

ORIGINAL ARTICLE

Prevalence of atopy and staphylococcal superantigen-specific immunoglobulin E (IgE) antibodies and total serum IgE in primary cutaneous T- and B-cell lymphoma

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ABSTRACT

The prevalence of atopy was investigated in 20 patients with Sézary syndrome (SS), 20 patients with plaque phase mycosis fungoides (MF), 9 patients with primary cutaneous marginal zone lymphoma (pcMZL) and 8 patients with primary cutaneous follicle center lymphoma (pcFCL) with the Phadiatop multi-allergen test. The relationship among serologic atopy, IgE reactivity against Staphylococcal enterotoxin superantigens, and serum total IgE (IgE-t) levels and their prognostic implications in SS was investigated. Phadiatop test was positive in 45%, 15%, 33% and 0% of samples of SS, MF, pcMZL and pcFCL, respectively. IgE-t levels were also increased in SS, pcMZL and marginally MF. No correlation was found with patients' history of atopic disorders. Staphylococcal superantigen-specific IgE ≥ 0.35 kUa/L, most often against toxic shock syndrome toxin-1, was detected in 40% of Sézary samples followed by MF (20%). In the absence of serologic atopy (negative Phadiatop test), IgE-t levels for patients with SS and MF were not significantly higher than controls whereas the levels for pcMZL remained high. Furthermore, even with a negative Phadiatop test, IgE-t values were higher in sera of patients with SSaIgE ≥ 0.35 kUa/L vis-à-vis < 0.35 kUa/L across all diagnostic categories including controls albeit the difference was statistically significant only for SS. The presence of specific IgE antibodies ≥ 0.35 kUa/L, IgE-t > 122 kU/L or eosinophils $> 500/\mu\text{L}$ had no impact on survival of patients with SS. These results indicate that a pathogenic link may exist between an atopic diathesis and development of SS and possibly pcMZL.

Key words: atopy, immunoglobulin E, lymphoma, Sézary syndrome, superantigens.

INTRODUCTION

Primary cutaneous T-cell lymphoma (CTCL) is comprised of two major variants: (i) Sézary syndrome (SS) which is characterized by diffuse erythroderma, high numbers of circulating lymphocytes with highly convoluted “cerebriform” nuclei (Sézary cells) and T-cell clonality; and (ii) mycosis fungoides (MF) including de novo erythrodermic CTCL (E-CTCL) lacking the blood criteria of SS.¹ Primary cutaneous B-cell lymphoma (pcBCL) likewise has two main clinical subtypes: (i) primary cutaneous marginal zone lymphoma (pcMZL); and (ii) follicle center lymphoma (pcFCL).

Like atopic dermatitis, the skin of most patients with SS (~60% of cases compared with 10% for the general population) are colonized by *Staphylococcus aureus*, and most staphylococcal isolates produce enterotoxins with superantigenic activity.^{2–5} These staphylococcal superantigens (SSAg) intensify skin inflammation in part through production of

immunoglobulin E (IgE) antibodies that react with SSaAg (SSaAg-IgE), and subsequently bind to mast cell Fc receptors in the skin. Total serum IgE (IgE-t) is also increased. Prudent antimicrobial treatment cannot only ameliorate cutaneous signs and symptoms of SS,³ but may also improve survival by reducing the risk of life-threatening infectious complications such as septicemia and pneumonia that are common in SS.^{6–9}

In addition, SSaAg may promote disease progression, thereby impacting disease-specific survival of patients with SS.¹⁰ One potential mechanism is the stimulation of neoplastic T cells to proliferate by SSaAg reacting with the appropriate V β -segment of the T-cell receptor (TCR-V β) region, for example, toxic shock syndrome toxin-1 (TSST1) with TCR-V β 2.^{2,11} SSaAg-secreting bacteria may also contribute to a perturbed TCR-V β repertoire on circulating T cells in CTCL and perhaps influence TCR-V β expression profiles of neoplastic T cells as well.^{12–14}

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Another proposed mechanism involves the contribution of SSAG to the pro-tumorigenic inflammatory milieu of SS.^{15,16} For example, staphylococcal enterotoxin A (SEA) has been shown to trigger signal transducer and activator of transcription (STAT)3 activation and interleukin (IL)-17 expression by neoplastic T cells.⁵ Dysregulated STAT3 is an important factor in neoplastic cell proliferation. Recently, the skin microbiota was found to be crucial for malignant transformation of T cells and progression of disease in a constitutively-activated STAT3 mouse model of CTCL.¹⁷

Despite these observations, it remains unclear whether prognosis is affected to a greater degree by SSAG-secreting *S. aureus* compared with non-SSAG-secreting strains. In this regard, Scala *et al.*¹⁸ studied 55 patients with SS for SSAG-specific IgE with a comprehensive panel of allergens that included SEA and staphylococcal enterotoxin B (SEB), but not TSST1. At least one positive IgE antibody specificity was detected in the sera of 24 (44%) patients, and these IgE-positive patients had a significantly worse prognosis (median overall survival, 2.9 years; 5-year survival rate, 26%) compared with IgE-negative patients (median overall survival, 8.9 years; 5-year survival rate, 82%). Notably, six of 24 (25%) SS had IgE reactivity against SEB and no patient had IgE reactivity against SEA. The prognostic implications of SEB-IgE per se were not reported. IgE-t levels had no prognostic significance.

In this study, we tested sera from patients with SS and plaque phase MF for SSAG-IgE and IgE specific to common airborne allergens (aero-IgE) using the Phadiatop test, which if positive, is regarded as serological evidence of atopy.¹⁹ We also studied two common variants of pcBCL to gain information about any possible connection with atopy and to compare with CTCL. Our findings indicate that patients with SS and possibly pcMZL, but not plaque phase MF, have an atopic diathesis that may contribute to high levels of SSAG-IgE and IgE-t in SS and IgE-t in pcMZL. The presence of either aeroallergen-specific IgE or SSAG-IgE in SS did not correlate with poor survival.

METHODS

Clinical data were obtained from a cutaneous lymphoma registry approved by the institutional review board at Johns Hopkins University. The SS cohort was comprised of seven men and 13 women, aged 31–94 years (median age, 69). All had erythroderma and absolute Sézary cell counts of 1.0 K/ μ L or more and T-cell clone in the blood confirmed by chromosome analysis and/or molecular genetic methods. No patient with SS had pre-existing MF, and three had evidence of large cell transformation. The clinical stages were IVA1 (12 patients) and IVA2 (eight patients). The MF group (13 men, seven women) had clinically typical, histopathologically confirmed plaque phase MF at stages IA–IIA. Patients with plaque phase MF were selected to avoid confusion with atypical clinical presentations or so-called spongiotic simulants. Tumor phase MF was also excluded to discount ulceration as a source of *S. aureus*. The pcBCL group included eight patients (four men, four

women) diagnosed with pcFCL and nine patients (four men, five women) with pcMZL limited to the skin. No patient with cutaneous T- or B-cell lymphoma had clinical evidence of infection at the time of clinical evaluation.

For this study, IgE-t and allergen-specific IgE on de-identified frozen sera from a previous study were measured in the Johns Hopkins Dermatology Allergy and Clinical Immunology Reference Laboratory using the ImmunoCAP method (ImmunoCAP; Thermo Fisher Scientific, Uppsala, Sweden). The Phadiatop multi-allergen test screens for IgE reactivity against 10 common aero-IgE in the northeastern USA and is considered the single best serological test to assess atopic status based on an NIH Biomarker Group Consensus.¹⁹ To determine the relationship between serological atopy and SSAG-IgE and its prognostic significance in SS, IgE specific for SEA, SEB and TSST1 were also measured individually. A positive test for aeroallergen-specific IgE or SSAG-IgE was defined as a concentration of 0.35 kUa/L or more. Sera collected from adult patients (22 men, 30 women; aged 24–78 years), who were evaluated for rhinosinusitis and had a negative Phadiatop test for aero-IgE, served as a serological non-atopic controls. Previously obtained IgE-t measurements from other reference laboratories were also available for most patients and used to compare SS with non-Sézary variants of E-CTCL.

Statistics

Results of IgE measurements are presented as a median value with a range. Because IgE-t, allergen-specific IgE and absolute eosinophil values tend to be skewed,^{20,21} the distribution of these variables was normalized by log transformation and the geometric mean, namely the anti-log of the mean log₁₀ IgE-t value, and its 95% confidence interval (CI) is also provided. The lower limit of detection (LLD) was 2.0 kU/L for IgE-t and 0.1 kU/L for allergen-specific IgE. For values less than the LLD, a fill value of LLD/ $\sqrt{2}$ was used for calculation purposes, namely 1.41 kU/L for IgE-t and 0.07 kU/L for allergen-specific IgE.²⁰ Absolute eosinophil counts were calculated from the total and differential leukocyte counts. For samples without eosinophils on manual leukocyte differential counts, fill value of 1 eosinophil/ μ L was used for calculation of log-transformed absolute eosinophil counts.

The non-parametric Kruskal-Wallis ranks (KW) test was used to test differences of median IgE values among independent groups. In addition, log-transformed IgE values were used to compare mean values using parametric tests (one-way ANOVA, Student's *t*-test with equal variances not assumed [*t*-test] and Dunnett's *t*-test for comparison against the control sera). Fisher's and Pearson's χ^2 - and exact tests were used to test categorical data in 2 by 2 and R by C tables, respectively. The influence of SSAG-IgE measurements and other variables on survival was evaluated using the Cox proportional hazards model and the difference in overall and disease-specific survival curves was determined using the Kaplan-Meier method and log-rank test of Mantel. The statistical software used in the study were SYSTAT10 and SPSS 13.0 for Windows (SPSS, Chicago, IL, USA) and StatXact-3 (Cytel, Cambridge, MA, USA).

RESULTS

Total serum IgE is increased in SS and pcMZL

Table 1 summarizes the results of IgE-t according to diagnostic categories in this study. IgE-t values were increased vis-à-vis controls for patients with SS, the MZL variant of pcBCL, and probably skin-limited plaque phase MF.

Correlation between clinical atopy and positive aeroallergen-specific IgE screen (Phadiatop)

A positive Phadiatop is serological evidence of atopy. It was recorded as positive in 45% of serum samples from the SS group, but only 15% of plaque phase MF and 18% of pcBCL (all with pcMZL or 33%). However, no correlation was found between serological evidence of atopy and the patient's history of an atopic condition in their medical records (Table 2). These observations suggest that the prevalence of atopy based on the patient's history of atopic disorders may be underestimated. It also suggests that a relationship may exist between the patient's atopic diathesis and the development of SS and possibly pcMZL.

Relationship between serological evidence of atopy and IgE-t levels

For each disease category, IgE-t levels were significantly higher for patients with serological evidence of atopy compared with patients without serological atopy (Table S1). IgE-t levels of seronegative patients did not differ according to diagnosis (KW, $P = 0.890$; ANOVA, $P = 0.762$). This observation suggests that atopic diathesis is more important than disease-related factors that potentially influence IgE production.

On the other hand, the IgE-t levels for patients who were seropositive for aero-IgE might have been higher for SS than the small number of patients in other diagnostic categories (KW, $P = 0.045$; ANOVA, $P = 0.147$). The influence of staphylococcal SSaG as a factor is investigated below.

Prevalence of SSaG-specific IgE

Measurable IgE reactivity (≥ 0.1 kU/L) against at least one SSaG was present in 55% of patients with SS, 45% patients with plaque phase MF and 35% specimens of patients with pcBCL compared with 12% of non-atopic control subjects (Table S2). However, at the concentration used to define serological atopy

(≥ 0.35 kUa/L), the detection frequency remained highest for SS (40%) followed by MF (20%), and lowest for pcBCL (6%) which was the same as controls (6%). At this threshold, TSST1-IgE was detected most often (all patients with SS usually along with SEA-IgE and SEB-IgE (20% of Sézary patients). TSST1-IgE exceeded the 0.35 kUa/L threshold in three control subjects (0.43, 0.47 and 0.93 kU/L), a finding that may reflect the selection of sera from patients evaluated for rhinosinusitis and therefore prone to nasal colonization by *S. aureus*. Compared with controls, the difference in frequencies of SSaG IgE of more than 0.35 kUa/L was statistically significant for SS ($P = 0.001$) and not quite significant for MF ($P = 0.088$). For TSST1-IgE alone, only SS was significantly higher ($P = 0.001$).

Relationship between SSaG-specific IgE and serological atopy

For patients with SSaG-IgE levels of 0.35 kUa/L or more, IgE-t was increased for SS and probably plaque phase MF compared with patients with SSaG-IgE levels of less than 0.35 kUa/L (Table 3). This trend was also seen in the control group. Only one patient with pcBCL had a high SSaG-IgE level. This finding illustrates that patients who have developed IgE antibody responses against SSaG have increased IgE-t levels, but does not address to what degree this is due to an atopic diathesis.

To investigate this issue, IgE-t levels are categorized for each disease according to Phadiatop and SSaG-IgE test results (Table 3). When both tests were negative (aero-IgE and SSaG-IgE, <0.35 kUa/L), IgE-t level for patients with SS and MF were not significantly higher than controls. On the other hand, IgE-t was increased in patients with pcBCL, specifically the MZL subset. This finding suggests that atopic diathesis and/or SSaG stimulation are of primary importance in modulating IgE-t in CTCL and that some other disease-related factor is important in pcMZL.

The possible contribution of SSaG in affecting IgE-t production was investigated by comparing IgE-t levels for serologically non-atopic patients with SS and MF (aero-IgE, <0.35) with or without SSaG-IgE. Despite the small number of patients in each cohort, IgE-t values were higher in sera with SSaG-IgE of 0.35 kUa/L or more vis-à-vis less than 0.35 kUa/L for both diagnostic categories although the difference was statistically

Table 1. Total serum IgE levels according to diagnostic categories

Diagnosis	No.	IgE-t median (range)	IgE-t GM (95% CI)	KW*	t-test*
SS	20	172 (1.4–3556)	110 (34.6–352)	0.001	0.002
MF, PQ	20	26.0 (1.4–490)	33.0 (14.3–75.9)	0.034	0.061
pcBCL	17	53.6 (1.4–3354)	50.5 (19.9–128)	0.006	0.013
FCL	8	29.6 (1.4–216)	21.5 (5.9–78.5)	0.396	0.483
MZL	9	193 (8.7–3354)	108 (27.8–418)	0.001	0.008
Controls	52	14.8 (2–187)	14.1 (10.0–19.9)	—	—

Bolding indicates statistical significance. * P -values for disease group vs control: KW, Kruskal–Wallis test and t -test. The difference in IgE-t between pcFCL and pcMZL subtypes of pcBCL approached statistical significance (KW, $P = 0.068$; t -test, $P = 0.063$). FCL, follicle center cell lymphoma; GM, geometric mean and its 95% confidence interval; IgE-t, total serum immunoglobulin E (kU/L); KW, Kruskal–Wallis; MF, PQ, mycosis fungoides, plaque phase; MZL, marginal zone lymphoma; pcBCL, primary cutaneous B cell lymphoma; SS, Sézary syndrome.

Table 2. Lack of correlation between patients' history (PHx) of an atopic condition and positive Phadiatop test for serological evidence of atopy (aero-IgE, ≥ 0.35 kUa/L)

Diagnostic group	Aero-IgE, <0.35 kUa/L	Aero-IgE, ≥ 0.35 kUa/L	P*
All SS	11	9 (45%)	
No PHx atopy	6	5	1.0
Yes PHx atopy	5	4	
All MF, PQ	17	3 (15%)	
No PHx atopy	12	2	1.0
Yes PHx atopy	5	1	
All pcBCL	14	3 (18%) [†]	
No PHx atopy	11	1	0.191
Yes PHx atopy	3	2	

*Fisher's exact test. [†]Phadiatop positive for three of nine (33%) pcMZL samples and no sample of pcFCL ($P = 0.206$). MF, PQ, mycosis fungoides, plaque phase; pcBCL, primary cutaneous B-cell lymphoma; SS, Sézary syndrome.

significant only for SS (Table 2). This finding supports the concept that colonization of the skin of SS by *Staphylococcus*-producing SSaG can augment IgE production in otherwise non-atopic individuals.

Finally, when both aero-IgE and SSaG-IgE are positive, the highest IgE-t values were recorded for SS, a finding consistent with a positive interaction between an atopic diathesis and stimulation by SSaG.

Influence of SSaG on survival of patients with SS

Of the 20 patients with SS, all are now deceased with all but four deaths attributed to their disease or treatment (disease-specific deaths). Consequently, overall and disease-specific survival curves were similar, with median survival time of 3.5 years (95% CI, 1.2–5.9) and 5-year survival rate of 35% for all causes of deaths and 3.6-year median survival (CI, 1.5–5.7) and 37% 5-year survival for disease-specific deaths. In the Cox proportional hazards model, none of the following variables was significantly associated with either overall or disease-specific death as the end-point: patient's age and personal history of atopy, IgE-t levels, presence of aero-IgE of 0.35 kUa/L or more (positive Phadiatop) or SSaG-IgE levels of 0.1 kU/L or more and 0.35 kUa/L or more. The impact of SSaG-IgE and aero-IgE on overall survival curves of patients with SS is shown in Figures 1 and 2.

Comparison of SS with non-Sézary E-CTCL

Measurements of IgE-t and absolute eosinophil counts were obtained as part of the initial laboratory evaluation of 85 patients diagnosed with E-CTCL (42 with SS and 43 with non-Sézary E-CTCL). The non-Sézary E-CTCL group was heterogeneous and composed of patients whose erythroderma followed clinically typical MF (three patients), erythroderma that developed de novo, and patients with relatively high numbers of Sézary cells in the blood, but not to the level required for the diagnosis of SS. Follow up indicated that 15 non-Sézary E-CTCL patients subsequently developed SS (examples of pre-SS or forme fruste SS). These patients had significantly higher

Table 3. Relationship among disease categories and presence or absence of aero-IgE and SSaG-IgE

Diagnosis	Cohort	Aero-IgE ^{†,‡}	SSaG-IgE [‡]	No.	Median IgE-t (range) [‡]	IgE-t GM (95% CI)
SS	1	Any	<0.35	12	28.4 (1.4–749)	34.1 (8.7–134)
	2	Any	≥ 0.35	8	1368 (29.1–3556)	642 (136–3029)
	3	<0.35	<0.35	7	9.1 (1.4–53.8)	8.1 (2.3–28.9)
	4	<0.35	≥ 0.35	4	172 (29.1–582)	139 (17.3–1111)
	5	≥ 0.35	<0.35	5	380 (35.3–749)	253 (59.6–1075)
	6	≥ 0.35	≥ 0.35	4	3202 (2153–3556)	2976 (2092–4235)
MF, PQ	7	Any	<0.35	16	20.1 (1.4–490)	23.2 (9.1–60.2)
	8	Any	≥ 0.35	4	214 (27.4–239)	131 (24.7–697)
	9	<0.35	<0.35	14	17.4 (1.4–490)	18.3 (6.6–50.3)
	10	<0.35	≥ 0.35	3	192 (27.4–236)	108 (5.6–2056)
	11	≥ 0.35	<0.35	2	151 (74.4–228)	130
	12	≥ 0.35	≥ 0.35	1	239	—
pcBCL	13	<0.35	<0.35	13	50.3 (1.4–3354)	38.6 (11.9–125)
	14	<0.35	≥ 0.35	1	25.7	—
	15	≥ 0.35	<0.35	3	206 (193–210)	203 (182–226)
	16	≥ 0.35	≥ 0.35	0	—	—
Control	17	<0.35	<0.35	49	13.5 (2.0–110)	12.5 (9.0–17.3)
	18	<0.35	≥ 0.35	3	132 (56.0–187)	111 (23.8–520)

[†]Positive Phadiatop test defined as aero-IgE of ≥ 0.35 kUa/L. [‡]Aero-IgE and SSaG-IgE concentrations in kUa/L and IgE-t in kU/L. Statistical comparison of cohorts using Kruskal–Wallis (KW) or *t*-tests: SS, cohort 1 vs 2: KW, $P = 0.011$ and *t*-test, $P = 0.005$; MF cohort 7 vs 8: KW, $P = 0.073$ and *t*-test, $P = 0.036$. SS, cohort 3 vs control cohort 17: KW, $P = 0.488$ and *t*-test, $P = 0.458$; MF, cohort 9 vs control cohort 17: KW, $P = 0.413$ and *t*-test, $P = 0.452$; pcBCL, cohort 13 vs control cohort 17: KW, $P = 0.036$ and *t*-test, $P = 0.064$. SS, cohort 3 vs cohort 4: KW, $P = 0.014$ and *t*-test, $P = 0.012$; SS, cohort 5 vs cohort 6: KW, $P = 0.014$ and *t*-test, $P = 0.008$; MF, cohort 9 vs cohort 10: KW, $P = 0.101$ and *t*-test, $P = 0.097$; Control cohort 17 vs cohort 18: KW, $P = 0.006$ and *t*-test, $P = 0.012$. GM, geometric mean and its 95% confidence interval (CI); IgE-t, total serum IgE; MF, PQ, mycosis fungoides, plaque phase; No., number in cohort; pcBCL, primary cutaneous B-cell lymphoma; SS, Sézary syndrome.

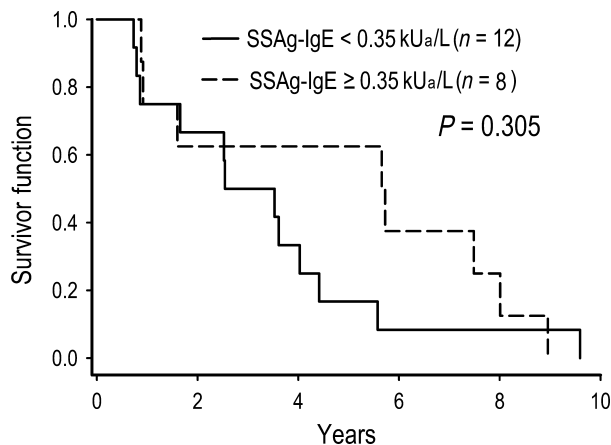


Figure 1. Overall survival curves of patients with Sézary syndrome grouped according to concentrations of staphylococcal superantigen-specific immunoglobulin E in the serum (SSAg-IgE).

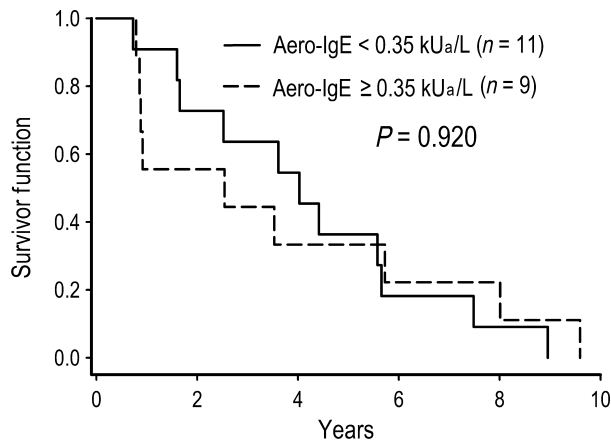


Figure 2. Overall survival curves of patients with Sézary syndrome grouped according to Phadiatop test results for immunoglobulin E reactivity against 10 airborne allergens in the serum (aero-IgE).

percentages of Sézary cells in the lymphocyte population (median, 27%; range, 0–65%) and absolute Sézary cell counts (median, 415/ μ L; range, 0–950/ μ L) than the other 28 E-CTCL patients (median, 13%, range, 0–40% and 132/ μ L; range, 0–648/ μ L). In terms of clinical stage, five of seven (71%) E-CTCL patients at stage IIIB progressed into SS compared with five of 30 (17%) at stage IIIA ($P = 0.009$).

Levels of IgE-t were significantly lower in patients with SS than other patients with E-CTCL (Table 4). Conversely, eosinophil counts were not different (Table S3). In addition, no differences in IgE-t levels or eosinophil counts were observed for subsets of SS and non-Sézary E-CTCL divided according to clinical stage or patients with non-Sézary E-CTCL that progressed to SS versus patients who did not.

Finally, 14 of 42 (33%) patients with SS had a personal history of an atopic disorder compared with nine of 43 (21%)

patients with non-Sézary E-CTCL; this difference in prevalence was not significant ($P = 0.229$). Antecedent atopic dermatitis was reported by only one patient with SS and two patients with non-Sézary E-CTCL.

DISCUSSION

In this study, 45% of patients with SS had a positive Phadiatop screening test and IgE-t was increased compared with non-atopic controls. These results indicate that a pathogenic link may exist between an atopic diathesis and development of SS. However, no correlation was apparent between serological evidence of atopy and the patient's history of an atopic condition for this small cohort of patients. This may be due to underreporting by patients (recall bias) and/or failure to adequately document atopic disorders in patients' charts. The clinical history alone is known to be unreliable as a sole indicator of atopic status.^{22,23} Additional studies on a larger cohort of patients may clarify this point.

The question of whether patients with atopy, in particular atopic eczema, have an increased risk of developing MF/SS was first raised by the Mycosis Fungoides Cooperative Group study in the 1970s.²⁴ An atopic skin disorder was recorded in 38 of 211 (18%) patients with MF including erythroderma. Twelve of these patients (5.7%) had childhood eczema. Hay fever and asthma were reported in 10.0% and 4.3%, respectively. These frequencies were suggested to be higher than the general population; however, there was no control group. The presence of each atopic condition individually as well as high IgE-t values did not correlate with prognosis.²⁵

Subsequently, Tuyp *et al.*²⁶ found that patients with MF had a significantly higher family history of atopic dermatitis (4.5 relative risk). However, the prevalence of atopic dermatitis, hay fever or asthma in MF patients versus controls was not different. Another larger European case-control study of MF showed a slight but non-significant association between atopic dermatitis or asthma and MF (odds ratio, 1.6 for both).²⁷

In an uncontrolled study at the Mayo Clinic, a history of at least one atopic disorder was documented for 12 of 102 (11.8%) patients with MF and 16 of 122 (13.1%) patients with SS plus two of 35 (6%) with "pre-SS", namely E-CTCL with circulating Sézary cells of less than 1000/ mm^3 . Atopic dermatitis per se was identified in only two (2%) patients with MF and four (3%) patients with SS.²⁸ The authors concluded that the prevalence of atopic dermatitis in patients with CTCL was not higher than what might be expected in the general population.

Conversely, a large study from the International Lymphoma Epidemiology Consortium (InterLymph) indicated that a subject's personal history of eczema, but not hay fever or asthma, was associated with an approximately twofold increased risk of MF/SS.²⁹ Because only a small number of SS patients were included in this study, the observed association was attributed primarily to MF. Furthermore, the possibility of confusing eczema with MF/SS was considered because the risk of MF/SS was higher for subjects with a history of eczema of less than 5 or 10 years before diagnosis compared with 10 years or more.

Table 4. Serum total IgE values for patients with Sézary syndrome and non-Sézary erythrodermic cutaneous T-cell lymphoma

Cohort (stage)	No.	No. increased (%) [†]	<i>P</i> [*]	IgE-t median (range)	IgE-t GM (95% CI)	<i>P</i> ^{***,‡}	<i>P</i> ^{****,‡}
SS	42	15 (36)	0.274	46 (1–8420)	65 (36–118)	0.035	0.036
Non-SS	43	21 (49)		108 (1–18 703)	176 (84–365)		
All E-CTCL	85	36 (42)		74 (1–18 703)	107 (67–173)		
Pre-SS	15	8 (53)	0.755	181 (2–3266)	132 (43–406)	0.769	0.542
Other E-MF	28	13 (46)		101 (1–18 703)	205 (76–554)		
SS (IVA1)	23	8 (35)	0.206	39 (1–3264)	57 (25–133)	0.767	0.871
SS (IVA2)	15	4 (27)		47 (3–8420)	71 (25–200)		
SS (IVB)	4	3 (75)		256 (3–420)	95 (2–3784)		
Non-SS (IIIA)	30	13 (43)	0.10	80 (1–18 703)	148 (59–372)	0.104	0.154
Non-SS (IIIB)	7	6 (86)		828 (80–11 645)	785 (166–3714)		
Non-SS (IVA2)	6	2 (33)		66 (2–765)	73 (8–663)		

Bolding indicates statistical significance. **P*-values for Fisher's or Pearson's χ^2 - and exact tests, **Kruskal-Wallis and ****ANOVA or *t*-tests. †Increased IgE-t defined as value exceeding the top normal value for adults from reference laboratory (GM + 2 SD = 122 kU/L). ‡Additional statistical comparisons for IgE-t: (i) SS at IVA1 vs IVA2: KW, *P* = 0.777, *t*-test *P* = 0.230; (ii) stages IIIA vs IIIB: KW, *P* = 0.057, *t*-test *P* = 0.334; and (iii) stages IIIA, IIIB and IVA1: KW, *P* = 0.017, ANOVA, *P* = 0.025 (significant between IIIB and IVA1). E-CTCL, erythrodermic cutaneous T-cell lymphoma; GM, geometric mean and its 95% confidence interval (CI); IgE-t, serum total immunoglobulin E (kU/L); No., number in cohort; Pre-SS, E-CTCL that evolved into SS; SS, Sézary syndrome.

In addition to the unreliability of patients' recollections of personal and family histories of atopic conditions,^{22,23} and the clinical problem of differentiating eczema from early MF, no distinction is made on epidemiological studies between various non-specific eczemas and the more specific diagnosis of atopic dermatitis. Accordingly, other methods to assess for atopy such as positive skin prick tests to allergens or measurement of allergen-specific IgE are more objective indicators of atopy. IgE-t levels are often, but not always, increased in atopic conditions and therefore are less reliable.

In this regard, Kural *et al.*³⁰ recently reported that the frequency of positive skin prick tests and detection of allergen-specific IgE in the blood as well as IgE-t levels was significantly higher in CTCL patients than controls, thereby providing objective evidence of atopy. Mean IgE-t levels were particularly high in erythrodermic CTCL, stage III (mean IgE-t, 1138 IU/mL; range, 166–2000 IU/mL) compared with controls (mean IgE-t, 140 IU/mL; range, 4–1151 IU/mL) and earlier clinical stages. In addition, 17 of 72 (24%) patients with MF and one of six (17%) patients with SS had a personal history of an atopic condition compared with nine of 77 (12%) dermatological controls. The difference in frequency between MF and controls was nearly statistically significant (*P* = 0.083).

Scala *et al.*³¹ also detected specific IgE reactive to one or more environmental or food allergens (including SSAG) in 24 of 55 (44%) patients with SS. This finding and our results suggest that atopy is common in SS. However, Väkevä *et al.*³¹ found no allergen-specific IgE in nine patients with SS, but did detect IgE antibody in four patients designated as having "atopic dermatitis/pre-SS". Moreover, none of the patients with SS had IgE antibodies to individual molecular component allergens using the ImmunoCAP ISAC microarray chip analysis, and not allergen extracts that are used in the singleplex ImmunoCAP analyses in our study. The reason for these discrepant results may stem from the fact that the ISAC chip does not contain the comprehensive number of allergenic molecules represented

in the multiple aeroallergen extracts used in the Phadiatop's allergosorbent.

Our findings also indicate that serological atopy may not be more prevalent in patients with MF, but more patients need to be studied to clarify this issue. In addition, like SS, chronic skin lesions of MF are prone to bacterial colonization (~25% of cases),³ and SSAG may enhance the level of both specific IgE responses as detected by the Phadiatop analysis and IgE-t.

It is generally accepted that the neoplastic cells of SS/MF are T-helper (Th)2 polarized,^{31–35} and capable of producing Th2 cytokines including IL-5, a cytokine that is a major maturation and differentiation factor for eosinophils, and IL-13, a cytokine that promotes IgE isotype switching of B cells. Interestingly, neoplastic T cells of MF/SS not only can produce IL-13, but also express its signaling receptor (IL-13R α 1), leading to proliferation via STAT6 signaling.³⁶ Thus, IL-13 may function like an autocrine growth factor similar to other cytokines reported to affect the growth of MF/SS cells (e.g. IL-2, IL-7, IL-9, IL-15, IL-21).^{37–40}

Consequently, blood eosinophil counts and IgE-t are frequently increased in SS and advanced MF. Tancrede-Bohin *et al.*⁴¹ reported that blood eosinophilia (>700 eosinophils/ μ L) was present in 10 of 13 (77%) patients with SS. Moreover, eosinophilia was associated with disease progression and disease-specific death in their patients with CTCL, but it was not indicated if this applied to the Sézary cohort. Hurabielle *et al.*⁴² subsequently found eosinophilia (>700 eosinophils/ μ L) in 10 of 64 (16%) patients with SS and confirmed these patients had a worse prognosis than patients without eosinophilia.

Conversely, Vidulich *et al.*⁴ found eosinophilia (>600 eosinophils/ μ L) in 31 of 122 (25%) patients with E-CTCL, and apparently found no correlation with overall survival. The first author's experience was similar to that of Vidulich *et al.* in that eosinophilia (>500 eosinophils/ μ L) occurred in 21% of patients and none had hypereosinophilia (>1500 eosinophils/ μ L; Table S3). Moreover, patients with SS and eosinophilia did

not have a poorer overall or disease-specific survival compared with patients without eosinophilia (Figure S1). The reason for the disparate results with eosinophil counts between groups in France and the USA is unknown, but conceivably could be related to underlying differences in the prevalence of atopy in these populations.⁴³

With regard to IgE-t, given the abundance of neoplastic T cells in SS, it is surprising that IgE-t in approximately one-third of reported patients with SS is normal or even decreased.^{27,30,32} In this study, IgE-t was increased in 36% of SS and IgE-t levels were significantly lower than patients with non-Sézary E-CTCL (Table 4). Could a low or even normal IgE-t level be used to differentiate non-Sézary E-CTCL from erythrodermic atopic dermatitis which almost always has a high IgE-t level?⁴⁴ Additional studies are needed to determine the diagnostic usefulness of IgE-t for patients with E-CTCL versus non-neoplastic erythrodermas.

In this study, IgE-t levels for MF/SS patients with serum aero-IgE or SSAg-IgE concentrations of less than 0.35 kUa/L were not increased compared with controls. This finding suggests that in the absence of serological evidence of an atopic diathesis or stimulation by SSAg, IgE-t production does not seem to be increased in SS or MF. In other words, the influence of IgE-promoting cytokines or other disease-related factors apart from staphylococcal colonization may not be a critical component in defining IgE-t levels.

If this assertion is valid, several factors may account for unexpectedly low IgE-t in some patients with SS. One possibility is that the cytokine profile of SS may switch from Th2 to a Th17 cytokine profile, thereby decreasing production of IL-13 (so-called neoplastic cell plasticity).¹⁶ Another possibility is the severe immunosuppression that characterizes patients with SS.⁴⁵ However, suppression of B-cell function seems unlikely because serum Ig levels are normal or increased in advanced CTCL.⁴⁶

In addition to atopic diathesis of the patients, colonization of the skin of SS by SSAg-producing *S. aureus* as implied by presence of SSAg-IgE is an important factor that mediates IgE-t levels. This has been extensively studied in patients with atopic dermatitis.^{47,48} It would be interesting to learn if IgE-t levels differ according to SSAg-producing versus non-SSAg-producing strains of *S. aureus* isolated from the skin. Perhaps a difference in the skin microbiome may explain why SSAg-IgE against SEA and SEB are detected twice as often as TSST1 in atopic dermatitis whereas TSST1-IgE was detected more often in our patients with SS.⁴⁹

With regard to prognosis, unlike Scala *et al.*'s experience,¹⁸ we found no evidence that prognosis of Sézary patients with serological evidence of either aero-IgE or SSAg-IgE is worse compared with patients without SSAg-specific IgE. However, our cohort of patients is relatively small. In this regard, bacterial colonization was not found to independently influence overall prognosis when subjected to multivariate analysis by the group that treats bacterial colonization aggressively.⁴

Another interesting observation is that IgE-t levels are increased in pcMZL, but not pcFCL. We studied pcBCL because to our knowledge, there are no reports of an

association between atopy and pcBCL. By contrasting results with CTCL, we hoped to provide insight as to the contribution to IgE-t levels by the inflammatory infiltrate minus Th2 polarized neoplastic T cells. However, a study by van Maldegem *et al.*⁵⁰ indicated that the common subtype of pcMZL has "class-switched" Ig and is associated with a Th2 inflammatory background. The neoplastic cells in a minority of these cases express IgE, and therefore may provide the source for high IgE-t levels. An atopic diathesis may be another factor because three of seven (43%) of our patients with pcMZL had evidence of atopy with a positive Phadiatop screening test for aeroallergen-specific IgE versus no patient with pcFCL. It is also worth noting that high IgE-t levels have been reported in IgG4-related cutaneous pseudolymphoma, which has many features that resemble pcMZL.⁵¹

In summary, we found that patients with SS and pcMZL, but not plaque phase MF, have high levels of SSAg-IgE and IgE-t. These results indicate that a pathogenic link may exist between an atopic diathesis and development of SS and possibly pcMZL. However, the presence of either aeroallergen-specific IgE antibody or SSAg-IgE in SS did not correlate with poor survival.

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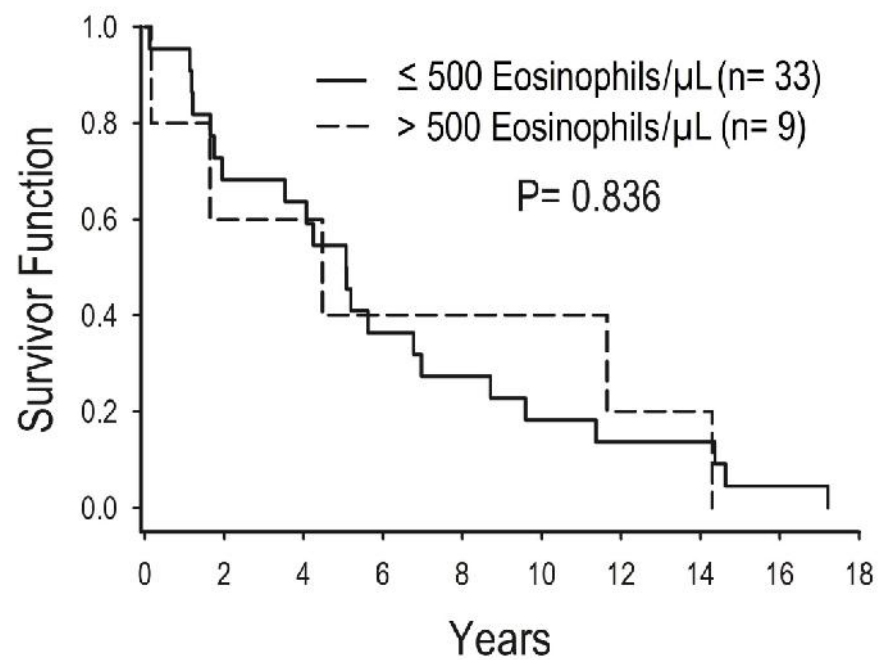
SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Overall survival curves for patients with Sézary syndrome grouped according to presence or absence of blood eosinophilia.

Table S1. Relationship among disease categories and Phadi-atop test results for aero-IgE **Table S2.** Distribution of positive staphylococcal superantigen-specific IgE at two concentrations (≥ 0.1 kUa/L and ≥ 0.35 kUa/L) according to disease categories **Table S3.** Absolute eosinophil counts for patients with Sézary syndrome and non-Sézary erythrodermic cutaneous T-cell lymphoma

Supplemental Figure 1. Overall survival curves for patients with Sézary syndrome grouped according to presence or absence of blood eosinophilia.



Supplemental Table 1. Relationship among disease categories and Phadiatop test results for aero-IgE.

Diagnosis	Aero-IgE†	No.	IgE-t Median (range)†	IgE-t GM (95% CI)	KW*	t-test*	Dunnett**
SS	< 0.35	11	21.5 (1.4-582)	22.8 (6.3-82.9)	0.001	< 0.001	0.812
	≥ 0.35	9	749 (35.3-3556)	757 (231-2480)			
MF, PQ	< 0.35	17	21.2 (1.4-490)	25.0 (10.0-62.3)	0.080	0.011	0.556
	≥ 0.35	3	228 (74.4-239)	159 (30.9-823)			
All pcBCL	< 0.35	14	41.9 (1.4-3354)	37.5 (12.7-111)	0.078	0.005	0.144
	≥ 0.35	3	206 (193-210)	202.9 (182-226)			
FCL	< 0.35	8	29.6 (1.4-216)	21.5 (5.9-78.5)	----	----	0.660
MZL	< 0.35	6	55.0 (8.7-3354)	78.5 (8.2-749)	----	----	0.010
Control	< 0.35	52	14.8 (2.0-187)	14.1 (10.0-19.9)	----	----	----

Abbreviations: SS, Sézary syndrome; MF, PQ, mycosis fungoides, plaque phase; pcBCL, primary cutaneous B cell lymphoma; FCL, follicle center cell lymphoma; MZL, marginal zone lymphoma; No., number patients in cohort; IgE-t, total serum IgE; GM, geometric mean and its 95% confidence interval.

† Aero-IgE concentration in kUa/L and IgE-t in kU/L.

* Comparison of IgE-t in disease subsets with aero-IgE < 0.35 versus ≥ 0.35 kUa/L by Kruskal-Wallis or t-test.

** Comparison of IgE-t in disease subsets with aero-IgE < 0.35 with control by Dunnett's t-test.

Supplemental Table 2. Distribution of positive Staphylococcal superantigen-specific IgE at two concentrations (≥ 0.1 kUa/L and ≥ 0.35 kUa/L) according to disease categories.

SSAg-IgE (≥ 0.1 kUa/L)	SS (n= 20)	MF, PQ (n= 20)	pcBCL (n= 17)	Control (n= 52)
TSST1 alone	3	5	1	3
TSST1+SEA	1	0	0	2
TSST1+SEB	1	1	0	0
TSST1+SEA+SEB	6	1	1	0
SEA alone	0	0	2	0
SEA+SEB	0	2	1	0
SEB alone	0	0	1	1
Any SSAg	11 (55%)	9 (45%)	6 (35%)	6 (12%)
P-value*	< 0.001	0.003	0.059	----
SSAg-IgE (≥ 0.35 kUa/L)	SS (n= 20)	MF,PQ (n= 20)	pcBCL (n= 17)	Control (n= 52)
TSST1 alone	2	2	1	2
TSST1+SEA	0	0	0	1
TSST1+SEB	1	0	0	0
TSST1+SEA+SEB	5	1	0	0
SEA alone	0	0	0	0
SEA+SEB	0	1	0	0
SEB alone	0	0	0	0
Any SSAg	8 (40%)	4 (20%)	1 (6%)	3 (6%)
P-value*	0.001	0.088	1.0	----

Abbreviations: SS, Sézary syndrome; MF, PQ, mycosis fungoides, plaque phase; pcBCL, primary cutaneous B cell lymphoma; SSAg-IgE, Staphylococcal superantigen-specific IgE; TSST1, toxic shock syndrome toxin-1; SEA/SEB, Staphylococcal enterotoxin A or B.

* Frequency of positive test, disease versus control groups, Fisher's exact test.

Supplemental Table 3. Absolute eosinophil counts for patients with Sézary syndrome and non-Sézary erythrodermic cutaneous T cell lymphoma.

Cohort (stage)	No.	No. Increased (%)†	P*	Eos Median (range)	Eos GM (95% CI)	P**‡	P***‡
SS	42	9 (21)	1.0	187 (0-1400)	204 (151-276)	0.778	0.669
Non-SS	43	10 (23)		196 (0-4692)	179 (104-307)		
All E-CTCL	85			187 (0-4692)	191 (141-259)		
Pre-SS	15	2 (13)	0.451	276 (67-824)	235 (156-354)	0.789	0.348
Other E-MF	28	8 (29)		171 (0-4690)	155 (68-350)		
SS (IVA1)	23	3 (13)	0.10	104 (0-940)	178 (121-261)	0.339	0.373
SS (IVA2)	15	6 (40)		249 (0-1400)	271 (146-504)		
SS (IVB)	4	0 (0)		210 (0-298)	158 (43-580)		
Non-SS (IIIA)	30	8 (27)	0.334	150 (0-4692)	147 (69-313)	0.361	0.487
Non-SS (IIIB)	7	0 (0)		302 (75-441)	228 (123-422)		
Non-SS (IVA2)	6	2 (33)		431 (146-824)	362 (181-722)		

Abbreviations: No., number in cohort; SS, Sézary syndrome; E-CTCL, erythrodermic cutaneous T cell lymphoma; Pre-SS, E-CTCL that progressed into SS; Eos, eosinophil count (/μL); GM, geometric mean and its 95% confidence interval.

† Increased eosinophil count defined as > 500 eosinophils/μL.

P-values for Fisher's or Pearson's chi-squared exact test*, Kruskal-Wallis** and ANOVA or t-tests***

‡ Additional statistical comparisons for absolute eosinophil counts:

(1) SS at IVA1 vs. IVA2: KW, P= 0.150, t-test, P= 0.230.

(2) Stages IIIA vs. IIIB: KW, P= 0.614, t-test P= 0.334.

(3) Stages IIIA, IIIB and IVA1: KW, P= 0.309, ANOVA, P= 0.774.