

Simplified Flow Cytometric Assessment in Mycosis Fungoides and Sézary Syndrome

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Abstract

By using flow cytometry with markers for CD3, CD4, CD26, and CD7, we examined the blood samples of 109 patients for abnormal T cells: 69 patients with mycosis fungoides (MF)/Sézary syndrome (SS), 31 hospitalized control subjects, and 9 patients with inflammatory skin disease. T cells were identified as quantitatively abnormal ($>15\%$ CD26 $-$ or CD7 $-$ T cells) or phenotypically abnormal (CD26 $-$ or CD7 $-$ T cells with bright or dim CD3 or CD4 or bright CD7). Patients were followed for a median of 82 months, and abnormal T cells were correlated with diagnosis, clinical outcome, and other laboratory parameters.

Abnormal T-cell populations were identified in 46% of patients with MF/SS (32/69) and correlated with disease extent. Quantitative abnormalities were more frequent than phenotypic abnormalities, and CD4 $+$ /CD26 $-$ T cells were more frequent than CD4 $+$ /CD7 $-$ T cells. CD26 $-$ T cells correlated better with disease extent than did CD7 $-$. Increasing numbers of abnormal T cells were associated with worsening disease. Flow cytometry provides valuable information for diagnosis, prognosis, and therapeutic efficacy in MF/SS.

Mycosis fungoides (MF) is the most common primary cutaneous T-cell lymphoma (CTCL), making up about 50% of all CTCLs.¹⁻³ It typically manifests an indolent course but may progress to an aggressive lymphoma.³⁻⁶ Sézary syndrome (SS), although much less frequent than MF, is defined as an erythrodermic and leukemic variant of CTCL and is associated with a much worse prognosis, with a 5-year overall survival of 10% to 20%.^{2,3,7} Both of these disorders are characterized by neoplastic, often large T cells with cerebriform, hyperchromatic nuclei and an aberrant T-cell phenotype.^{1,3} T-cell abnormalities in MF/SS can be detected by using flow cytometry and may be classified as phenotypic, with markers expressed at abnormal intensities, or quantitative, with expansion of T-cell subsets that presumably include the neoplastic cell population.

The diagnosis of MF and SS frequently requires more than 1 diagnostic modality,^{1,8-13} and assessing prognosis and response to therapy can be difficult in both disorders. Because it detects phenotypic abnormalities, flow cytometry is a useful tool to evaluate T cells in the peripheral blood of patients with MF/SS. For example, flow cytometry can identify atypical T cells with altered expression of pan T-cell antigens (typically dimmer) when compared with normal T cells.¹⁴⁻³⁰ However, diminished expression of some antigens, such as CD2, CD3, and CD4, is more useful than others; decreased intensity of expression of CD7 and CD5 may be observed in reactive processes, limiting their use as markers of phenotypic aberrancy.^{18,31}

When phenotypic abnormalities are not encountered, many patients with MF/SS have quantitative abnormalities in lymphocyte subsets, suggesting that the increased subset includes the neoplastic T-cell clone along with normal cells.

In this regard, most attention has been given to increased numbers of CD4⁺ T cells that lack expression of CD26 (CD4⁺/CD26⁻) or CD7 (CD4⁺/CD7⁻).^{1,19,22,28-30,32-40} CD26 (dipeptidyl peptidase IV) is a membranous protease present on most peripheral T cells.⁴¹ It serves as a negative regulator of epidermotropism by cleaving and inactivating skin-homing chemokines and down-regulating their corresponding receptors.⁴¹⁻⁴⁵ Furthermore, CD26 has been linked to T-cell activation (as reviewed by Ohnuma et al⁴⁶) and to oncogenesis (as reviewed by Pro and Dang⁴⁷ and Havre et al⁴⁸). CD7, a member of the immunoglobulin gene superfamily, is expressed on the majority of peripheral T cells.^{31,49} It functions as a costimulatory molecule for T cells⁵⁰ and as a ligand for the proapoptotic and widely expressed receptor galectin-1.^{51,52} Thus, absent or diminished CD7 may provide resistance to apoptosis and, consequently, confer a survival advantage.

Diminished expression of CD26 and/or CD7 may be seen in a subset of normal T cells and may be increased with T-cell activation.^{29,32,49,53} For this reason, these markers are not generally considered acceptable as phenotypic markers of T-cell aberrancy. By contrast, quantitative abnormalities in CD26^{28,33,35,39,54} or CD7^{28-30,34,36-39,55} T-cell subsets have been used as diagnostic and prognostic aids and have been used to assess therapeutic response. Currently, the International Society for Cutaneous Lymphomas/European Organization of Research and Treatment of Cancer (ISCL/EORTC) and the American Joint Committee on Cancer recommend assessment of T-cell immunophenotype, including CD26 and CD7, by peripheral blood flow cytometry during the staging workup of patients with MF/SS.^{56,57} However, precise criteria for immunophenotypic abnormalities are not defined and can be difficult to assess given the heterogeneity of immunophenotypic abnormalities between patients²⁸ and the overlap with normal and inflammatory conditions.^{29,31,32,49}

In this retrospective study, we reviewed our experience with quantitative and phenotypic abnormalities by flow cytometry in the peripheral blood of 69 patients with MF/SS, 31 hospitalized control subjects, and 9 patients with inflammatory skin disease (ISD). By using a simple, 4-color, single-tube approach, we assessed the expression of CD26 and CD7 in CD3⁺ and CD4⁺ T cells, quantifying the percentage of these T cells with complete loss of CD26 or CD7. We also examined T cells for their intensity of expression of CD3, CD4, and CD7 and for size. Finally, we correlated flow cytometric findings with Sézary cell counts, CD4/CD8 ratios, molecular studies, chromosomal analysis, and clinical outcome. We sought first to evaluate whether detection of abnormal peripheral blood T cells by flow cytometry could facilitate the diagnosis of MF/SS and, second, whether T-cell abnormalities correlated with patient outcomes and therapeutic response. We show that these markers are abnormal in the majority of patients with MF/SS and that they can provide

useful information for diagnosis, prognosis, and monitoring of therapeutic efficacy.

Materials and Methods

This study included 81 consecutive patients referred to the cutaneous lymphoma program at Johns Hopkins Hospital, Baltimore, MD, between July 2002 and August 2003, for suspected or confirmed MF/SS. All patients had peripheral blood flow cytometry as part of their initial evaluation. All patients were evaluated by one of us (E.C.V.), and the final diagnosis was based on a combination of histologic, clinical, and ancillary findings. In addition to flow cytometric studies, patients underwent skin biopsy (all patients), peripheral blood Sézary cell counts (65 with MF/SS; 8 with ISD), determination of T-cell receptor clonality in the skin (61 with MF/SS; 9 with ISD) and blood (all patients), chromosomal analysis (12 with MF/SS), and determination of CD4/CD8 ratios (all patients).

Of the 81 patients, 61 had histopathologically confirmed MF and 11 were diagnosed with erythrodermic CTCL (E-CTCL). The patients with E-CTCL included 4 with primary SS (no antecedent MF), 1 with erythrodermic MF, and 6 with E-CTCL, not otherwise specified (NOS).⁹ Three patients were excluded from the study: 1 was diagnosed with peripheral T-cell lymphoma, NOS, and 2 had evidence of a coexisting lymphoproliferative disorder. Three patients had histologic evidence of transformation to aggressive large cell lymphoma, 2 with tumor phase MF and 1 with E-CTCL, NOS.

Of the 81 patients, 9 were ultimately diagnosed with non-neoplastic ISD and served as a control group. They included 1 patient each with nummular eczematous dermatitis, atopic dermatitis, lichen planus, chronic urticaria, subacute prurigo, drug eruption, and idiopathic erythroderma and 2 patients with parapsoriasis en plaques, small digitate lesion type. In addition, our control subjects included 31 arbitrarily selected, anonymous hospitalized patients who had CBCs within normal limits. These studies were performed under a protocol approved by the Johns Hopkins University School of Medicine Institutional Review Board.

The disease in patients with MF/SS was retrospectively staged according to the recently revised tumor-node-metastasis-blood classification for CTCL.^{56,57} Notably, the revised peripheral blood criteria for B2, leukemic disease, requires a clonal, peripheral blood T-cell receptor gene rearrangement and more than 1,000 Sézary cells/ μ L. In place of the Sézary cell count, it also permits 2 flow cytometric findings as supplemental criteria for B2: (1) increased circulating CD4⁺ or CD3⁺ T cells with a CD4/CD8 ratio of 10 or more and (2) increased CD4⁺ cells with an abnormal phenotype. For purposes of assigning a B2 blood rating, we used the suggested

criteria of 40% or more CD4+/CD7– T cells and 30% or more CD4+/CD26– T cells to define an abnormal phenotype.^{56,57}

Collected peripheral blood samples were stained with the 4-color combination CD3-allophycocyanin/CD4–peridinin chlorophyll protein/CD26-phycoerythrin/CD7–fluorescein isothiocyanate and lysed with FACSlyse following the manufacturer's directions (Becton Dickinson [BD], San Jose, CA). After washing, cells were analyzed on a FACSCalibur flow cytometer (BD). Photomultiplier tube voltages were established using QC3 beads (Bangs Laboratories, Fishers, IN) per the manufacturer's instructions. Compensation was set manually before acquisition, and voltages and compensation were validated using a known normal sample. A minimum of 10,000 ungated events was acquired, but in all cases, an acquisition gate was set on displays of CD3 vs side scatter to acquire at least 2,500 CD3+ T cells. Sample analysis of list mode data was performed using Paint-a-Gate software (BD). T cells were first identified on displays of CD3 vs side scatter, CD4+ T cells were subsequently identified on a CD3/CD4 display, and, finally, a correlated display of CD26 and CD7 was used to identify populations of abnormal CD3+/CD4+ T cells without CD26 and/or CD7. An autofluorescent control or examination of the distribution of these antigens on gated CD3+/CD4+ populations was used to identify cells negative for CD7, CD26, or both. There was no substantial difference in these 2 assessments.

T-cell populations were evaluated for quantitative and phenotypic abnormalities. A specimen was considered to have a quantitatively abnormal population when the percentage of CD3+/CD4+ T cells lacking CD26 or CD7 was at least 3 SD larger than the mean of such populations in hospitalized control subjects. We also evaluated the clinical significance of increased subsets of CD3+/CD4+ T cells with loss of CD26 and/or CD7, including CD7+/CD26–, CD7–/CD26+, and CD7–/CD26– T cells. However, these subsets did not provide additional diagnostic sensitivity or specificity or additional prognostic information and were not investigated further.

Phenotypically abnormal T-cell populations were identified as CD3+/CD4+/CD26– or CD3+/CD4+/CD7– T cells with (1) diminished (dimmer) or increased (brighter) CD3 or CD4 relative to normal T cells or (2) increased expression of CD7. We also noted T cells with increased forward scatter indicating larger T cells. Finally, we examined dim or bright expression of CD26 and/or an abnormal CD26 distribution (specifically, an unusually homogeneous “cluster” of antigen-positive cells that was seen in some cases), but these alterations of CD26 did not provide useful information and were not further evaluated.

To determine the relationship between quantitative and phenotypic T-cell abnormalities and MF/SS, several parameters were investigated, including tumor (T), lymph node (N), and blood (B) ratings; clinical stage; and patient

survival. The Student *t* test was used to compare percentages of CD26– and CD7– T cells with T rating (T1 vs T2-T4), N rating (N0 vs N3), B rating (B0 vs B1-B2), and clinical stage (I-IIA vs IIB-IVB). CD26– and CD7– T cells were correlated with Sézary cell counts using regression analysis. Survival probabilities were calculated by using the Kaplan-Meier method, and survival curves were compared by using the Mantel log-rank test. Survival was determined from the date of flow cytometry at initial evaluation to the date of death (uncensored observation) or last known date alive (censored observation). Death attributed to MF/SS or its treatment was used to define MF/SS-related survival. Statistical analysis was performed using Excel (Microsoft, Redmond, WA), SPSS 13.0 for Windows (SPSS, Chicago, IL), SYSTAT 10 (SPSS), StatXact 6 (Cytel, Cambridge, MA), SigmaStat 3.0 (Systat Software, Point Richman, CA), and SigmaPlot 9.0 (Systat Software).

Results

Patient Characteristics

Adults with MF/SS had a median age of 54 years (range, 28-87 years) and included 40 women and 28 men. One patient with patch phase MF was a 10-year-old boy. Patients with MF/SS were separated into groups based on clinical diagnosis, T rating, B rating, and stage (Table 1). Overall, 53 (77%) of 69 patients evaluated during this interval had clinically early MF (patch or plaque phase MF at stage I to IIA). Because of limited numbers of cases for certain clinical stages, especially stages IIA and IIB, we grouped cases into clinically early (stages I to IIA) and late (stages IIB to IV) categories for analysis purposes. Patients with ISD (7 women, 2 men) had a median age of 58 years (range, 32-78 years). Clinical follow-up was available for all patients with MF/SS and 8 of 9 patients with ISD and ranged from 2 weeks to 95 months (median, 82 months) with 86% of patients with MF/SS having at least 2 years of follow-up and patients with ISD having a minimum of 40 months of follow-up.

CD4+/CD26– and CD4+/CD7– T-Cell Populations in Hospitalized Control Subjects

The mean and SD for the percentage of CD26– and CD7– T cells were determined in hospitalized control subjects (Image 1). We defined the minimum threshold for quantitatively abnormal T-cell populations as the mean value + 3 SD (CD26, 9.0% + 6.0%; CD7, 7.2% + 7.8%). None of the hospitalized control subjects had CD26– and/or CD7– T-cell populations above this threshold. In addition, no phenotypic abnormalities, as defined, were identified in hospitalized control subjects.

Abnormal T-Cell Populations in Patients With MF/SS and Patients With ISD

Nearly half of the patients with MF/SS had quantitative or phenotypic abnormalities in CD3+/CD4+ T-cell populations (Table 1). Phenotypic abnormalities in the expression

of CD3 and/or CD4 were seen in 17 patients (25%), one of whom also had bright expression of CD7 (Image 2) and Image 3. In 12 patients (17%), we found increased forward light scatter. Quantitative abnormalities were more common, occurring in 31 patients (45%) (Image 4). Expansion of

Table 1
Quantitative and Phenotypic Abnormalities in MF/SS and ISD*

Group	No. of Cases	Abnormal T-Cell Populations							Any Abnormality
		Quantitative			Phenotypic				
		CD26	CD7	Either	CD3	CD4	↑ CD7	Any	
Diagnosis									
MF/SS	69	28	14	31	9	13	1	17	32
ISD	9	3	1	4	0	0	0	0	4
Clinical diagnosis									
MF, patch	26	3	2	3	1	0	0	1	3
MF, plaque	29	13	4	15	1	4	0	5	16
MF, tumor	3	2	2	3	2	1	0	2	3
MF, erythrodermic	1	1	1	1	1	1	0	1	1
SS	4	4	3	4	1	4	0	4	4
E-CTCL, NOS	6	5	2	5	3	3	1	4	5
T rating									
T1	40	10	4	12	2	3	0	5	13
T2	15	6	2	6	0	1	0	1	6
T3	3	2	2	3	2	1	0	2	3
T4	11	10	6	10	5	8	1	9	10
B rating									
B0	37	10	4	12	3	2	1	4	12
B1	20	8	4	9	2	3	0	4	10
B2	8	8	5	8	4	7	0	8	8
Clinical stage									
Early (I-IIa)	53	14	6	16	2	3	0	5	17
Late (IIb-IVb)	16	14	8	15	7	10	1	13	15

E-CTCL, NOS, erythrodermic cutaneous T-cell lymphoma, not otherwise specified; ISD, inflammatory skin disease; MF, mycosis fungoides; SS, Sézary syndrome.

* Cases of MF/SS were separated by clinical diagnosis, T rating, B rating, and clinical stage. Quantitative abnormalities include >15% CD26+ T cells, >15% CD7+ T cells, and either quantitative abnormality. Phenotypic abnormalities include dim or bright CD3 or CD4, bright CD7, and any phenotypic abnormality. T (tumor) ratings are as follows: T1/T2, <10%/≥10% body surface area covered by patches or plaques; T3, cutaneous tumors; T4, generalized erythroderma. B (blood) ratings were as follows: B0, ≤5% Sézary cells; B1, >5% Sézary cells, but not meeting the criteria for B2; B2, detectable T-cell clone and an absolute Sézary cell count of ≤1,000/μL. In place of a Sézary cell count of ≥1,000/μL, 2 additional criteria may be used for the B2 blood rating: (1) increased circulating T cells with a CD4/CD8 ratio of at least 10; or (2) increased CD4+ T cells with an aberrant flow cytometric immunophenotype (suggested ≥30% CD26+ T cells or ≥40% CD7+ T cells).^{56,57} Per the updated International Society for Cutaneous Lymphomas/European Organization of Research and Treatment of Cancer classification, the breakdown by clinical stage (No. of patients in group) was as follows: IA, 38 (T1 N0 M0 B0/1); IB, 15 (T2 N0 M0 B0/1); IIA, 1 (T2 N1 M0 B1); IIB, 1 (T3 N0 M0 B0); IIIA, 1 (T4 N0 M0 B0); IIIB, 2 (T4 N0 M0 B1); IVA, 3 (1, T1 N0 M0 B2; 2, T4 N0 M0 B2); IVA, 6 (1, T1 N3 M0 B1; 2, T3 N3 M0 B0; 3, T4 N3 M0 B2); and IVB, 3 (1, T4 NX M1 B0; 2, T4 N3 M1 B2); N is the nodal rating, as follows: N0, clinically uninvolved; N1, clinically enlarged, without significant histologic involvement; N2, clinically and histologically involved with preservation of lymph node architecture; N3, clinically and histologically involved with effacement of lymph node architecture; M rating is as follows: M0/1, absence/presence of visceral disease.^{56,57}

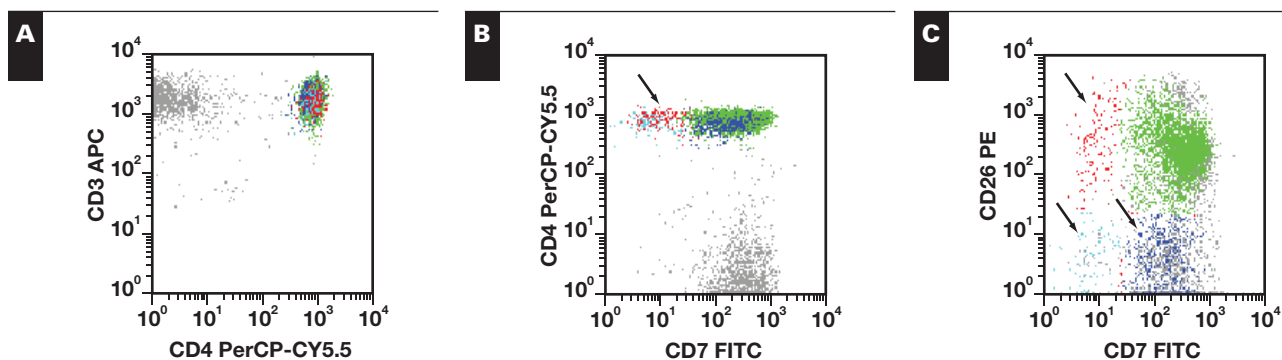


Image 1 CD26 – and CD7– T cells in a hospitalized control subject. Distribution of CD3+/CD4+ T cells (A), CD7– T cells (B and C; arrows, red and cyan events), and CD26– T cells (C; arrows blue and cyan events). APC, allophycocyanin; CY5.5, cyanine 5.5; FITC, fluorescein isothiocyanate; PE, phycoerythrin; PerCP, peridinin chlorophyll protein.

CD26– T cells was more common than expansion of CD7– T cells, occurring in 28 vs 14 patients, respectively. Among the patients with MF/SS who had a quantitative abnormality, the median value and range were similar for CD26– and CD7– T cells (% of lymphocytes, median [range], CD26, 22.7%

[15.1%-98.6%]; CD7, 21.8% [15.7%-98.6%]). A peripheral blood T-cell clone was detected in 57% of patients (16/28) with abnormally expanded CD26– T cells and 79% of patients (11/14) with abnormally expanded CD7– T cells. Patients with ISD had quantitative abnormalities in a frequency similar

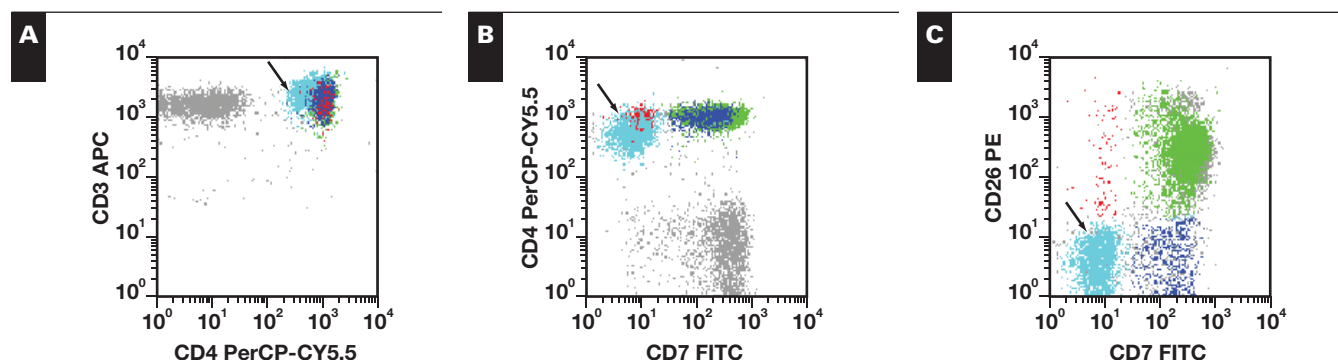


Image 2 Quantitatively and phenotypically abnormal CD3+/CD4+/CD26–/CD7– T cells in a patient with mycosis fungoides with plaques. An increased proportion of CD3+/CD4+ T cells (**A**) display dim CD4 (**A** and **B**; arrows, cyan events) and lack CD26 and CD7 (**C**; arrow, cyan events). APC, allophycocyanin; CY5.5, cyanine 5.5; FITC, fluorescein isothiocyanate; PE, phycoerythrin; PerCP, peridinin chlorophyll protein.

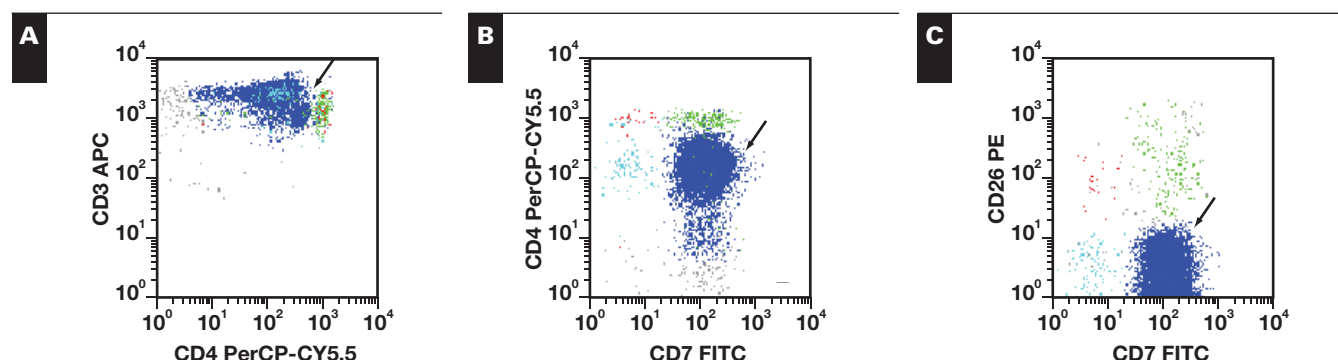


Image 3 Quantitatively and phenotypically abnormal CD3+/CD4+/CD26–/CD7+ T cells in a patient with Sézary syndrome. The majority of CD3+/CD4+ T cells (arrows, blue events) are CD3 bright (**A**), CD4 dim (**A** and **B**), CD26– (**C**), and CD7+ (**B** and **C**). APC, allophycocyanin; CY5.5, cyanine 5.5; FITC, fluorescein isothiocyanate; PE, phycoerythrin; PerCP, peridinin chlorophyll protein.

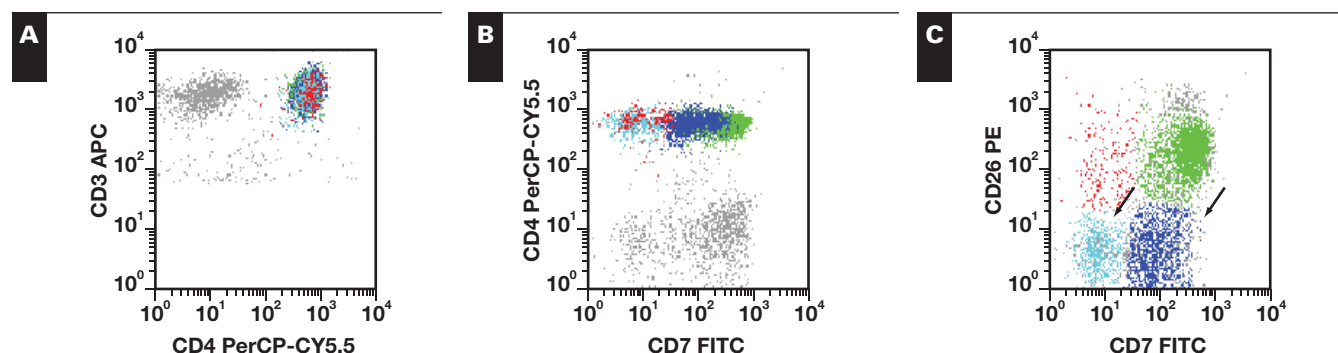


Image 4 Quantitatively abnormal CD26– T cells in a patient with erythrodermic cutaneous T-cell lymphoma, not otherwise specified. An increased proportion (>15%) of CD3+/CD4+ T cells (**A** and **B**) display absence of CD26 (**C**; arrows, blue and cyan events). APC, allophycocyanin; CY5.5, cyanine 5.5; FITC, fluorescein isothiocyanate; PE, phycoerythrin; PerCP, peridinin chlorophyll protein.

to that of patients with MF/SS; however, they had much lower percentages of abnormal T cells (ISD, 15.1%-19.7% vs MF/SS, 15.1%-98.6%) and never showed a phenotypic abnormality. Quantitative abnormalities of other CD3+/CD4+ T-cell subsets, including CD7+/CD26+, CD7+/CD26-, or CD7-/CD26-, did not provide significant additional information.

Abnormal T-Cell Populations vs Clinical Diagnosis and Stage

Although we consecutively studied patients with different clinical diagnoses and stages, only small numbers of patients were seen in some groups (Table 1). Abnormalities were uncommon in patch phase MF (3/26), slightly more common in plaque phase MF (16/29), and nearly universal in patients with advanced disease (13/14); the latter group included patients with tumor phase MF and E-CTCL. Similarly, abnormalities were less common in patients with stage I disease (11/38 stage IA, 6/15 stage IB) but nearly universal in patients with stages II, III, and IV disease (15/16). Patients with SS generally had the largest percentages of circulating abnormal T cells (mean $\pm$ SD, 89% $\pm$ 12%, with all patients having at least 70% CD26- T cells). There was a significant correlation between the number of abnormal T cells detected by flow cytometry and morphologic Sézary cells in all patients with MF/SS ($r = 0.99$; $P = .01$), although the former was nearly twice the latter. In particular, the correlation between absolute numbers of Sézary cells and CD26- T cells was excellent ($r = 0.98$; $P < .001$) but was slightly less strong for CD7- T cells ($r = 0.92$; $P < .001$). Twelve patients had abnormally high

forward light scatter, including 2 of 3 patients whose disease transformed to large cell lymphoma and 10 of 16 patients with late-stage disease (stages IIB-IVB).

Abnormal T-cell populations correlated with stage, increasing in frequency and size with advancing T rating, B rating, and clinical stage (Table 1). Loss of CD26 more closely correlated with measures of progressive disease in patients with MF/SS than loss of CD7. Specifically, CD26- T cells more closely correlated with the T rating ($P = .003$ vs $P = .009$; Student t test) and B rating ($P < .0001$ vs $P = .01$). A similar trend was identified for N rating and stage.

Abnormal T-Cell Populations and Prognosis

At last contact, 14 (20%) of 69 patients had died of MF/SS (Table 2). Patients dying of MF were much more likely to have demonstrated a quantitative (14/14) or phenotypic (10/14) abnormality than were patients who did not die (17/55 quantitative; 7/55 phenotypic). Survival of patients with phenotypically abnormal T cells was significantly worse than of patients who lacked such abnormalities (Figure 1A). The presence of an abnormal T-cell population by flow cytometry was a more sensitive indicator of death of disease than was the presence of a detectable T-cell clone in the peripheral blood by polymerase chain reaction, although it should be noted that at the time these studies were performed, the clonality assay had a sensitivity of only approximately 70%. In addition, 4 of 5 patients with a chromosomal abnormality in peripheral blood lymphocytes and 4 of 5 patients with a Sézary cell count of more than 1,000/ μ L died of disease.

Table 2
Characteristics of Patients Dying of Disease

Case No.	T Cells (%)		Phenotypic Abnormalities		Clinical Diagnosis	Rating			Stage	No. (%) Sézary Cells/ μ L	PB T-Cell Clone	Chromosomal Abnormality	Interval (mo)
	CD26-	CD7-	CD3	CD4		T	N	B					
1	7.9	16.6	+	-	MFT [†]	3	3	0	IVA ₂	0 (0)	-	ND	35
2 [‡]	14.3	22.2	-	-	MFPQ	1	0	1	IA	135 (20)	+	ND	15
3	15.1	21.1	+	+	MFT [†]	3	3	0	IVA ₂	28 (2)	+	-	1
4 [§]	15.3	9.9	-	+	E-CTCL, NOS [†]	4	X	0	IVB	48 (4)	-	-	14
5	16.7	3.5	-	-	MFPQ	1	0	1	IA	41 (2)	-	ND	58
6	18.8	12.0	-	-	MFT	3	0	0	IIB	42 (3)	-	ND	19
7	21.6	14.2	-	-	MFPQ	1	3	1	IVA ₂	79 (9)	-	ND	28
8	28.4	19.1	+	+	E-CTCL, NOS	4	0	1	IIIB	120 (18)	+	-	10
9	38.2	21.5	+	+	MFE	4	3	2	IVA ₂	106 (44)	+	ND	3
10	65.8	64.1	+	+	E-CTCL, NOS	4	3	2	IVA ₂	416 (15)	+	-	53
11	84.7	43.4	-	+	SS	4	3	2	IVB	13,474 (39)	+	+	10
12	87.7	4.3	-	+	MFPQ	1	0	2	IVA ₁	1,211 (71)	+	+	63
13	97.9	1.2	-	+	SS	4	3	2	IVB	7,477 (66)	-	+	58
14	98.6	98.6	-	+	SS	4	0	2	IVA ₁	13,456 (70)	+	+	1

E-CTCL, NOS, erythrodermic cutaneous T-cell lymphoma, not otherwise specified; ISD, inflammatory skin disease; MF, mycosis fungoides; MFE, MF, erythrodermic; MFPQ, MF, plaque; MFT, MF, tumor; ND, not done; PB, peripheral blood; SS, Sézary syndrome; +, positive or present; -, negative or absent.

* Of the patients studied, 14 died of disease. The PB T-cell clone was determined by polymerase chain reaction. The interval refers to the length of time from initial evaluation to death. The table is organized by the percentage of CD26- T cells.

[†] Disease was transformed in these cases.

[‡] The patient experienced chemotherapy-induced leukopenia but had extensive disease in the central nervous system and bone marrow involvement and eventually died.

[§] The patient had a CD4-/CD8- neoplastic T-cell phenotype that was not well detected by the flow cytometric parameters used in this study.

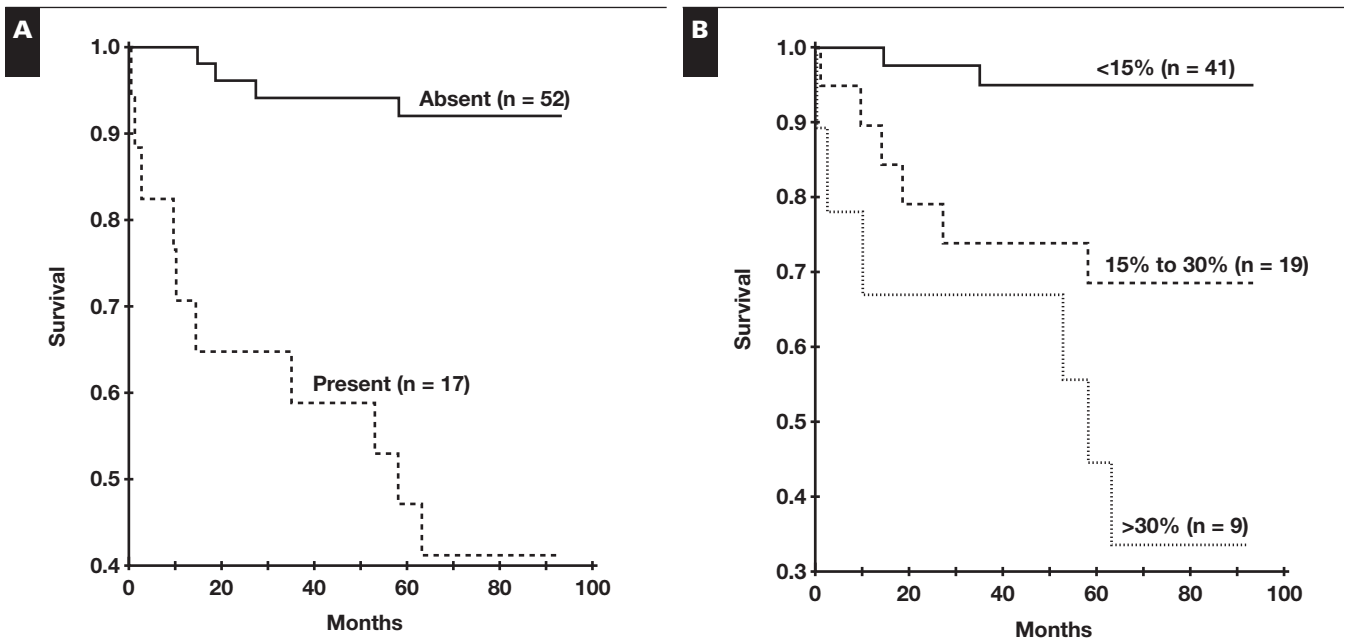


Figure 1 Disease-related survival curves of patients with mycosis fungoides/Sézary syndrome with or without phenotypically abnormal cells in the peripheral blood (**A**; $P < .001$) and by percentages of CD4+/CD26- T cells (**B**; $P < .001$).

The 16 patients with a quantitative and phenotypic abnormality encompassed nearly all patients with other unfavorable features. They included 13 of 16 patients with late-stage disease, 12 of 17 patients with a peripheral blood T-cell clone, all patients with a CD4/CD8 ratio of 10 or more, 4 of 5 patients with a chromosomal abnormality, and all patients with a Sézary cell count of more than 1,000/ μ L. Of 14 patients who died of disease, 10 had both abnormalities. Of the 16 patients with both abnormalities, 8 met the ISCL/EORTC criteria for the B2 blood rating. The ISCL/EORTC staging and classification system suggests thresholds of CD4+/CD7- more than 40% and CD4+/CD26- more than 30% as criteria for B2. However, in this study, patients with more than 15% but fewer than 30% CD4+/CD26- T cells had an adverse prognosis, although patients with more than 30% such cells seemed to fare even worse (Figure 1B).

Follow-up of Patients With Abnormal T-Cell Populations

Flow cytometric analysis of peripheral blood samples was done more than once (range, 2-31) for 17 patients. Of 12 patients with increasing numbers of CD26- T cells, 9 (75%) experienced a clinical decline, including lymph node involvement, systemic spread, or death. In 4 patients, we found decreasing numbers of abnormal T cells; 2 of these patients experienced an improvement in their disease. These 2 patients included a patient with E-CTCL, NOS who experienced a complete remission with extracorporeal photopheresis and a patient with SS who responded partially to chemotherapy but eventually died of disease. An additional patient experienced a decline in the number of abnormal T cells in the setting of

chemotherapy but had extensive disease involving the central nervous system and bone marrow and eventually died. The remaining patient with a decreasing number of abnormal T cells was in clinically stable condition. Finally, 1 patient had stable, negative findings on repeated flow cytometry and showed a complete response to methoxsalen-ultraviolet A photochemotherapy.

Discussion

Blood studies to detect and measure circulating neoplastic T cells have become an integral part of the evaluation and monitoring of patients with MF/SS.^{9,56-59} In this study, we used a simplified, 4-color flow cytometric approach to examine T cells in the peripheral blood of 69 patients with MF/SS. We found that nearly half of patients with MF/SS (46% [32/69]) displayed an abnormal T-cell population. Although abnormalities were found in about 30% of patients with clinically early MF (patch or plaque phase MF at stage I to IIA), quantitatively and phenotypically abnormal T-cell populations increased in frequency and size with measures of disease extent, including clinical diagnosis, T rating, B rating, and stage. Quantitative abnormalities were more frequent than phenotypic ones. Furthermore, CD26- T cells were more frequently abnormal and more closely related to measures of disease extent than CD7- T cells. Overall, patients with abnormal populations had significantly shorter survival than patients without. Moreover, nearly all patients who died of disease had abnormal T cells detected by flow cytometry,

even when this was determined at a single time long before death occurred.

Several prior studies have demonstrated that loss of CD26 is a reliable marker of the neoplastic T cells in MF/SS. CD26– T cells correlate with morphologically identifiable neoplastic T cells in the peripheral blood of patients with MF/SS^{28,32} and are sensitive and specific for the diagnosis of leukemic MF and SS.^{19,32,33} However, not all studies have shown this finding.²⁷ Analysis of gene expression profiles corroborates the results of flow cytometry, and underexpression of CD26 in peripheral blood mononuclear cells on complementary DNA arrays helps to identify patients with SS.⁶⁰

Nevertheless, there is currently no consensus on the criteria that are most useful for diagnosis. The ISCL/EORTC has suggested a threshold of 30% CD4+/CD26– T cells for staging purposes in MF/SS.⁵⁶ Such a high threshold may have some diagnostic specificity, as it would not have identified any of our cases of inflammatory dermatoses as abnormal. However, it is not a very sensitive indicator of disease, as only 8 patients with MF/SS met this criterion. Moreover, it did not identify all patients with erythroderma or all patients with identical T-cell clones in the peripheral blood and skin. Even in patients with established diagnoses of MF/SS, our results suggest that it might be too high a threshold for an optimal staging tool. We used a cutoff of 15% CD26– T cells, calculated as 3 SD above the mean of hospitalized patient control subjects without MF. This cutoff for distinguishing patients with MF/SS from healthy subjects is similar to what has been seen in other studies.^{25,29,31,32,34,37,38,40,49} In support of the validity of this cutoff, we found that patients with between 15% and 30% CD4+/CD26– cells had inferior clinical outcomes compared with patients with levels in the normal range. Further assessment of the best prognostic cutoff will require additional study with more patients.

In contrast with the situation with CD26, loss of CD7 is variable in MF/SS and does not correlate as well as CD26– T cells with circulating Sézary cells, clonal T cells, or an increased CD4/CD8 ratio.^{19,22,27,28,32–37,39,61} Although loss of CD7 is common in MF/SS,^{36,38} it does not identify all patients with leukemic MF or SS.^{22,27,32,35–37,39} In other studies, it has been associated with clinical evidence of disease progression³⁷ and an adverse prognosis.³⁹ In this study, CD7 was most likely to be lost in patients with extensive disease and poor outcomes but was not as reliable a marker as CD26.

In addition to numeric abnormalities in T-cell populations, other studies have identified circulating T-cell populations with abnormal phenotypes in patients with leukemic MF or SS, including altered expression of CD2, CD3, and CD4.^{22,27–29,32,34} As in this study, these abnormalities were not observed in patients with benign dermatoses.^{27,29,62} We found that phenotypic abnormalities were most likely to be present in patients who also had a quantitative abnormality. In

addition, the 16 patients with a quantitative and a phenotypic abnormality had many other unfavorable features and a particularly poor prognosis, although only 8 met criteria for the B2 blood rating. Many more patients will have to be studied to determine the relative importance of these abnormalities in the context of other adverse prognostic factors. At this time, it is unknown whether the flow cytometric findings presented herein provide independent prognostic information when stage is taken into account. Further studies are necessary to validate the specific cutoffs we propose as prognostic criteria.

Although we had limited longitudinal measurements of phenotype in our patients, our results suggest that changes in abnormal populations parallel changes in a patient's clinical course. Others have also shown that changes in abnormal T cells, in particular levels of CD26– T cells,³⁵ correlate with therapeutic interventions and clinical outcome.^{25,33,35,54,55,63}

Our study has some limitations. We defined abnormalities in a complex manner, and the definition of phenotypic abnormalities is somewhat subjective. Most significant, we have not defined criteria that are sensitive or specific for the diagnosis of MF/SS. The most specific abnormalities, phenotypically abnormal T-cell populations, failed to identify many patients with MF/SS, while the most sensitive indicator, an increase in CD4+/CD26– T cells, was also abnormal in 3 of 9 patients with ISD. Moreover, the known heterogeneity in phenotype in MF/SS limits our approach. For example, 3 patients with MF in our study had an abnormal T-cell population that was CD26+ and would not have been identified by looking for loss of CD26 alone. Similarly, our approach would not work in the rare MF/SS case with CD8+ or CD4–/CD8– phenotype or for some patients with tumor stage MF, who may have low percentages of CD26– or CD7– T cells in peripheral blood despite aggressive disease. In addition, it would not detect defects limited to other T-cell markers, such as CD2 and CD5.

It is possible that we could improve our definitions by using higher order flow cytometry to look at clonal populations of T cells within our abnormal populations using T-cell receptor V β reagents^{16,25,30,63} or more specific markers of neoplastic T cells such as KIR3DL2.^{26,30,64} Nevertheless, our study defined a readily applicable, cost-effective, single-tube, 4-color flow cytometric approach that detects abnormalities in patients with MF/SS and that can be used as a tool to identify patients with a poor prognosis and for patient monitoring. Moreover, although we have not systematically compared new combinations or approaches with the method described herein, we continue to find this single tube to be the most useful in our panel for evaluating patients with MF/SS.

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