Serum Immunoglobulins in Recurrent Aphthous Stomatitis

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Abstract

Immunoglobulin estimations by the single radial diffusion technique were performed using serum from 100 subjects comprising 50 patients with recurrent aphthous stomatitis, 25 with Behçet's syndrome and 25 normal controls. The data were subjected to multivariate analysis methods including Hotelling $T^2$ statistics and the Roy-Bose simultaneous confidence interval. Raised levels of IgA and IgM were found in patients with recurrent aphthous stomatitis in comparison with normal controls, but there was no difference in IgG. In Behçet's syndrome, only the IgM titers showed a difference, IgG levels being insignificant.

Introduction

Aphthous stomatitis is a collection of shallow mucosal ulcers with flat, fairly even borders surrounded by erythema. The ulcer is often covered with a pseudomembrane. Recurrent aphthous stomatitis (RAS) usually occurs alone but sometimes may be seen as an oral manifestation of Behçet's syndrome.

It has never been adequately demonstrated that RAS is due to a virus or any other chemical, physical or microbial agent. In recent years significant findings have raised the possibility that an immune or presumably autoimmune reaction may be responsible for the oral aphthous lesions. The ulcers are often painful, one or more may be present, and they tend to be recurrent. Stresses of various types have also been shown to be contributory. RAS occurs at approximately 4-week intervals and healing, which usually occurs in 1–3 weeks, may be only slightly accelerated with treatment.

The existence of Behçet's syndrome as a separate entity has served to divide what some clinicians consider to be a spectrum of disease. Patients suffering from Behçet's syndrome also possess a variety of antibodies to tissue extracts, namely fetal mucosa, skin and colon. In Behçet's disease thrombosis may occur and a vasculonecrotic response may also be seen in delayed hypersensitivity reactions where the tissue damage is apparently due to lymphocyte activity.

Today, multivariate analysis is accepted as an important field of statistics and when we consider a variable P then we also have to consider the means and variances of P and P (P−1)/2 covariances. These are the basic parameters of the
multivariate normal distribution.

The purpose of the present study was to estimate the levels of serum IgA, IgG and IgM in patients with RAS and Behçet's syndrome and to examine the data using multivariate analysis.

Materials and Methods

Fifty patients with RAS and 25 patients with Behçet's syndrome were included in this study. The control group consisted of 25 subjects. Ig classes were investigated by the single radial diffusion technique.\[7\]

Procedure: Molten agarose (Agarose Litex 1.0 g, Tris/barbitone 100 ml) in a 25-ml test tube was placed in a 55°C water-bath, and a 10 x 10-cm glass slide was placed on a level surface nearby. Then, 15 ml of the agarose was pipetted out onto the glass slide. Origin wells were made in the agarose and 5 ml of each sample was applied to each well. The slide was incubated at room temperature until diffusion was complete, then the ring sizes of the precipitates were measured.

Hotelling $T^2$ Statistics: Suppose that two independent random samples of observations on some multidimensional variate have been obtained under different experimental or environmental conditions. We shall assume that in either condition the variates have a multivariate normal distribution with the same, though unknown, covariance matrix of full rank $p$. However, it follows from the conditions or other evidence that the distributions may not necessarily have the same location parameters and we now desire to test a null hypothesis

\[ H_0 : \mu_1 = \mu_2 \] ...............................(1)

that the population mean vectors are identical, as opposed to the alternative hypothesis

\[ H_1 : \mu_1 \neq \mu_2 \] ...............................(2)

of different means. From the data, mean vectors have been computed and the pooled estimate $S$ of the common covariance matrix has been obtained. By application of the union intersection or likelihood-ratio principles, the statistic for testing the hypothesis is

\[ T^2 = \frac{N_1N_2}{N_1+N_2}(\bar{x}_1-\bar{x}_2)'S^{-1}(\bar{x}_1-\bar{x}_2) \] ...............................(3)

where $N_1$ and $N_2$ are the sizes of two samples, $\bar{x}_1$ and $\bar{x}_2$ are the mean vectors of the two samples, and $S$ is the pooled covariance matrix.

The quantity

\[ F = \frac{N_1+N_2-P-1}{(N_1+N_2-2)P}T^2 \] ...............................(4)

has the variance ratio $F$ distribution with $P$ degrees of freedom and $N_1+N_2-P-1$. The decision rule for testing the $\alpha$ level has this form:

Accept $H_0 : \mu_1 = \mu_2$ if

\[ T^2 \leq \frac{(N_1+N_2-2)P}{N_1+N_2-P-1}F_{\alpha}; \ P, N_1+N_2-P-1 \] ...............................(5)

and reject otherwise.\[10\]

The 100 $(1-\alpha)$ percent simultaneous confidence intervals for all linear compounds $a'\delta$ of the mean differences are defined by ROY-BOSE as shown below:
\[ a' (\bar{x}_1 - \bar{x}_2) - \sqrt{a'^2 \frac{N_1 - N_2}{N_1 N_2} T_a}; \text{ } P, N_1 + N_2 - P - 1 \leq a' \delta \]
\[ \leq a' (\bar{x}_1 - \bar{x}_2) + \sqrt{a'^2 \frac{N_1 - N_2}{N_1 N_2} T_a}; \text{ } P, N_1 + N_2 - P - 1 \]  
\[ \text{where } \delta \text{ is the population mean of } \bar{x}_1 - \bar{x}_2^{[0]}. \]

**Results**

Table I shows the arithmetic means, standard errors and ranges for the serum IgA, IgG and IgM variables of the three groups examined.

The Hotelling \( T^2 \) statistics were applied to analyze the difference between the mean vectors of Ig classes in the control and Behçet’s syndrome groups. The two-sample \( T^2 \) statistic had a value of 17.933; the associated \( F \) was 5.663. This result was greater than the table value of \( F_{0.01; \ 3,36} \) (4.38). Therefore we rejected the null hypothesis, which was \( H_0: \mu_1 - \mu_2 = 0 \) (the mean vectors of groups were equal). The arithmetic mean vectors of the two groups were significantly different (\( P < 0.01 \)).

Although we rejected the null hypothesis, it was not clear which of the three mean differences might have contributed to the significant \( T^2 \).

The Roy-Bose simultaneous confidence intervals were used to test the individual differences using a confidence coefficient of 0.95. The results are shown in Table 2.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>The arithmetic means, standard errors and ranges for the serum IgA, IgG and IgM variables of the three groups</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Ig's</td>
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<tr>
<td><strong>Control</strong></td>
<td></td>
</tr>
<tr>
<td>IgA</td>
<td>100.55</td>
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<tr>
<td>IgM</td>
<td>106.85</td>
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<tr>
<td>IgG</td>
<td>741.5</td>
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<tr>
<td><strong>Behçet’s Syndrome</strong></td>
<td></td>
</tr>
<tr>
<td>IgA</td>
<td>123</td>
</tr>
<tr>
<td>IgM</td>
<td>167.85</td>
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<tr>
<td>IgG</td>
<td>588.65</td>
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<tr>
<td><strong>RAS</strong></td>
<td></td>
</tr>
<tr>
<td>IgA</td>
<td>151.2</td>
</tr>
<tr>
<td>IgM</td>
<td>166.05</td>
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<tr>
<td>IgG</td>
<td>720.4</td>
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</tbody>
</table>

\( \bar{x} \): Arithmetic mean  
\( \pm SE \): Standard error  
R: Range

Since zero was included in the intervals IgA and IgG, we concluded at the 5% joint significance level that the arithmetic means were not different. The other (IgM) 95% simultaneous confidence interval had not included zero, and so the arithmetic means of IgM were found to be significantly different (\( P < 0.05 \)).

The Hotelling \( T^2 \) statistics were applied for testing the difference between the means of the control and RAS groups.

The two-sample \( T^2 \) statistic had a value of 25.1509. The value of \( F \) (8.0945)
was found to be greater than the table value of $F_{0.01} = 3.56$ (4.13). Therefore we were able to say that the arithmetic means of the two groups were significantly different ($P < 0.01$).

The Roy-Bose simultaneous confidence intervals were used to test which of the three mean differences might have contributed to the significant $T^2$. The results are shown in Table 2.

Since zero was included in the intervals for IgG we were able to say that the arithmetic means were not different ($P > 0.05$). The other IgA and IgM intervals did not include zero, and so the arithmetic means were different ($P < 0.05$).

<table>
<thead>
<tr>
<th>Table 2 The results of individual differences</th>
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</thead>
<tbody>
<tr>
<td>Confidence Intervals</td>
</tr>
<tr>
<td>Behçet’s S.</td>
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<tr>
<td>IgA  $= \bar{x}_1 - \bar{x}_2 = 46.26328$</td>
</tr>
<tr>
<td>IgM  $= \bar{x}_2 - \bar{x}_2 = 109.5314$</td>
</tr>
<tr>
<td>IgG  $= \bar{x}_3 - \bar{x}_2 = 281.7807$</td>
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</tbody>
</table>

**Discussion**

Immunoglobulin levels in oral diseases have so far not been studied comprehensively. In order to determine whether patients with different types of recurrent oral ulcers show any abnormality in their serum immunoglobulin levels, the latter were estimated.

Brody and Silberman\(^{[1]}\) compared laboratory findings with the clinical condition of patients and found lowered serum IgA in aphthous patients. Lehner\(^{[9]}\) reported raised levels of serum IgA and IgG in patients with recurrent aphthous lesions as compared with controls. Adinolfo and Lehner reported no change in serum IgG in cases of aphthous lesions. Hannah et al.\(^{[3]}\) found an insignificant difference for serum IgA. Serum IgA levels were similar in aphthous patients and in healthy persons, as reported by Lehner\(^{[9]}\). Greenspan and Boackle\(^{[11]}\) reported that aphthous patients have a raised level of circulating antibody to a saline extract of fetal and mucous membrane. Slightly raised levels of the same antibody have also been found in ulcerative conditions, but at lower titers. Dolby\(^{[8]}\) reported a parallel change in the titer of antibody with the severity of disease in Behçet’s syndrome. Recently, Güven reported no significant difference between the serum Ig values in patients with aphthous lesions, those with Behçet’s syndrome and the values in healthy individuals.

In our present study we predicted the parameters for multivariate analysis methods, i.e., Hotelling $T^2$ statistics and Roy-Bose simultaneous confidence intervals, which are more precise for the study of this subject. Statistical analysis revealed that there was no significant difference between the serum IgA and IgG levels in patients with Behçet’s syndrome and those in the control group. However, serum IgM values were found to be significantly different. The IgG level was similar in aphthous patients and in healthy persons.
References


