Efficacy of serum angiopoietin-1 measurement in the diagnosis of early rheumatoid arthritis

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Abstract

Objective

Previous studies showed that angiopoietin-1(Ang-1) expression was increased in the synovium in early rheumatoid arthritis (RA) patients. The present study was therefore designed to examine whether determination of serum Ang-1 might be effective in diagnosis of early RA.

Methods

One hundred and five serum samples of RA (21 males, 84 females) were studied for serum Ang-1 level. Serum samples were also collected from other collagen diseases, including 35 cases of SLE, 29 cases of systemic sclerosis, 16 cases of polymyositis/dermatomyositis. Serum samples were additionally obtained from 34 patients who visited our clinic for evaluation of symmetrical polyarthritis with morning stiffness. After one year of follow-up, those patients who satisfied the ACR 1987 classification criteria for RA were defined as "early RA". Serum Ang-1 levels were measured by sandwich ELISA using anti-angiopoietin-1 antibodies (both monoclonal and polyclonal antibodies). Serum anti-CCP antibody and rheumatoid factor (RF) were measured by ELISA and by laser nepherometry, respectively.

Results

Serum Ang-1 in RA patients was significantly higher than those in other collagen diseases. Serum Ang-1 levels in 50 normal healthy individuals were 5.8 ± 0.31 pg/ml (mean ± SEM). There was no significant difference in CRP and serum RF at the first visit between early RA patients and non-RA patients, whereas serum Ang-1 levels at the first visit were significantly higher in early RA (58.7 ± 17.9 pg/ml [mean ± SEM]) than those in non-RA (8.2±4.5 pg/ml). ROC analysis revealed that serum Ang-1 (cut-off 23.91 pg/ml) could diagnose early RA at sensitivity 57.1% and specificity 84.6%, providing comparable area under the curve (0.71, 95% CI: 0.54–0.88) to that of serum anti-CCP antibody (0.72, 95% CI: 0.53–0.92). There was no significant correlation between anti-CCP antibody and Ang-1.

Conclusion

These results indicate that serum Ang-1 is as useful a marker for the diagnosis of early RA as serum anti-CCP antibody.

Key words

Early rheumatoid arthritis, angiopoietin-1, anti-CCP antibody

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Introduction

In recent years, the ability to predict the development of rheumatoid arthritis (RA) in patients with an early-onset undifferentiated arthritis is highly required in order to obtain adequate responses to the treatment and achieve remission. Thus, considerable efforts have been made to predict the development of RA in patients with an early-onset undifferentiated arthritis from various aspects (1), including examination with power Doppler ultrasonography (2). In the meantime, additional markers would also help to predict the development of RA.

It should be noted that RA is a chronic inflammatory disease characterised by hyperplasia of synovial lining cells (3). One of the characteristic features in RA synovium is angiogenesis (4). Thus, a number of studies have shown that persistent neovascularisation is a crucial support to the continuous proliferation of the synovium, through delivery of nutrients and recruitment of inflammatory cells and lymphocytes in to the synovium (4, 5).

Angiopoietin-1 (Ang-1), a ligand for the TIE-2 receptor, is expressed in pericytes and a portion of periendothelial support cells to enhance either remodeling of blood vessels or survival of endothelial cells (6, 7). Previous studies have shown that the levels of Ang-1 and TIE-2 are increased in rheumatoid synovial tissue (8-10). In addition, it has been also disclosed that experimental collagen-induced arthritis was significantly inhibited after treatment with antagonistic soluble TIE-2 protein or forced expression of antagonistic soluble TIE-2 receptors by viral vector (11, 12).

Of note, it has been shown that angiogenesis takes place in the very early stage of RA in the absence of hyperplasia of synovial lining cells (13). Previous studies also showed that Ang-1 expression was increased in the synovium in early RA patients (14). The present study was therefore designed to examine whether determination of serum Ang-1 might be effective in predicting the development of RA in patients with an early-onset undifferentiated arthritis.

Patients and methods

Patients

One hundred and five serum samples of RA (21 males, 57.5±8.5 years old [mean ± SEM], 84 females, 54.8±13.2 years old) were studied. These 105 patients showed DAS28-CRP 4.5±1.3 (mean ± SEM), and RF 112.5±220.7 U/ml. for angiopoietin-1 level. Serum samples were also collected from other collagen diseases, including 35 cases of systemic lupus erythematosus (SLE; 4 males, 32.7±17.1 years old, 31 females, 30.8±11.2 years old), 29 cases of systemic sclerosis (SSc; 4 males, 50.3±0.1 years old, 25 females, 44.8±8.9 years old), 16 cases of polymyositis/dermatomyositis (PM/DM; 6 males, 56.8±9.8 years old, 10 females, 51.2±13.2 years old) and 50 normal healthy volunteers (22 males, 28.5±8.8 years old, 28 females, 28.0±10.6 years old).

In addition, serum samples were obtained from 34 patients who visited our clinic for evaluation of polyarthritis and gave informed consent (7 males, aged 59.7±6.7, 27 females, aged 59.0±14.8. The inclusion criteria at the first visit was as follows: 1) the presence of morning stiffness of at least 30 minutes of duration; 2) the presence of soft tissue swelling in any of proximal interphalangeal joints, metacarpophalangeal joints, wrists, elbows, knees, ankles, and metatarsophalangeal joints; 3) symmetrically joint involvement. Patients were followed up with possibly best treatment. After one year of follow-up, those patients who satisfied the ACR 1987 classification criteria for RA (13) and required treatment with disease modifying anti rheumatic drugs (DMARDs) including methotrexate (MTX) were defined as "early RA" and those who did not were defined as "non-RA " polyarthritis retrospectively.

Angiopoietin-1 measurement

Serum Ang-1 was measured by sandwich enzyme-linked immunosorbent assay (ELISA) as previously described (14). Briefly, wells of a 96-well microtiter plate (Maxisorp, Nunc, Roskilde, Denmark) was coated with 1.0 μg/ml goat polyclonal anti-human Ang-1 antibody (R&D, Minneapolis, MN)

in phosphate buffered saline (PBS, pH 7.40) at 4ºC overnight. Following overcoat with 1% bovine serum albumin in PBS(PBS-BSA) at room temperature for 2 hours, the wells were incubated with various concentrations of recombinant human Ang-1(R&D) or serum samples diluted at 1:200 in PBS at room temperature for 2 hours. Then, the wells were incubated with 0.5 μg/ml mouse monoclonal anti-human Ang-1 antibody (R&D) in PBS-BSA at room temperature for 2 hours, followed by incubation with 0.5 μg/ml alkaline phosphatase conjugated-goat anti-mouse IgG antibody (Zymed, South San Francisco, CA) at room temperature for 2 hours. After the incubation, the enzymatic activity was measured with substrate solution (Diethanolamine Buffer Solution, Phosphate Substrate, p-Nitrophenyl Phosphate Hexahydrate Disodium Salt Tablets; pNPP) using microtiter plate reader (BIORAD MICROPLATE READER Model680). Plates were read at absorbance of wavelength 405nm. Detection limit of this assay was 5 pg/ml.

Anti-CCP antibody and rheumatoid factor

Serum anti-CCP antibody levels were measured by ELISA(MEASACUP® CCP, MBL Co. Ltd, Nagoya, Japan). Serum RF levels were measured by laser nephelometry.

Statistical analysis

Comparison between early RA and non-RA was carried out by Mann-Whitney U-test. Diagnostic efficacy of various parameters for early RA was examined by ROC analysis. Correlation between anti-CCP antibody and Ang-1 or between DAS28 and Ang-1 were evaluated by Spearman's rank correlation test.

Results

Serum Ang-1 levels determined by ELISA

The ELISA could detect Ang-1 as little as 5 pg/ml. Serum Ang-1 in RA was 148.1±296.9 pg/ml(mean ± SEM). Overall, serum Ang-1 in RA patients were significantly higher than those in other collagen diseases, including SLE, SSc and PM/DM. Serum Ang-1 in SLE were 56.4±201.3 pg/ml (p<0.07), SSc were 97.6±360.5 pg/ml (p<0.003) and PM/DM were 26.1±46.3 pg/ml (p<0.02) (Fig. 1). Serum Ang-1 in 50 normal healthy individuals was 5.8±0.31 pg/ml(mean ± SEM).

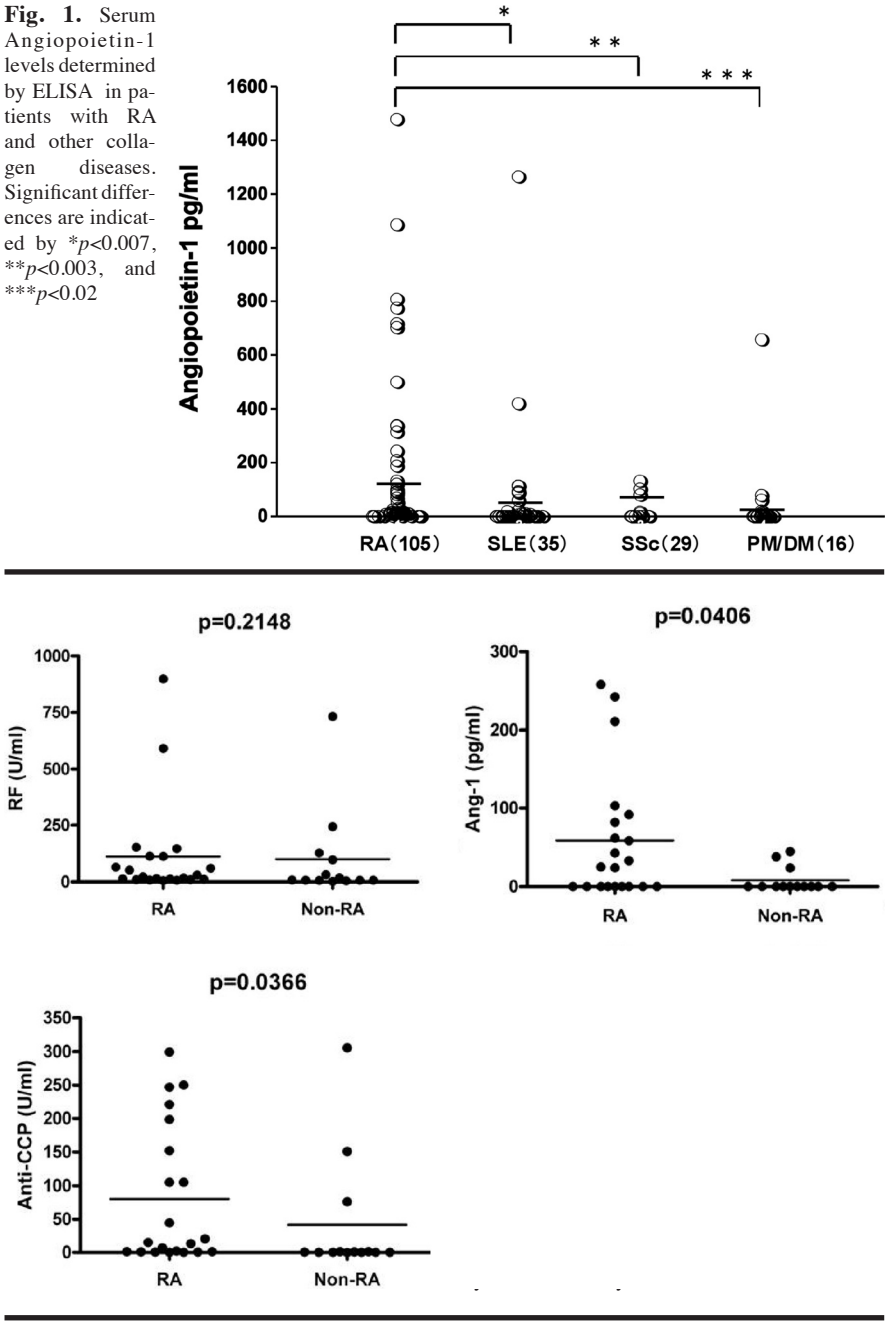

Fig. 1. Serum Angiopoietin-1 levels determined by ELISA in patients with RA and other collagen diseases. Significant differences are indicated by *p<0.007, **p<0.003, and ***p<0.02

Serum Ang-1 in early RA patients

In 34 patients with polyarthritis who had been suspected for RA, 21 patients and 13 patients were confirmed as early RA (3 males and 18 females, 56.8±13.9 years old, DAS28 –CRP 3.9±1.8 [mean ± SEM]) and non-RA (4 males and 9 females, 62.9±12.1 years old), respectively. Nineteen out of 21 early RA patients were receiving MTX 6–8mg/week. Four of 21 patients receiving DMARDs (salazosulfapyrizin 2 patients, bucillamine 2 patients. None of early RA patients were receiving corticosteroids. None of the non-RA patients were receiving MTX, DMARDs or corticosteroids. As shown in Figure 2, there was no significant difference in CRP (data not shown) and serum RF at the first visit between early RA patients and non-RA patients. By contrast, serum anti-CCP antibody levels at the first

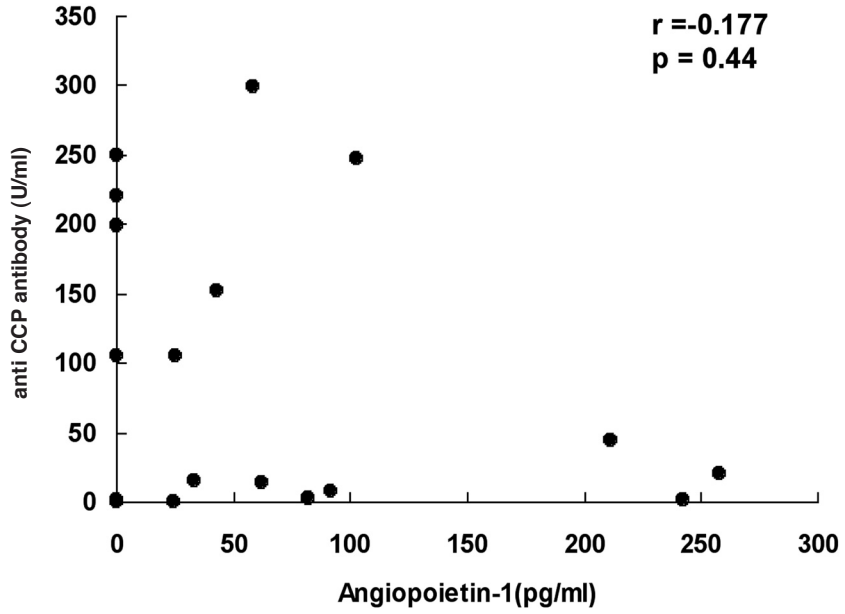

Fig. 3. Correlation with serum Angiopoietin-1 and serum anti CCP antibody in patients with early RA. There was no significant correlation between anti-CCP antibody levels and serum Ang-1 levels at the first visit in early RA patients (p=0.44).

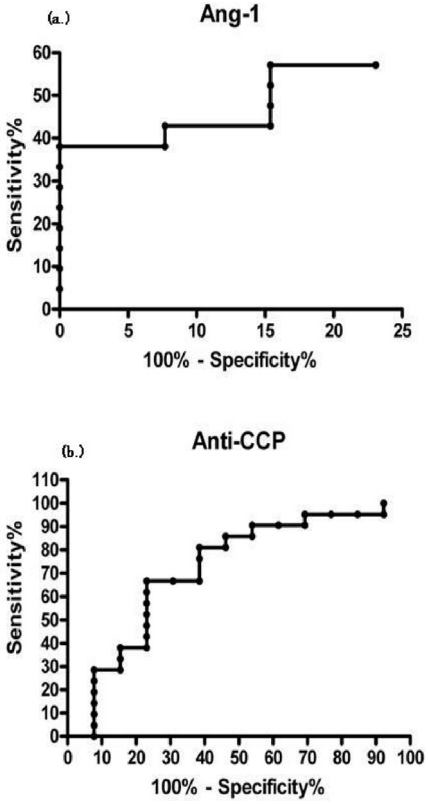

Fig. 4. ROC analysis of serum Ang-1 and anti-CCP for diagnosis of early RA (early RA = 21 patients, non-RA = 13 patients).
a. The sensitivity and specificity of serum Ang-1 were 57.1% and 84.6%, respectively, at the cut-off value of 23.91 pg/ml.
b. The sensitivity and specificity of serum anti-CCP were 61.9% and 76.9%, respectively, at the cut-off value of 4.7 U/ml.

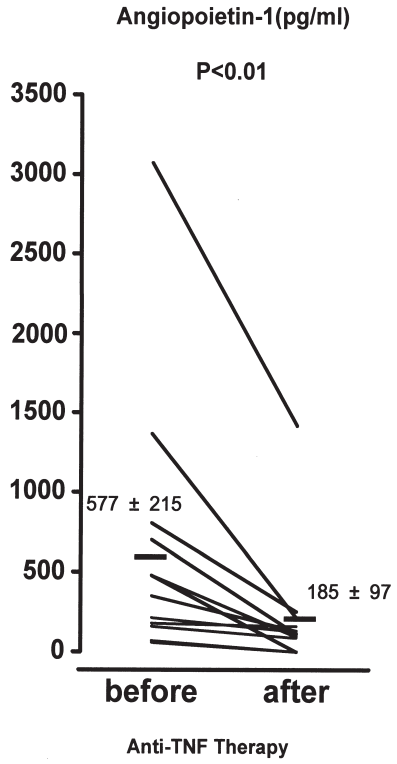

Fig. 5. Serum Ang-1 level in 11 RA patients treated with anti-TNF drugs (9/11 infliximab, 2/11 etanercept). The mean and SEM level of Ang-1 before treatment was 577±215 pg/ml, whereas that after treatment (20-24 weeks) was 185±97 pg/ml. Thus, significant reduction in serum Ang-1 levels was seen after 20-24 weeks (p<0.01).

visit were significantly higher in early RA (80.3±22.6 U/ml [mean±SEM]) than those in non-RA (41.5±25.3 U/ml). Of note, serum Ang-1 levels at the first visit were also significantly higher in early RA (58.7±17.9 pg/ml [mean±SEM]) than those in non-RA (8.2±4.5 pg/ml). However, there was no significant correlation between anti-CCP antibody levels and serum Ang-1 levels at the first visit in early RA patients (Fig. 3), whereas DAS28 correlated with Ang-1 (p<0.003, data not shown).

We next examined the efficacy of serum Ang-1 for differential diagnosis of early RA and non-RA using ROC analysis. As can be seen in Figure 4a, serum Ang-1 (cut-off 23.91 pg/ml) could diagnose early RA at sensitivity 57.1% and specificity 84.6%. The results indicate that serum Ang-1 is a sensitive and specific marker for a diagnosis of early RA. As can be seen in Figure 4b, serum anti-CCP antibody (cut-off 4.7 U/ml) could also diagnose early RA at sensitivity 61.9% and specificity 76.9%. Thus, the area under the curve for serum Ang-1(0.71, 95%CI: 0.54–0.88) was comparable to that for anti-CCP antibody (0.72, 95%CI: 0.53–0.92).

Sequential Ang-1 level in patients with RA treated by anti-TNF drugs

We examined sequentially serum Ang-1 levels in 11 RA patients treated with anti-TNF drugs (9/11 infliximab, 2/11 etanercept). The levels of Ang-1 before treatment were 577±215 pg/ml (mean±SEM), whereas those after treatment (20-24 weeks) were 185±97 pg/ml. Thus, significant reduction in serum Ang-1 levels was seen after 20-24 weeks (p<0.01) (Fig. 5).

Discussion

In the present study, we performed a retrospective analysis of serum Ang-1 in patients with RA and other collagen diseases. Overall, serum Ang-1 levels in RA patients were significantly higher than those in other collagen diseases. These results confirm that Ang-1 expression is upregulated in active RA.

Recently, the need for the ability to predict the development of RA in patients with an early-onset undifferentiated ar-

thritis has been highly appreciated (1, 2). Therefore, we also performed analysis of serum Ang-1 in patients with an early-onset undifferentiated arthritis, in whom serum Ang-1 and anti-CCP levels had been examined at the first visit. The results clearly indicate that serum Ang-1 is increased specifically in patients with early RA compared with patients with non-RA. Thus, ROC analysis showed that serum Ang-1 at the cut-off value of 23.91pg/ml could discriminate early RA from non-RA patients with reasonable sensitivity and specificity. It makes sense that serum RF or CRP could not discriminate these 2 groups of patients (data not shown). A number of studies have confirmed that serum anti-CCP antibody is a useful marker for RA compared with RF (17, 18). Accordingly, anti-CCP was also helpful for differential diagnosis between early RA and non-RA in the present study.More interestingly, serum Ang-1 appeared to be as effective as anti-CCP antibody for the diagnosis of early RA in our series of patients. It is therefore suggested that serum Ang-1 might be as useful a marker for the diagnosis of early RA as anti-CCP. However, since the sample size of our study was small, further studies with a larger numbers of patients would be necessary to confirm this point. Furthermore, in early RA patients, there was no significant correlation between anti-CCP antibody levels and serum Ang-1 levels at the first visit. Therefore, it is likely that these two makers might be mutually independent risk factors for those patients developing RA.

Recent studies have revealed that Ang-1 was also strongly expressed *in vivo* at the invasive front of rheumatoid pannus and was responsible for enhanced cartilaginous matrix degradation (19). Thus, the contributions of Ang-1 and TIE-2 to arthritic joint destruction have been appreciated (19). In fact, we found that serum Ang-1 was significantly decreased after successful treatment with anti-TNF agents (data not shown). It is therefore suggested that serum Ang-1 might be also useful for the evaluation of disease activities in RA.

Of note, it has been shown that synovial angiogenesis takes place in the very early stage of RA in the absence of hyperplasia of synovial lining cells or any other inflammatory changes (13). It is therefore suggested that elevation of serum Ang-1 in early RA might not be secondary to systemic inflammation, but a primary abnormality intrinsic of RA. Accordingly, there was no significant difference in serum CRP between early RA and non-RA in the present study. In this regard, the source of serum Ang-1 in early RA might be different from that in established RA, in which Ang-1 expression is induced by active inflammation mediated through TNF-α in synovial fibroblasts (9, 10). Ang-1 is expressed constitutively in pericytes and a portion of periendothelial support cells to enhance either remodeling of blood vessels or survival of endothelial cells (6, 7). It is therefore possible that some early events in RA might involve the production of Ang-1 in pericytes and a portion of periendothelial support cells. Therefore, further studies to investigate into the mechanism of the elevation of Ang-1 expression would be important for delineation of the pathogenesis of RA.

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