

Prospective study of the clinical value of determining circulating IgA- α_1 -antitrypsin complex using a prototype ELISA kit in patients with rheumatoid arthritis

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Abstract

Objective—To evaluate the clinical value of determining circulating IgA- α_1 -antitrypsin (IgA-AT) complex in rheumatoid arthritis.

Methods—The IgA-AT complex was assayed by a prototype ELISA kit using a specific monoclonal antibody against the complex.

Results—The median level of serum IgA-AT complex in rheumatoid patients (2.26 AU ml⁻¹) was significantly higher than in osteoarthritis patients (1.37 AU ml⁻¹, $P < 0.05$) and healthy volunteers (1.03 AU ml⁻¹, $P < 0.001$). The concentration of IgA-AT complex in rheumatoid arthritis patients at baseline was correlated with the number of painful joints ($P < 0.05$), number of swollen joints ($P < 0.01$), erythrocyte sedimentation rate ($P < 0.05$), and modified Lansbury index ($P < 0.01$). The median serum level of IgA-AT complex in rheumatoid patients at baseline was higher than that at three months ($P < 0.01$), six months ($P < 0.01$), and 12 months ($P < 0.01$) after the start of treatment. The difference and ratio of IgA-AT complex levels before and after treatment were significantly associated with radiographic progression.

Conclusions—The findings validate the usefulness of determining IgA-AT complex using ELISA in the management of rheumatoid arthritis.

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Rheumatoid arthritis is a chronic progressive disease characterised by excessive cellular proliferation of the synovium and destruction of both cartilage and bone, eventually causing disability. Although routinely measured laboratory variables such as erythrocyte sedimentation rate (ESR), C reactive protein, and rheumatoid factors have been shown to be helpful in the diagnosis and management of patients with rheumatoid arthritis, it is generally accepted that they sometimes fail to fulfil all requirements.

Immunoglobulin A (IgA)- α_1 -antitrypsin (AT) complex is a "non-immune" complex

formed by disulphide bonding between thiol reactive IgA and α_1 -antitrypsin. Previous clinical studies using two dimensional immunoelectrophoresis have shown that the serum concentration of IgA-AT complex increases in rheumatoid arthritis and is associated with joint erosions.¹

We have measured serum IgA-AT complex concentrations in rheumatoid arthritis patients by enzyme linked immunosorbent assay (ELISA) using monoclonal antibodies and have examined the relevance of this to disease activity indices.

Methods

This 12 month study was conducted prospectively on outpatients attending the rheumatology clinic at St Marianna University School of Medicine. Forty rheumatoid arthritis patients satisfying the 1987 ACR (American College of Rheumatology) criteria for the classification of rheumatoid arthritis² were entered into the study provided that they were not already receiving corticosteroids or disease modifying antirheumatic drugs on the first visit. Twenty two patients with osteoarthritis and 21 healthy age and sex matched volunteers were also included. The basic characteristics of the study population are summarised in the table.

The clinical and laboratory variables of 25 of the 40 initial cases could be followed. The clinical variables evaluated were (1) the number of joints which were painful or tender on pressure or passive motion, (2) the number of swollen joints, (3) the duration of morning stiffness (min), (4) grip strength (mm Hg), and (5) a modified Lansbury index which was scored by excluding the times of fatigue onset and aspirin requirement from the original method.³

The serum IgA-AT complex was measured by an ELISA using a monoclonal antibody against specific epitopes on the IgA-AT complex. The kits were supplied by Dr D R Stanworth (Rheumatology and Allergy Research Unit, Birmingham University, UK). The results were expressed as arbitrary units (AU) ml⁻¹.

Hand and wrist x rays taken at the baseline and after 12 months were scored according to a modification of the methods described by

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Table 1 Characteristics of patients with rheumatoid arthritis (RA) and osteoarthritis (OA)

	RA (n=40)	OA (n=22)
Age, years		
Median (range)	50.5 (22-75)	53.5 (38-83)
Sex		
Male:female	10:30 (25%:75%)	2:20 (9%:91%)
Disease duration, (months) ^a		
Mean (range)	31.3 (0.5-247.0)	36.8 (1.0-121.0)
Class (n=37)		
1	2 (5%)	
2	25 (68%)	
3	10 (27%)	
4	0 (0%)	
Positive RF ^b	31/40 (78%)	
Stage (n=15)		
I	6 (40%)	
II	3 (20%)	
III	1 (7%)	
IV	5 (33%)	
JSN		
Baseline		
Mean (range)	6.1 (0-23)	
At 12 months		
Mean (range)	10.1 (0-26)	
Erosion		
Baseline		
Mean (range)	6.3 (1-21)	
At 12 months		
Mean (range)	10.7 (1-25)	
Total		
Baseline		
Mean (range)	12.5 (1-40)	
At 12 months		
Mean (range)	20.9 (3-47)	
Drug treatment (n=25)		
PSL only	1 (4%)	
Buc only	4 (16%)	
PSL + Buc	17 (68%)	
PSL + SASP	2 (8%)	
PSL + Buc + MTX	1 (4%)	

^a Time since the appearance of symptoms.

^b RF = rheumatoid factor, > 15 IU ml⁻¹ by laser nephelometric assay and/or > 40× by RAHA. JSN, joint space narrowing; PSL, prednisolone; Buc, bucillamine; SASP, sarazosulphapyridine; MTX, methotrexate.

Sharp *et al.*⁴ The same areas as those in their report were scored for erosion and joint space narrowing (JSN). Erosion was given a score of 0 if none was present, 1 if it was suspected, and

2 if it was evident; collapsed bone (mutilans type) was scored as 3 (maximum). Similarly, joint space narrowing was given a score of 0 if none was present, 1 if it was suspected, and 2 if it was evident; ankylosis was scored as 3 (maximum). Erosion scores, joint space narrowing scores, and total scores (sum of the erosion and joint space narrowing scores) were calculated. The changes in score were evaluated using the formulas: [post-pre], [post/pre] [(post-pre)/M], and [(post-pre)/pre], where pre = the scores estimated at the baseline, post = those estimated at 12 months after initiation of therapy, and M = months of observation.

Results

Baseline median serum levels of IgA-AT were 2.26 AU ml⁻¹ in rheumatoid arthritis, 1.37 AU ml⁻¹ in osteoarthritis, and 1.03 AU ml⁻¹ in healthy volunteers. The levels of IgA-AT complex in rheumatoid arthritis were significantly higher than those in healthy volunteers and osteoarthritis ($P < 0.001$ and $P < 0.05$ respectively; Mann-Whitney U test). The mean + 2 SD of the value for healthy volunteers (1.60 AU ml⁻¹) was chosen as the cut off level. The percentage of rheumatoid arthritis patients with raised serum IgA-AT complex levels (≥ 1.60 AU ml⁻¹, 28/40, 70%) was significantly higher than that of osteoarthritis patients (9/22, 41%, $P < 0.05$, χ^2 test) and healthy volunteers (0/21, 0%, $P < 0.001$).

As shown in fig 1A, the baseline concentration of serum IgA-AT complex in rheumatoid arthritis was correlated with clinical indices such as the number of painful joints ($r = 0.392$, $P < 0.05$), the number of swollen joints ($r = 0.496$, $P < 0.01$), and the modified Lansbury index ($r = 0.418$, $P < 0.01$). There was also a significant correlation with laboratory variables such as IgA ($r = 0.895$, $P < 0.001$), IgG ($r = 0.548$, $P < 0.001$), and ESR ($r = 0.333$, $P < 0.05$). However, the level of α_1 antitrypsin was not correlated with that of IgA-AT complex.

The median serum concentrations of IgA-AT at three, six, and 12 months after the start of treatment (1.58 AU ml⁻¹, $P < 0.01$; 1.83 AU ml⁻¹, $P < 0.01$; and 1.37 AU ml⁻¹, $P < 0.01$, respectively) were found to have decreased significantly compared to those at baseline (2.54 AU ml⁻¹, Wilcoxon T test). Serum IgA-AT complex tended to reflect the articular findings in most rheumatoid cases. The changes in the concentrations of serum IgA-AT complex at baseline and at 12 months corresponded well to those of the modified Lansbury index (fig 2).

As shown in fig 1B, IgA-AT [pre-post] was correlated with JSN [post/pre] ($r_s = -0.709$, $P < 0.05$; Spearman rank correlation [post/pre] test). IgA-AT was correlated with JSN [post/pre] ($r_s = 0.867$, $P < 0.01$), and IgA-AT [pre-post] was correlated with total [post-pre] ($r_s = -0.540$, $P < 0.05$).

Discussion

IgA-AT complex is a "non-immune" complex formed by disulphide bonding between thiol-reactive IgA and α_1 antitrypsin. The latter is produced by hepatocytes and released

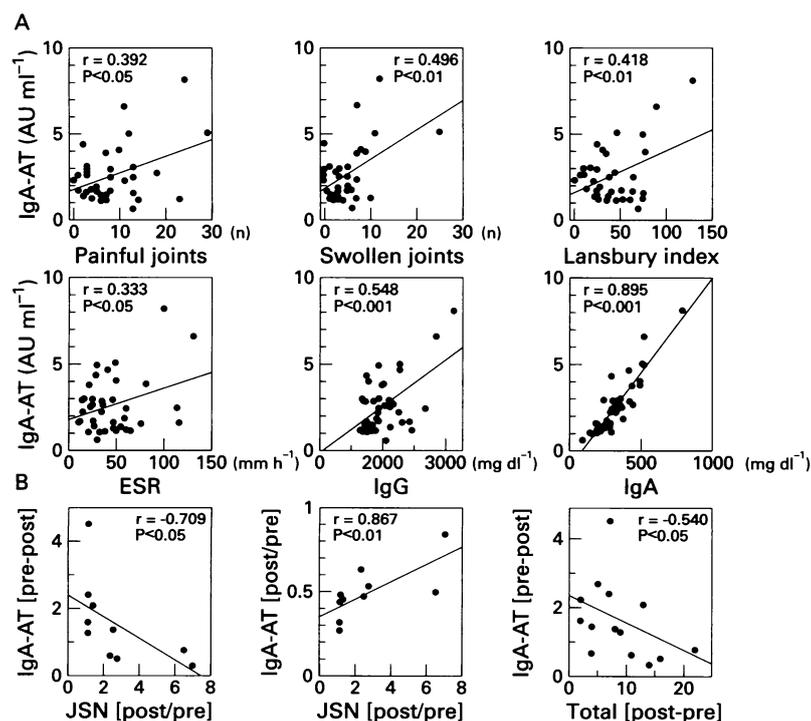


Figure 1 (A) Relation of baseline concentrations of serum IgA-AT complex with clinical and laboratory indices in rheumatoid arthritis. (B) Relation of changes in serum IgA-AT complex and radiographic findings.

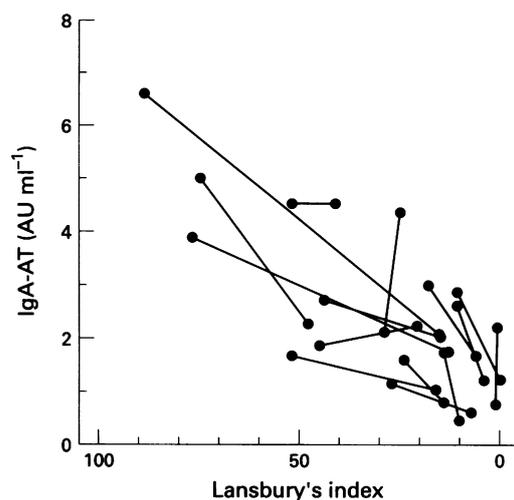


Figure 2 Changes in serum concentrations of IgA-AT complex at baseline and at 12 months in relation to the modified Lansbury index.

into the circulation. It is also released into synovial fluid and is known to protect the joints from destruction by inhibiting the action of protease.

Several laboratory indices including ESR and C reactive protein have been used to evaluate the efficacy of therapeutic drugs in rheumatoid arthritis, as discussed in ACR core disease activity measures for rheumatoid arthritis in Boston-6 and Maastricht-8.⁵ However, the therapeutic efficacy must be adequately evaluated, with emphasis on the long term outcome. Thus, indices capable of predicting radiographic changes in rheumatoid arthritis joints have long been awaited. Using two dimensional immunoelectrophoresis, the level of IgA-AT complex in rheumatoid arthritis was useful in predicting the development of erosions.¹ Therefore we prospectively evaluated the values of determining the IgA-AT complex using a more quantitative ELISA.

At baseline, serum concentrations of IgA-AT complex and the percentage of raised serum IgA-AT complex values in rheumatoid arthritis patients were higher than in osteoarthritis patients and healthy volunteers. Moreover, the baseline level of serum IgAAT complex in rheumatoid arthritis was correlated with the number of painful and swollen joints and the modified Lansbury index, reflecting its clinical relevance in comparison with existing indices. The baseline complex level was also correlated with the level of IgA, but not that of α_1 antitrypsin. The complex level in rheumatoid arthritis was shown to decrease longitudinally after treatment, and in most cases paralleled both the articular findings and the modified Lansbury index. The reduction of IgA-AT [pre-post] showed a significant inverse relation with the change in radiographic score [JSN [post/pre] and total [post-pre]]. The rate of decrease of IgA-AT [post/pre] also showed a significant positive relationship with the change in joint space narrowing. These findings suggest that the decrease in the IgA-AT complex is closely linked with the postponement of articular damage, particularly joint space narrowing. Lack of correlation

between the change in the level of IgA-AT complex and the change in erosions might be due to the shorter duration of follow up, the variability in disease duration,⁶ or the smaller cohort of patients.

Possible mechanisms responsible for the overproduction of IgA-AT complex in rheumatoid arthritis patients are: (1) increased production of IgA by synovial plasma cells, (2) increased production of α_1 antitrypsin as acute phase protein, (3) decreased production of J chain, or (4) a defect of B lymphocyte sulphhydryl oxidase mediating the linking of the J chain to IgA.⁷

With regard to the role of the IgA-AT complex in the pathogenesis of rheumatoid arthritis, the formation of complexes leads to the removal of α_1 antitrypsin, which is a potent protease inhibitor. This exposes the joints to harmful degrading proteolytic enzymes. At the in vitro level, the purified IgA-AT complex has been shown to be capable of eliciting the cytolytic release of lysosomal enzymes from mouse peritoneal macrophages, and the generation of a C3b ligand by activation of the alternative pathway.⁸ The IgA-AT complex has been reported to inhibit natural killer cell cytotoxicity⁹ and to induce the release of tumour necrosis factor from a murine macrophage cell line.¹⁰

Bucillamine [*N*-(2-mercapt-2-methyl-propanoyl)-*L*-cysteine], which has recently been developed in Japan and is similar to D-penicillamine in structure except that it contains two free sulphhydryl groups, has proved to be one of the most potent disease modifying antirheumatic drugs to date.¹¹ Levels of serum IgA-AT complex were reported to decrease in rheumatoid patients treated with D-penicillamine or gold sodium aurothiomalate.¹²⁻¹⁴ IgA has a reactive sulphhydryl group in the C-terminal region of the heavy chain, and this is the site of binding to α_1 antitrypsin, which also has a reactive sulphhydryl group. The newly synthesised IgA molecules have a free sulphhydryl group when they are released from plasma cells, and as soon as they meet reactive α_1 antitrypsin, combination takes place. However, when the newly formed IgA molecules come into contact with D-penicillamine, they bind with it, leaving no reactive site for the α_1 antitrypsin.¹³ Similarly, in the presence of bucillamine, thiol reactive IgA may bind to bucillamine instead of α_1 antitrypsin, resulting in competitive inhibition of IgA-AT complex formation.

In conclusion, a new ELISA which estimates the level of serum IgA-AT complex using specific anticomplex monoclonal antibodies has been found useful in the clinical evaluation of rheumatoid arthritis patients, because the values obtained are correlated with the postponement of articular damage that follows the introduction of disease modifying antirheumatic drugs. Further studies are required to explore the effects of other drugs on IgA-AT complex formation.

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