

Prognostic Implications of Blood B Ratings for Erythrodermic Cutaneous T cell Lymphoma

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Abstract

Objective: The magnitude of blood involvement in cutaneous T cell lymphoma (CTCL) has prognostic significance. Several groups have proposed hematologic criteria for B ratings for use in clinical staging of CTCL, but in practice B ratings are only relevant for the staging of erythrodermic CTCL without lymph node or visceral involvement, i.e., T4N0-2M0B0 defines sub-stage IIIA, T4N0-2M0B1 defines sub-stage IIIB and T4N0-2M0B2 defines sub-stage IVA1. This retrospective study examines the effect of various B rating criteria on prognoses of patients with erythrodermic CTCL.

Methods: At initial presentation, 152 patients diagnosed with E-CTCL had quantitative Sézary cell counts performed on blood smears and CD4/CD8 ratios determined in the blood by flow cytometry. In addition, 39 patients had percentages of CD4⁺CD26⁻ and CD4⁺CD7⁻ lymphocytes directly measured. The Kaplan-Meier method and Cox proportional hazards model were used to estimate mean overall and CTCL-specific survival and to compare survival curves of sub-stages of erythrodermic CTCL.

Results: With B2 rating defined as absolute Sézary cell counts ≥ 1.0 K/ μ L or CD4/CD8 ratio ≥ 10 , a better separation of survival curves between sub-stages IIIA and IIIB was observed if the threshold for B1 was defined as Sézary cells $\geq 20\%$ and CD4/CD8 ratio defined as ≥ 4 plus confirmation of blood involvement. With either criterion, the survival of patients at sub-stages IVA1 and IVA2 were not statistically different. There was also a suggestion that percentage of CD4⁺CD26⁻ or CD4⁺CD7⁻ cells (which ever was higher) might be used to define B1 and B2 thresholds ($\geq 30\%$ and $\geq 60\%$, respectively) as an alternative to absolute counts. Maximum CD4⁺CD26⁻/CD4⁺CD7⁻ $\geq 30\%$ plus confirmation also might provide a hematologic definition of leukemic CTCL.

Conclusion: Additional studies on larger number of patients and evaluation with other measures of blood tumor burden are indicated.

Keywords: Cutaneous lymphoma; Erythroderma; B rating; Prognosis; Survival

Introduction

The presence of neoplastic T cells in the blood of patients with cutaneous T cell lymphoma (CTCL), which includes predominantly mycosis fungoides (MF) and Sézary syndrome (SS), is recognized to have prognostic significance. Once atypical lymphocytes of SS were demonstrated to have hyperconvoluted “cerebriform” nuclei (Sézary cells), [1,2] the magnitude of neoplastic involvement in the blood has been estimated by counting the percentage of Sézary cells (SzC) within the lymphocyte population and their absolute number per microliter (μ L) [3-5]. Based on work at the Mayo clinic in the 1970s, SS was defined by an absolute SzC count of ≥ 1.0 K/ μ L [6]. In addition, other investigators showed that SzC counts $\geq 20\%$ were usually not encountered in non-neoplastic inflammatory skin diseases and therefore might have diagnostic importance [3,7]. It was subsequently reported that for patients with confirmed CTCL, SzC counts $\geq 20\%$ correlated strongly with erythroderma (T4 skin rating) and provided no additional prognostic import in the T (tumor)-N (node)-M (metastasis) system for clinical staging of CTCL [3]. For this reason, B

ratings were not incorporated in the original TNM staging classification proposed by the Mycosis Fungoides Cooperative Group/National Cancer Institute in 1979 [8].

Today, it is recognized that non-neoplastic lymphocytes also may have hyperconvoluted nuclei and that SzC counts exceeding $\geq 20\%$ and ≥ 1.0 K/ μ L may be encountered with certain drug eruptions and severe photosensitivity disorders that can mimic the clinical and histopathologic features of erythrodermic CTCL (E-CTCL) including SS [9,10]. Visual identification of SzCs, particularly those with small diameters, on blood smears may be especially difficult. Investigators at one center coupled SzC counts with molecular genetic evidence of T cell clones in an attempt to recognize cases with high numbers of non-neoplastic SCs [11]. Indeed, the detection of a T cell clone in the blood per se identifies patients with a worse prognosis than patients without a clone especially if the clone is identical to the clone in involved skin or lymph node [12,13]. However, because of the high detection sensitivity of modern PCR-based methods (e.g., Biomed-2 protocol), the primary role of molecular genetics is to confirm that neoplastic cells are present in the blood rather than quantifying them [14].

At other centers, quantitative SzC counts have been abandoned in favor of more objective measures of neoplastic cell blood involvement

using flow cytometry. The observation that expanded CD4⁺ lymphocytes with a CD4/CD8 ratio ≥ 10 differentiated E-CTCL from other erythrodermic skin disorders formed the basis for one of the International Society for Cutaneous Lymphomas-European Organization for Research Treatment of Cancer (ISCL-EORTC) criteria for B2 blood ratings [15]. Furthermore, because neoplastic T cells often lose expression of CD7 or CD26 (about 60% and 90% of Sézary cases, respectively) [16-18], the ISCL-EORTC suggests that CD4⁺CD7⁻ $\geq 40\%$ and CD4⁺CD26⁻ $\geq 30\%$ may provide another criterion for B2 rating [19].

As a result of these studies, the ISCL-EORTC revised the TNM staging classification to include B ratings [19]. If SzC counts are available, B0 is defined as $<5\%$ SC and is further subdivided into B0a and B0b based on absence or presence of a T cell clone, respectively. Similarly, B1, which is defined as $\geq 6\%$ SC but absolute count <1.0 K/ μ L, is subdivided into B1a (clone absent) and B1b (clone present). If molecular genetic studies are not available, then a SC $\geq 20\%$ may be used to define B1b. If SzC counts are not available, B2 may be defined by flow cytometry as an expanded number of CD4⁺ or CD3⁺ cells with a CD4/CD8 ratio ≥ 10 or abnormal immunophenotype including loss of CD7 or CD26. The definition of "expanded" CD4⁺ or CD3⁺ cells was not provided.

Since then, Gibson proposed a modification of the ISCL-EORTC B ratings [20]. In lieu of SzC counts, B0 was defined absence of T cells with immunophenotypic alterations or TCR-V β restricted PCR⁺ clones. B1 was defined as CD4/CD8 ratio ≥ 5 but <10 or CD4⁺CD7⁻ $\geq 20\%$ but $<40\%$ or CD4⁺CD26⁻ $\geq 20\%$ but $<40\%$ with evidence of clonality by PCR or TCR-V β restriction. B2 was defined as CD4/CD8 ratio ≥ 10 or CD4⁺CD7⁻ $\geq 40\%$ or CD4⁺CD26⁻ $\geq 30\%$ with evidence of clonality. It was also implied that the absolute count of T cells with immunophenotypic alterations might also be used to define B ratings.

Recently, the EORTC cutaneous lymphoma task force proposed the B ratings be defined using absolute counts of these lymphocyte subsets, specifically B0: CD4⁺CD7⁻ or CD4⁺CD26⁻ <250 cells/ μ L, B1: ≥ 250 to $<1,000$ cells/ μ L, and B2: $\geq 1,000$ cells/ μ L plus a T cell clone [21]. Although I support the concept [18], the fact that absolute counts are less stable than relative counts and may be suppressed by treatments such as corticosteroids, additional criteria for B ratings using relative counts may be useful.

In this manuscript, I shall review my experience with SzC counts, CD4/CD8 ratios and CD4⁺CD26⁻ counts from patients with E-CTCL and propose modifications to B rating criteria.

Materials and Methods

The patient population was composed of 152 patients (76 men, 76 women; 129 White, 20 Black and 3 other racial background) diagnosed to have E-CTCL (T4 skin rating) and blood studied by flow cytometry for CD3, CD4 and CD8 as part of the immunophenotypic panel. Prior to June 1997, 92 samples were studied by single antibodies as previously reported [22]. Thereafter, 60 samples were studied by multiple antibodies using CD45 and side scatter to define the lymphocyte gate. In addition, at least 100 lymphocytes on blood smears were examined for SzCs by one experienced technician and these were classified according to their cell diameter as small (8-11 μ m), large (12-14 μ m) or very large (>14 μ m) [5,7].

The diagnosis of CTCL was based on (1) a diagnostic skin histopathology or (2) compatible histopathologic findings plus

evidence of a T cell clone in the skin and/or (3) blood findings that were diagnostic of leukemic involvement. According to ISCL recommendations [23], the E-CTCL patients were further classified as SS/T4B2 (62 patients), erythrodermic MF (6 patients) or E-CTCL, not otherwise specified (84 patients including one patient with a papuloerythroderma presentation). The clinical stages at presentation were IIIA (53 patients), IIIB (25 patients), IVA1 (35 patients), IVA2 (34 patients) and IVB (5 patients) [19].

Statistics

Results of laboratory studies are given as mean values ± 1 standard error of the mean (SEM) or median value with a range. Fisher's and Pearson's chi-square exact tests were used to test categorical tables. Kruskal-Wallis test was used to compare median values of independent groups. Spearman's correlation coefficient was used to test the strength of correlations. The Kaplan-Meier method with log rank statistic was used to determine estimated mean survivals and to compare overall and CTCL-specific survival curves. The Cox proportional hazards model with -2 log maximized likelihood statistic was used to compare alternative criteria for B ratings² [24]. The statistical software used for data analysis were SYSTAT10 and SPSS 13.0 for Windows, SPSS, Inc., Chicago, IL., and StatXact-3 and EGRET for Windows, Cytel, Inc., Cambridge, MA.

Results

Prognostic considerations

The patients' ages ranged from 27 to 96 years (median 69 years; mean 66.23 ± 1.14 years). In the Cox model, patients' age or age categorized into two groups (<60 years, ≥ 60 years) correlated significantly ($P<0.001$) with overall, but not CTCL-specific survival. Accordingly, age was entered as a covariate in Cox models that compared B ratings. Gender and race (White, non-White) were not significant. For 107 patients studied by PCR, a T cell clone was detected in the blood of 52 (49%) patients. A clone was detected in 32/72 (44%) specimens studied by PCR-DGGE, 8/15 (53%) studied by PCR-SSCP, and 12/20 (60%) by PCR-GeneScan. The detection frequencies of T cell clones among the 3 methods were not statistically different ($P=0.436$). In addition, 24/44 (55%) patients had a clone detected by Southern blot analysis of TCR V β gene.

PCR evidence of a blood clone identified 52 patients with a worse mean overall survival (4.71 ± 0.78 years) compared to 55 patients without a clone (mean, 9.44 ± 1.12 years; $P=0.001$). The prognosis of 25 patients with a clone detected with the Southern blot technique was slightly worse (mean, 4.42 ± 0.77 years), presumably a reflection of the lower sensitivity of Southern blot compared to PCR. When 6 additional patients with a clone demonstrated by chromosome analysis were combined with both molecular genetic methods, the mean survival of 90 patients with any clone (4.53 ± 0.53 years) was shorter than 51 patients without a clone (9.95 ± 1.15 years; $P<0.001$).

In addition to absolute SzC counts, the percentage of SzCs in the lymphocyte population correlated significantly with both overall and CTCL-specific survival in the Cox model with or without age as a covariate ($P<0.001$). A plot of SzC percentage versus estimated mean survival shows that as the percentage of SzCs increases among lymphocytes, the mean overall and CTCL-specific survival rates decrease (Figure 1). In addition, at $\geq 20\%$ SzCs, the mean overall survival of patients (4.73 ± 0.60 years) is nearly identical to that of

patients with a positive clone by PCR (4.72 ± 0.78 years; $P=0.991$). This supports the ISCL-EORTC proposal that a 20% SzC count can be used as a substitute criterion for B1 for patients not studied by PCR [19]. Moreover, at $\geq 40\%$ SzCs, all deaths are CTCL-specific.

The relationship between SzC count and molecular genetic results is shown in Figure 2. A significantly higher percentage of SzCs was observed in PCR positive patients (mean, $34.7 \pm 3.4\%$; median, 31%, range 0-92%) compared to PCR negative patients (mean, $19.2 \pm 3.2\%$; median, 14%, range 0-85%; $P=0.001$). However, it is disconcerting that 30 (55%) and 21 (38%) of the 55 PCR-negative patients had SzC counts $\geq 6\%$ and $\geq 20\%$, respectively. This includes 8 patients with absolute SzC count ≥ 1.0 K/ μ L.

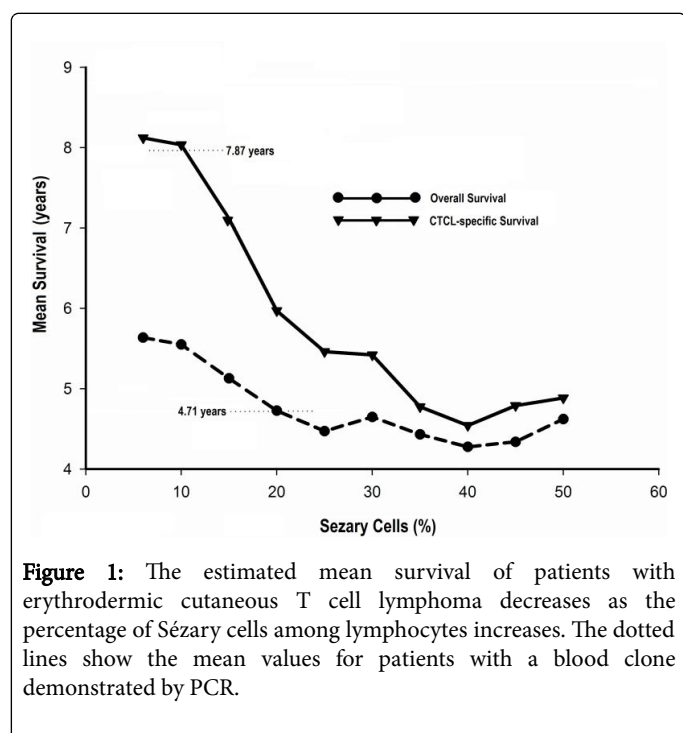


Figure 1: The estimated mean survival of patients with erythrodermic cutaneous T cell lymphoma decreases as the percentage of Sézary cells among lymphocytes increases. The dotted lines show the mean values for patients with a blood clone demonstrated by PCR.

Some factors contribute to this finding. Firstly, the PCR methods available at our center had a lower detection sensitivity compared to modern methods. For example, we have encountered PCR-negative results in 6 of 26 (23%) of Sézary patients with a chromosomally-abnormal clone.

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Some factors contribute to this finding. Firstly, the PCR methods available at our center had a lower detection sensitivity compared to modern methods. For example, we have encountered PCR-negative results in 6 of 26 (23%) of Sézary patients with a chromosomally-abnormal clone. This suggests the “false-negative” rate for PCR

exceeded 20%. Secondly, the SzC count includes non-neoplastic SzCs and this might result in a higher percentage compared to other centers. When the count was restricted to larger SzCs ($\geq 12 \mu$ m diameter) that are more likely to be neoplastic than reactive, the number of larger SzCs $\geq 6\%$ and $\geq 20\%$ in PCR-negative patients decreased to 23 (42%) and 8 (15%), respectively. Of interest, a plot of the percentage of larger SzCs versus mean survival indicated that patients with $\geq 6\%$ larger SzCs had a mean overall survival (4.76 ± 0.60 years) comparable to 20% total SzCs and a positive clone by PCR discussed above and at $\geq 25\%$ larger SzCs, all deaths are CTCL-specific (Supplemental Figure 1). This observation suggests that $\geq 6\%$ larger SzCs may serve as another surrogate for the B1 threshold.

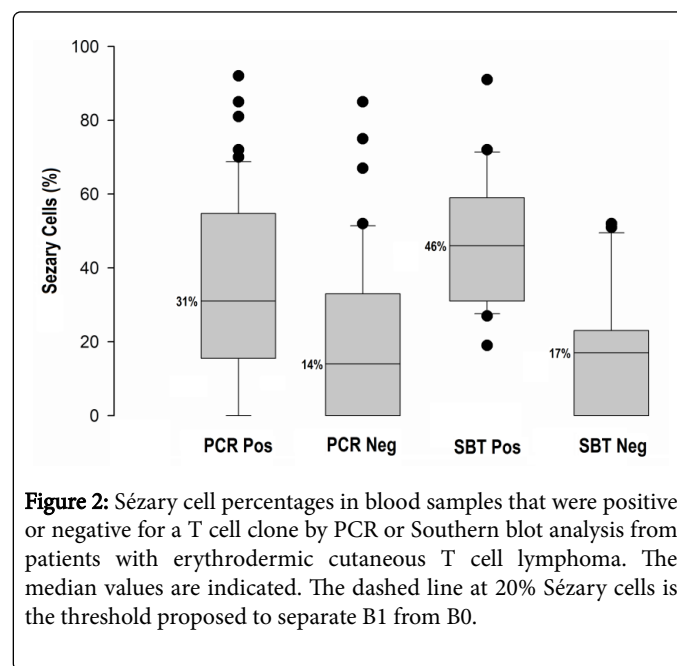


Figure 2: Sézary cell percentages in blood samples that were positive or negative for a T cell clone by PCR or Southern blot analysis from patients with erythrodermic cutaneous T cell lymphoma. The median values are indicated. The dashed line at 20% Sézary cells is the threshold proposed to separate B1 from B0.

Modification of ISCL-EORTC staging criteria

For this retrospective review, the ISCL-EORTC requirement of “plus clonality”, which was intended to confirm that neoplastic cells are present in the blood, was augmented because PCR methods available at the time sometimes failed to detect a blood clone in patients with bona fide SS or were not performed in 17 patients. For these reasons, the confirmation criterion was expanded to include not only PCR or Southern evidence of a blood clone, but also (1) evidence of a clone by chromosome analysis, (2) very large SzCs with diameters $>14 \mu$ m, and (3) T cells with diminished expression of CD2, CD3, CD4 or CD5 relative to normal cells in the sample [18]. Of 16 PCR negative patients, 14 had confirmation by other means, most often by very large SzCs. Additional confirmatory tests might be $CD4^+CD26^- \geq 30\%$ or $CD4^+CD7^- \geq 40\%$, but these would have changed B0a to B0b for only one patient with erythrodermic MF with no SzCs, a CD4/CD8 ratio of 2.77 and no clone by PCR.

Problems with B rating assignments

Three patients with SzC ≥ 1.0 K/ μ L (1,050, 1,082, and 1,336 SzC/ μ L) and 4 patients with $CD4/CD8 \geq 10$ (10.6, 12.0, 160.0, and 23.0) did not have confirmed blood involvement as defined above. The question then is what B rating should be assigned to these patients? The patients with high absolute SzCs had percentages that exceeded 20% (35%, 52% and

38%, respectively) which the ISCL-EORTC accepts as confirmation if molecular genetic studies are not available. They also had percentages of larger SzCs >10%. Therefore, these patients could be assigned a B2 blood rating. However, their clinical course was favorable compared to other patients with SS although one patient subsequently developed a localized tumor successfully treated with radiation. This suggests that their SzC counts might have been composed of many non-neoplastic SzCs. Conversely, all patients with high CD4/CD8 ratios had percentages of SzC <20%. CD7 was partially expressed by CD4+ T cells from one patient (CD3: 80%, CD4: 74%, CD8: 7%, CD7: 22%) and was fully expressed on cells from the other 3 patients. Two of these patients including the patient with diminished CD7 expression had a relatively favorable clinical course and two died of unrelated causes shortly after evaluation.

Because of this uncertainty, these patients were assigned to B0a, B0b, B1a, B1b and B2 subsets and the resulting combinations compared in the Cox model. For SzCs, the threshold for B1 was set at SzC ≥ 20%, B2 was defined as SzC ≥ 1.0 K/μL plus confirmation and stages IVA2 and IVB were excluded. The best fit for both overall and CTCL-specific survival was when the 3 unconfirmed patients with absolute SzC ≥ 1.0 K/μL were assigned to the B0a category (data not shown). A similar result was observed using Gibson's proposed criteria (CD4/CD8 ratio ≥ 5 for B1 threshold and ≥ 10 plus confirmation for B2) although the 4 patients with unconfirmed CD4/CD8 ≥ 10 could alternatively be assigned to the B1a rating. The slightly better fit with CTCL-specific deaths with B0a versus B1a and to maintain consistency with unconfirmed SzCs, these patients were staged at IIIA in subsequent models.

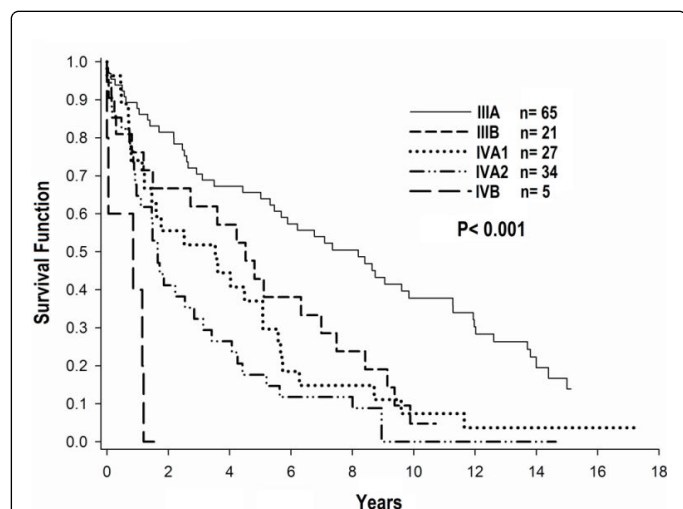


Figure 3: Overall survival curves of patients with erythrodermic cutaneous T cell lymphoma according to clinical stage with B1 and B2 thresholds defined as Sézary cells ≥ 20% and ≥ 1.0 K/μL, respectively. See Supplemental Table 1 for mean and 5 year survival rates and comparison between curves.

Clinical stages based on Sézary cell counts

Based on a prior analysis [17], the author suggested that the threshold for B1 using SzC counts should be increased to ≥ 20%. With this cohort of patients with E-CTCL, the resulting clinical stages based on this alternative B1 threshold with B2 defined as SzC ≥ 1.0 K/μL

were compared (Supplemental Table 1). Patients with SzC counts that satisfied the criteria for B1 or B2 but without additional confirmation of neoplastic cells in the blood were classified as B0/stage IIIA. The change to ≥ 20% increased the number of patients at sub-stage IIIA from 56 to 65 patients with corresponding decrease in sub-stage IIIB from 30 to 21 patients. The number of patients at sub-stage IVA1 (n=27), sub-stage IVA2 (n=34) and stage IVB (n=5) was unaffected.

In the Cox model, a better fit for both overall and CTCL-specific deaths as endpoints was observed using SzC ≥ 20% as the B1 threshold instead of SzC ≥ 6% (Supplemental Table 1). Comparison of sub-stages IIIA and IIIB showed a significant difference for the overall survival curves with B1 threshold at SzC ≥ 20% (P=0.019) whereas overall survival was not significantly different for the SC ≥ 6% threshold (P=0.236). The difference in CTCL-specific survival curves between IIIA and IIIB was highly significant for either threshold of B1, but the difference between sub-stages IIIB and IVA1 was only significant with B1 defined as SzC ≥ 6%. This was because the mean CTCL-specific survival of sub-stage IIIB patients with B1 defined as SzC ≥ 20% (7.22 ± 1.35 years) was closer to the mean survival of sub-stage IVA1 (5.67 ± 1.22 years) than patients with sub-stage IIIB using B1 defined as SzC ≥ 6% (9.91 ± 1.31 years). The survival curves for patients with B1 and B2 thresholds at SzC ≥ 20% and ≥ 1.0 K/μL are shown in Figures 3 and 4.

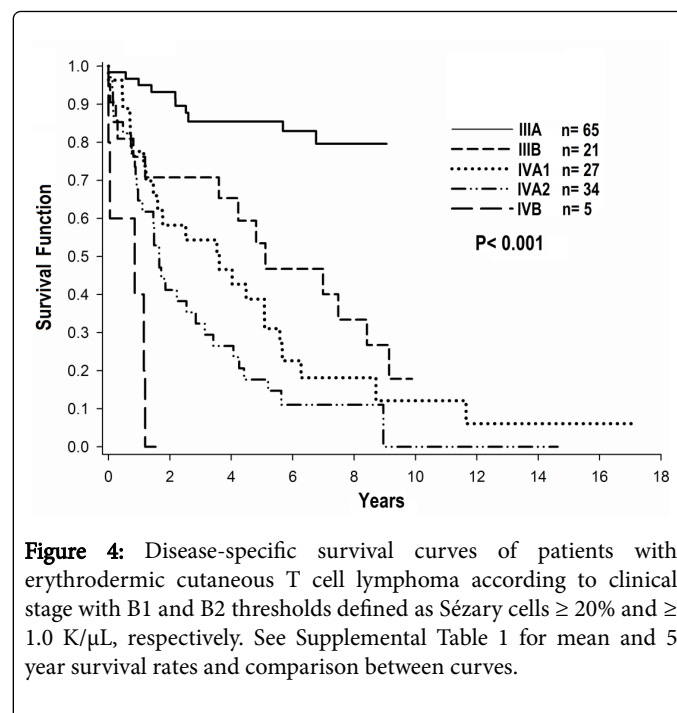


Figure 4: Disease-specific survival curves of patients with erythrodermic cutaneous T cell lymphoma according to clinical stage with B1 and B2 thresholds defined as Sézary cells ≥ 20% and ≥ 1.0 K/μL, respectively. See Supplemental Table 1 for mean and 5 year survival rates and comparison between curves.

CD4/CD8 ratio as criteria for B ratings

A confounding factor for defining B ratings using CD4/CD8 ratios was the aberrant expression of CD4 or CD8 by circulating neoplastic cells. In this series, 3 patients had circulating neoplastic cells with loss of CD4, one patient had cells expressing a CD4⁻CD8⁺ phenotype and one patient had cells co-expressing CD4 and CD8. These patients were excluded from CD4/CD8 analysis. In addition, high CD4/CD8 ratios may occur because of low CD8 values rather than an increase in CD4⁺ cells. To check for this possibility, for patients studied by single antibodies, the sum of CD4⁺ and CD8⁺ percentages was compared against the percentage of CD3⁺ cells and found to correlate well in all

samples except those with neoplastic cells expressing diminished expression of CD3 and one patient with a CD4/CD8 ratio of 23.0 that could be due to a diminished expression of CD4 and/or an erroneously low CD8⁺ percentage (CD3⁺ 82%, CD4⁺ 69%, CD8⁺ 3%). This patient plus 3 others with a CD4/CD8 \geq 10 had no confirmation of neoplastic cells in the blood. Their B rating was ultimately downgraded from B2 to B0.

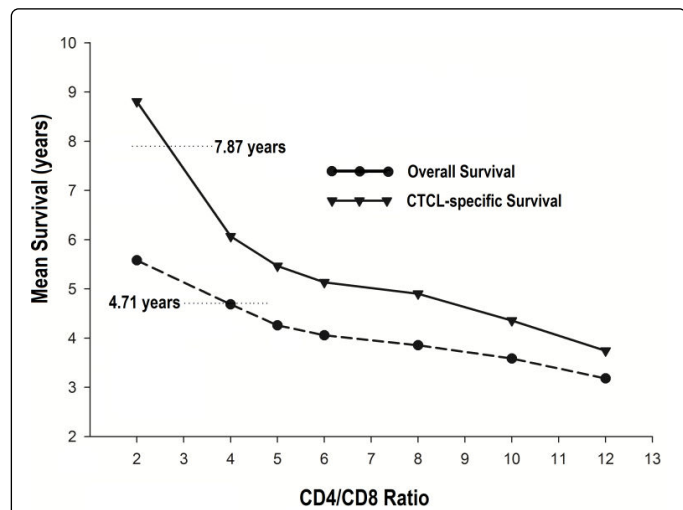


Figure 5: The estimated mean survival of patients with erythrodermic cutaneous T cell lymphoma decreases as the CD4/CD8 ratio in the blood increases. The dotted lines show the mean values for patients with a blood clone demonstrated by PCR.

A plot of CD4/CD8 ratios versus mean survival for each group is shown in Figure 5. At a CD4/CD8 ratio \geq 4, the estimated mean overall survival (4.69 ± 0.51 years) approximates the mean survival for patients with a clone in the blood by PCR (4.71 ± 0.77 years). This suggests that CD4/CD8 \geq 4 might provide a threshold for B1.

Clinical stages based on CD4/CD8 ratios

Gibson at Yale University proposed B ratings based on CD4/CD8 ratios: B0: CD4/CD8 <5; B1: CD4/CD8 \geq 5 but <10, and B2: CD4/CD8 \geq 10 plus confirmation of abnormal cells [20]. As with SzC counts, for this cohort of patients with E-CTCL, confirmation requires evidence of positive clone, very large SCs, or T cells with aberrant T cell marker expression and absence of confirmation will be designated as B0 for purpose of staging.

The clinical stages with B ratings thus defined and the corresponding mean survivals are shown in Supplemental Table 2. The difference in survival curves for 71 patients at sub-stage IIIA and 15 patients at sub-stage IIIB for both overall and CTCL-specific was statistically significant. No difference was found between sub-stages IVA1 and IVA2.

The B1 threshold was then changed to CD4/CD8 \geq 4 which resulted in 4 patients reassigned from B0 to B1. The fit in the Cox model improved slightly (data not shown). The B2 threshold was then changed from CD4/CD8 \geq 10 to \geq 12 which further improved the fit, particularly for CTCL-specific survival as the endpoint (Supplemental Table 2). However, these modifications to Gibson's criteria are not profound, but might be considered if the goal is to provide a B2 rating

that makes survival of sub-stages IVA1 and IVA2 nearly identical. The survival curves for clinical stages using Gibson's criteria for B1 and B2 are shown in Supplemental Figures 1 and 2.

Maximum CD4⁺CD26⁻ or CD4⁺CD7⁻ percentages

In this cohort, only 39 patients had percentages of CD4⁺CD26⁻ and CD4⁺CD7⁻ lymphocytes directly measured. In most cases, the percentage of CD4⁺CD26⁻ cells exceeded that of CD4⁺CD7⁻ cells. However, for 2 patients with SS, the percentage of CD4⁺CD7⁻ cells was 14% and 60% higher than CD4⁺CD26⁻ cells, presumably due to partial expression of CD26 by neoplastic cells. The maximum value of either subset, referred here on as max-CD4⁺CD26⁻/7⁻, was used to define B ratings.

According to recent guidelines of the EORTC cutaneous lymphoma task force [21], 22 (56%) patients had absolute max-CD4⁺CD26⁻/7⁻ counts \geq 1.0 K/ μ L and perforce B2. Of the remaining patients, 6 had counts \geq 250/ μ L (B1) and 11 had counts <250/ μ L (B0). Of interest 6 of 13 patients with B0 and all the B1 and B2 patients had confirmed involvement by methods described above. If the threshold for B1 was changed to \geq 30% based on a prior study [18], only one patient at B0 would be reassigned to B1. This patient at stage IVA2 had low absolute lymphocyte count as a consequence of prior systemic chemotherapy but a CD4/CD8 ratio of 15.7 (CD3⁺CD4⁺: 94%, CD3⁺CD8⁺: 6%).

The proposed EORTC B ratings were used to define clinical stages (Supplemental Table 3). The difference in survival curves for overall and CTCL-specific deaths was not statistically significant, most likely because of small numbers of patients in sub-stages IIIA (n=9) and IIIB (n=3). The survival curves of patients at sub-stages IVA1 and IVA2 were nearly identical.

Although the small number of patients available for analysis could contribute to this result, the author wondered if absolute counts might have been suppressed by treatments or other factors. Therefore, the possibility that alternative B ratings might be defined using percentages of max-CD4⁺CD26⁻/7⁻ lymphocytes instead of absolute counts was investigated.

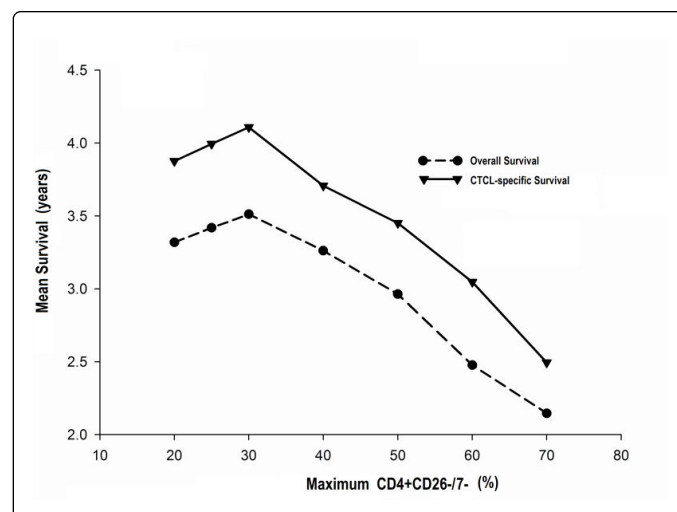


Figure 6: The estimated mean survival of patients with erythrodermic cutaneous T cell lymphoma decreases as the percentage of max-CD4⁺CD26⁻/7⁻ lymphocytes in the blood increases.

Figure 6 shows the relationship between estimated mean survival rates and various percentages of max-CD4⁺CD26⁻/7⁻ cells for 23 patients with stages IVA2 and IVB excluded. Like SzC counts and CD4/CD8 ratios, as the proportion of these cells among lymphocytes increases, the corresponding overall and CTCL-specific mean survival rates progressively decrease above the 30% threshold and eventually converge. All patients with max-CD4⁺CD26⁻/7⁻ percentages $\geq 20\%$ had confirmed blood involvement by other methods. This indicates that increased percentage of this subset of lymphocytes in patients with E-CTCL indicates the presence of circulating neoplastic cells.

Various combinations of max-CD4⁺CD26⁻/7⁻ percentages were used to define alternative B ratings for staging and tested in the Cox model for comparison with the EORTC recommendations (Supplemental Table 4). Notably, for this small cohort of patients, a better fit was observed for any cause of death or CTCL-specific death as endpoints if the B1 threshold was changed from max-CD4⁺CD26⁻/7⁻ $\geq 250/\mu\text{L}$ to $\geq 20\text{-}30\%$ and/or B2 changed from max-CD4⁺CD26⁻/7⁻ $\geq 1.0 \text{ K}/\mu\text{L}$ to $50\text{-}60\%$ (Supplemental Table 4). Although B1 $\geq 20\%$ and B2 $\geq 60\%$ thresholds provided the best fits in the Cox model, the author recommends that $\geq 30\%$ and $\geq 60\%$ be used until more data becomes available because it retains the ISCL-EORTC suggested recommendation for B2.

Correlation max-CD4⁺CD26⁻/7⁻ with other measures

Of the 39 patients studied, the max-CD4⁺CD26⁻/7⁻ percentage correlated strongly with both SzC percentages ($\rho=0.893$, $P<0.001$) and CD4/CD8 ratios ($\rho=0.792$, <0.001). Twenty-two of these patients had one of the major ISCL-EORTC criteria for B2 [19]. Specifically, 20 (91%) patients had a CD4/CD8 ratio ≥ 10 , and 14 (64%) patients had an absolute SzC count $\geq 1.0/\mu\text{L}$ plus confirmation. Of note, 22 patients had an absolute max-CD4⁺CD26⁻/7⁻ count of $\geq 1.0/\mu\text{L}$, which was proposed for B2 by the EORTC task force [21]. All but 2 of these patients also met the ISCL-EORTC criteria for B2. These results suggest that SzC counts underestimate the magnitude of blood involvement compared to other methods; consequently, the absolute SzC count $\geq 1.0/\mu\text{L}$ threshold for B2 is often not reached.

Increasing percentages of max-CD4⁺CD26⁻/7⁻ cells was also correlated to the frequency of patients that fulfilled the ISCL-EORTC criteria for B2 (Supplemental Table 5). For the 28 patients with a max-CD4⁺CD26⁻/7⁻ percentage $\geq 30\%$, 21 patients (75%) had either an absolute SzC count $\geq 1.0/\mu\text{L}$ alone (1 patient), a CD4/CD8 ratio ≥ 10 alone (8 patients) or both together (12 patients). The corresponding proportion of patients with absolute max-CD4⁺CD26⁻/7⁻ $\geq 1.0/\mu\text{L}$ was 79% (22 of 28 patients). Increasing the max-CD4⁺CD26⁻/7⁻ percentage to $\geq 40\%$ increase the proportion of patients at B2 by a few percentages and the highest proportions occurred at about 45% (Supplemental Table 5).

Percentages of max-CD4⁺CD26⁻/7⁻ cells were also used to define various thresholds that separate B0, B1 and B2. A better fit with survival endpoints in the Cox model with B1 threshold defined as max-CD4⁺CD26⁻/7⁻ $\geq 30\%$ was observed with B2 defined as max-CD4⁺CD26⁻/7⁻ $\geq 60\%$ than with max-CD4⁺CD26⁻/7⁻ $\geq 50\%$. An even better fit occurred if the B1 threshold was lowered to max-CD4⁺CD26⁻/7⁻ $\geq 20\%$ (Supplemental Table 4). This change accentuated the difference in prognosis between sub-stages IIIA and IIIB although still not statistically significant. Perhaps a larger patient population would clarify this issue.

Discussion

Various methods have been utilized to determine the magnitude of neoplastic T cell involvement in the blood of patients with CTCL. These include counts of SzCs and flow cytometry using markers that are often lost by neoplastic cells (CD7, CD26) or expressed by neoplastic cells (TCR-V β , CD158k/KIR3DL2, CD164). However, these markers are not entirely specific for neoplastic cells because small numbers of lymphocytes with the same characteristics can be identified in the blood of healthy individuals. Furthermore, in certain severe inflammatory skin disorders, lymphocytes bearing these properties may increase in the blood. Therefore, for the purpose of identifying blood involvement in patients with E-CTCL, values are chosen that exceed what might be encountered in non-neoplastic erythrodermic inflammatory diseases. Some examples are SCs $\geq 20\%$ [3], CD4/CD8 ratio ≥ 10 [15], CD4⁺CD7⁻ $\geq 40\%$ [16], and CD4⁺CD26⁻ $\geq 30\%$ [25]. However, exceptions are still possible and such cases may be misdiagnosed as E-CTCL and even SS, particularly given that non-specific skin histopathologic findings occur in about 25% of patients with bona fide SS [26]. For this reason, the ISCL-EORTC recommends that the presence of neoplastic T cells in the blood be substantiated by demonstrating a T cell clone by molecular genetic techniques [19]. Other confirmatory methods include demonstration of clonality by TCR V β restriction or chromosome analysis, presence of SCs with very large diameters, and aberrant T cells with altered expression levels of T cell antigens.

The hematologic criteria used to define B ratings continue to be revised. For centers that utilize SzC counts to assess blood tumor burden, this retrospective review suggests that SC $\geq 20\%$ rather than $\geq 6\%$ plus confirmation of blood involvement be used as the threshold for B1 if an absolute count $\geq 1.0 \text{ K}/\mu\text{L}$ defines B2. This assumes that patients with SC $\geq 20\%$ without confirmation are assigned to B0. This provides for a significant separation of survival patterns between sub-stages IIIA and IIIB and no difference between IVA1 and IVA2 (Figures 3 and 4, Supplemental Table 1).

The main objection to SzC counts pertains to its subjective nature and we have addressed this problem by having one experienced technician perform the counts. Nevertheless, although strongly correlated with other measures used to define B2, a smaller proportion of patients with E-CTCL met the SzC criterion for B2 (i.e., SzC $\geq 1.0 \text{ K}/\mu\text{L}$ plus confirmation) compared to CD4/CD8 ≥ 10 or absolute max-CD4⁺CD26⁻/7⁻ $\geq 1.0 \text{ K}/\mu\text{L}$. This suggests either that not all SzCs are being recognized visually and/or that not all neoplastic cells have hyperconvoluted nuclei.

At other centers, investigators prefer to use the CD4/CD8 ratio to define B ratings. For example, Gibson at Yale University recently proposed CD4/CD8 ≥ 5 but <10 plus confirmation as the criterion for B1 [20]. In my cohort of patients, with CD4/CD8 ≥ 10 used to define B2, the separation between sub-stages IIIA and IIIB are highly significant for CTCL-specific survival and not quite significant for overall survival (Supplemental Table 2). This was because patients with CD4/CD8 ≥ 5 but <10 and unconfirmed involvement were placed into the B0/sub-stage IIIA group. Moreover, 4 patients with CD4/CD8 ≥ 10 but unconfirmed blood involvement were also placed into sub-stage IIIA. The difference between stages IVA1 and IVA2 was not significant. It is possible that, with more sensitive PCR methods available today, some of these "unconfirmed" B1 and B2 patients would be reassigned to a higher B rating.

With either SzC percentage or CD4/CD8 ratios, dividing B0 and B1 into groups based on absence (i.e., B01a and B1a) or presence (i.e., B0b and B1b) of neoplastic T cells in the blood indicated that B1b alone could be used to define sub-stage IIIB. The importance of demonstration of circulating neoplastic cells per se explains why an alternative B rating system with B1 defined as CD4/CD8 ratios <10 plus confirmation seems to provide similar results as Gibson's criteria. This might prove useful if the T cell clone in the blood is shown to be identical to that in involved skin or lymph node.

With the recognition that neoplastic T cells have diminished expression of CD26 in about 90% of Sézary patients, the percentage and absolute numbers of CD4⁺CD26⁻ lymphocytes have recently been proposed to define B ratings [18,21]. However, because CD26 may be variably expressed (some neoplastic cells positive, some negative), it is useful to also measure CD4⁺CD7⁻ cells and to use this value if it exceeds the percentage of CD4⁺CD26⁻ cells [27].

In this cohort of patients with E-CTCL, 39 were studied for CD4⁺CD26⁻ and CD4⁺CD7⁻. According to B criteria proposed by the EORTC cutaneous lymphoma task force [21], 9 erythrodermic patients were at sub-stage IIIA, 3 at sub-stage IIIB, 11 at sub-stage IVA1, 13 at sub-stage IVA2, and 3 at stage IVB. Unexpectedly, the overall difference in survival curves was not statistically significant. In particular, the mean survival rates for IIIB, IVA1 and IVA2 were almost identical. An attempt to use percentages of max-CD4⁺CD26⁻/7⁻ cells to avoid reliance on absolute lymphocytes yielded similar results. Additional studies with larger numbers of patients may clarify this issue.

Ultimately, better methods than discussed in this retrospective review may be required to quantify neoplastic involvement in the blood and B ratings for staging. Measurement of the killer immunoglobulin-like receptor KIR3DL2/CD158k on T cells has promise in this regard, but is not always expressed by neoplastic cells [28]. Molecular genetic methods such as next generation sequencing of clones may serve this purpose if they can be made cost effective [29].

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