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Cutaneous T cell lymphoma: Current practices in blood assessment and the utility of T-cell receptor Vβ chain restriction

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Abstract

Background—Accurate quantification of malignant cells in the peripheral blood of patients with cutaneous T cell lymphoma (CTCL) is important for early detection, prognosis, and monitoring disease burden.

Objective—Determine the spectrum of current clinical practices; critically evaluate elements of current ISCL B_1 and B_2 staging criteria; and assess the potential role of TCR-Vβ analysis by flow cytometry.

Methods—We assessed current clinical practices by survey, and performed a retrospective analysis of 161 patients evaluated at Yale (2011-2014) to compare the sensitivity, specificity, PPV, and NPV of parameters for ISCL B_2 staging.

Results—There was heterogeneity in clinical practices among institutions. ISCL B_1 criteria did not capture five Yale cohort patients with immunophenotypic abnormalities who later progressed. TCR-Vβ testing was more specific than PCR and aided diagnosis in detecting clonality, but was of limited benefit in quantification of tumor burden.

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Conflicts of interest: Dr. Hussong is the Director of Laboratory for ARUP Laboratories, Salt Lake City, UT; is on the Executive Committee for ARUP Laboratories, Salt Lake City, UT. Mr. Mohl is a technical supervisor for ARUP Laboratories, Salt Lake City, UT. Ms. Hill is a hematologic flow cytometry specialist for ARUP Laboratories, Salt Lake City, UT.

Limitations—Because of limited follow-up involving a single center, further investigation will be necessary to conclude whether our proposed diagnostic algorithm is of general clinical benefit.

Conclusion—We propose further study of "modified B_1 criteria": CD4/CD8 ratio ≥5, %CD4+/CD26- ≥ 20%, %CD4+/CD7- ≥ 20%, with evidence of clonality. TCR-Vβ testing should be considered in future diagnostic and staging algorithms.

Keywords

Cutaneous T cell lymphoma; mycosis fungoides; Sézary syndrome; flow cytometry; TCR-Vβ; peripheral blood analysis

Introduction

Cutaneous T-cell lymphoma (CTCL) is a relatively rare and heterogeneous group of indolent skin-homing neoplasms of memory CD4+ T cells that can variably involve the lymph nodes and peripheral blood.[1] Leukemic involvement has been found to be an independent prognostic factor in CTCL, and identification and quantification of blood involvement is important for early detection of disease as well as for monitoring tumor burden over time.[2-4]

In 2007, the ISCL/EORTC published revised guidelines for the assessment of peripheral blood involvement. B_0 and B_1 ratings are defined by Sézary counts (SCs), (B_0 < 5% SCs; B_1 > 5% SCs but either less than 1.0K/μl absolute SCs or absence of a clonal TCR rearrangement, or both) while the current B_2 rating is defined by clonal rearrangement of the TCR in conjunction with any one of the following: 1) >1000 SCs/μl, 2) increased CD4+ or CD3+ population with CD4/CD8 >10, or 3) an increase in CD4+ cells with an abnormal phenotype (i.e. >40% CD4+/CD7-; >30% CD4+/CD26-).[5]

Current guidelines continue to utilize the identification and enumeration of the morphologically atypical Sézary cells. The sensitivity of SCs is limited by inter-observer subjectivity and the fact that many patients with blood involvement will not manifest SCs.[5] SCs may also be found in patients with benign inflammatory conditions as well as in normal individuals, albeit usually in smaller quantities.[6]

As our understanding of cell surface markers and atypical phenotypes in CTCL has improved, flow cytometry has proven to be more robust than Sézary counts. However, there is no single marker that is both sensitive and specific for blood involvement. [7] An elevated CD4/CD8 ratio, and loss of CD7 and/or CD26 on CD3+CD4+ T cells, have commonly been cited as occurring in malignant T cells of CTCL but are also observed physiologically and in benign erythrodermas.[8-10,11,12] Central to the diagnosis of peripheral blood involvement is the identification of T cell clonality by PCR. Yet despite its superior sensitivity, PCR in CTCL patients is relatively non-specific, with some studies even concluding that "clonality" demonstrated by PCR was more common in patients with benign conditions than in patients with SS.[13-15]

Flow cytometric analysis using antibodies directed against the T cell receptor (TCR)β chain has been shown to demonstrate clonality in between 79% to 100% of patients with blood involvement.[16,17] However, TCR-Vβ has been found in previous studies to be less sensitive

than PCR.[14] Thus, to improve the sensitivity of TCR-Vβ analysis, some groups have begun to analyze Vβ expression on subset T cell populations after gating (i.e. first selecting cells for further flow cytometric analysis), such as on CD4+CD7- T cells or other identified phenotypic aberrancies.[17]

Commercially available Vβ antibodies are capable of recognizing only 70% of the TCR-Vβ repertoires in normal T-cells.[18] A known clone by PCR may also demonstrate "non-reactivity," where a non-detectable, dominant clone suppresses the usage of other Vβ families, leading to a restricted TCR repertoire. [18,19] Hence, several studies have suggested that failure of at least 70% of a gated T cell population to react to the panel of Vβ antibodies is indicative of clonality. [20]

Despite the recommendations by the ISCL/EORTC, there is a paucity of data examining the performance parameters of individual criteria for detecting blood involvement and there are no guidelines for testing for clonality by TCR-Vβ flow cytometric analysis. Herein, we endeavored to determine the current spectrum of clinical practices and then aimed to (1) identify which, if any, of the markers most frequently used to identify abnormalities in the peripheral blood possess superior performance measures, (2) determine the utility of TCR-Vβ testing for detection of blood involvement in CTCL and situations in which this test should be used, and (3) suggest recommendations for B1 criteria that are not based on SC enumeration.

Methods

Survey

A survey study of six multiple-choice questions (SurveyMonkey) was sent by email to members of the USCLC and the MDS to characterize clinical practices for analyzing blood in CTCL. Topics included indications for testing for blood involvement, stage(s) at which blood was tested, diagnostic tools, T cell markers tested during flow cytometric analysis, laboratories to which specimens were sent for analysis, and contexts in which Vβ analysis was performed.

Patient selection

A retrospective analysis was carried out including 161 patients evaluated at the Yale Cutaneous Lymphoma Center between 2011 and 2014 who were tested for TCR-Vβ restriction. All patients were retrospectively staged according to the current ISCL guidelines and tumor burden was assessed using the modified severity weight assessment tool.[62] The diagnosis of CTCL was confirmed in 133 patients by biopsy, two patients were diagnosed based on blood involvement in constellation with clinical features, and all other patients were diagnosed based on clinical presentation. Yale University's IRB granted exempt status for this retrospective case series.

Flow cytometry

In the course of patients' treatment at the Yale Cutaneous Lymphoma Center, peripheral blood samples were obtained at various time points. Flow cytometry analysis was performed

using fluorochrome-conjugated antibodies for the following antigens: CD2, CD3, CD4, CD5, CD7, CD8, CD25, CD26, CD45, CD45RA, and CD45RO. Blood was longitudinally assessed for CD4/CD8 ratio, CD4+CD7- and CD4+CD26- phenotype percentages, and CD3+CD4+ absolute number. Yale Laboratory Medicine does not perform Sézary counts due to the aforementioned limitations. If atypical lymphocytes were noted based on immunophenotypic alterations, this percentage was multiplied by the absolute lymphocyte count to approximate the number of "Sézary cells", allowing for qualification for B_2 or B_1 criteria based on the absolute number of atypical circulating cells. Blood involvement was defined by the ISCL B_2 and B_1 ratings. [5]

Polymerase chain reaction

Genomic DNA was extracted from peripheral blood samples and tested for TCR beta and gamma gene rearrangements. Regions of the gene were amplified enzymatically, and the amplified products were characterized for the presence of PCR products in the 75-110bp range on acrylamide gels. The primers V-γ-11, V-γ -101, J-γ-1, and Jp-γ-12 were used for TCR-γ gene rearrangement amplification.[21] The primer set D2 and J2 was used for TCR-β gene rearrangement.[22]

TCR-Vβ testing

Testing for TCR-Vβ restriction was performed for all patients by ARUP Laboratories, (Salt Lake City, Utah). Briefly, evaluation of the TCR-Vβ expression was performed using 5-color immunophenotyping including primarily antibodies to CD3 (ECD, Coulter Immunotech), CD4 (PC-7, Coulter Immunotech), CD7 (PC-5, Coulter Immunotech) as well as fluorochrome (FITC and PE) labeled antibodies to the various TCR-Vβ's within the IOTest Beta Mark TCR-Vβ Repertoire kit (Beckman Coutler). The IOTest Beta Mark TCR-Vβ Repertoire kit evaluates 24 different TCR-Vβ utilizing 8 tubes. Each tube in conjunction with CD3, CD4, and CD7 evaluates 3 different TCR-Vβ's labeled with FITC, PE or FITC plus PE.

Selective gating using CD3, CD4 and CD7 allowed analysis of the total T-cell population (CD3+) as well as various CD3+ T-cell subset populations such as CD3+, CD4+, CD7-. A T-cell population was considered clonal if greater than 50% of the analyzed population expressed a single TCR-Vβ or if the analyzed population expressed less than a total of 20% of the evaluated TCR-Vβs. The latter would indicate that 80% or more of the analyzed population failed to react with any of the TCR-Vβs antibodies, presumptively due to expression of a TCR-Vβ not recognized by the antibody panel in the repertoire kit. We defined an "equivocal" test as one that showed 20-50% reactivity. The number of circulating atypical cells was calculated using TCR-Vβ by multiplying the percentage of atypical T cells by the TCR-Vβ expression in that population by the total number of T cells (absolute CD4+CD3+).

Statistical Analysis

The student t test was used to compare percentages of CD4+CD7-, CD4+CD26-, and CD4/CD8 ratios between B_0/B_1 and B_2 groups. The number of patients in each group

meeting each criterion for B_2 involvement was compared using the Fischer exact test for comparison of proportions. A p value<0.05 was considered statistically significant.

Results

Current clinical practices

Of 33 institutions surveyed, there was substantial variation with regard to the CTCL stage at which blood involvement was assessed, tools utilized for assessment of blood involvement, and T cell receptor markers tested by flow cytometry (Table 1). 51.4% reported obtaining peripheral blood smears for SC counting and only 14.3% reported utilizing Vβ assays for detection of a clonal T cell population.

ISCL B1 staging

Twelve individuals satisfied ISCL B_1 criteria by having greater than 5% circulating atypical cells, but <1k/μl (4 T_4, 1 T_3, 5 T_2, 2 T_1). Ten of these twelve had TCR gene arrangements by PCR of which six had TCR-Vβ restricted clones, and two had “equivocal” TCR-Vβ results.

Analysis of current B2 criteria

161 patients were identified who were tested for TCR-Vβ restriction between 2011 and 2014. Five patients had concurrent CLL, 3 patients had follicular mucinosis, 2 patients had PCSM-TCL, and 6 patients had folliculotropic MF.

Thirty-four patients met current B_2 ISCL criteria. This group included patients with greater tumor burden as assessed by T stage and mSWAT at time of TCR-Vβ testing who required a greater number of systemic therapies (Table 2). Patients fulfilling B_2 criteria had higher median values for %CD4+CD7-, %CD4+CD26-, and CD4/CD8 ratios than those fulfilling B_0/B_1 criteria ($p<0.05$ in all cases). The proportion of patients who satisfied individual components of the ISCL criteria for B_2 blood rating was greater in those with blood involvement of B_2 rating than in the those without B_2 blood involvement ($p<0.001$). Additionally, the proportion of patients with a Vβ-detected clonal T cell population was greater in patients with blood involvement than in those without ($p<0.0001$) (Table 3).

The sensitivity, specificity, PPV, and NPV of individual components of the ISCL criteria for blood involvement and for Vβ analysis were calculated and compared (CD4/CD8 ratio ≥ 10, $CD4^+/CD7^-$ ≥ 40%, $CD4^+/CD26^-$ ≥ 30%, positive PCR for clonality) (Table 4). $CD4^+/CD7^-$ ≥ 40% possessed the highest specificity for blood involvement while $\%CD4^+/CD26^-$ ≥ 30% possessed the highest sensitivity and NPV. Both had comparable PPVs. Consistent with other studies, patients with blood involvement demonstrated a stable phenotype over time.

TCR-Vβ analysis

When gated on an atypical population (defined by abnormalities in CD3, CD4, and/or CD7) TCR-Vβ testing revealed clonal expansions in 36 individuals. TCR-Vβ clonal restriction was identified in 25 of the 34 B_2 patients (73.5%) and allowed for the identification of clonal expansion in patients with as low as 1% circulating atypical cells. Five of these 25 patients (20.0%) met TCR-Vβ criteria of clonality through non-reactivity (<70% reactivity).

TCR-Vβ testing demonstrated lower sensitivity (73.5%) compared to PCR (by definition 100%) but was significantly more specific for blood involvement (90.6% vs. 64.0%). Twelve individuals had TCR-Vβ restricted clones in the non-B_2 population (9.4%). In contrast, when gated on all CD3+ cells, TCR-Vβ testing only revealed clonal populations in thirteen patients (12 B_2 patients, 1 non-B_2).

Discussion

Assessment of blood involvement in CTCL offers prognostic significance yet guidelines for blood analysis are sparse, and Sézary cell enumeration is subjective yet remains a component of ISCL B_2 and B_1 criteria. We conducted this study with the goal of exploring a new approach for the assessment of blood involvement in CTCL.

Current Practices

We found significant heterogeneity among institutions in the assessment of peripheral blood involvement in CTCL. Over half of respondents indicated use of peripheral smears for SC quantification despite the impracticality of the tool and evidence of drawbacks, including subjectivity of results and nonspecific nature of morphologic assessment. [5-7] While nearly 90% of respondents checked for CD7 expression, only about 40% checked for loss of CD26. Analysis of TCR-Vβ was also infrequently utilized and institutions that indicated performing TCR-Vβ analysis used the test solely to demonstrate clonality in the blood.

Analysis of ISCL B_2 criteria

We next aimed to work towards a new approach for the assessment of blood involvement. We first analyzed each individual component of the current ISCL B_2 criteria. We found significant differences in all components of the ISCL criteria between ISCL B_2 and non-B_2 groups. Loss of CD26 was the most sensitive marker for blood involvement and only five individuals did not meet the $CD4^+/CD26^-\geq 30\%$ criterion. With a goal to improve the sensitivity of current B_2 criteria, we assessed the utility of the parameter $\%CD4^+/CD26^- \geq 25\%$. This change increased the parameter's sensitivity without a great cost to its specificity (Table 4). In contrast to other studies which found loss of CD26 to be more specific in CTCL than loss of CD7, our study found the criteria for CD4+CD7->40% to be the most specific of the ISCL parameters.[23]

Modified B_1 criteria

To help formulate B_1 criteria that are based on immunophenotypic data instead of morphology, we applied the following conditions to our current group: CD4/CD8 ratio ≥5, %CD4+/CD26- ≥ 20%, %CD4+/CD7- ≥ 20%. We identified eighteen individuals with evidence of clonality, all with TCR gene rearrangements, four with TCR-Vβ restriction, and four with an “equivocal” TCR-Vβ result. Four patients had T_4 disease and required systemic therapy; five had T_2 disease with progression of blood involvement necessitating systemic therapy; and one had T_3 disease and developed metastatic lesions. Sixteen patients were observed longitudinally – twelve progressed (75.0%) and four (3 T_1, 1 T_2) improved and eventually did not meet the revised B_1 criteria.

Our modified B_1 criteria identified 10 individuals not captured by current ISCL criteria. Five had advanced stage disease (oneT_4, one T_3, and 3 T_2 disease) that required systemic therapy. Importantly, the three ISCL B1 individuals who did not meet our modified criteria had either indolent disease (T_1, not requiring systemic therapy) or met B_2 criteria using TCR-Vβ restriction as evidence for clonality; thus, implementation of these criteria would not omit clinically significant patients.

Role of TCR-Vβ testing

We next attempted to clarify the role of TCR-Vβ testing in assessing blood involvement in CTCL. In line with other investigators, we found that TCR-Vβ testing on gated atypical populations was more specific yet less sensitive than PCR. In our group, testing for clonality using TCR-Vβ would allow one patient to meet criteria for B_2 blood involvement. Consistent with other studies, in patients where a TCR-Vβ clone is detectable, it may be useful to continue to monitor tumor burden by TCR-Vβ expression. [20,24,25]

Regarding the gating on a small atypical population, it has been postulated the test may be overly sensitive and lead to false positives. In our study, eleven “false positives” were identified (TCR-Vβ restricted in non-B_2 group). However, nine patients had some evidence of blood involvement with PCR testing; six had other flow cytometric abnormalities (CD4/CD8>5, %CD4+/CD26-≥ 20, %CD4+/CD7- ≥ 20). Of the two patients who had TCR-Vβ restricted clones without concordant PCR positivity, one was lost to follow up and one developed PCR positivity, qualifying her for B_2 blood involvement and later requiring systemic therapy.

Conversely, there were nine “false negatives,” patients who had blood involvement by the ISCL criteria but nonetheless failed to demonstrated TCR Vβ-restriction by flow cytometry. Four patients demonstrated equivocal TCR-Vβ restriction (30-47%), and the other five patients had atypical CD4+CD26- populations. Thus, it is possible that if gated on CD4+CD26-, these patients too would have TCR-Vβ restricted populations. Currently, ARUP Laboratories does not use loss of CD26 to gate on atypical populations. We would propose gating on CD4+CD26- populations in order to increase the sensitivity of TCR-Vβ testing.

“Equivocal” TCR-Vβ testing

Four B_2 patients had TCR-Vβ “equivocal” testing (greater than 20% and less than 50%). Three of these four were followed on average for 7.6 months and all required systemic therapy, suggesting the utility of TCR-Vβ testing to upstage higher risk individuals. In one patient, an equivocal test occurred during a transition between two malignant clonal populations. In contrast, equivocal TCR-Vβ testing in ISCL B_1-B_0 patients (n=15) was not clinically significant and no patients had disease progression.

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Abbreviations and acronyms

CTCL	Cutaneous T cell lymphoma
MF	mycosis fungoides
SS	Sézary syndrome
SC	Sézary counts
TCR-Vβ	T-cell receptor Vβ
ISCL	International Society for Cutaneous Lymphomas
EORTC	European Organisation for Research and Treatment of Cancer
USCLC	United States Cutaneous Lymphoma Consortium
MDS	Medical Dermatology Society
IRB	Institutional Review Board
PPV	positive predictive value
NPV	and negative predictive value
PCSM-TCL	Primary cutaneous CD4 positive small/medium T cell lymphoma
CLL	chronic lymphocytic leukemia
mSWAT	Modified Skin Weighted Assessment Tool

Capsule summary

- Accurate assessment of blood involvement in cutaneous T cell lymphoma (CTCL) is important for disease detection and monitoring.
- We examine the potential of use of TCR-Vβ analysis to inform B_1 staging criteria.
- We provide a novel algorithm to aid the diagnosis and monitoring of blood involvement in CTCL.

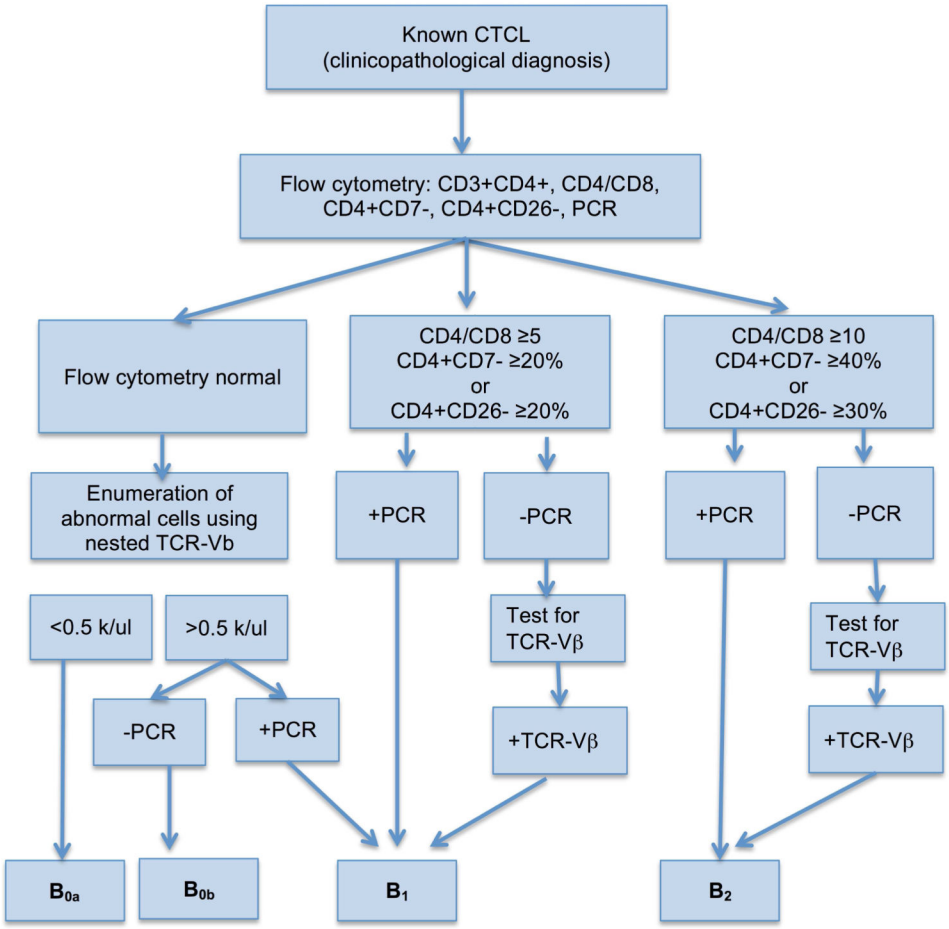

Figure 1. Proposed algorithm for assessment of blood involvement in CTCL

This algorithm may aid in further research investigating the prognostic significance of our proposed modified B_1 criteria and "equivocal" TCR-Vβ tests. As loss of CD26 was the most sensitive parameter of the current B_2 criteria, we would suggest investigating the potential for TCR-Vβ testing in CD4+CD26- populations. B1 and B2 were defined by ISLC criteria (if atypical lymphocytes were noted based on immunophenotypic alterations only, this percentage was multiplied by the absolute lymphocyte count to approximate the number of "Sézary cells" where no such counts were performed/available).

Table 1

Results of Survey Study

Question	Answer choice	Percentage respondents (%)
Stage(s) at which assessment of peripheral blood for malignant involvement is initiated.	Stage I	60
	Stage II	80
	Stage III	86
	Stage IV	74
Testing utilized to assess for peripheral blood involvement	PCR for TCR clonal gene rearrangement	83
	TCR V-beta analysis	14
	Peripheral blood smear for SC	51
	None of the above	14
Purpose of TCR-Vβ analysis	Assess clonal expansion	100
	Assess tumor quantity	0
	To monitor tumor burden during treatment	0
Laboratory to which blood specimens were sent for analysis.	Home institutional	86
	Outside laboratory	14

Table 2
Comparison of clinical characteristics between ISCL B_2 and non-B_2 groups

ISCL	B_2+	B_2-	*p* values
No.	34	127	
No. systemic therapies	2 (0-7)	0 (0-6)	<0.0001*
mSWAT	40 (4-100)	15 (0-180)	<0.0001*
T_1 (patches/plaques <10% BSA)	4/34 (11.8%)	55/120 (45.8%)	0.0002**
T_2 (patches/plaques ≥10% BSA)	14/34 (41.2%)	45/120 (35%)	0.6948**
T_3 (tumor(s) present)	1/34 (2.9%)	7/120 (5.8%)	1.000**
T_4 (erythroderma)	13/34 (38.2%)	10/120 (8.3%)	0.0002**
No. with circulating atypical cells	27/34 (79%)	12/127 (9.4%)	0.0001**

Table 2 footnote: BSA, body surface area; B1 and B2 were defined by ISLC criteria (if atypical lymphocytes were noted based on immunophenotypic alterations only, this percentage was multiplied by the absolute lymphocyte count to approximate the number of “Sézary cells” where no such counts were performed/available)

Table 3
Comparison of flow cytometric and molecular data between ISCL B_2 and non-B_2 groups

ISCL	B_2+	B_2-	*p* values
No.	34	127	
CD4/CD8 ratio	5.4 (1.3-67)	2.7 (0.6-24.3)	<0.0001*
Abs CD4 (K)	1.17 (0.06-7.8)	0.87 (0.21-2.5)	<0.0001*
CD45RO+	43.6 (12.9 -83.1)	30.3 (9.4-77.7)	<0.0001*
% CD4+/CD7-	22.3 (4.1-90)	8.8 (1.8-44.3)	<0.0001*
% CD4+/CD26-	35.4 (11.9-71.6)	10.7 (2-38.8)	<0.0001*
Abs lymphocyte	1.9 (0.13-10.4)	1.6 (0.5-3.9)	<0.0001*
CD4/CD8 ≥ 10	9/34 (26%)	2/127 (1.6%)	<0.0001**
CD4+/CD7-≥ 40%	8/33 (24%)	1/125 (0.8%)	<0.0001**
CD4+/CD26- ≥ 30%	25/30 (83%)	3/126 (2.4%)	<0.0001**
PCR +	34/34(100%)	46/126 (37%)	<0.0001**
TCR-VB +	25/34 (74%) 5 by non-reactivity	11/127 (10.2%) 4 by non-reactivity	<0.0001**
TCR- VB+ (including equivocal)	29/34 (85%)	26/128 (20%)	<0.0001**

*student t-test

**Fischer exact test p-value

Table 3 footnote: B1 and B2 were defined by ISLC criteria (if atypical lymphocytes were noted based on immunophenotypic alterations only, this percentage was multiplied by the absolute lymphocyte count to approximate the number of “Sézary cells” where no such counts were performed/available)

Table 4

Comparison of individual ISCL performance parameters

Parameter	Sensitivity	Specificity	PPV	NPV
CD4/CD8	26.5	98.4	81.8	83.3
CD4+CD7- ≥ 40%	23.5	99.2	88.9	83.2
CD4+CD26-≥ 30%	83.3	97.6	89.2	96.0
CD4+CD26-≥ 25%	93.3	90.5	70	98.2
CD4+CD26-≥ 40%	50	100	100	89.4
PCR+	100	63.4	42.5	100
TCR-Vβ > 50% or total reactivity <20%	73.5	90.6	67.6	92.8