Evaluation of the Association between CD4, CD8 and CD25 Cell Counts and SLE in Active Disease and in Remission

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Abstract

Aim: To evaluate the correlation between the levels of CD4, CD8 and CD25 cells and SLE disease in active phase and in remission.

Method: A total of 25 SLE patients, aged between 18-60 years, and fulfilling the ACR criteria with preferential Renal and CNS involvement were included in this study. Baseline CD4/CD8 and CD25 counts, lab parameters etc were conducted. Approximately at the end of 6 months with the settlement of the disease activity blood sample was drawn for the CD4, CD8 and CD25 counts and other lab parameters.

Results: ESR showed a statistical significant decrease while the SLEDAI score and proteinuria showed a decreasing trend as the patients underwent remission. The C3 showed an increasing trend, while the C4 showed more or less a stable pattern. Rise in %CD4 and %CD25 count was statistically significant. There was negative correlation between % CD4 count and SLEDAI score, while positive correlation between % CD25 count and SLEDAI score.

Conclusion: %CD4 count is a sensitive, specific, reliable and valid marker of active disease in SLE and can be used to follow disease activity. %CD25 count can also be used as a marker to follow disease activity.

Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease and it results in the activation of both the innate as well as the adaptive immunity. SLE results due to a disturbance in immune tolerance, which is most likely initiated by a pathogen challenge, which in presence of genetic defects results in autoimmune hyperactivity.1 SLE is associated with a number of symptoms due to nephritis, nervous system inflammation, arthritis, leukopenia and skin rashes.2 Immune homeostasis is maintained mainly by self tolerance. CD4, CD8 and CD25 cells which consist of 5-10% of the peripheral blood count prevent autoimmunity.3 There is recent evidence that show breaks in immune tolerance to self-antigens most likely result in the commencement of end organ disease. Several factors like autoantibodies, immune complex deposition, activation of T cell result in organ specific presentation of the condition.4 It has been previously shown that when CD4, CD25 regulatory T cells have been experimentally depleted by thymectomy, there is an increment in auto-reactive T cells and hastened autoantibody production. On the other hand, supplementation of the CD4, CD25 regulatory T cells isolated from normal mice has been shown to decrease the development of autoimmunity.5

SLE is marked by frequent relapses and remissions. Even though clinical features are important markers of disease activity, several laboratory parameters like the Hb, ESR, C3, C4 levels serve as more objective markers.6 Recurrent defects in immune tolerance leads to disease flares in SLE which is marked by CD4 and CD25 lymphocytopenia.7

However, the role of CD4 and CD25 cells in the maintenance of immune tolerance and as a marker

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of disease activity in SLE is not explored. Hence in this study we have tried to evaluate the correlation between the levels of CD4, CD25 cells and SLE in disease activity and in remission.

**Materials and Methods**

The study was initiated after receiving approval from the Institutional Review board. A total of 25 SLE patients, aged between 18-60 years, and fulfilling the ACR criteria with preferential Renal and CNS involvement were included in this study. Patients suffering from other rheumatological disorders and overlap syndromes were excluded from the study. Patients fulfilling the inclusion criteria were enrolled in the study after taking their informed consent. These study participants were clinically examined and all the routine investigations were conducted. A 4cc peripheral venous sample was collected and processed as per standard protocol for CD4/CD8 and CD25 counts. The study participants were followed up to study overall disease activity, treatment regimen, and laboratory parameters. Approximately at the end of 6 months with the settlement of the disease activity blood sample was drawn for the CD4, CD8 and CD25 counts.

Baseline study participant characteristics was described using descriptive statistics. Parametric data if it passed the tests of normality was analyzed using parametric tests or else non-parametric tests were used for its analysis. Categorical data was analyzed using Chi-square test. Parametric correlation analysis was done using Pearson correlation test while non-parametric correlation analysis was done using Spearman correlation test.

**Results**

The mean age of the patients included in this study was 26.8 ± 7.1 (mean ± SD) years. Of the 25 patients in this study, 22 were females and 3 were males. The major organ involvement in lupus patients on presentation was such that, 14 patients had renal involvement only, 2 had CNS involvement only and 9 patients had both renal and CNS involvement.

The mean number of ACR criteria at the beginning of the study and the end of the study was 6.16 ± 1.92 and 1.32 ± 0.62 (mean ± SD) respectively. Applying the Wilcoxon matched pair test, it is found that the number of ACR criteria at the end of the study was significantly lesser than the number of criteria at the beginning of the study (p<0.0001).

The mean total leucocyte count (TLC) at the beginning of the study and at the end was 6936 ± 4774 and 7284 ± 2291 (mean ± SD) cells/mm³ respectively. Applying the Wilcoxon matched pair test, it is found that even though there is an increase in the TLC it is not statistically significant (p=0.33).

The mean absolute lymphocyte count (ALC) at the beginning of the study and at the end was 2131 ± 1707 and 2430 ± 846 (mean ± SD) cells/mm³ respectively. Applying the Wilcoxon matched pair test, it is found that even though there is an increase in the ALC it is not statistically significant (p=0.12).

The mean ESR at the beginning of the study and at the end was 52.48 ± 16.25 and 30.08 ± 12.39 (mean ± SD) respectively. Applying the paired t test, it is found the ESR showed a statistical significant decrease by the end of the study (p 0.0001).

The mean serum creatinine level at the beginning of the study and at the end was 1.19 ± 0.56 and 1.24 ± 0.75 (mean ± SD) mg/dL respectively. Applying the Wilcoxon matched pair test, it is found that even though there is an increase in the serum creatinine level it is not statistically significant (p 0.81).

After treatment for the study duration, of the 25 patients included in this study, 21 entered remission while 4 did not. The induction of remission was defined according to the SLEDAI score (Systemic Lupus Erythematosus Disease Activity Index). According to this definition, an SLEDAI score <3 indicates remission while a score of ≥ 3 indicates active disease.

The mean SLEDAI score at 0, 3 and 6 months was 23.48 ± 10.8, 8.32 ± 3.30 and 2.48 ± 1.82 (mean ± SD) respectively. Applying the Friedman test the difference is statistically significant (p<0.0001). Post hoc Dunn test showed that the difference was statistically significant for the difference in proteinuria between 0 & 6 months, 0 and 3 months and 3 and 6 months. The SLEDAI score showed a decreasing trend as the patients underwent remission due to treatment.

The mean proteinuria at 0,3 and 6 months was 1908 ± 1306, 1044 ± 891 and 444 ± 400 (mean ± SD) mg/24 hrs. Applying the Friedman test the difference is statistically significant (p<0.001). Post hoc Dunn test showed that the difference was statistically significant for the difference in proteinuria between 0 & 6 months, 0 and 3 months and 3 and 6 months. The proteinuria, showed a decreasing trend after treatment.

The mean C3 levels at 0, 3 and 6 months was 54.46 ± 21.20, 63.34 ± 20.88 and 97.96 ± 15.93 (mean ± SD) mg% respectively. Applying the Friedman test the difference is statistically significant (p<0.0001). Post hoc Dunn test showed that the difference was statistically significant for the difference in C3 levels between 0 & 6 months, and 3 and 6 months. The mean C3 levels at 0, 3 and 6 months was 23.48 ± 10.8, 8.32 ± 3.30 and 2.48 ± 1.82 (mean ± SD) mg% respectively. Applying the Friedman test showed that the difference was statistically significant (p<0.001). Post hoc Dunn test showed that the difference was statistically significant for the difference in proteinuria between 0 & 6 months, 0 and 3 months and 3 and 6 months. The proteinuria, showed a decreasing trend after treatment.
pattern as the patients underwent treatment for the study duration.

The %CD4 count is the absolute CD4 count expressed as percentage of normal CD4 count which is the lower value of the normal range. The %CD4 count at the beginning of the study was 55.84 ± 44.70 and was 107.69 ± 45.44 by the end of the study. Applying the Wilcoxon matched pairs test showed that the rise in %CD4 count was statistically significant (p<0.0001).

The %CD8 count is the absolute CD8 count expressed as percentage of normal CD8 count which is the lower value of the normal range. The %CD8 count at the beginning of the study was 113.61 ± 70.39 and was 134.39 ± 90.43 by the end of the study. Applying the paired t test showed that the rise in %CD8 count was statistically comparable (p = 0.37).

The %CD25 count is the absolute CD25 count expressed as percentage of normal CD25 count which is the lower value of the normal range. The %CD25 count at the beginning of the study was 4.76 ± 4.33 and was 11.12 ± 4.75 by the end of the study. Applying the Wilcoxon matched pairs test showed that the rise in %CD25 count was statistically significant (p = 0.0006).

Correlation analysis between % CD4 count during active lupus and SLEDAI score at baseline showed that there is a negative correlation between % CD4 count and SLEDAI score with the Spearman correlation coefficient being -0.5554. The two-tailed P value is 0.0039, which is statistically significant.

Correlation analysis between % CD4 count at remission and SLEDAI score at baseline showed that there is a negative correlation between % CD4 count and SLEDAI score with the Spearman correlation coefficient being -0.6397. The two-tailed P value is 0.0006, which is statistically significant.

Correlation analysis between % CD8 count at flare and remission with patients divided according to the number of positive ACR criteria at baseline showed that there is a positive correlation between % CD8 count and SLEDAI score at baseline.

Correlation analysis between % CD8 count during active lupus and SLEDAI score at baseline showed that there is a negative correlation between % CD8 count and SLEDAI score with the Spearman correlation coefficient being -0.08. The two-tailed P value is 0.68, which is statistically non-significant.

Correlation analysis between % CD8 count at remission and SLEDAI score at remission showed that there is a positive correlation between % CD8 count and SLEDAI score with the Spearman correlation coefficient being 0.50. The two-tailed P value is 0.0096, which is statistically significant.

If the patients were grouped into 3 subgroups according to the number of ACR criteria positive at diagnosis, and the % CD4 count of these patients were compared at both baseline and at remission, it can be seen that there is a steady increase in %CD4 count as the patients went into remission. So we can state that the CD4 count is most depressed in patients with severe flare as indicated by low CD count when 8-11 ACR criteria were present.
Fig. 3: Correlation between %CD25 count at flare and remission with patients divided according to the number of positive ACR criteria at baseline

Table 2: Correlation between %CD count at flare and remission with patients divided according to the SLEDAI score at baseline

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<th>Parameters</th>
<th>Flare</th>
<th>Remission</th>
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<td></td>
<td>SLEDAI &gt;12</td>
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<tr>
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</tr>
<tr>
<td></td>
<td>SLEDAI &gt;12</td>
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</tr>
<tr>
<td>%CD25 count</td>
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<td>3</td>
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<td></td>
<td>SLEDAI &gt;12</td>
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Table 3: Correlation between %CD count at flare and remission with patients divided according to the WHO class of nephritis

<table>
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<td></td>
<td>Class IV (WHO)</td>
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<tr>
<td>%CD8 Count</td>
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<td></td>
<td>Class IV (WHO)</td>
<td>104.85</td>
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<tr>
<td>%CD25 count</td>
<td>Class II (WHO)</td>
<td>3</td>
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<tr>
<td></td>
<td>Class IV (WHO)</td>
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Discussion

Twenty five patients with active lupus were included in this present study. The same patients in remission acted as their own controls. Of the 25 patients, 3 were males and 22 were females. This sex distribution is similar to the known sex distribution of SLE which is female: male 9:1.6 Of all the patients, lupus nephritis was the mode of presentation in 9 of the patients, 14 had both CNS and renal involvement on admission and only CNS involvement was seen in 2 patients. This correlates well with the usually described incidences of renal (30-50%) and CNS (50%) involvement.6

Out of the total of 25 patients in this study, 5 patients had leucopenia at the onset as shown by a total leucocyte count < 4000 cells/mm³, while it was normal (4000-11000 cells/mm³) in 17 patients (Table 1). The reported incidence of leucopenia in active Lupus varies between 20-70% in different case series. Santiago et al reported the incidence of leucopenia as 75%.8 In another study conducted by Lertchaisataporn et al the incidence of leucopenia was around 50%.9 The apparent discrepancy in different studies with respect to leucopenia appears mainly due to treatment with steroids and immunosuppressive agents prior to the inclusion of these patients in the study. In the present study all the included patients were treatment naïve and hence the reported incidence of leucopenia (20%) may reflect a more accurate and unbiased view.
Lymphopenia is defined as ALC <1500 cells/mm³. In our study lymphopenia was seen in 9 patients, while normal lymphocyte count was seen in 16 patients (Table 1). This correlates well with the work done by Ng et al who showed the incidence of lymphopenia to be about 42% in active lupus patients.

The mean serum creatinine level at the beginning of the study was 1.19 ± 0.56 mg/dL. This is comparable to most of the other studies where the serum creatinine level was <1.5. This shows that renal function of majority of patients is well preserved at presentation. The statistical non-significant change in serum creatinine may be because of the fact that majority of the patients had near normal creatinine levels on presentation.

The proteinuria showed a descending trend on treatment which was statistically significant. This result correlated well with the case series published by Brinmingham et al and Touma et al. The C3 levels showed an increasing trend during the treatment period which well correlates with the study conducted by Ziakas et al. C4 is a marker of disease activity and should rise as patient enters remission. However, in the present study different results were obtained. The C4 levels were more or less stable during the treatment duration. This low sensitivity and specificity of C4 levels in predicting relapse and remission observed in our study is similar to that seen in another study conducted by Bombardier et al.

The SLEDAI score showed a decreasing trend over the study duration. This is similar to a study conducted by Mirzayan et al which shows that SLEDAI decreases over treatment. In our study the %CD25 count showed an increasing trend on treatment. This was similar to a study conducted by Miyara et al which showed a decrease in the CD25 count.

Correlation analysis between %CD4 count during active lupus and SLEDAI score at baseline showed that there is a negative correlation between %CD4 count and SLEDAI score. This was in line with the study conducted by Ester Rosarion Ben et al in which CD4 +CD25+ Foxp3+Treg cell depletion was reported in 25 patients of SLE which correlated with higher SLEDAI scores. Also Shah et al have shown that the CD4 T cell count was decreased in SLE patients.

Correlation analysis between % CD8 count during active lupus and SLEDAI score at baseline showed that there is a negative correlation between %CD8 count and SLEDAI. This was similar to the study conducted by Dolf et al which showed that there is increase in CD8 cells during active disease. Correlation analysis between % CD8 count and SLEDAI score at remission showed no correlation. However this was in contrast with existing literature where Dolf et al have shown that the CD8 count decreases in remission.

Conclusion

Our data indicates that leucopenia is not a very sensitive marker of disease activity since it is present in about 20% patients with flare even though it has been described as a traditional marker. ESR was high in all patients with flare making it a very sensitive marker albeit a less specific one since it was high even in patients of remission. Creatinine levels were not good markers since patients with active disease had normal creatinine levels. C3 is a very sensitive marker of flare as compared to C4 levels.

Our study results show that the CD4 count has an inverse relation with the SLEDAI score, number of positive ACR criteria and class of lupus nephritis. It is most depressed in patients with severe flare as compared to mild or moderate flare. The %CD4 count rises with the settling of disease activity and reaches to normal on treatment. This proves that %CD4 count to be a sensitive, specific, reliable and valid marker of active disease and can be used to follow disease activity. Our study results show that %CD8 count has got no correlation with the disease activity in mild and moderate as well as severe lupus flares and cannot be used to follow disease activity in lupus. The %CD25 counts tend to be low in all lupus flares and rises to normal in patients with remission. It also correlates inversely with SLEDAI score and number of positive ACR criteria. Hence %CD25 count can also be used as a marker to follow disease activity.

References


