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High soluble CD30, CD25 and IL-6 may identify patients with worse survival in CD30+ cutaneous lymphomas and early mycosis fungoides

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Abstract

Histopathology alone cannot predict outcome of patients with CD30+ primary cutaneous lymphoproliferative disorders (CD30CLPD) and early mycosis fungoides (MF). To test the hypothesis that serum cytokines/cytokine receptors provide prognostic information in these disorders, we measured soluble CD30 (sCD30), sCD25, and selected cytokines in cell cultures and sera of 116 patients with CD30CLPD and 96 patients with early MF followed up to 20 years. Significant positive correlation was found between sCD30 levels and sCD25, CD40L, IL-6, and IL-8, suggesting CD30+ neoplastic cells secrete these cytokines, but not Th2 cytokines. In vitro studies confirmed sCD30, sCD25, IL-6 and IL-8 are secreted by CD30CLPD-derived cell lines. CD30CLPD patients with above normal sCD30 and sCD25 had worse overall and disease-related survivals, but only sCD30 retained significance in Cox models that included advanced age. High sCD30 also identified patients with worse survival in early MF. Increased IL-6 and IL-8 correlated with poor disease-related survival in CD30CLPD patients, We conclude that: (1) neoplastic cells of some CD30CLPD patients do not resemble Th2 cells, (2) high serum sCD30, sCD25, IL-6, and perhaps IL-8 levels may provide prognostic information useful for patient management.

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Conflict of Interest Disclosures

MEK and ECV state no conflicts of interest. JCD and IP are salaried employees of ARUP National Reference Laboratories where cytokine measurements were done.

Introduction

Some investigators have suggested that current clinical and histologic criteria are inadequate to predict outcome in CD30+ primary cutaneous lymphoproliferative disorders (CD30CLPD).(Bekkenk et al., 2000) Most patients with CD30CLDP have an excellent prognosis, but up to 20% progress to systemic involvement or develop a second lymphoma such as mycosis fungoides (MF) or Hodgkin lymphoma (HL). (Bekkenk et al., 2000; (Gruber et al., 2006). At presentation, the natural history of CD30CLDP is often not apparent and may be modified by subsequent molecular/genetic events (Kadin, 2006). Clinical monitoring is essential for patient management. We hypothesize that measurement of serum cytokines/cytokine receptors could improve ability to predict prognosis of CD30CLPD.

CD30/TNFRSF8 is a co-stimulatory molecule expressed on activated T and B cells commonly used as a marker for neoplastic cells of HL, systemic anaplastic large cell lymphoma (sALCL) and CD30CLPD.(Borchmann, 2008; Stein et al., 1985) Serum levels of sCD30 correlate with tumor burden and normalize following successful treatment of HL(Nadali et al., 1994) and sALCL.(Zinzani et al., 1998) Similarly, serum levels of the soluble truncated α-chain of the interleukin-2 receptor (sCD25) expressed by activated immune cells correlate with disease activity and prognosis in HL,(Gause et al., 1992) sALCL(Gause et al., 1992) and MF. (Gause et al., 1992; Wasik et al., 1996) However, the clinical significance of sCD25 levels has not been reported in CD30CLPD. Herein we report our retrospective analysis of serum levels of sCD30, sCD25 and other cytokine/cytokine receptors for patients with CD30CLPD and early MF.

Results

sCD30

Our primary objective was to measure sCD30 levels in cases of CD30CLPD and correlate results with clinical observations including prognosis. Of 116 cases of CD30CLPD, sCD30 was increased above normal reference laboratory values in 10 (9%) cases (Table 1). Within this group, 2 of 15 (13%) cases of lymphomatoid papulosis (LyP) type C and 2 of 13 (15%) cases of primary cutaneous anaplastic large cell lymphoma (pcALCL) had increased sCD30 compared to 6 of 87 (7%) LyP types A or B. Median sCD30 levels also tended to be higher in LyP-C and pcALCL cases compared to LyP-A or B cases. Although not statistically significant, this observation suggests a relationship between the density of CD30+ cells in evaluated skin lesions and sCD30 levels.

In absence of banked sera from concurrent normal controls, inflammatory skin disease (ISD) cases with absent to rare CD30+ cells in the dermal infiltrate were compared against other disease categories (Table 1). As expected, no ISD case had sCD30 values that exceeded 29 ng/mL, the top normal reference value. Compared to CD30-low ISD, significantly higher levels of sCD30 were observed for all subtypes of CD30CLPD as well as patients with early MF and patients with CD30CLPD co-existing with early MF (CD30CLPD/MF). Sixteen of 19 (84%) patients with various advanced CD30+ cutaneous lymphomas (CD30-high

controls) had above normal sCD30 levels, supporting the correlation of increased sCD30 and disease severity.

Correlation of sCD30 with Th2 cytokines

Considering that CD30 may be a marker of Th2 polarized CD4+ cells,(Del Prete et al., 1995) we correlated sCD30 with Th2 and other cytokines in CD30CLPD. Statistically significant positive correlations were found between sCD30 and sCD25 (Spearman's correlation rho = 0.510, P< 0.001), sCD40L (rho = 0.411, P< 0.001), IL-6 (rho = 0.252, P = 0.007), and IL-8 (rho = 0.322, P< 0.001), but not IL-4 (rho = 0.116, P = 0.214), the prototypic Th2 cytokine, nor IL-5 (rho = 0.164, P = 0.079) and IL-13 (rho = 0.130, P = 0.164). These results suggest that CD30-secreting cells do not secrete abundant Th2 cytokines other than IL-6 (and perhaps IL-10 which was not studied due to technical problems), and by inference non-neoplastic cells may be a source of Th2 cytokines in CD30CLPD.

CD30CLPD co-existing with MF

Compared to CD30-low ISD controls, the CD30CLPD/MF group had significantly higher sCD30 levels (P< 0.001), but not sCD25 (Tables 1 and 2). Patients with CD30CLPD/MF also had significantly lower levels of IFN-γ (P = 0.029), IL-12 (P = 0.034), IL-4 (P = 0.027), and possibly IL-2 (P = 0.057). Compared to CD30-low MF cases, no significant differences in any biomarker were observed. These findings suggest that the cytokine profile of patients with CD30CLPD/MF is not skewed toward either Th1 or Th2 polarized profile.

sCD25

Compared to ISD controls, sCD25 levels were significantly higher for cases of CD30CLPD and CD30CLPD/MF as well as the CD30-high control group (Table 2). Although above normal serum values (>1033 pg/mL) occurred in only 8% of the CD30CLPD cases, sCD25 was more often increased in cases with numerous CD30+ cells in lesions, i.e., LyP type C (13%) and pcALCL (23%) compared to LyP-A or B (5%) (Pearson exact $\chi 2$, P = 0.036). Because sCD25 and sCD30 levels correlated strongly (rho = 0.510), this suggests the possibility of a common cellular source.

IL-6 and IL-8

IL-6 and IL-8 levels were significantly increased in cases of CD30CLPD compared to ISD controls (Table 3; Table S1). IL-6 levels were above normal in 27% of CD30CLPD patients (about 40% of LyP-C and pcALCL cases versus 25% of LyP-A or B cases). IL-8 was above normal in only 10% of CD30CLPD cases and correlation with histopathologic subsets was not apparent.

Effect of storage time on serum cytokine/cytokine receptor levels

These studies were performed on samples stored for 6 to 21 years (median, 11 years). If cytokine/cytokine receptor levels deteriorated over time, one might expect a negative correlation with storage time. However, only TGF-β had a significant negative correlation

for the entire dataset (rho = −0.145, P = 0.015); the correlations for sCD30 and sCD25, and IL-6 were rho = 0.058, −0.035, and 0.044, respectively.

Effect of CD30 ligand on sCD30

To investigate whether sCD30 serum levels might be suppressed by its ligand, we correlated sCD30 with sCD30L/CD153 in 77 samples covering a wide range of sCD30 values (range 0–2798.6 ng/mL). No significant correlation was found between these cytokines (rho = −0.035, P = 0.760). This observation suggests that sCD30 levels are not inhibited or removed by high sCD30L/CD153 in the blood.

Repeat specimens on patients responding to treatment

Blood samples obtained at baseline and after a complete response to treatment were available on 8 patients with CD30CLPD (3 LyP-A, 2 LyP-C, 3 pcALCL) and 10 patients with early MF (Table 4). Of the cytokines studied, only sCD25 levels of patients with CD30CLPD showed a significant decrease post-treatment (P = 0.023, Wilcoxon test). For these 8 patients, sCD30 levels did not correlate with treatment response.

Cytokine levels in supernatants of CD30+ cell lines

As shown in Table 5, abundant sCD30 and sCD25 was detected in supernatants of CD30CLPD-derived cell lines Mac1 and Mac2A and systemic ALCL cell line JB6. Compared to Mac 1 cells, Mac 2A cells secreted larger amounts of IL-6 and IL-13. Mac 2A and JB6 cells also secreted small amounts of IL-8, but neither cell line secreted prototypic Th2 cytokines (IL-4, IL-5, IL-10). JB6 systemic ALCL cells secreted abundant IL-10. These results indicate that CD30CLPD-derived cells are capable of secreting various cytokines other than Th2 type cytokines.

Prognostic Implications

In this cohort, only 1 of 87 (1.2%) patients presenting with LyP-A or B, 2/16 (12.5%) patients with LyP-C and 2/13 (15.4%) patients with pcALCL experienced documented disease progression and related death (P = 0.033). No patient developed HL. Details are provided in Tables S2 and S3. Because information about the specific cause of death was not available for 20 patients, the actual disease-related death rate may be higher than 5/116 (4.3%).

The relationship between clinical parameters, levels of cytokines/receptors and survival of patients with CD30CLPD were analyzed using Cox and Kaplan-Meier models. With all causes of death as the event, significant differences were found for several clinicopathologic parameters: patients' age (continuous variable and <60 vs. >60 years, P< 0.001), race (white vs. other, P = 0.018), and histopathologic subtype (P = 0.024). The difference in survival was significant between pcALCL and LyP-A or B, but not between pcALCL and LyP-C. (Figure S1) For disease-related deaths, significant differences in survival occurred between both pcALCL and LyP-C versus LyP-A or B whereas no difference was observed between pcALCL and LyP-C. Although the frequency of CD30CLPD-related deaths was only 4.3%, it is consistent with other studies [Bekkenk et al., 2000]. Furthermore, the LyP-C subset may

have a greater potential for disease progression than LyP-A or B although an impact on overall survival was not demonstrated.

Likewise, univariate analysis identified several biomarkers as being significantly associated with worse overall survival when categorized into clinically useful normal versus above normal results: increased sCD30, sCD25, and IL-8 but not IL-6 (nor lactate dehydrogenase [LDH] in a limited model (Table S4). Interestingly, an above normal level of TGF-β correlated with improved survival. When analyzed using disease-related death as the event, increased sCD30, sCD25, IL-6, (and LDH) but not IL-8 were significantly associated with adverse prognosis. Survival curves for sCD30, sCD25 and IL-6 are shown in Figures 1 and 2 and Figure S2, respectively).

Multivariate analysis was then performed using the following categorized variables: age <60, >60) years, race (white, other), histopathologic subsets (LyP, pcALCL) and sCD30, sCD25, IL-6 and IL-8 (normal, above normal) levels. For all causes of death as the event, advanced age, race other than white, and above normal sCD30 levels were identified as independently significant prognostic indicators (Table S5). For a model in which age, histopathologic subset and sCD30 were analyzed together, only age and sCD30 were significant. Increased sCD30 significantly correlated to overall survival in most other models, but increased IL-8 also was independently significant in some (data not shown).

A similar multivariate analysis using disease-related death as the event identified age and above normal IL-6 levels (hazard ratio, 9.206) to provide more prognostically important information than increased sCD30, sCD25 and IL-8 levels (or LDH). (Figure S2) The reason appears to be that 4 of the 5 patients with disease-related deaths had above normal IL-6 levels compared to only 2 patients for sCD30 and sCD25 and only one for IL-8 (Supplemental Table 2). Finally, no difference in survival was found for patients with CD30CLPD/MF compared to CD30CLPD.

The prognostic implication of sCD30 and other markers was similarly investigated for the 96 patients with early MF. Univariate analysis identified patients' age, sCD30 and sCD25 levels to be correlated significantly with prognosis (all causes of death). However, with age as a covariate, high sCD30 but not sCD25 levels retained significance (Table S5, Figure S3). Although only 4 (4.2%) early MF patients died of disease, high sCD30 levels remained independently significant (hazard ratio, 11.644) with regard to disease-related survival.

Discussion

We investigated the clinical significance of sCD30 and other biomarkers in 116 patients with CD30CLPD and 96 patients with early MF. Considering that CD30 and CD25 are expressed by the atypical cells in skin lesions of LyP, particularly histologic subtypes A and C, and pcALCL (El Shabrawi-Caelen et al., 2004; Willemze and Beljaards, 1993; Willemze et al., 2005) it was not surprising that serum levels of these markers in CD30CLPD patients were significantly higher than in CD30-low ISD cases. We also found increased sCD30 and sCD25 levels were associated with worse overall and disease-related survivals, suggesting the levels might reflect the number of neoplastic cells in the skin and identify patients with

an increased risk for disease progression. In a Cox model that included advanced age (≥ 60 years), increased sCD30 levels were a better predictor of prognosis than either sCD25 or histopathologic classification of CD30CLPD lesions.

In this regard, Bekkenk reported their experience with 110 Dutch patients with LyP and 79 with pcALCL. In their study, 2/118 (1.7%) of LyP patients and 4/79 (5.1%) with pcALCL died as a result of lymphoma compared to 3/103 (2.9%) LyP patients and 2/13 (15.4%) pALCL in our study. Death rates among LyP-A or B, LyP-C and pcALCL in Bekkenk's study were not significantly different ($P = 0.536$, $\chi 2$ test) whereas the difference was significant in our study ($P = 0.033$). In addition, they did not identify any clinical or histopathologic finding that correlated significantly with tumor progression by univariate analysis including age or CD30CLPD subtype. Although the median ages for LyP and pcALCL at the time of study entry for each patient population was nearly identical, the median follow up times were considerably longer for our patients (118 months for LyP and 114 months for pcALCL for our patients compared to 77 months for LyP and 61 months for pcALCL for Bekkenk's patients). Because disease progression and the development of systemic involvement often requires years to occur, (Bekkenk et al., 2000; Gruber et al., 2006) this could account for the difference in the observed death rates for the two patient populations.

Our study showed sCD30 and sCD25 are increased in early MF and high sCD30 but not sCD25 levels correlated with worse overall and disease-related survival in a Cox model that included advanced age. The risk of death from progressive disease in this MF cohort was quite low (~ 4%), but similar to what other groups have reported. (Kim et al., 1996; van Doorn et al., 2000) Edinger reported that frequent dermal CD30+ cells were an independent adverse prognostic factor in non-transformed MF. (Edinger et al., 2009) Because we studied only CD30-low early MF cases, we could not confirm the prognostic significance of CD30+ cell numbers in skin lesions of the MF cohort. It is likely that increased sCD30 levels in early MF cases are derived in part from CD30-expressing non-neoplastic cells, and the observation that sCD30 values did not correlate with clinical response suggests skin lesions are not the only source of sCD30 in MF.

Our study showed a significantly positive correlation between sCD30 levels and sCD25, IL-6, and IL-8 as well as sCD40L, suggesting that CD30+ cells are secreting these proteins, but not IL-4 nor other Th2 cytokines. In vitro studies confirmed that sCD30, sCD25, IL-6 and to a lesser degree IL-8, but not CD40L were secreted by CD30CLPD-derived cells. Absence of IL-4 and IL-5 in CD30CLPD-derived supernatants and lack of concordance between sCD30 and IL-4 or IL-5 levels in patient sera suggest that CD30+ neoplastic cells do not closely resemble Th2 cells.

Despite the variable histology between individual lesions of patients with CD30CLPD, we found significant correlation between sCD30 levels and histopathologic subtypes in our patients, possibly due to selection of clinically advanced lesions for biopsy. If sCD30 were secreted by neoplastic cells in vivo, one might expect serum levels to reflect the total number of CD30+ neoplastic cells in skin infiltrates which depend on (1) the number of

lesions, and (2) the average density of CD30+ cells. Neither variable can reliably be determined in clinical practice, suggesting the need for a serologic biomarker.

Our studies indicate that IL-6 is increased in CD30CLPD cases and in Mac2A cells which have a 20-fold amplification of IL-6 gene expression compared to Mac1 cells from earlier disease.(Li et al., 2001) Several studies indicate CD30+ neoplastic cells from systemic ALCL secrete IL-6 and that IL-6 can act as an autocrine growth factor.(Siebert et al., 2007) Therefore, high serum IL-6 levels may portend a poor prognosis, and in our study, an above normal level of IL-6 identified CD30CLPD patients with a worse survival from disease-related death but not overall death as the event. However, the number of patients with progressive CD30CLPD was small and further studies are required to confirm this finding.

Our observation that IL-8 serum levels correlate significantly with sCD30 levels in both CD30CLPD (rho = 0.322) and early MF (rho = 0.435), and that 35% of CD30-expressing tumor controls had elevated IL-8 values and absolute values significantly higher than other groups suggests that IL-8 is secreted by CD30+ neoplastic cells. Furthermore small amounts of IL-8 were secreted by CD30CLPD-derived cells in vitro. A previous study by one of the authors (MEK) revealed tumor cell secretion of IL-8 and increased serum levels in cases of neutrophil-rich pcALCL. (Burg et al., 2003).

One hypothesis to explain the correlation between increased sCD30 levels and adverse prognosis is that high sCD30 (and sCD25) levels reflect a general state of immune activation, perhaps to a persistent antigen, that predisposes susceptible individuals to development and/or progression of CD30CLPD and MF. (Chechlinska et al., 2010); (Vendrame and Martinez-Maza, 2011); (Burg et al., 2001) A possible mechanism is that cross-linking of cell surface CD30 causes up-regulation of NF-kB, (Levi et al., 2000) which activates anti-apoptotic C-FLICE in cutaneous ALCL cells (Braun et al., 2010). A similar mechanism may be operative in MF in which NF-kB, a pro-survival transcription factor, is constitutively expressed (Izban et al., 2000).

A prospective study is needed to confirm or refute our hypothesis that patients with CD30CLPD and above normal serum values for sCD30, sCD25 or IL-6 (and early MF patients with high sCD30 levels) are at increased risk for disease progression or development of an associated lymphoma. Accordingly, we suggest that sCD30, sCD25 and IL-6 be measured as part of the baseline evaluation and if any biomarker is increased, such patients should undergo staging procedures (e.g. PET scan) and be more closely monitored by clinical examinations than might otherwise be done. In this context, it is important to exclude other possible explanations for a rise in sCD30 or sCD25 such as autoimmune diseases. (Kadin, 2000) We also suggest biomarker levels be measured at regular intervals (6 to 12 months) to determine if an increased baseline value persists or a value changes from normal to persistently high during follow up. This might prompt repeat staging procedures.

Our observations on a small number of patients (eight) suggest that there was an inconstant correlation between observed clinical responses and serum levels of sCD30 and sCD25 before and after treatment. Nevertheless, persistent elevation of sCD30, sCD25 or IL-6 in the face of a favorable treatment response might identify patients at risk for poor outcome.

Conceivably, a high baseline sCD30 could even be a harbinger of B-lymphoproliferative disorders known to be associated with MF (Barzilai *et al.*, 2006; Purdue *et al.*, 2009) (Hallermann *et al.*, 2007; Herro *et al.*, 2009)

Materials and Methods

Sera separated and frozen/stored in 1 ml vials at −80°C within one hour of phlebotomy during clinical evaluation of 103 patients with LyP, 13 patients with pcALCL, and 16 patients with LyP or pcALCL co-existing with patch or plaque phase MF were retrieved from the cutaneous lymphoma tissue repository at Johns Hopkins University and studied for cytokine expression at ARUP laboratories. This study was conducted in accordance with the Declaration of Helsinki Principles and approved by the Institutional Review Board at Johns Hopkins Medical Institute. Patient consent was not required because studies were performed on de-identified residual sera. LyP cases were sub-classified as types A, B or C (Willemze et al., 2005). Because concurrent frozen samples from normal volunteers were not available, sera from 96 patients with early MF (stage IA) and 22 patients with benign ISD and absent or rare CD30+ cells in the dermal infiltrate, were selected to represent CD30-low controls. Sera from 19 patients with various CD30-expressing cutaneous tumors (20–90% positivity by IHC) were studied as a CD30-high control group. Detailed composition of control groups is provided in the legend of Table 1.

Test reference intervals for 36 healthy individuals established at ARUP laboratory were used to define an abnormally high result for each cytokine or cytokine receptor, except sCD30, for which 151 healthy donors were used.(Pavlov *et al.*, 2009) Normal ranges measured using the multi-analyte fluorescent detection method are as follows: IL-1β (0–36 pg/mL), IL-2 (0–12 pg/mL), IL-2R/sCD25 (0–1033 pg/mL), IL-12 (0–6 pg/mL), and IL-4, IL-5, IL-6, IL-8, IL-13, and IFN-γ (0–5 pg/mL). Normal ranges for other markers measured by ARUP Luminex-based assays were sCD30 (1–29 ng/mL), sCD40L (0–244 pg/mL), and TGF-β (3.5–13.9 ng/mL). For correlation with sCD30 findings, sCD30L/CD153 was measured using ELISA (Bender MedSystems, Inc., Burlingame, CA).

Three CD30+ ALCL lines (Mac-1, Mac-2A, JB-6) were examined for secretion of cytokines/cytokine receptors in vitro. Cultures were seeded with 10^6 viable tumor cells and incubated in tissue culture media. At 24 and 48 hours, aliquots of conditioned medium were collected, frozen and sent to ARUP labs for analysis. Mac-1 and Mac-2A are ALK negative lines were derived from early and advanced disease, respectively, of a patient with progressive CD30CLPD. JB6 is an ALK+ cell line derived from systemic ALCL.

Statistics

Because most continuous variables did not have a normal distribution, non-parametric Kruskal-Wallis analysis of variance on ranks test was used to test differences of median values among independent groups. Fisher's and Pearson's chi-square exact tests tested categorical data in 2×2 and 2×3 tables, respectively. Strength of association between pairs of cytokines/cytokine receptors was analyzed using Spearman's rank correlation coefficient. Wilcoxon signed rank exact test was used for differences in median values in pre- and post-treatment samples. Prognostic significance of measured values was tested in the Cox

proportional Hazards Model and differences in survival were determined using the Kaplan-Meier method and log-rank test of Mantel. Survival was determined from time of blood sampling (usually at presentation) to last contact. Both disease-related and all causes of death were used as non-censored events. The Holm-Sidak Test was used for pairwise comparisons among multiple survival curves. Statistical packages used for data analysis were SYSTAT10 and SPSS 13.0 for Windows, SPSS, Inc. (Chicago, IL); StatXact 3 and EGRET for Windows, Cytel, Inc. (Cambridge, MA); and SigmaStat3, Systat Software, Inc. (Point Richman, CA).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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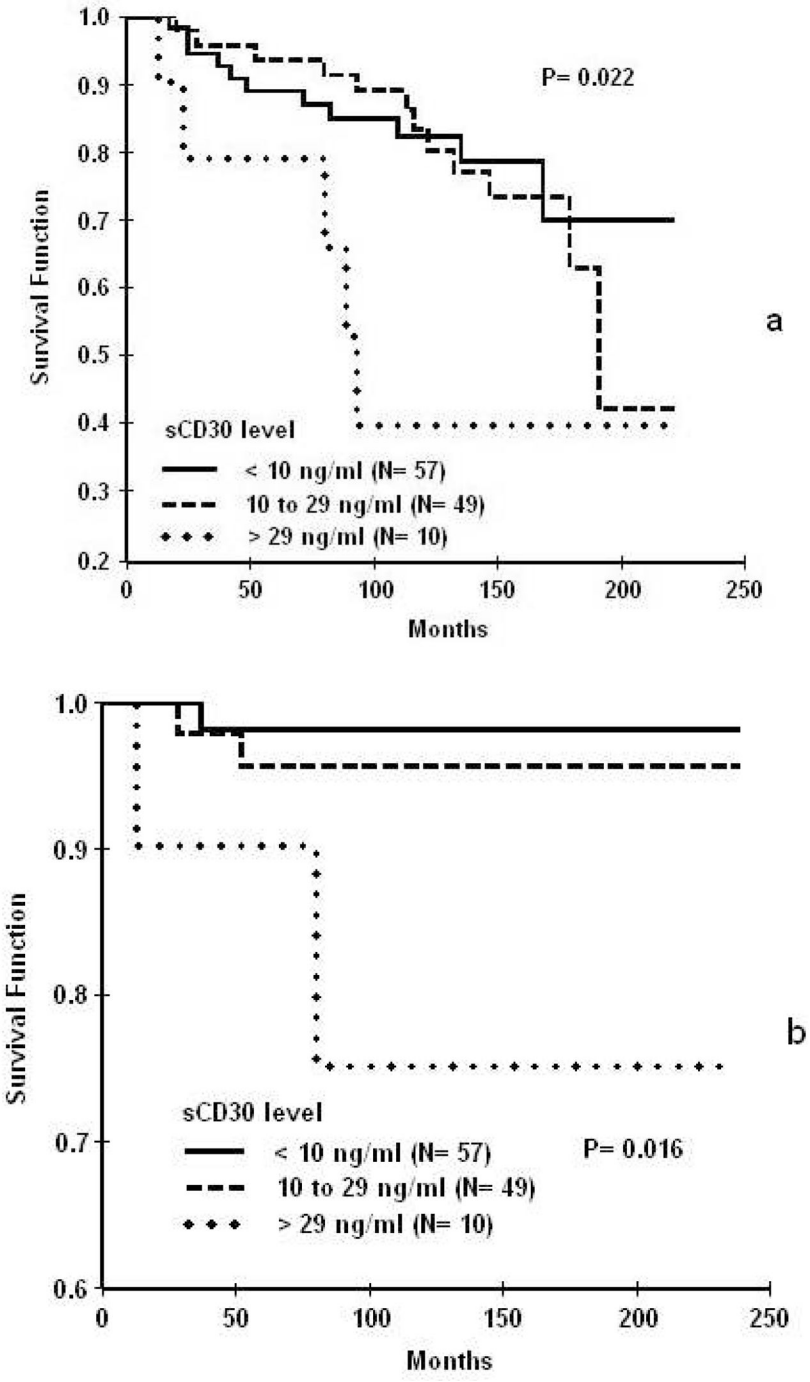
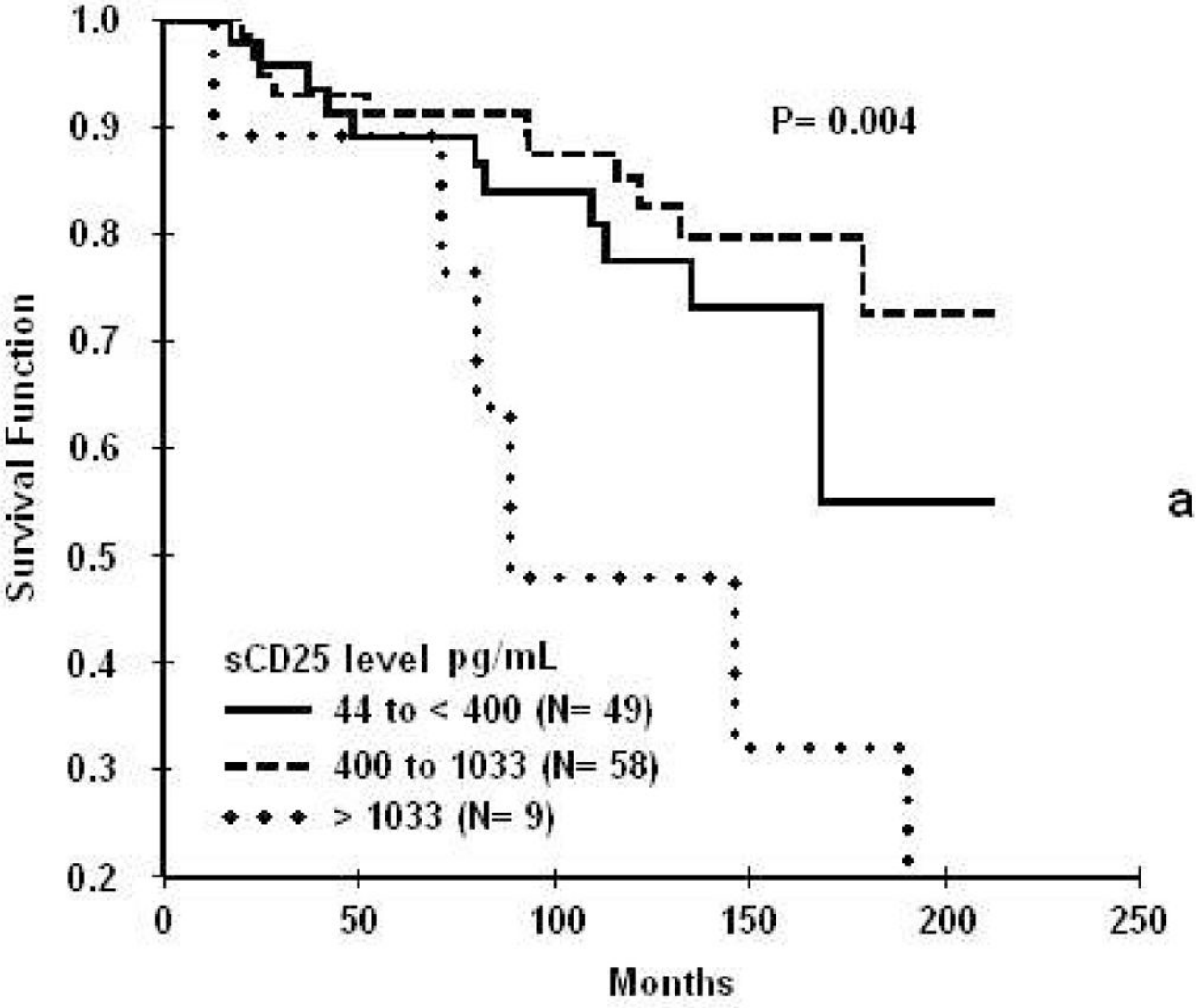


Figure 1.

Survival of patients with CD30+ primary cutaneous lymphoproliferative disease according to low normal, high normal and above normal sCD30 levels. a. all causes of death; b. disease-related death.



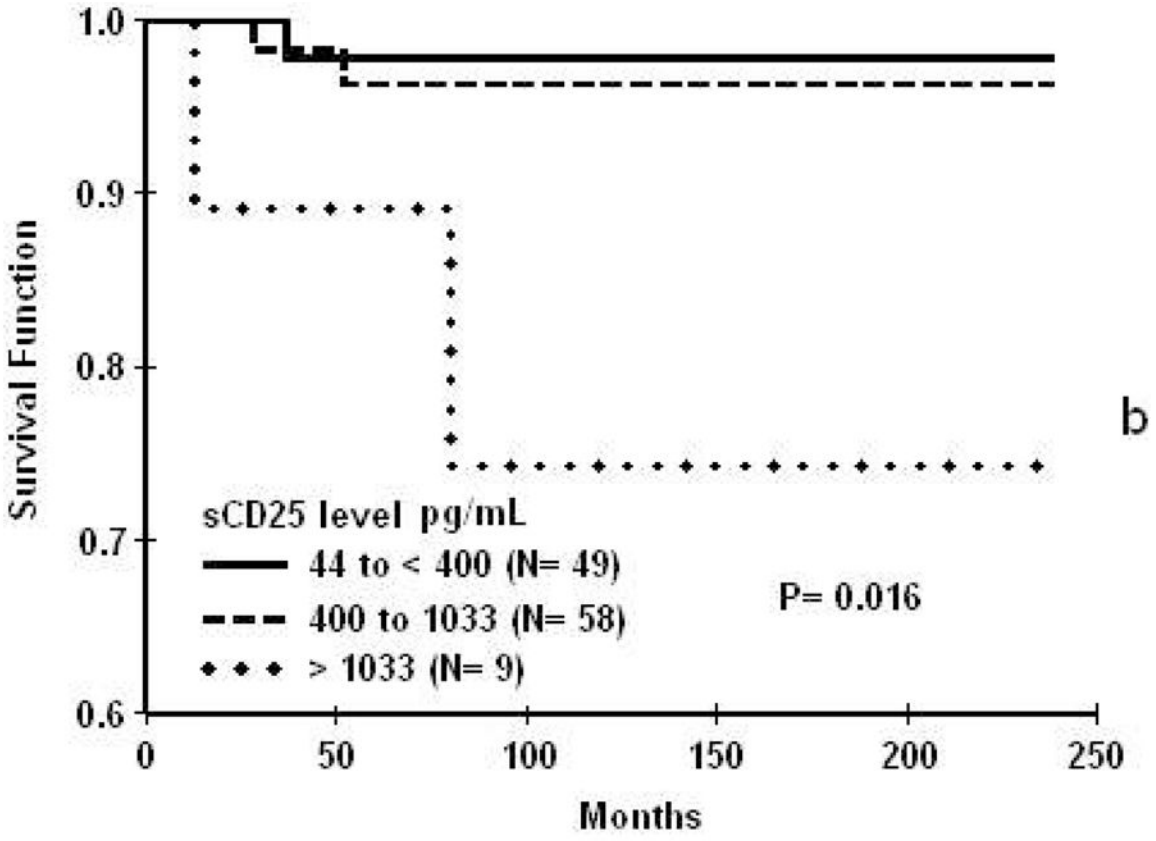


Figure 2.

Survival of patients with CD30+ primary cutaneous lymphoproliferative disease according to low normal, high normal and above normal sCD25 levels. a all causes of death; b. disease-related death.

Table 1

Serum sCD30 levels in patients with CD30+ primary cutaneous lymphoproliferative disease and early mycosis fungoides.

Diagnosis (No. in cohort)	No. High sCD30 (%)[1]	Median (Range)	Mean ± SD	P-value[7]	P-value[8]
CD30CLPD (116)	10 (9)	10.1 (0.1–599.2)	20.8 ± 59.6	< 0.001	0.606
LyP (103)	8 (8)	10.0 (0.1–207.2)	15.9 ± 26.0	< 0.001	0.798
LyP-A or B (87)	6 (7)	9.5 (0.1–121.9)	14.1 ± 18.8	0.001	0.917
LyP-C (16)	2 (13)	12.5 (0.6–207.2)	25.9 ± 49.5	0.002	0.239
pcALCL (13)	2 (15)	13.0 (2.6–599.2)	59.3 ± 162.6	0.002	0.243
CD30CLPD/MF[2] (16)	1 (6)	14.3 (2.1–70.0)	16.4 ± 15.6	< 0.001	0.084
CD30 High Controls[3] (19)	16 (84)	109.7 (10.9–2798.6)	377.9 ± 650.8	< 0.001	< 0.001
CD30-low Controls[4]					
Early MF[5] (96)	6 (6)	9.5 (0.1–102.2)	12.3 ± 13.2	< 0.001	
ISD[6] (22)	0 (0)	5.2 (0–11.7)	5.2 ± 3.7		< 0.001

Abbreviations: CD30CLPD, CD30+ primary cutaneous lymphoproliferative disease; LyP, lymphomatoid papulosis; pcALCL, primary cutaneous anaplastic large cell lymphoma; MF, mycosis fungoides; ISD, inflammatory skin disease.

[1] Normal laboratory range for sCD30, 1 to 29 ng/mL.

[2] Early MF co-existing with LyP (13 cases) or pcALCL (3 cases).

[3] Includes 15 cases of advanced CD30+ CTCL (13 large cell transformation), one case of CD30+ pleomorphic T cell lymphoma with mild erythroderma, one case of extensive pagetoid reticulosis expressing CD30 and CD25, and one case of adult T cell lymphoma with CD30+ papules and plaques and lymph node involvement.

[4] Absent or rare CD30+ cells in dermal infiltrate.

[5] Includes unilesional (14 cases), hypopigmented (3 cases), purpuric (3 cases), folliculotropic (2 cases), and palmoplantar (2 cases) variants.

[6] Includes digitate parapsoriasis en plaques (13 cases), pityriasis lichenoides (6 cases), and erythema annulare centrifigum (3 cases).

[7] Compared against CD30-low ISD control group using Kruskal-Wallis test.

[8] Compared against CD30-low MF control group using Kruskal-Wallis test.

Table 2

Serum sCD25 levels in patients with CD30+ primary cutaneous lymphoproliferative disease and early mycosis fungoides.

Diagnosis (No. in cohort)	No High sCD25 (%)[1]	Median (Range)	Mean ± SD	P-value[2]	P-value[3]
CD30CLPD (116)	9 (8)	444 (44–7285)	581 ± 724	0.009	0.898
LyP (103)	6 (6)	427 (44–7285)	562 ± 746	0.015	0.783
LyP-A or B (87)	4 (5)	436 (44–7285)	551 ± 779	0.019	0.685
LyP-C (16)	2 (13)	421 (49–2306)	617 ± 545	0.051	0.784
pcALCL (13)	3 (23)	548 (108–1801)	733 ± 523	0.014	0.114
CD30CLPD/MF (16)	2 (13)	467 (25–1212)	528 ± 341	0.076	0.790
CD30 High Controls (19)	14 (74)	1468 (334–133066)	9325 ± 30198	< 0.001	< 0.001
CD30-low Controls					
Early MF (96)	7 (7)	449 (11–2042)	523 ± 372	0.015	
ISD (22)	0 (0)	233 (10–947)	329 ± 286		0.015

See Table 1 legend for abbreviations.

[1] Normal laboratory reference for sCD25, 0 to 1033 pg/mL

[2] Compared against CD30-low ISD control group using Kruskal-Wallis One-Way Analysis of Variance.

[3] Compared against CD30-low MF control group using Kruskal-Wallis One-Way Analysis of Variance.

Table 3

Serum IL-6 levels in patients with CD30+ primary cutaneous lymphoproliferative disease and early mycosis fungoides.

Diagnosis (No. in cohort)	No High IL-6 (%)[1]	Median (Range)	Mean ± SD	P-value[2]	P-value[3]
CD30CLPD (116)	31 (27)	1.6 (0–238.8)	13.1 ± 38.6	0.003	0.002
LyP (103)	26 (25)	1.5 (0–238.8)	13.8 ± 40.8	0.004	0.003
LyP-A or B (87)	20 (23)	1.5 (0–238.8)	13.3 ± 39.3	0.008	0.009
LyP-C (16)	6 (38)	2.0 (0–200.7)	16.7 ± 49.4	0.005	0.015
pcALCL (13)	5 (38)	2.6 (0–36.9)	7.7 ± 11.5	0.023	0.080
CD30CLPD/MF (16)	3 (19)	0.9 (0–26.4)	4.3 ± 8.7	0.415	0.794
CD30 High Controls (19)	8 (42)	4.1 (0.2–33.9)	9.8 ± 10.6	< 0.001	< 0.001
CD30-low Controls					
Early MF (96)	14 (15)	0.9 (0–110.1)	4.5 ± 14.1	0.273	
ISD (22)	0 (0)	0.6 (0–4.3)	1.0± 1.2		0.273

See Table 1 legend for abbreviations.

[1] Normal laboratory reference for IL-6, 0 to 5 pg/mL

[2] Compared against CD30-low ISD control group using Kruskal-Wallis test.

[3] Compared against CD30-low MF control group using Kruskal-Wallis test.

Table 4

Comparison of sCD30, sCD25 and IL-6 values in selected patients who achieved a complete response (CR) from treatment.

Cohort	No. Pts.	Cytokine	Baseline	CR	% Change Mean ± SD	P-Value[1]
CD30CLPD	8	sCD30	10.3 ± 7.6	11.1 ± 4.4	+51.0 ± 121.1	0.742
	8	sCD25	711 ± 557	419 ± 268	−34.5 ± 28.3	0.023
	8	IL-6	5.8 ± 7.5	3.6 ± 4.2	+3.1 ±126.4	0.547
MF	10	sCD30	19.7 ± 23.0	16.9 ± 19.9	−4.8 ± 27.7	0.383
	10	sCD25	1255 ± 1877	1257 ± 2350	+1.5 ± 54.3	0.695
	10	IL-6	7.6 ± 11.4	3.2 ± 3.2	−45.9 ± 48.4	0.148

Abbreviations: CD30CLPD, CD30+ primary cutaneous lymphoproliferative disease; MF, mycosis fungoides

[1]Change in cytokine median values tested using Wilcoxon test

Table 5

Cytokine levels in CD30CLPD-derived cell cultures.

Cell line	sCD30 ng/ml	sCD25 pg/ml	IFN-γ pg/ml	IL-4 pg/ml	IL-6 pg/ml	IL-8 pg/ml	IL-10 pg/ml	IL-13 pg/ml
RPMI/FBS*	0	0	<10	0	0	0	0	0
Mac-1	2757	>10240	25	0	1	4	20	2
Mac-2A	>9000	>10240	<10	0	7395	17	0	3067
JB-6	4002	>10240	<10	0	0	56	>10240	0

Cell cultures were seeded at 10^6 cells/ml and incubated for 48 hr in RPMI/FBS. Conditioned media was centrifuged to remove cells and supernatants collected and frozen immediately at −20°C.

*RPMI/FBS = Roswell Park Medium with 10% fetal bovine serum.