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C3 AND C4 COMPLEMENT – BIOMARKERS FOR PROGNOSIS OF TREATMENT OF TNF-BLOKERS

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Abstract: *Introduction:* The complement system adds antibodies and helps phagocytic cells to destroy pathogens from the body. This is part of a congenital immune system that is not adaptive and does not change over the individual life. However, complement can be "triggered" by the adaptive immune system. The complement system is the main effector of the humoral patr of the immune system. Activation of complement results in opsonization, chemotaxis and cytolysis. Regulation of the complement system can control inflammatory diseases including psoriatic arthritis and vice versa, complement fixation disorders can lead to illness. Treatment with anti-TNF blokers complement activity in patients with inflammatory joint diseases.
Objective: To investigate C3, C4 fractions of complement, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and complement the response prediction and monitoring of anti-TNF treatment in patients with psoriatic arthritis.
Materials and Methods: 36 patients were included sequentially before treatment with TNF-a-blokers. C3, C4, ESR, CRP were assessed at baseline at 6 and 12 months after initiation of treatment with TNF-α blockers. The activity of the disease is measured by the DARSA disease activity scale, the responses being compared with a control group of persons similar in gender and age. Statistical data processing was performed using the SPSS v25.
Results and conclusions: According to the results obtained, C3 and C4 were significantly higher than controls at initiation of treatment (C3 111.3 ± 30.8, C4 91.9 ± 12.4 mg / dl, controls 19.1 ± 8.3 mg / dl, 10.2 ± 5.6 mg / dl p = 0.001, p = 0.001). At 6 and 12 months of follow-up, 76.2% of patients had a reduction in the level of C3 and C4 (p = 0.002, 0.001, respectively). It was found that higher baseline levels of C3 were associated with higher DARSA values at 6 and 12 months.
Conclusion: The study of the C3 and C4 complement fractions can be used as biomarkers to estimate the prognosis of TNF-blocker therapy.
Keywords: C3 and C4 complementq psoriatic arthtitis, TNF-α blockers

INTRODUCTION

The complement system is a major effector of the humoral immune response (1). It consists of proteins synthesized by the liver, tissue macrophages, blood monocytes, epithelial cells of the urogenital pathways and the gastrointestinal tract, circulating as inactive precursors (1). Under the influence of trigger mechanisms, the proteases in the system cleave specific proteins, thereby activating them and thus triggering a long cascade of events, the ultimate result of which is a massive enhancement of the response and activation of a complex that attacks the cell membrane and leads to its death (2). The complement system consists of serum proteins, serous proteins and cell membrane receptors (2). The main functions of the complement system are lysis of microbiological agents, phagocytosis of antigens, immune complexes and soluble receptors as well as enhancement of chemotaxic function of macrophages and leukocytes (3).
The classical activation pathway of the complement system begins after binding of the first Clq component to antigen-antibody complexes, cellular proteins or phage proteins as the C-reactive protein or serum amyloid P. This process proceeds by attaching the serine proteases Clr and C1s to Clq and subsequent cleavage of the C4 and C2 complement fractions, respectively, and activation of the C3 complement fraction (1, 4). C3 binds to its receptor located on the surface of immune cells and stimulates their activation. The lectin pathway is activated when bound to mannan-binding lectin (MBL), fibrinolytes and/or collectives which are elements of the cellular structures of microorganisms (5).
The alternative pathway for complement activation begins by spontaneous hydrolysis of C3 or by binding of propperdine to the cell surface of microorganisms. The hydrolyzed C3 binds to Factor B (FB), which is subsequently activated and this leads to the formation of C3 convertase that cleaves C3 and thus re-stimulates the activation of immune cells. C3 converts can be stabilized with factor P (bovidein) (1, 6).

The complement system has regulatory properties in inflammatory and immune processes in the human body (1, 2, 3, 5).

Skin is an active immune organ with complex interactions between cellular components and various mediators. Synthetic autoantibodies and invasive microorganisms activate the complement system. Genetic changes that lead to changes in the complement system contribute to disruption of immune response and the development of diseases such as psoriasis, psoriatic arthritis, rheumatoid arthritis, supraventricular hydradyenitis and others (4, 6).

Keratinocytes are capable of producing several complementary proteins, including C3 and C4 complement fractions, the synthesis of which is regulated and enhanced by interleukin-1α, interferon-γ (IFN-γ) and tumor necrosis factor-TNF-a (7, 8). IFN-γ increases the production of FH and FI locally, thus preventing epidermal damage that may be caused by locally produced C3, C4 and FB (6, 7). Keratinocytes and melanocytes express cell-bound complement regulatory DAF, MCP and CD59 proteins, making these cells less vulnerable to autologous complement attack (7, 8). Psoriasis is a chronic skin disease affecting about 2-4% of the human population (9). The disease has multifocal aetiology, and genetic, environmental and behavioral factors play a role in the pathogenesis and course of the disease (9).

In the last years In several research groups analyzed extracts of psoriatic lesions and found that psoriatic scales had chemotactic and chemokinetic properties (9, 10). Tagami and Ofuji, found the presence of C3a in the skin lesions of patients with psoriasis (11). The deposition of C3b in the presence of immunoglobulins in the corneal layer of the skin of patients with psoriasis suggests activation of the classical pathway (11).

There are several hypotheses that can explain the activation of complement and the generation of C5a in psoriatic lesions. The first focuses on the presence of autoantibodies against carbohydrate antigens in the stratum corneum and subsequent deposition of IgG, C3b and / or C4b, which initiates a classic pathway of complement activation (2, 11). According to the second hypothesis, serine proteases present in the stratum corneum cleave C5 and thus activate the complementary pathway for complement activation (2, 11, 12). According to the third hypothesis, microorganisms that colonize the skin and lead to exacerbation of psoriasis directly activate the complement system.

As a result of all these processes, complement elements are activated in the dermis, penetrate into the epidermis and cause neutrophil migration and epidermal cell damage (12).

Psoriasis is an autoimmune disease and therefore psoriasis therapy focuses primarily on the immune system. According to the complex immunopathology of psoriasis, during immune activation, plasmacytogenic dendritic cells and keratinocytes synthesizing IL-1 and TNF-a are activated in response to dendritic toll-like receptor activation by DNA (5). In this initial phase, neutrophil migration is mainly due to the release of IL-1β and TNF-α. Anaphylaxin C5a is the strongest chemoattractant for neutrophils, monocytes and macrophages. According to the authors, in the early stages of the psoriatic lesion development, neutrophils predominate and the complement system is activated. Stratum corneum releases C5a, which activates T-lymphocytes and keratinocytes. C5a migrates to the dermal layer, activating endothelial cells and degranulating fat cells, resulting in capillary dilation characteristic of early psoriasis (5).

Following autoinflammatory initiation, the reaction is shifted to autoimmune activation including IL-23 / IL-17 activation (5, 10).

In recent years, bimodal immune activation has been reported in psoriasis - auto-inflammatory activation of neutrophils, together with T-lymphocyte autoimmune activation (12).

The complement system is this leading factor that binds innate autoimmunity and adaptive immunity to psoriasis patients (12).

THE AIM

Our aim was to investigate C3, C4 fractions of complement, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and their role for prognosis and monitoring of anti-TNF blocker therapy in patients with psoriatic arthritis .

MATERIALS AND METHODS

36 patients with psoriatic arthritis were enrolled sequentially before initiating treatment with etanercept or adalimumab. C3, C4, ESR, CRP were assessed at baseline at 6 and 12 months after initiation of treatment with TNF-α blockers. The activity of the disease is measured by the DARSA disease activity scale, the responses being compared with a control group of persons similar in gender and age. The statistical processing of the data was done using the statistical program SPSS v25, with a confidence level $p < 0.05$.

The C3 and C4 complement fractions were tested by the immuno-gravimetric method using company test kits, used by AMP Diagnostics BR-5420-S. The ESR has been investigated through Westergreen method, which measures the

rate of precipitation of the erythrocyte column by measuring the height of the plasma column, in mm/h at the 1st and 2nd hour, the norm for men over 50 ages is 0-15 mm/h and women over 50 years is 0-20 mm/h. The C-reactive protein was tested by the immuno-turbidimetric method using the company's AMP Diagnostics BR-5420-S high sensitivity C-Reactive Protein (hs-CRP) test kits, up to 10 mg/l. DAPSA is based on DAREA (Disease Acitvity Index for Reactive Arthritis) and covers about 3 times more joints-66/68. DAPSA (Disease Activity for Psoriatic Arthritis) is a result showing PsA activity and is calculated as the sum of painful and swollen joints, EWS value and patient's assessment of their own conditions and pain. As a result of DAPSA less than 4, the patient is in clinical remission.

INCLUSION CRITERIA

1. Patients with proven psoriatic arthritis according to currently available diagnostic and classification criteria.
2. Patients starting treatment with a biological agent - adalimumab (Humira) or etanercept (Enbrel) due to a therapeutic need.
3. Patients with good mental health, contact, adequate.
4. Patients have their consent to participate by signing an informed informed consent approved by the local ethics committee.

EXCLUSION CRITERIA

1. Patients who refused to give informed informed consent
2. Patients with rheumatic diseases, other than psoriatic arthritis, treated with biological agents other than adalimumab or etanercept.
3. Patients incapable, according to the researcher, to handle the procedures planned for each visit
4. Patients who, according to the investigator, are inappropriate to participate in the trial

RESULTS

36 patients with psoriatic arthritis were included in the study. The demographic characteristics of the patients are presented in Table 1

Table 1 Characteristics of patients included in the study prior to initiation of treatment with anti-TNF-α blockers

Characteristics	Patients receiving adalimumab	Patients receiving etanercept	Total
Male (n, %) Female (n, %)	8 (22,22%) 6 (16,66%)	12(33,33%) 10(27,77%)	20(55,55%) 16(44,44%)
Age, years	55,8 (34-69,8)	51,3 (31-68,2)	53,5 (32,4-68,9)
Concomitant MTX	12 (85,57)	20 (90,90%)	32 988,88%)
MTX, mg/week	12,5 (7,7-20)	12,5 (7,7-20)	12,5 (7,7-20)
Disease duration, years	13 (3-18)	12,7(4-22)	12.8 (3-22)
CRP, mg/ml	43,7 (28-71)	51.8 (31-88)	47,7 (28-88)
ESR mm	45,9 (27-79)	40,2 (30-76)	42,8 (27-79)
DAPSA	16,3 (9-22)	18,2 (11-31)	17.2 (9-31)

Mean values of C3 and C4 significantly higher than controls at baseline (C3 111.3 ± 30.8 and C4 91.9 ± 12.4 mg / dl, controls C3 - 19.1 ± 8.3 mg / dl, C4 - 10.2 ± 5.6 mg / dl, C3 p = 0.001, C4 p = 0.001). At 6 and 12 months of follow-up, 76.2% of patients had a reduction in the level of C3 and C4 (p = 0.002, 0.001 respectively) as shown in Table 2

Table 2 Level of C3 and C4 complement fractions in patients with psoriatic arthritis

	Before starting treatment	6 months after treatment	12 months of treatment
C3 mg/dl	111,3 ± 30,8	71,24 ± 3,12	29,66± 5,71
C4 mg/dl	91,9 ± 12,4	54,11 ± 2,51	20,48±2,89
C3 - Control group healthy subjects	19,1± 8,3	15,71± 1,4	16,1± 1,5
C4 - Control group healthy subjects	10,2± 5,6	9,56± 1,2	10,8± 1,8
DAPSA	17.2 (9-31)	5,2± 1,2	4,9± 1,3

In the analysis of the patients, there was a positive correlation between the decrease of C3 and C4 levels and the mean values of DAPSA ($r_{x,y} = 0.75$, $p = 0.002$).
When analyzing the individual values of psoriatic arthritis patients, it was found that higher baseline levels of C3 were associated with higher DARS values at 6 and 12 months in patients who did not achieve clinical remission ($r_{x,Y} = 0, 81$, $p = 0.001$).

DISCUSSION

The complement system is much more than an initial idea that it functions as a non-specific protective mechanism against microorganisms. In recent years, the knowledge of the complementary functions and relationships of immune response elements has been significantly expanded. The complement system undoubtedly has a protective function in the human body, but its inappropriate activation can lead to damage to tissues and structures. Local and systemic activation of the complement cascade has been demonstrated in psoriatic arthritis, but its relevance to the pathogenesis of the disease is still ongoing.
Preliminary results from clinical studies have shown promising results using the study of C3 and C4 complement fractions as biomarkers for assessing disease activity and evaluating the effect of treatment. Our results show that there is a strong correlation between these two complement fractions and the DARSA disease rating scale. We therefore recommend that the study of complement fractions be used as biomarkers to evaluate the activity of psoriatic arthritis.

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