

## **Evaluation of albumin/globulin ratio as a diagnostic marker for relapsing ocular Behçet's disease**

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### **Abstract**

**Background:** The aim of this study was to evaluate the albumin/globulin (A/G) ratio as a diagnostic marker for relapsing ocular Behçet's disease.

**Methods:** This study was conducted using retrospective design. Patients of ocular Behçet's disease under infliximab infusion therapy were registered. The parameters for remission, i.e., total protein, albumin, serum protein fraction, serum amyloid A (SAA), C reactive protein (CRP), erythrocyte sedimentation rate (ESR), were monitored. The inflammatory proteins;  $\alpha$ 1-antitripsine,  $\alpha$ 1 acid glycoprotein, retinol binding protein, haptoglobin, ceruloplasmin, and  $\alpha$ 2 macroglobulin were measured during the pre- and post- relapse phase in three patients.

**Results:** Fourteen occurrences of relapse among 10 patients were confirmed during the study period. The A/G ratio decreased to  $0.10 \pm 0.11$  (mean  $\pm$  SD), the increase in the mean percentage of  $\alpha$ 1 and  $\alpha$ 2 globulin fractions was statistically significant, and analysis of three patient samples showed an increase in  $\alpha$ 1 acid glycoprotein ( $p = 0.04$ ) and a decrease in retinol binding protein ( $p = 0.04$ ). Comparison of the A/G ratio with other markers, ESR, SAA, and CRP, between the pre- and post- relapse phase showed that all four factors changed significantly.

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**Conclusions:** The A/G ratio appears to be a useful marker for relapsing ocular Behçet's disease.

**Keywords:** albumin/globulin ratio, Behçet's disease, infliximab

## 1 Background

Behçet's Disease (BD) is an unknown disease, which is characterized by repeating inflammatory disorders. The four major symptoms of BD have been identified as repeating stomatitis, dermatitis, genital ulceration, and ocular inflammation. Ocular BD is manifested by alternating relapse and remission phases during the clinical course. Repeating relapse of ocular disease has the risk of progression to loss of eyesight. The therapeutic procedures for ocular BD have changed over time, especially the use of tumor necrosis factor alpha (TNF- $\alpha$ ) inhibitor which is a promising drug for maintaining remission status for ocular BD [1,2]. For control of ocular BD inflammation, early detection of disease activity for effective therapeutic intervention is essential.

There are no appropriate laboratory tests for the detection of active BD. The erythrocyte sedimentation rate (ESR) or C reactive protein (CRP) is seldom elevated during either the acute or relapse phase of BD. Leukocytosis or hyper-gammaglobulinemia is also evident in some cases of active BD. However, these markers do not correlate strongly with disease activity alone [3,4]. To find biomarkers for evaluation of BD activity, several studies have been conducted, in which neopterin [5], homocysteine [6], nitric oxide (NO), endothelin-1 [7], and arufa-1 acid glycoprotein [8] were introduced as biomarkers reflecting disease activity. Mao et al. [9] showed that haptoglobin and serum amyloid A (SAA) are useful for the evaluation of disease activity by means of the 2-DE and MALDI-TOF/TOF-MS method. In addition, CXCR-2 (IL8-BP) [10], leptin [11], or regulatory T cells [12], have also been reported as candidate marker. Most of these biomarkers have been investigated in experimental research, and are difficult to test in clinically. As there is no consensus biomarker to identify relapsing ocular BD which may lead to serious ocular manifestation, a different evaluative index needs to be explored for determination of effective therapeutic intervention for active ocular BD, which can be used in the clinical setting.

The albumin/globulin (A/G) ratio is an index calculated using albumin and total protein. A decrease in the A/G ratio commonly occurs in the following diseases: nephritic syndrome, chronic infection, liver cirrhosis, hyper-gammaglobulinemia, or collagen disease. This index has been tried for the evaluation of the severity in burn patients' condition [13], the extent of effusion in otitis media [14], or the clinical stage of sarcoidosis [15]. We applied the A/G ratio as a diagnostic marker for relapsing BD and specifically investigated its usefulness as a diagnostic marker for relapsing ocular BD.

## 2 Methods

### Patients and samples

This study was scheduled and conducted using a retrospective design. The candidate patients were admitted to the internal medicine department's outpatient clinic of Ogihara Misaki Hospital in Kobe Japan between January 2009 and December 2011. The candidates needed to meet the following criteria: 1) diagnosis of ocular BD by an ophthalmologist. 2) administration of infliximab (TNF- $\alpha$  inhibitor) infusion at regular intervals, and attainment of remission status for BD including its ocular manifestation. The 10 patients who met these criteria were enrolled in this study. The patient characteristic at the time of entry are listed in Table 1. Patient data, i.e., age, gender, infliximab dose interval, status of cyclosporine, colchicine and prednisone, as well as methotrexate prescription details, were collected from medical records. During the study period, 14 occurrences of relapse (eight ocular attacks, four oral ulcers, and two cases of erythema nodosum) observed among the 10 patients while they were undergoing infliximab therapy. The cases of oral ulcer and erythema nodosum were confirmed by a rheumatologist, and those of ocular disease by an ophthalmologist. The laboratory data obtained before and two weeks after the onset of BD relapse was collected from the medical records. The relevant parameters, i.e., total protein (TP), albumin (Alb), serum protein fraction, serum amyloid A (SAA), C reactive protein (CRP) and erythrocyte sedimentation rate (ESR), were measured by an outsourcing company (BML, Inc., Tokyo, Japan). The A/G ratio was calculated by dividing the value for albumin (mg/dl) by that for total protein (mg/dl) minus albumin (mg/dl).

The serum samples remaining after the laboratory test were frozen. The samples which could be kept from before and two weeks after the relapse were used to measure the markers, i.e.,  $\alpha$ 1-antitrypsin ( $\alpha$ 1AT),  $\alpha$ 1 acid glycoprotein ( $\alpha$ 1AGP), retinol binding protein (RBP), haptoglobin (HPT), ceruloplasmin (CP), and  $\alpha$ 2 macroglobulin ( $\alpha$ 2MG). These markers were measured in the Department of Laboratory Medicine, Kobe University Hospital.

### Statistical analysis

R version 2.14 (R Foundation for Statistical Computing, Vienna, Austria) was used for all of the statistical analyses. Values for each item for every patient were compared by means of the paired t-test and expressed as mean  $\pm$  standard deviation (mean  $\pm$  SD). Comparisons between pre- and post- relapsing events were evaluated by using the paired t-test or Wilcoxon signed rank test as appropriate. For all analyses, p values  $<$  0.05 were considered statistically significant.

Table.1: Patient characteristics

Patient number	Age / gender	Infliximab dose / interval	Cyclosporine (mg/day)	Colchicine (mg/day)	Immune suppressant
1	49/M	5mg/kg, 8wi	100	-	-
2	37/M	5mg/kg, 8wi	-	1	-
3	54/M	5mg/kg, 8wi	100	1	-
4	42/M	5mg/kg, 8wi	75	-	-
5	36/M	5mg/kg, 8wi	-	1	PSL 5mg/day
6	60/M	5mg/kg, 8wi	50	-	-
7	34/M	5mg/kg, 8wi	100	-	-
8	14/M	5mg/kg, 8wi	-	0.5	MTX 15mg/week
9	47/M	5mg/kg, 8wi	100	0.5	-
10	42/F	5mg/kg, 8wi	25	-	-

Abbreviations: M, male; F, female; wi, weeks interval; PSL, prednisolone; MTX, Methotrexate

### 3 Results

Ten patients who met the aforementioned criteria were enrolled in this study. The clinical features of the patients at the entry are listed in Table 1. All patients were treated with an infliximab-based protocol based on the diagnosis of ocular BD. Fourteen occurrences of relapse were observed during the study period. The changes in TP, Alb, and the A/G ratio before and after relapse of BD are shown in Fig.1-a, and amounted to  $-0.05 \pm 0.43$  (mean  $\pm$  SD),  $-0.14 \pm 0.22$ , and  $-0.10 \pm 0.11$ , respectively. The percentage change for each of the globulin fractions associated with disease relapse is shown in Fig.1-b. The mean percentage change in the  $\alpha 1$  and  $\alpha 2$  globulin fractions a statistically significant increase, while the percentage change in the  $\beta$  and  $\gamma$  globulin fractions was not significant.

The finding that the percentage of  $\alpha 1$  and  $\alpha 2$  globulin fraction increased with a relapse of BD prompted us to examine which proteins in the globulin fraction had increased. Three pairs of samples (from patients No. 4, 5 and 8), which could be kept and prepared just before and after the relapse, were used to measure the following inflammatory proteins:  $\alpha 1$ -antitripsine ( $\alpha 1$ AT),  $\alpha 1$  acid glycoprotein ( $\alpha 1$ AGP) and retinol binding protein (RBP) for the  $\alpha 1$  globulin fraction, and haptoglobin (HPT), ceruloplasmin (CP) and  $\alpha 2$  macroglobulin ( $\alpha 2$ MG) for the  $\alpha 2$  globulin fraction.

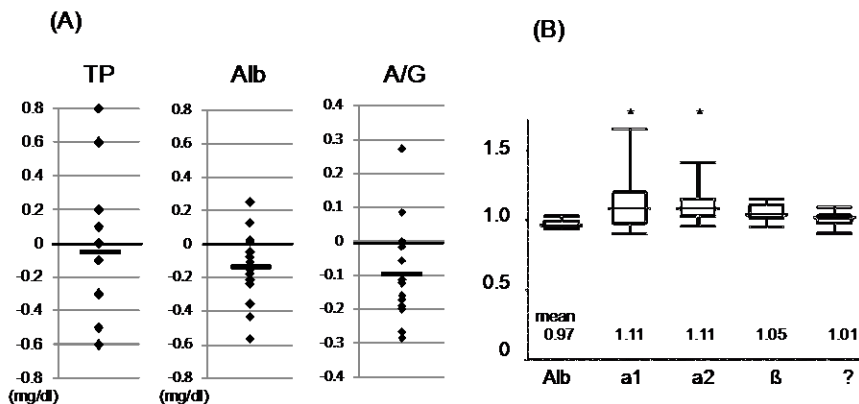


Figure 1

(A) Changes in total protein, albumin, and A/G ratio during relapse.

Fourteen events in ten patients are represented in each graph. Black bar: mean value; TP: total protein; Alb: albumin; A/G: albumin/globulin ratio.

(B) Percentage change in each globulin fraction with relapsing BD.

Data were analyzed with the t-test. (\*) statistically significant ( $<0.05$ ). The increase in the mean percentage of  $\alpha 1$  and  $\alpha 2$  globulin fractions was statistically significant, but the percentage of  $\beta$  and  $\gamma$  globulin fractions did not change significantly.

The results shown in Fig.2 demonstrate that the increase in  $\alpha 1$ AGP and the decrease in RBP (both  $p = 0.04$ ) were statistically significant, while CP showed a tendency to increase ( $p = 0.06$ ). There were no statistically significant changes in  $\alpha 1$ AT, HPT or  $\alpha 2$ MG. Considering the protein scale altering with,  $\alpha 1$ AGP accounted for a much larger volume of protein than did RBP in the  $\alpha 1$  globulin fraction (a difference of approximately 2 logs), leading us to assume that  $\alpha 1$ AGP might be the major contributor to the increase of the  $\alpha 1$  globulin fraction.

Since these findings indicated that the A/G ratio decreased BD relapsed as a result of a decrease in albumin concentration and an increase in globulin including  $\alpha 1$ AGP, we next compared the A/G ratio with other, conventional inflammation markers, i.e., ESR, SAA, and CRP for the pre- and post-relapse phases. The result is shown in Fig.3, that is, significant changes in all four markers (CRP:  $p = 0.03$ ; ESR:  $p < 0.01$ ; SAA:  $p < 0.01$ ; A/G ratio:  $p = 0.01$ ).

## 4 Discussion

Protein electrophoresis is used for the evaluation of inflammatory activity in rheumatic disease, with a decrease in albumin or an increase in globulin concentration indicating acute inflammatory status [16]. Because the A/G ratio is

calculated by dividing the value for albumin by that for total protein minus albumin, this ratio is theoretically decreases in the presence of inflammatory status including that associated with active BD. However, the A/G ratio had not been investigated for the evaluation of BD activity before, so that we conducted a retrospective study to examine its usefulness for the evaluation of ocular BD patients treated with an infliximab-based regimen, and found that a decrease in the A/G ratio may function as a clinical marker for relapsing ocular BD.

In our study, the absolute albumin concentration showed a decrease during relapse, which is explained by a change in albumin consumption. We also detected an increase in the  $\alpha 1$  and  $\alpha 2$  globulin fractions, which are usually elevated in the presence of acute inflammation. We followed specific protein measurements for three items per fraction:  $\alpha 1$ AT,  $\alpha 1$ AGP and RBP for the  $\alpha 1$  globulin fraction, and HPT, CP and  $\alpha 2$ MG for the  $\alpha 2$  globulin fraction, and found that  $\alpha 1$ AGP may be the protein largely responsible for inflammatory ocular BD.  $\alpha 1$ AGP has an anti-inflammatory or inhibitory effect on chemotactic response [16], leading to immunomodulative activity [17]. This protein can block an TNF- $\alpha$  induced apoptosis cascade, but not an anti-Fas-induced cascade [17]. Because the inflammation during TNF- $\alpha$  inhibition with infliximab maintenance therapy may be caused by the activation of a TNF- $\alpha$  cascade resulting from insufficient blocking of this drug,  $\alpha 1$ AGP may constitute a biomarker suitable for monitoring during infliximab therapy. With a focus on  $\alpha 1$ AGP for monitoring disease activity in rheumatic disease, some studies have reported its usefulness for active rheumatoid arthritis [18, 19]. With respect to BD, Lehner et al. [8] showed that  $\alpha 1$ AGP increases mainly in cases with ocular type inflammation, which is in line with our findings.

Our study compared as marker the A/G ratio with other conventional inflammatory laboratory markers, i.e., CRP, ESR and SAA, and that each of the four factors constituted a significant index [Fig. 3]. The median CRP level for the post-relapse phase of BD in our study was 0.7 mg/dl. Since the cut-off level for CRP is usually defined as 0.6 mg/dl, CRP will be slightly higher during relapsing BD. For the same reason, the median ESR and SAA levels of post-relapse BD were 12.5 mm/hr and 13.3  $\mu$ g/ml, respectively, values which are close to the cut-off levels for each of these two indices (10-15mm/hr for ESR; 8  $\mu$ g/ml for SAA). This means that the practical usefulness of these three biomarkers for BD will be limited. On the other hand, our findings indicate that the A/G ratio decreased proportionally with the relapse of ocular BD. Since this ratio comprises albumin and total protein value, it is a basic laboratory index and can be determined in a conventional clinic by dry chemistry thus making it highly practical. However, the A/G ratio as an inflammatory marker for rheumatic diseases has been discussed in only a few reports [15, 20]. As there is no relevant laboratory test for detecting relapse of ocular BD, we propose the A/G ratio as a potentially useful biomarker for this disease in the clinical setting.

For the practical application of this index, we used the changes in the A/G ratio for evaluating disease activation. While the basal A/G ratio levels during

remission differed because albumin and globulin concentrations varied from one patient to the next, we established that the A/G ratio decreased to  $-0.10 \pm 0.11$  (mean  $\pm$  SD) [Fig. 1-a]. We hope to demonstrate the usefulness of the A/G ratio in the clinical setting to obtain warning signs for the detection of ocular inflammation. As this study used a small number of samples, a study with more samples will be needed for a more accurate evaluation.

To conclude, the A/G ratio decreased during relapse of ocular BD patients undergoing infliximab therapy. We propose that monitoring A/G ratio values can yield a biomarker for detecting relapse of ocular BD. A tendency of the A/G ratio to decrease may constitute a warning sign of inflammatory ocular BD.

## 5 Conclusions

The A/G ratio appears to be a useful marker for relapsing ocular Behçet's disease. A tendency of the A/G ratio to decrease may constitute a warning of relapsing ocular Behçet's disease.

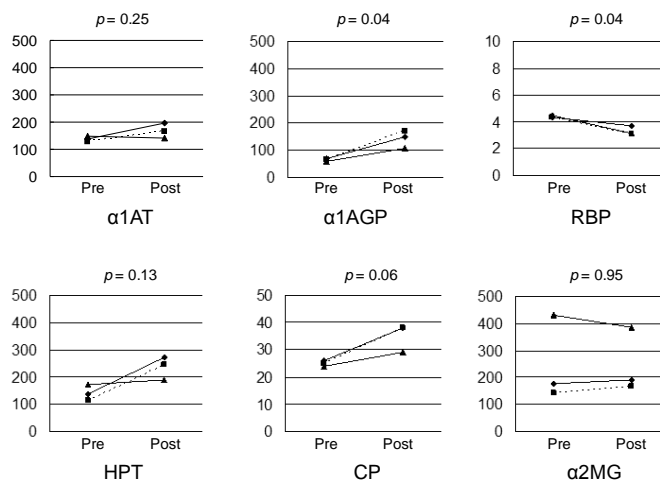


Figure 2. Comparison of inflammatory protein in the pre- and post- relapse phases. Three pairs of samples obtained before and after inflammatory events were compared. P value was calculated with the t-test.  $\alpha$ 1AT:  $\alpha$ 1-antitripsine;  $\alpha$ 1AGP  $\alpha$ 1: acid glycoprotein; RBP: retinol binding protein; HPT: haptoglobin; CP: ceruloplasmin;  $\alpha$ 2MG  $\alpha$ 2: macroglobulin. The unit of each protein (Y axis) is mg/dl.

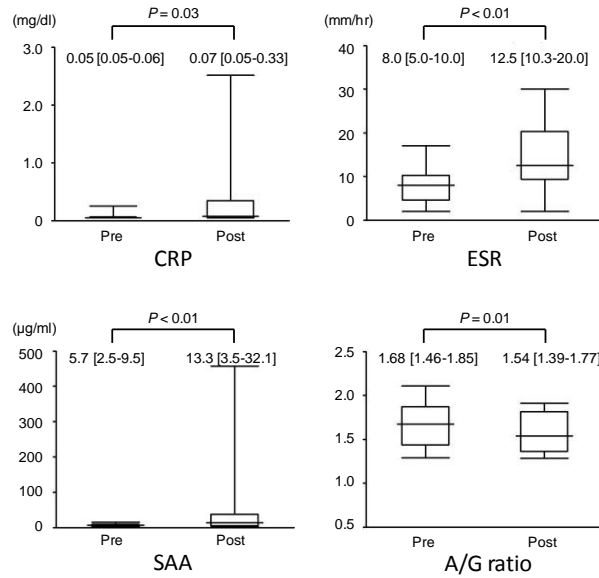


Figure 3. Comparison of the A/G ratio as an inflammation marker and other laboratory tests (CRP, ESR, and SAA) in the pre- and post- relapse phases. Values are expressed as median [25% tile - 75% tile]. Data were analyzed with the Wilcoxon signed rank test.

## 6 Competing Interest

The authors declare that they have no competing interest.

## 7 Authors' Contribution

TS and SK designed and analyzed data. KM, SM and JS provided support for the data analysis. NH tested the chemical samples, DS was performed the statistical analysis, and AM supervised the analysis.

## 8 Acknowledgement

The authors wish to thank all the staff members of the outpatient clinic at Ogihara Misaki Hospital for their assistance.



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