however, TCR analysis has been successfully combined with other markers to improve identification of SzS.[44]

The prognostic value of a cell surface marker may vary with treatment. Novelli et al.,[50] found SzS patients who had a presence of a variable proportion of CD26+ atypical cells at diagnosis showed a statistically significant higher overall survival. However, in their retrospective cohort study of eleven MF/SzS patients who had undergone treatment, Vandersee et al.[51] calculated a low positive predictive value for changes in CD26 expression and clinically meaningful events. CD26-cell number has also been shown to vary with chemotherapeutic and immunomodulating treatments irrespective of concurrent clinical response.[51,52] Thus, CD26 status may confer prognostic information at diagnosis but may not be indicative of treatment response or disease progression. Chemotherapeutic treatment regimens increase cell turnover, and immunomodulating ones can alter the presence of surface markers related to immune function. As a result, treatment conditions must be carefully considered when trending a surface marker for prognosis.

6 GENETIC AND EPIGENETIC MARKERS

Gene transcription in MF/SzS has been studied extensively using blood samples or skin biopsies with microarray platforms, transcriptome sequencing and quantitative reverse transcriptase PCR (qRT-PCR).[53] A review of this work by Wong highlighted a number of key genetic markers with altered expression in MF/SzS patients.[54] We build on that work and present a list of genes identified in multiple expression studies in Table 2.

Clinically, upregulated or downregulated genes can be identified with qRT-PCR, and genes translated to proteins can be identified with Western blotting, ELISA, IHC or flow cytometry. In the case of biomarkers, the best candidate genes are those which can identified with little additional processing of the patient sample, that is whole blood rather than CD4+CD7−T cells.[53] In addition to the genes presented in Table 2, a number of the cell surface markers in Table 1 including KIR3DL2 and CD26 are also altered at the gene

expression level; however, cell surface markers can be up-or downregulated at the transcription, translation or localization level, so gene expression does not always correlate with presence on the cell surface.[55]

A panel of genes can easily be tested simultaneously on a single qRT-PCR plate. Two such panels have been proposed. Nebozhyn et al.[56] identified a five gene SzS panel including *STAT4*, *GATA3*, *PLS3*, *CD1D* and *TRAIL* which displayed an average accuracy of 90% over 1000 resamplings of PBMCs from 49 SzS patients and 65 healthy controls. Michel et al.[57] demonstrated qRT-PCR analysis of *PLS3*, *Twist1*, *CD158k/KIR3DL2* and *NKp46* accurately classified 100% of 81 SzS patients; however, qRT-PCR detection of *CD158k/KIR3DL2* and *NKp46* in PBMCs may be complicated by expression of these two markers on NK cells of healthy patients. Instead, the authors propose flow cytometry analysis could be used on these two markers in combination with qRT-PCR analysis. Both of these expression panels were tested in single-centre efforts, and future study of these panels or others would benefit from multicentre, international trials. Prognostic panels based on gene expression have also been proposed; Litvinov et al. identified a group of 17 genes including IL2RA, CCR4, STAT5A and TOX that could identify patients at risk of progression and distinguish MF from SzS (Litvinov et al. 2015).

Non-coding RNAs represent another set of possible biomarkers in MF/SzS, and differential expression of microRNAs (miRNAs) and long non-coding RNAs (lnRNAs) has been studied. Ballabio et al.[58] found an increased level of miR-223 in CD4+ T cells was 90% accurate with 91% specificity and 90% sensitivity in correctly predicting diagnosis of SzS compared with MF or healthy controls. Ralfkiaer et al.[59] reported a three-miRNA panel (miR-155, miR-203 and miR-205) that distinguished CTCL from benign skin diseases with a classification accuracy of 95%. Narducci et al.[60] identified miR-214 and miR-486 overexpression in the majority of SzS patients as well as a signature of 14 miRNAs including miR-21, a miRNA upregulated in a number of cancers, which grouped SzS patients into favourable vs unfavourable outcomes. Benner et al.[61] identified differential expression of five miRNAs, including miR-155, between tumor-stage MF and primary cutaneous anaplastic large-cell lymphoma (cALCL). Conversely, Sandoval et al.[62] identified upregulated miR-155 in both tumor-stage MF and cALCL as well as upregulation of miR-42-5p and miR146a in MF. MiRNA expression has also been correlated with transformation of MF[63] and disease progression to advanced stage.[64] Lee et al.[65] identified 12 long non-coding RNAs in 3 SzS patient samples with transcriptome sequencing and confirmed the presence of long non-coding RNAs in MF/SzS tumors. While the number of studies of miRNAs and lnRNAs in MF/SzS is still relatively small, the results are promising.

Potential biomarkers also exist at the chromosomal level where translocations, duplications or deletions can be detected clinically using fluorescent in situ hybridization (FISH). To date, detected chromosomal imbalances in SzS have included gains of 17p11.2–q25.3 and 8q24.1–8q24.3 and losses of 17p13.2–p11.2, 10p12.1–q26.3 which each occurred in >40% of SzS cases.[66–68] Chromosomal alterations have been more commonly identified in SzS than MF, although duplication of 17q11.2 approximately q12 was identified in both.[66] Individual chromosomal aberrations have not been correlated with SzS prognosis, but an

increasing number of chromosomal gains or losses does correlate with decreased survival.[67] Interestingly, TCR loci chromosomal translocations have been described in several T-cell malignancies but were not identified by FISH in MF/SzS.[69] Gains of *TCRB* and *TCRG* genes were, however, observed in 23% of SzS and 50% of tumor-stage MF.[69] Gains or losses of additional individual gene loci have also been demonstrated, and FISH panels to screen for genetic changes have been proposed.[70] Analysis of SzS cases with FISH using an *IgH*/*BCL2* probe revealed loss of at least one copy of *BCL2* in 83% of samples,[71] and digital droplet PCR showed gains the *TNFRSF1B* (TNFR2) locus in 10 of 73 SzS/MF patients.[72] In a comprehensive study of chromosomal alterations in SzS, gain of cMYC and loss of cMYC antagonists (MXI1 and MNT) was observed.[68]

In the last year, several reports of exome and whole-genome sequencing in MF and SzS have emerged. Frequent somatic mutations (single nucleotide variants or small insertion and deletions) were reported in TCR/NFκB signalling (NFKB2 truncations, TNFAIP3, PLCG1, PRKCQ and TNFAIP3 mutations),[73,74] Th2 differentiation (ZEB1),[75] cell survival and fate (PDGFR, ERK, JAK/STAT, MAPK),[74–76] epigenetic regulation (DNMT3A, ASLX3, TET1-3),[77] homologous recombination (RAD51C, BRCA2, POLD1)[74] and cell-cycle control (TP53).[75,77] These pathways represent new targets for treatment, and mutated genes represent potential biomarkers particularly for prognostication or monitoring of disease activity in a patient already diagnosed with MF/SzS.

7 WHERE DO WE GO FROM HERE?

To take the discovery of possible biomarkers through the process of validation, collaboration amongst groups will be key. We suggest a group of molecules for future studies based on the various functions of biomarkers in MF/SzS (Table 3). Head-to-head comparisons of various biomarkers in multicentre large-cohort studies will be necessary to select those markers with the highest clinically meaningful applications. Fortunately, collaborative efforts have already begun. The European Organization for Research and Treatment of Cancer evaluated 57 SzS and 40 erythrodermic inflammatory dermatoses cases to determine which histopathological features were the best indicators. CD7 loss, increased small cerebriform lymphocytes, decreased CD8+ lymphocytes and increased proliferation (Ki-67+ lymphocytes) were the best features for differentiation.[78] The Cutaneous Lymphoma International Consortium collected data at 29 specialist centres on 1275 patients diagnosed with advanced-stage MF/SzS examining and identified four independent prognostic markers for a worse survival: stage IV, age>60 years, large-cell transformation and increased LDH.[10] Moving forward, continued work of this type that incorporates new available technological platforms or possibly combinations of platforms along with rigorous biostatistical analysis will allow for additional integration of the bench discovery work summarized in this review with the clinical care of MF/SzS patients.

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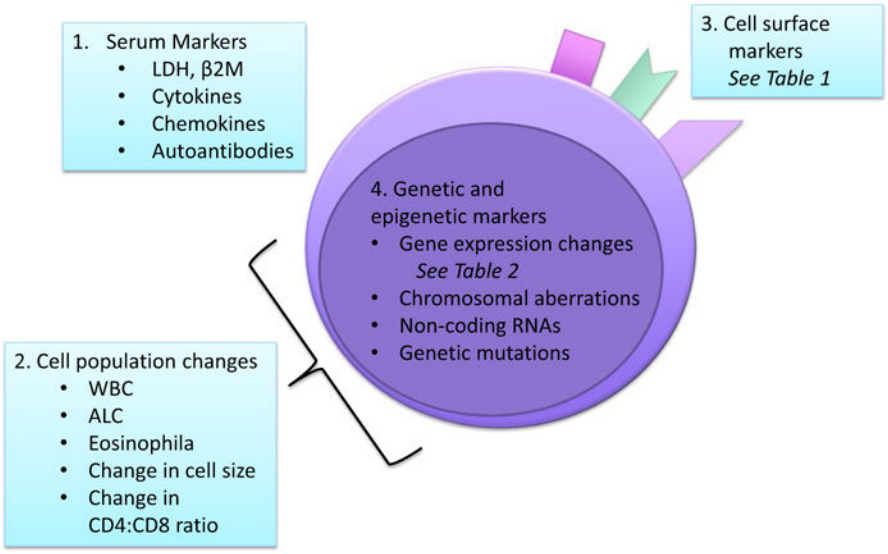

FIGURE 1.

Biomarker Classes in MF/SzS—Putative markers include[1] molecules measured in serum samples,[2] leucocyte cell population changes measured by whole blood or peripheral mononuclear cell analysis,[3] cell surface markers measured by flow cytometry or immunohistochemistry and[4] genetic and epigenetic markers measured by a variety of molecular techniques. Biomarkers may belong to several categories at the same time. LDH, lactate dehydrogenase; β2M, beta-2 microglobulin; WBC, white blood cell count; ALC, absolute lymphocyte count

TABLE 1

Measurement of cell surface markers in SzS or MF patient samples

Candidate biomarkers	Sample size and type[a]	Method[b]	Diagnostic value/outcome[c]	Ref
CD26	52 SzS, 151 MF (14 stage B1), 88 IE, 72 HD	Flow	CD4+CD26−>30% of PBLs in 93% of B1-MF cases and >40% in all SzS; CD4+CD26−<30% PBLs in 100% of HD, IE and B0-MF	[79]
	107 SzS	Flow	89.7% of SzS patients demonstrate loss of CD26	[50]
$CD3^{dim}$	17 SzS, 11 HD	Flow	76% of SzS patients with >1000 CD4+CD7−$CD3^{dim}$ cells μL^{-1}, 0% of HD	[44]
	107 SzS	Flow	76.6% of SzS patients demonstrate dim CD3	[50]
CD27	40 SzS, 137 IE, 63 HD	Flow, IHC	40% of PBLs from IE or HD were CD4+CD27−compared with 80.5% of PBLs from SzS	[80]
CTLA-4	9 SzS, 9 MF	IHC	89% staining in MF, 100% staining in SzS	[81]
CD45R0 vs CD45RA	29 SzS	IHC	75% of SzS with 75%–100% of infiltrate CD45RO+	[18]
	215 MF	IHC	8.7% of MF are CD45RA+ with higher # of other pathologic abnormalities	[82]
	6 SzS, 30 MF, 18 HD	Flow	Increase in % and # of CD4+CD45R0+ CD45RA−in late stage MF/SzS compared to early disease or HD	[35]
Vimentin	14 MF, 3 SzS, 2 other CTCL	SEREX, qRT-PCR	qRT-PCR found vimentin in 73% of CTCL samples, also expressed in 80% of normal tissues	[83]
	87 CTCL	2D WB + MS	Vimentin identified at multiple sites on 2D blot suggesting splicing variants or variable isoforms	[31]
CD158k/KIR3DL2	17 SzS, 11 HD	Flow	65% of SzS with CD158k+ cells, 0% of HD	[44]
	33 SzS	Flow	96.9% of SzS with CD158k+ cells, these CD158k+ restricted to phenotypically abnormal T cells	[84]
	34 SzS, 6 IE, 10 HD	Flow	Positive correlation between %CD158k+ and %atypical circulating cells in SzS, malignant clone expresses CD158k	[85]
	25 SzS	Flow	CD158k+ associated with clinical flare	[86]
NKp46/CD335	17 SzS, 5 MF, 10 IE, 4 HD	Flow, immunoblotting	CD4+NKp46+ tumoral cells identified in all SzS patients, no significant CD4+NKp46+ in other groups	[87]
Ganglioside GD3/CD60	6 SzS, 30 MF, 18 HD	Flow	Significant increase in % and # of CD4+CD60+ in late stage MF/SzS compared to early disease or HD	[35]
	62 SzS, 180 MF, 6 BCL, 19 AD	Flow	Higher # of CD60+ circulating CD4+ T cells at SzS presentation associated with lower probability of survival	[88]
Syndecan 4 (SD-4)	6 SzS, 3 MF, 4 HD, 3 AD, 3 psoriasis	Flow	All SzS patients had high expression of SD-4 on CD4+ cells, significantly higher than all other samples	[89]
Sialomucin (CD164)	59 SzS, 10 MF, 6 AD, 14 HD	Flow	% CD4+CD164+ T cells significantly increased in SzS compared to MF, AD, HD; CD164+ cells disappear with disease remission	[90]
PD-1/CD279	7 SzS, 4 MF, 5 HD	Flow	Significant increase in CD4+PD-1+ cells in SzS group compared with MF and HD	[91]
	27 SzS, 60 MF	IHC	89% of SzS cases had >50% neoplastic cells PD-1+, 13% of MF cases had >50% neoplastic cells PD-1+	[92]

Candidate biomarkers	Sample size and type[a]	Method[b]	Diagnostic value/outcome[c]	Ref
	25 SzS, 30 IE	IHC	>50% of infiltrating T cells PD-1+ in 92% of SzS cases (where PD-1+ cells were CD4+) and of 13% IE cases (where PD-1+ cells were CD8+)	[93]
CD52	16 CTCL	Flow	87.5% of CTCL samples were CD52+	[94]

PBLs, peripheral blood lymphocytes.

[a]Sample type abbreviations include Sézary syndrome (SzS), mycosis fungoides (MF), inflammatory erythroderma (IE), healthy donors (HD), cutaneous T-cell lymphoma (CTCL), B-cell lymphomas (BCL) and atopic dermatitis (AD).

[b]Candidate biomarkers have been studied primarily using peripheral blood samples or skin biopsies with application of flow cytometry (flow) or immunohistochemistry (IHC). Other methods include serological identification of antigens by recombinant expression cloning (SEREX), Western blotting (WB) and mass spectrometry (MS).

[c]Peripheral blood leucocytes (PBLs).

TABLE 2

Measurement of gene expression in SzS or MF patient samples

Candidate genes	Sample size and type[a]	Method[b]	Diagnostic value/outcome	Ref
T-plastin (*PLS3*)	PBMCs: 18 SzS, 9 Th2-skewed controls	Microarray, qRT-PCR	Increased 14-fold in SzS compared with controls based on array and 479-fold based on qRT-PCR	[95]
	PBMCs: 49 SzS, 69 HD, 3 IE	qRT-PCR	Increased 520-fold in SzS compared with controls	[56]
	PBMCs: 10 SzS, 10 HD	Microarray, qRT-PCR	0.89 AUC for discrimination between SzS and controls with sensitivity 0.82 and specificity 0.91	[96]
	CD4+ T cells: 81 SzS, 12 HD	qRT-PCR	Increased 145-fold in SzS compared with HD samples and positive in 87% of SzS patients	[57]
	CD4+ T cells: 9 SzS, 4 HD; skin biopsies: 2 SzS, 4 MF, 9 psoriasis	qRT-PCR, IHC	Increased 270-fold in SzS compared with controls, PLS3 detected in infiltrate on MF/SzS biopsy but not psoriasis	[97]
JUNB	Peripheral blood ± skin biopsies: 26 SzS, 13 MF	Real-time PCR, IHC	26% of SzS/MF had increased copy number of JUNB, 91% of SzS/MF had strong nuclear staining of JUNB	[71]
	PBMCs: 18 SzS, 9 Th2-skewed controls	Microarray, qRT-PCR	Increased fivefold in SzS compared with controls based on array and 10-fold based on qRT-PCR	[95]
	PBMCs: 49 SzS, 69 HD, 3 IE	qRT-PCR	Increased 4.3-fold in SzS compared with controls	[56]
GATA3	PBMCs: 49 SzS, 69 HD, 3 IE	qRT-PCR	Increased 6.4-fold in SzS compared with controls	[56]
	PBMCs: 18 SzS, 9 Th2-skewed controls	Microarray, qRT-PCR	Increased 2.5-fold in SzS compared with controls based on array and sevenfold based on qRT-PCR	[95]
SATB1	CD4+CD7− T cells: 9 SzS; CD4+ T cells: 9 HD	Microarray, qRT-PCR, WB	Decreased 4.2-fold in SzS samples compared with controls based on array, confirmed by qRT-PCR and WB	[98]
	PBMCs: 10 SzS; CD4+ T cells: 5 IE, 3 HD	Microarray, qRT-PCR	Decreased 4.6-fold in SzS compared with controls based on array, confirmed by qRT-PCR	[99]
	Skin biopsies: 90 MF, 19 benign lesional skin	IHC	Higher expression of SATB1 in MF compared with controls, and SATB1 higher in patients with lymph node involvement	[100]
STAT4	PBMCs: 49 SzS, 69 HD, 3 IE	qRT-PCR	Decreased 4.7-fold in SzS compared with controls	[56]
	PBMCs: 18 SzS, 9 Th2-skewed controls	Microarray, qRT-PCR	Decreased 3.7-fold in SzS compared with controls based on array and 4.5-fold based on qRT-PCR	[95]
	Skin biopsies: 60 MF/SzS, 19 benign lesional skin, 6 HD, 19 benign lesional skin	qRT-PCR, WB	STAT4 was expressed in MF/SzS lesional skin but not normal skin or benign lesions, loss of STAT4 was associated with progressive disease	[101]
Twist1	PBMCs: 10 SzS; CD4+ T cells: 5 IE, 3 HD; skin biopsies: 9 MF, 24 benign lesions	Microarray, qRT-PCR	Increased 19.8-fold in SzS compared with controls based on array, Overexpressed in all SzS and 4/9 MF samples by qRT-PCR but not control lesional skin	[99]
	CD4+ T cells: 81 SzS, 12 HD	qRT-PCR	Increased 150-fold in SzS compared with HD samples and positive in 91% of SzS patients	[57]
	Skin biopsies: 68 MF/SzS, 3 HD, 3 psoriasis, 3 SCC; CD4+ T cells: 5 SzS	IHC, qRT-PCR	Twist was found in 12.5% of T1, 33.3% of T2, 50.0% of T3, 84.6% of T4 biopsies by IHC. All SzS and no HD had expression of Twist by qRT-PCR in CD4+ T cells	[102]

Candidate genes	Sample size and type[a]	Method[b]	Diagnostic value/outcome	Ref
Fas	CD4+ T cells: 16 MF, 4 SzS, 25 benign lesions, 15 HD	Flow cytometry	Fas expression was lower in MF/SzS compared with controls, Fas increased after response to treatment	[103]
	Skin biopsies: 23 MF, 10 LyP, 10 CD30+ LTCL, 9 CD30-LTCL	IHC	Fas expression present in 100% of plaque-stage MF, LyP and CD30+ LTCL, but only 33% of tumor-stage MF. Decrease in Fas was observed with progression.	[104]
TOX	CD4+ cells: 9 SzS, 4 HD; skin biopsies: 2 SzS, 4 MF, 9 psoriasis	qRT-PCR, IHC	Increased sevenfold in SzS compared with HD on qRT-PCR, Strong nuclear staining of TOX on IHC in MF/SzS but not psoriasis	[97]
	Skin biopsies: 21 MF, 15 benign lesional skin, 21 HD	Microarray, qRT-PCR, IF, IHC	Increased 10.3-fold in MF compared with HD, confirmed with qRT-PCR, highly specific staining of TOX in MF biopsies on IHC and IF	[105]

[a]Sample type abbreviations include Sézary syndrome (SzS), mycosis fungoides (MF), inflammatory erythroderma (IE), healthy donors (HD), peripheral blood mononuclear cells (PBMCs), lymphomatoid papulosis (LyP), large T-cell lymphoma (LTCL).

[b]Methodology abbreviations include quantitative reverse transcription polymerase chain reaction (qRT-PCR), immunohistochemistry (IHC), Western blotting (WB), immunofluorescence (IF).

TABLE 3

Selected promising biomarkers for future validation studies

Diagnostic biomarkers	Biomarkers of advanced disease	Biomarkers of aggressive disease	Biomarkers of active disease
TOX (MF) T-plastin (SzS) Twist1 (SzS) CD158k/KIR3DL2 (SzS)	LDH CD4+CD45R0+CD45RA- SATB1 ganglioside GD3/CD60 STAT4 (loss) IL-13 TARC/CCL17	LDH ganglioside GD3/ CD60+ Fas (decrease) β2M IgE	CD158k/ KIR3DL2 sialomucin (CD164) TCR clone