Complement system in psoriatic arthritis: a useful marker in response prediction and monitoring of anti-TNF treatment

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Abstract

Objective

Treatment with anti-TNF agents is well established in psoriatic arthritis (PsA). Anti-TNF agents are capable of modulating complement activity in vitro but there are no data on the in vivo effect. Anti-TNF have high costs and potential risks, thus, there is an urgent need for accurate predictors of response. We aimed at studying the usefulness of erythrocyte-sedimentation rate (ESR), C-reactive protein (CRP), and complement for response prediction and monitoring of anti-TNF treatment in PsA patients.

Methods

Fifty-five patients were included consecutively before starting etanercept or adalimumab. ESR, CRP, plasma complement C3, C4, and C3 and B cleavage fragments were evaluated at baseline and after 22 weeks of anti-TNF treatment. Disease activity was measured with DAS28 and response to therapy with EULAR criteria. Complement was evaluated at baseline in 30 healthy subjects as well.

Results

At baseline, C3 and C4 levels were significantly higher than in controls (C3 126.9±22 vs. 110±25 mg/dl, p=0.000002; C4 31.2±9.2 vs. 22.7±8.3 mg/dl, p=0.0003). After anti-TNF therapy, C3 and C4 levels were significantly reduced to normalization (p=0.0009 and 0.0005, respectively) and ESR, CRP and DAS28 showed a significant reduction (p=0.002, 0.004 and 0.0001, respectively). Split products of C3 and B were not observed at baseline and after 22 weeks. Higher baseline C3 levels were associated with EULAR non-response (p=0.011).

Conclusion

PsA patients with moderate to severe disease show elevated C3 and C4 levels, reverted by anti-TNF treatment. High C3 may be considered a hallmark of inflammation and C3 revealed the highest predictive value for response to anti-TNF.

Key words

psoriatic arthritis, C3, complement, anti-TNF, disease activity
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Introduction
Psoriatic arthritis (PsA) is a chronic, inflammatory arthritis commonly associated with psoriasis. Ten to 30% of the patients with psoriasis develop PsA. Skin involvement precedes joint symptoms in most cases; however, articular involvement at disease onset without skin lesions occurs in 15% of PsA patients. The disease can be often debilitating affecting the entheses, the small and large joints, and the axial skeleton (1). More than a half of the patients exhibits progressive erosive arthritis associated with functional impairment (1). PsA pathogenesis is still incompletely understood, but a role for innate immunity has been recognised (2). The skin may secrete pro-inflammatory mediators, such as TNF, proximal or distal to the affected joints. The interruption of this pathological communication may represent a therapeutic target (3). Similar to the skin, the joint and surrounding structures are a source of both endogenous ligands of the innate immune system and soluble mediators of inflammation (4).

The complement system is part of the innate immune defense, it recognises microbes and unwanted host molecules to enhance phagocytosis, and it is fundamental in immune complex clearance. Complement activation results in the formation of C3 convertase, with cleavage of C3, production of biologically active complement fragments resulting in opsonisation, chemotaxis, and cytolysis (5). Regulation of the complement system may control inflammatory diseases, including arthritis (5), but, conversely, disturbances to the complement regulation can lead to disease (5). During joint inflammation, many different products of complement activation can contribute to tissue damage. Complement activation fragments can be found in the synovial fluid (SF) of patients affected with other inflammatory arthritis, such as rheumatoid arthritis (RA), where they may participate in the inflammatory process (6-8). Large randomised controlled trials in PsA have shown convincing results with TNF antagonists Adalimumab (9, 10), Etanercept (11, 12) and Infliximab (13) on many aspects of the disease, including the axial manifestations (14). These agents demonstrated to be effective by providing clinical benefit on skin lesions, enthesitis, and dactylitis, highlighting the similar pathogenesis of the disease in cuts, tendons, and synovial membrane (15). It has been recently demonstrated that none of these drugs is able to induce in vitro complement dependent cytotoxicity, suggesting that their different clinical efficacy profiles are not explained by differences in complement lysis (16). Anti-TNF drugs can modify cellular and molecular networks with an overall decrease of the inflammatory process. Nonetheless, given the high costs and the potential risks of treatment with TNF blockers, the patients suitable for this kind of treatment should be carefully selected and there is an urgent need for accurate predictors of response. Differently from RA, the sensitivity of erythrocytes sedimentation rate (ESR) and C-reactive protein (CRP) as biomarkers of disease activity is controversial in PsA as they are poorly associated with disease activity, even if may help clinicians to predict the response on TNF blockers. Since the very first report from Helliwell et al. it appeared that ESR rather than CRP was the best laboratory guide to clinical disease activity in PsA (17).

To the best of our knowledge, no data on the modifications of the complement system in PsA patients treated with anti-TNF have been reported so far. Therefore, we studied the complement system in patients affected by moderate to severe PsA and its changes after 22 weeks of anti-TNF treatment. Then, we explored the usefulness of ESR, CRP, and complement C3 and C4 for monitoring inflammation in PsA patients treated with anti-TNF along with the association between these inflammatory markers, the DAS28, and the EULAR response over time. We found that higher C3 levels are associated with PsA patients suitable for this kind of treatment should be carefully selected and there is an urgent need for accurate predictors of response.

Patients and methods
Fifty-five Caucasian patients [28 females (51%), and 27 males (49%); age 48.7±12.8 (range 20–72 years), mean
disease duration 6.5±3.8 years], affected with PsA diagnosed according to CASPAR criteria (18), were enrolled between 2006 and 2008 in the Unit of Rheumatology, Policlinico Tor Vergata, University of Rome Tor Vergata. Thirty healthy Caucasian subjects, age- and sex-matched [15 males (50%), and 15 females (50%), mean age 33.67±8.9 years] served as controls.

Before the enrollment, all the patients were naïve for biologic therapy and started anti-TNF therapy because inadequate responders or contraindicated to conventional DMARDs including cyclosporine (CyA), leflunomide (LFN), methotrexate (MTX), and sulphasalazine (SSZ). The patients were randomly assigned with treatment with either Adalimumab (40 mg every other week, subcutaneously, Humira, Abbott Immunology, USA, n=28, 51%) or Etanercept (50 mg once a week, subcutaneously, Enbrel, Wyeth, USA, n=27, 49%). Twenty percent of patients received anti-TNF as monotherapy, while the remaining (80%) were treated with other DMARDs: MTX (45.4%), SSZ (15.6%), and CyA (19%). Only two patients were assuming prednisone at the dosage of 10 mg per day, one together with adalimumab, and the other with etanercept. In all patients, DMARD therapy was maintained stable during the follow-up.

Clinical assessment
Patients were evaluated at baseline (T0) and after 22 weeks of follow-up (T22).

Enrollment screening procedures included chest radiography, laboratory tests (including screening for hepatitis A, B, and C viruses), and tuberculin skin test. No patients showed signs or symptoms of acute or chronic infection, and patients’ laboratory tests evaluated haematological, renal, and hepatic functions were within normal ranges. Data were collected into a standardised computerised electronically-filled form including: demographic characteristics, date of diagnosis, co-morbidities, past and present medications. Clinical evaluation included swollen and tender joints count, patient’s and physician’s global assessment using a visual analogue scale (VAS, range 0–100 mm). Disease activity was measured with the Disease Activity Score in 28 joints (DAS28) (19), with the Psoriasis Area and Severity Index (PASI) (20), and quality of life with the Spondyloarthritis modified Health Assessment Questionnaire (SpAHAQ) (21). Response to therapy was evaluated at T22 according to the European League Against Rheumatism (EULAR) response criteria (22).

Laboratory analysis
Blood samples were obtained from all subjects at T0 and T22 two hours before the anti-TNF injection. Sera were collected using standard protocols and stored at -70°C until use. The local medical ethics committee in compliance with the Helsinki declaration approved the collection and use of patients’ samples. All the subjects provided informed consent. All subjects were evaluated for plasma complement C3 and C4, and patients also on erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP). ESR was determined with the Westergren method. Serum CRP was measured by nephelometry with an automatic analyser according to the manufacturer’s instructions. Value ranges were ESR <20 mm/h and CRP <0.5 mg/dl.

Complement assays. C4 concentrations were studied by means of radial immunodiffusion (mg/dl) and C3 by nephelometry according to standard methods (mg/dl) (23, 24). Complement C3 and C4 were determined also in 30 healthy subjects who served as controls.

Immunohistochemical analysis. The presence of split products (SP) of C3 and B in the sera of patients was investigated with immunoelectrophoresis (IEP) and counterimmunoelectrophoresis (CIE). IEP was performed as previously reported (25) in 1% agarose containing 10 mM EDTA, for 120–150 min at 5 mA constant current/slide. CIE was carried out in 0–6% agarose containing 40 mM EDTA (25). The antisera employed in IEP were anti-β1C/β1A and anti-factor B; the antisera employed in CIE was anti-β1C/β1A. These antisera were found to contain antibodies directed against the native molecules as well as the respective SP (25).

Statistical analysis
The statistical calculations were performed using Graph Pad Prism 5.0 statistical software (GraphPad Prism, San Diego, CA, USA). Normally distributed variables were summarised using the mean ± standard deviation (SD), and non-normally distributed variables by the median and range. Wilcoxon’s matched pairs test and paired t-test were performed accordingly. Univariate comparisons between nominal variables were performed by chi-square (χ²) test or Fisher’s test where samples were <100. One-way analysis of variance (ANOVA) was used to compare mean C3 levels in patients with no or mild, moderate and good EULAR response at T22. Bonferroni post-test was used accordingly (P). A correlation matrix between clinical and biochemical variables was derived from the data. For assessment of the correlation between two continuous variables, Pearson’s product moment correlation coefficient and Spearman’s rank correlation coefficient for normal and non-normal variables were used (respectively r and rs). Logistic regression analysis was performed to investigate which of the following independent variables at the start of the treatment were associated with EULAR response: sex, age, disease duration, ESR, CRP, C3, C4, DAS28, PASI, SpAHAQ. Two-tailed p-values were reported, p-values ≤0.05 were considered significant.

Results
Clinical and laboratory features of the PsA patients are shown in Table I. At baseline, levels of ESR, CRP, C3 and C4 and clinical parameters (DAS28, SpAHAQ, PASI) resulted homogeneous between patients who were taking Adalimumab or Etanercept. We then stratified the patients according to the previous treatment they received but no significant differences were observed in complement levels. Disease severity was assessed as moderate to severe disease in all patients according to DAS28 (mean at baseline 4.6±1.2). Complement levels of C3 and C4 at baseline were significantly higher compared with those from the 30 healthy donors (p=0.0003 and 0.000002, respectively; Table I).
All of the inflammatory markers (ESR and CRP) decreased significantly after starting anti-TNF therapy (p=0.002 and 0.004, respectively, Table I). Complement C3 and C4 were significantly reduced after 22 weeks of anti-TNF therapy with both Adalimumab and Etanercept (C3 126.9±22 mg/dl at T0 vs. 110.7±21.8 mg/dl at T22, p=0.0009; and C4 31.2±9.2 mg/dl at T0 vs. 24.5±7.9 mg/dl at T22, p=0.0005). These differences were also independent from the co-medication used. Data are summarised in Table I. Interestingly, the levels of C3 and C4 at T22 in the PsA anti-TNF treated group were comparable with those from the control group (p=NS for both comparisons, Table I). A parallel reduction of all clinical parameters (DAS28, PASI and SpAHAQ) was observed after 22 weeks of anti-TNF therapy as shown in Table I (p<0.0001, p=0.009 and 0.001, respectively).

No or mild EULAR response was achieved in 16 patients (29.1%), moderate in 19 patients (34.5%) and good in 20 patients (36.4%) after 22 weeks of anti-TNF therapy. Dichotomising EULAR response, 29.1% of the patients achieved no response and 70.9% had any response to anti-TNF therapy.

**Monitoring of clinical response**

Analysis of the data suggested that C3 and, at a minor extent, ESR and CRP can be useful in monitoring the clinical response. Indeed, the Pearson’s correlation analysis showed that the baseline C3 levels positively correlated with both ESR and CRP levels (r=0.414, p=0.012, and r=0.380, p=0.046, respectively), as well as the C3 levels at T22 correlated with the ESR levels at the same time point (r=0.374, p=0.029). Notably, C3 levels at baseline as well as ESR positively correlated with the DAS28 outcome at T22 (r=0.399, p=0.0289, and r=0.32, p=0.0468, respectively, Figure 1). Furthermore, the reduction in ESR and CRP levels positively correlated with an improvement (decrease) in the DAS28 scores (r=0.551, p=0.001, and r=0.461, p=0.012, respectively). No other significant correlations were observed.

**Prediction of clinical response**

Analysis of baseline characteristics, suggested that C3 and, at a minor extent, CRP are predictors of EULAR response. We performed a semi-quantitative analysis transforming variables as summarised in Table II using Z scores of each variable.

Patients with an elevated baseline level of C3 (>135 mg/dl) did not achieve EULAR response at T22 significantly more often than patients with normal or low baseline C3 levels (low vs. high C3 levels in no/mild EULAR response: 12.7% vs. 16.4%; moderate response: 14.5% vs. 20%; and good response: 36.4% vs. 3.6%, χ²=11.8, p=0.0026). When EULAR response was dichotomised, lower C3 baseline levels (<135 mg/dl) showed a tendency to be associated with good EULAR response at T22 (OR=0.469 (low vs. high), p=0.056) as well as CRP>0.5 mg/dl (OR=3.333 (high vs. low), p=0.061).

Multiple adjustment for the different variables included in the study showed that a good model built by a backward stepwise selection regression method contained the following co-variables: sex, age, disease duration, ESR, CRP, C3, C4, DAS28, PASI, and SpAHAQ. When this model was adopted, the multiple logistic regression, performed...
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with EULAR-good-response as the dependent variable, showed that the only independent predictor of major clinical response was C3 ($\chi^2=9.044$, $p=0.011$), whereas sex, age, disease duration, ESR, CRP, C4, DAS28, PASI, SpAHAQ were without statistical significance (all $p>0.05$). Within these independent variables, only CRP had a tendency to be associated with clinical response ($\chi^2=4.878$, $p=0.087$).

ROC curves were created for baseline ESR, CRP, C3 and C4 and DAS28 as predictors of EULAR response (Fig. 2). The sensitivity and specificity of C3 levels for prediction of the EULAR response were 0.7 and 0.6, likelihood ratio 1.75, while the sensitivity and specificity of CRP levels were 0.65 and 0.67, likelihood ratio 1.96, respectively. The ROC curve for ESR and C4 (Fig. 2) showed that determination of these variables may not be of additional value in predicting the EULAR response.

Nonetheless, we divided baseline levels of complement C3 according to the 22 weeks EULAR response. We observed a statistically significant difference in the mean C3 levels between patients with no or mild response [16 patients (29.1%)], and those with good response [20 patients (36.4%)], using one-way ANOVA with Bonferroni correction (C3 levels 135.5±19.6 mg/dl vs. 116.1±25.2 mg/dl, $p<0.05$, Fig. 3).

No differences were found between patients with either no/mild response or good response and those with a moderate EULAR response (125.6±22.1 mg/dl) (Fig. 3).

**Imunochemical analysis**

We therefore analysed whether these high baseline levels of C3 and C4 and their subsequent decrease after anti-TNF therapy could be due to complement activation after TNF antagonist therapy or rather to normalisation of previously high levels. To address this issue, we evaluated the release of SP of C3 and B. These cleavage fragments were not detected in the patients at baseline, nor at 22 weeks.

**Discussion**

In our paper we demonstrated that PsA patients with a moderately to severely active disease show higher baseline C3 and C4 levels compared with a group of healthy subjects. A significant improvement of disease activity and laboratory features was observed after 22 weeks of anti-TNF therapy and was associated with a significant decrease in all the inflammatory markers including complement C3 and C4. Such a decrease was not ascribed to complement activation, rather to the normalisation
of previously abnormally high levels (as testified by the absence of complement cleavage fragments of activation). We also found that higher baseline C3 levels were associated with a worse EULAR response after 22 weeks of anti-TNF therapy.

The paper has raised several aspects of interest. Data on the role of complement in PsA are poor in the literature. Early studies on psoriatic patients have shown normal or elevated serum complement levels in most patients (24). Relatively high levels of synovial C3 and C4 were also observed in patients with PsA. Partsch and his coworkers have shown that SF from patients with PsA exhibits a relatively low percentage of the C3c cleavage product, in similar amounts than in patients with osteoarthritis (OA) (26). In accordance with our results, and in contrast to these low percentages of the C3c split product, these authors showed that PsA SF displays the highest C3 concentration when compared with RA and OA. Thus, synovial C3 may help in the differential diagnosis of PsA versus RA (27).

It has been previously reported that in inflammatory conditions the presence of abnormally, pathologically elevated complement levels of C3 may reflect the presence of an underlying inflammatory process. Indeed, complement proteins contribute to the acute phase response, and their improvement was demonstrated in chronic untreated inflammation (28). It has been suggested that high plasma amounts of component C3 could be due to spill over from the joints reflecting an in situ over-expression, or to an abnormal production of complement proteins due to inflammation itself (29, 30). In this view, in our study it is not surprising to find elevated baseline C3 and C4 levels in patients with a moderate to severe disease activity. Furthermore, the observed reduction in complement C3 levels after anti-TNF therapy, in the absence of complement activating cleavage fragments, may be considered an improvement of a pre-existing pro-inflammatory status exerted by treatment. Anti-TNF drugs have an outstanding anti-inflammatory potential that could be responsible for part of their mechanism in the disease control. No differences in complement reduction were observed in patients treated with both Adalimumab and Etanercept, suggesting that the intrinsic structure of these drugs does not influence complement activity. In vitro studies have already shown that these agents do not differ in complement lysis capacity (16). We cannot forget that in some cases it has been described the exacerbation of skin lesions in patients with psoriatic arthritis receiving anti-TNF therapy (31), even if the mechanisms underlying this undesirable effect are still to be elucidated.

How TNF antagonists are capable of reducing complement levels remains of debate. These drugs can reduce CRP, as demonstrated by large controlled trials and confirmed by our results as well (32). CRP is capable to interact with complement system, resulting in the activation of the classical complement cascade (33). TNF can induce CRP, leading to inflammation associated with complement activation (34). We demonstrated that there was a significant reduction in complement C3 and C4 levels as well as in CRP levels after with anti-TNF agents. It is possible that the reduction of complement C3 and C4 could be due to CRP modulation subsequent to drug-induced TNF reduction (35). Our data may suggest that complement C3 is acting as an acute phase reactant, given as the split products are not elevated and the C3 levels correlate with ESR, CRP, as well as with the DAS28.

We provided evidence that higher baseline C3 levels are associated with a worse EULAR response. This may suggest that elevated C3 levels may be considered a negative predictive factor for disease outcome and response to anti-TNF therapy in PsA patients. Furthermore, due to the fluctuation of ESR and CRP levels during disease course, and the fact that these inflammatory indexes are not always increased even in moderately to severely active phases (35, 36), complement C3 dosage may provide an additional tool in monitoring disease activity during treatment with anti-TNF.

Still, ESR and CRP appear to be good indicator of articular activity and severity in this study, as changes in their levels after anti-TNF therapy correlated with DAS28 score. This is agreement with previous studies (17, 37). On the other side, they seem to have less clinical value in selecting the patients most likely to benefit from treatment with TNF-inhibitors. In previous studies, it was showed that an increased CRP level at baseline was the factor most likely to predict clinical response in PsA (37-40). Similar results have been reported in studies in patients with RA and other spondylarthropathies (41, 42).

However, CRP failed to have an association with EULAR response. Probably, in our logistic regression model, the introduction of the independent variable C3 could have lowered the power of CRP in the prediction of the clinical response. Indeed, when we excluded C3 from the analysis, CRP was again the sole predictive variable with statistical significance ($\chi^2=5.551, p=0.018$). Acute phase proteins levels, such as those of CRP and C3, reflect systemic inflammation in patients with rheumatic disease and they might distinguish those patients with an active inflammatory disease capable of responding to an appropriate anti-inflammatory/disease modifying therapy. In this view, it should be underlined that the DAS28 score, rather than PASI and SpHAQ, was more sensitive to the fluctuations of the inflammatory laboratory variables studied, reflecting the capacity of this index to monitor, not only the articular, but also the serological status of patients with PsA.

Saber et al. aimed at assessing the predictor of remission in PsA, finding male gender, HAQ. Patient global VAS and early morning stiffness as independently associated with increased remission, while the HAQ was the sole predictor of DAS28 at one year in a multivariate analysis (43). In ankylosing spondylitis treatment response is usually assessed by means of the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), which does not incorporate serological evaluation. This, together with the fact that CRP and ESR are less frequently high in these patients, may
explain the lower sensitivity of the se-rological indexes in the monitoring of disease response.

To the best of our knowledge, this is the first study that addresses the role of complement in PsA patients treated with anti-TNF. Altogether, in this prospective cohort of PsA patients, measurement of inflammatory markers, in particular C3 and CRP served as a powerful tool not only for monitoring the efficacy of anti-TNF therapy, but also for the selection of PsA patients with a high likelihood of responding to anti-TNF treatment. The relevance of adopting comple-ment C3 as a marker of prediction and response to anti-TNF is linked with the feasibility of the assessment method, which is widely available, relatively cheap, fast, and highly standardized. Specific modulation and inhibition of local complement production could be an attractive target for PsA therapy. We believe that our data may contribute to the unveiling of the mechanisms exerted by complement on PsA pathogenesis and to the everyday management of these patients.

Key messages
• Complement C3 levels are elevated in PsA patients;
• C3 correlates with disease activity in PsA;
• C3 is useful in response prediction and monitoring of anti-TNF treatment in PsA patients.

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