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REVIEW ARTICLE

# Acute Phase Reactants

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*Received 02 September 2012; accepted 24 January 2013*

ABSTRACT

Acute phase response is the sum of the systemic and metabolic changes occurred by release of acute phase proteins in response to inflammatory stimulus. The most important ones of these acute phase reactants are erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), fibrinogen and ferritin. The most widely used ones are ESR and CRP while fibrinogen and ferritin are rarely used and other acute phase reactants have no place in routine clinical use. Although ESR is clinically the most commonly used test, it is fairly nonspecific. Even though CRP is more specific than ESR, because of the high cost of the analysis it has clinically limited usage.

**Key words:** Acute phase reactants, erythrocyte sedimentation rate, C-reactive protein

Acute phase reaction is a general term attributed to a group of systemic and metabolic changes that occur within hours of an inflammatory stimulus. The most important component of this response comprises the acute phase proteins, which are a heterogeneous group of plasma proteins. Most of the components of the acute phase response reflect the defense and adaptation mechanisms, which take place before the body gives an immunological response. Acute phase response takes place, by changes in a heterogeneous group of proteins which consists of around 30 proteins in response to bacterial infection, trauma, myocardial infarction, collagen tissue disorders which result in the production of IL-1, IL-6, TNF-α [1,2]. If the inflammatory response is self limiting or treated, the level of acute phase proteins returns to normal within days or weeks. The stronger the stimulus for inflammation, the greater is the change in the concentration of acute phase proteins, and will continue the high levels as long as the stimulus remains [3]. The most significant proteins in this group are Erythrocyte Sedimentation Rate (ESR), C-reactive protein (CRP), fibrinogen, ferritin, serum amyloid protein A, alpha-1 antichymotripsin, alpha-1 antitripsin, haptoglobulin, alpha-1 acid glucoprotein, ceruloplasmin and C3, C4 [3] (Table 1).

While the levels of CRP and serum amyloid protein A increase a few thousand folds, the levels of alpha-1 acid glycoprotein, alpha-1 proteinase inhibitor, haptoglobulin and fibrinogen increase a few folds, and the levels of ceruloplasmin and complement proteins increase around 50%. On the other hand, the levels of albumin decrease during an acute inflammation process [4].

All acute phase proteins may not rise in all inflammatory pathologies. For example, in systemic lupus erythematosus, sedimentation rate increases, while CRP level decreases. Similarly, in patients suffering from the same disease, the levels of acute phase proteins do not always change in the same way. Similarly, in a patient with fever, the CRP levels may be normal, just as there might be different changes in the plasma concentrations of the other acute phase proteins [5].

The acute phase proteins most commonly used in clinical practice are sedimentation rate and CRP. Fibrinogen and serum ferritin levels are used less frequently. The others do not have routine use,

**Table 1.** Human Acute Phase Proteins

| Increased proteins | |
|---|---|
| Complement System | C3, C4, C9<br>Factor B<br>C1 inhibitor<br>C4b binding protein<br>Mannose binding lectin |
| Coagulation and Fibrinolytic System | Fibrinogen<br>Plasminogen<br>Tissue plasminogen activator<br>Urokinase<br>Protein S<br>Vitronectin<br>Plasminogen Activator Inhibitor-1 |
| Antiproteases | Alpha1-Protease inhibitor<br>Alpha1-Antichymotripsin |
| Transport proteins | Seruloplasmin<br>Haptoglobulin<br>Hemopexin |
| Inflammatory Responders | Phospholipase A2<br>Lipopolysaccharide binding protein<br>İnterleukin-1 receptor antagonist<br>Granulocyte colony stimulating factor |
| Others | CRP<br>Serum amyloid protein A<br>Alpha1-asit glycoprotein<br>Fibronectin<br>Ferritin<br>Angiotensinogen |
| Decreased proteins | Albumin<br>Transferrin<br>Transthyretin<br>Alpha 2-HS glycoprotein<br>Alpha –feto protein<br>Thyroxin binding protein<br>Insulin like growth factor 1<br>Factor 12 |

(Adapted from NEJM, Gabay C, Kushner I. Acute Phase Proteins, 1999)

currently. Difficulties in measurements, variance in levels within the population and long duration for level changes limit their uses. The other acute phase reactants are used solely for research purposes. These proteins, which are not used routinely in clinical practice, will be mentioned briefly.

## Erythrocyte Sedimentation Rate (ESR)

Although not used for establishing a clinical diagnosis in the clinic, it is a commonly used and cost efficient test, which can assist in following-up with the progress and response to treatment of a disease. It indirectly reflects the increased concentrations of the acute phase proteins. The major determinant of sedimentation rate, is the rouleaux formation of erythrocytes, in which cells are lined up in a single axis [6]. The aggregation of erythrocytes is determined by the electrostatic forces. The erythrocytes are normally negatively charged and repel each other. On the other hand, many of the plasma proteins are positively charged, and neutralize the charge on the erythrocyte membrane, therefore lessening the repellant force, contributing to aggregation [6]. The proteins, which contribute most to erythrocyte sedimentation are fibrinogen, albumin, alpha and beta globulin. Among these proteins, fibrinogen with an asymmetric molecular structure has the highest contribution [6, 7]. A slight increase in the fibrinogen levels can bring about a great increase in ESR. An increase in the monoclonal immunoglobulin levels, as in multiple myeloma, may also increase ESR, independent of an acute phase reaction. Thus, ESR may not always reflect an acute phase reaction correctly (Table 2). Polycythemia vera, secondary polycythemias, sickle cell disease, hereditary spherocytosis, acanthocytosis, microcytosis, cachexia, and hipofibrinogenemia due to disseminated intravascular coagulation and massive hepatic necrosis may cause a decrease in ESR. Anemia and macrocytosis increase ESR (Table 3).

Erythrocyte sedimentation rate varies according to gender and age. In men, the value roughly equals to age (in year) /2, in women [age (in year) +10] /2. In men, 15 mm/hour and in women, 20 mm/hour are considered as normal values [8]. Although there are quite a few ways to measure sedimentation rate, the most commonly used techniques are the Westegren and Wintrobe methods [8]. Erythrocyte sedimentation rates can be falsely measured lower or higher due to a variety of reasons (Table 2).

Many reasons can cause increases and decreases in ESR (Table-3).

ESR may increase both in symptomatic and asymptomatic patients. Sometimes, the only finding in asymptomatic patients may be an increased sedimentation rate. For illuminating the etiology in these patients a complete blood count, biochemistry panel, urine test, serum protein electrophoresis, occult fecal blood test and chest x-ray may be beneficial [6]. If the increased sedimentation rate cannot be explained with further clinical evaluations, the sedimentation rate test must be repeated within a month. In a study where 43 patients had increased sedimentation rate without any clear explanation,

 

**Table 2.** Factors causing false changes in Erythrocyte Sedimentation Rate

| Factors causing false increases | Factors causing false decreases |
|---|---|
| Increased fibrinogen, globulin, cholesterol levels | Cachexia |
| High room temperature | Coagulation of the blood sample |
| Macrocytic anemia | Increase in bile salts |
| Menstruation | Increase in phospholipids |
| Pregnancy | Making the sedimentation sample wait more than two hours |
| Tilting or lying down of the ESR tube | Increase in adrenal steroids |
| Drugs: Dextrane, methyldopa, methysergide, penicillamine, procainamide, teophylline, trifluoperidole, vitamin A | Hypofibrinogenemia |
| | Hyperglycemia |
| | Hyperalbuminemia |
| | Leukocytosis |
| | Microcytic anemia |
| | Drugs: ACTH, cortisone, ethambutol, quinine, salicylates |

(Adapted from A Textbook of Natural Medicine, Pizzorno and Murray, 1992)

**Table 3.** Factors affecting Erythrocyte Sedimentation Rate (ESR)

| Increased ESR | Decreased ESR |
|---|---|
| Acute Heavy Metal Poisoning | Congestive heart failure |
| Collagen Vascular Disease | Polycythemia |
| Carcinomas | Sickle Cell Anemia |
| Cell or tissue injury | |
| Gout arthritis | |
| Infections | |
| Inflammatory disorders | |
| Leukemia | |
| Myocardial infarction | |
| Nephritis | |
| Syphilis | |

(Adapted from A Textbook of Natural Medicine, Pizzorno and Murray, 1992)

32 had normal rates within a month. The remaining 11 were followed up for 10 year; two developed malignancies, one person had benign dysproteinemia, 8 were never diagnosed with any disease [6].

Sedimentation rate might sometimes increase over 100 mm/hour. In general this is caused by infections, malignancies or collagen tissue disorders. Rarely, despite such high levels of ESR, there may not be an underlying disease [6, 7, 9].

In symptomatic patients, although physical examination and history may direct to a specific disease, a high ESR value may be used to justify the diagnosis. Although ESR by itself is not a specific test, along with the history, physical examination and laboratory values, it can assist in establishing a specific diagnosis [6].

ESR may sometimes decrease. In polycythemia, the increased number of erythrocytes decreases the rigidity of the rouleaux that in turn causes a decrease in ESR. Also, sickle cell anemia, spherocytosis, anisocytosis, hemoglobinopathies may decrease ESR. Low fibrinogen level caused by hepatic necrosis, cachexia, anorexia and malnutrition may also lead to a decreased ESR value. Also, anti-inflammatory drugs, high dose steroids may lead to a decrease in ESR value [6, 9, 10].

ESR and CRP are valuable in the follow up of rheumatological disorders, rather than the diagnosis. In polymyalgia rheumatica, it is the exact demonstrator of the disease activity and diagnostic

criteria. In rheumatoid arthritis (RA) and juvenile rheumatoid arthritis (JRA) there is a good correlation between the number of effected joints and ESR. Sometimes, when there is a very high ESR value that is not consistent with the clinical progression, in RA and JRA, amyloidosis must be considered. There is little correlation between systemic lupus erythematosus (SLE) and ESR, clinically. In osteoarthritis ESR is generally normal, however, as ESR will increase with age in older patients, it cannot be used to differentiate RA and osteoarthritis [8].

## C-Reactive Protein (CRP)

CRP is the prototype of human acute phase proteins and the most frequently studied one [11]. It has been named as C-reactive protein because it adheres to the "capsule" antigen of pneumococcus [12]. Plasma CRP production occurs via the stimulation of IL-6 in the liver. It assists in the recognition of damaged host cells and foreign pathogens, and their removal. When CRP binds to its ligand, it activates the complement system via the classical pathway and increases phagocytosis [11]. While it is present in minute quantities in the plasma, after an acute inflammatory stimulation, it rises within a few hours. It peaks within 2-3 days. Its half-life is 19 hours [12]. The increase in CRP levels is proportional to the inflammatory stimulus. With a greater stimulus, a higher and longer lasting level of CRP will be measured. After the inflammatory stimulus is removed, the CRP levels will quickly decrease [4, 11, 12]. The causes for increased CRP levels are summarized in Table 4.

In healthy individuals the CRP level is generally below 0.2 mg/dl. Due to micro-traumas that occur during the day, this level can increase up to 1 mg/dl. After a single stimulus it can increase up to 5 mg/dl within 6 hours, and can reach a peak value within 48 hours. While a value between 1-10 mg/dl is considered as mild, and any value above 10 mg/dl is considered a very high increase [3, 4, 11-13]. CRP is not specific for a certain disease. It shows inflammation and its degree. Although not always,, it mostly demonstrates inflammation and the degree of tissue damage, and the acute phase reaction more than any other parameter with more accuracy. Acute phase CRP reaction does not show diurnal variation, and is not affected by diet [11-13, 14]. CRP is mainly useful for following the response to treatment, hence the decrease in inflammation in organic disorders accompanied by mainly inflammation.

CRP reflects an inflammatory process and its degree. Therefore, it is very important in the follow up of rheumatologic disorders. In RA, high CRP values are almost always seen, and its titer correlates with the disease activity index [15]. A high CRP value at the initial presentation implies that a progressive erosive course is possible, and hence, guides the treatment selection [15]. In RA, CRP levels do not decrease with the use of non-steroidal anti-inflammatory drugs (NSAIDs), and only decreases with drugs that provide remission [3]. In only 2-30% of the SLE patients, an increased CRP is observed. Because of this, CRP level is not helpful in following up disease activity in SLE. It has been shown that an elevated CRP level in a SLE patient usually is seen if there is accompanying synovitis and serositis. If there is a CRP increase from the very beginning, then perhaps the CRP level may be used to evaluate the disease activity. If a patient, whose CRP level is known to be negative beforehand, afterwards becomes CRP positive, especially if the patient is admitted to a hospital, it should bring to mind a possible bacterial infection. The reason why CRP is not elevated in SLE is

**Table 4.** Different conditions affecting C-reactive protein levels.

| Normal/Insignificant (1 mg/ dl <) | Mild (1-10 mg/dl) | Very high (>10 mg/dl) |
|---|---|---|
| Heavy exercise | Myocardial Infarction | Acute bacterial infections (%80-85) |
| Influenza | Malignancy | Major trauma |
| Pregnancy | Pancreatitis | Systemic vasculitis |
| Gingivitis | Mucosal Infections (bronchitis, cystitis) | |
| Cerebrovascular accident | Collage tissue diseases | |
| Stroke | | |
| Angina pectoris | | |

not exactly known, however it is theorized that there is a post-receptor level resistance to the binding of IL-6 to its receptor on the liver. In rheumatologic diseases other than RA and SLE, CRP can be a good indicator of disease activity [3, 4].

As soon as the relationship between acute coronary syndromes and CRP was established, CRP has once more, become a subject of interest [11]. It is thought that it might be a good prognosis indicator after acute MI. However, in patients with stable and unstable angina, opposing results have also been seen [11]. However, there are studies which have also shown that in stable and unstable angina, CRP may be useful to correlate with an acute coronary event [16- 18].

## FIBRINOGEN

One of the acute phase proteins that originate from the liver is fibrinogen. Fibrinogen plays two important roles in the body. Firstly, it is an important component of the common pathway of coagulation. Secondly, it takes part in the acute phase response after tissue inflammation and damage [3].

High fibrinogen levels are also seen in heart or circulatory system disorders. Additionally, in stomach, breast and renal system malignancies, and inflammatory diseases such as rheumatoid arthritis, high fibrinogen levels may be observed. Fibrinogen is also used as a disease activity marker in Familial Mediterranean fever [19-21]. Additionally, exogenous use of estrogen and oral contraceptives is also associated with high levels [22].

Low levels of fibrinogen may be seen in liver diseases, prostate and lung cancers, bone lesions, malnutrition and some bleeding disorders. Afibrinogenemia, hypofibrinogenemia and dysfibrinogenemia are congenital diseases which are characterized with the lack or low levels of fibrinogen. In obstetric complications and traumas, low levels may be seen, too. The plasma fibrinogen levels may also decrease in response to massive amounts of transfusion due to blood provided from the banks. Drugs such as steroids, androgens, phenobarbital, urokinase, streptokinase and valproic acid may also cause low levels of fibrinogen.

Plasma fibrinogen level is normally around 200-400 mg/dl. It is not a test that initially reflects the acute phase response. Its delayed increase, long half life, prolonged high levels after inflammation has passed, lack of stability in frozen plasma and preserved plasma, are some of the disadvantages of this test [3].

## FERRITIN

Normally it reflects the iron stores in the body. Its normal values are 27-329 ng/ml for men and 9-125 ng/ml for women. However, in the presence of inflammation, it may increase as an acute phase response. It increases in cases of liver damage and malignancies [3].

### Other Acute Phase Proteins

Although serum amyloid A is known to be one of the major acute phase proteins, its role in the acute phase response has not been elucidated. Serum amyloid A is a member of the apolipoprotein family. During inflammation, it binds to high-density lipoproteins and alters the cholesterol metabolism. There are some studies which show that serum amyloid A may be effecting the adhesion of phagocytic cells and lymphocytes, and is responsible of their chemotaxis, causes the oxidation of high-density lipoproteins, hence playing a role in the development of inflammation in atherosclerotic coronary arteries [5]. Another serum amyloid protein is serum amyloid P. It has a pentameric structure. It comprises about 14% of the amyloid deposits. It has a role in the inflammation process [22].

Mannose binding lectin (MBL), is a member of the type C lectin super family, and is a protein, a member of natural immune system. It has a role in the first step immunity via recognition of microorganisms such as bacteria, viruses and fungi [23].

Many acute phase proteins are responsible for the initiation of inflammation and its continuation. Many of the classical complement pathway components are acute phase proteins, and have pro-inflammatory roles in the formation of immunity. Complement activation plays role in chemotaxis, increase of plasma proteins in the inflammation region, opsonization of the infectious agents and damaged cells [5].

Some acute phase proteins have anti-inflammatory properties. For example, haptoglobulin and hemopexin have antioxidant properties, and have protective roles against reactive oxygen species. Alpha 1-protease inhibitor and alpha 1-chymotrypsin inhibit proteolytic enzymes. Also, alpha 1-antichymotrypsin inhibits the production of superoxide anions. Two acute phase proteins play a role in wound healing. Fibrinogen, assists in the adhesion of endothelial cells, proliferation and dissemination, whereas, haptoglobulin helps wound healing by stimulating angiogenesis [5].

In conclusion, acute phase alterations demonstrate the presence and severity of inflammation. They have been used as a clinical guide to diagnosis and management for a long time. CRP and sedimentation are still frequently used in the clinic. Clinical studies are in progress for the use of others.

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