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Concordant Message of Different Inflammatory Markers in Patients with Rheumatoid Arthritis

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ABSTRACT

The acute phase reaction is an unspecific response to inflammatory stimuli characterized by alterations in the concentration of several plasma proteins.

It is of great clinical value to monitor the inflammatory state in patients with rheumatoid arthritis. The erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) are the assays most widely used to measure the acute phase response, but there are also several other inflammatory markers (e.g. fibrinogen, haptoglobin, α_1-acid glycoprotein, α_1-antitrypsin, interleukins (IL), serum amyloid component A (SAA)).

We have studied the interrelationships between several of these markers (ESR, Haptoglobin, Fibrinogen, CRP, SAA and IL-6) in rheumatoid arthritis patients. There was a good correlation between all acute phase markers in serum ($p<.01$). We found especially strong correlations between S-CRP and SAA ($p<.000001$) and between ESR and P-fibrinogen ($p=.000004$). The strong correlation indicates that P-fibrinogen could be used instead of ESR in monitoring rheumatoid arthritis patients. This would increase the specificity of the examination as ESR may be influenced by several factors other than the inflammatory response. There were no significant correlations between acute phase markers in serum or plasma and clinical index.

INTRODUCTION

Following tissue injury or infection, the concentrations of several plasma proteins are altered substantially (11). The characteristic pattern of this change is termed the acute phase response, and can be observed in many different inflammatory situations, including surgical trauma, injury, infections, tissue infarction and several immunologically mediated states such as temporalis

arteritis, polymyalgia rheumatica and rheumatoid arthritis. The most widely used assays for the acute phase response in humans are C-reactive protein (CRP) (14, 22) and the erythrocyte sedimentation rate (ESR) (9, 10).

Assessment of plasma (P) fibrinogen is an interesting alternative to ESR for evaluating the acute phase response as this protein has been shown to largely influence the ESR in inflammatory response (18). However, the ESR is influenced by several factors other than the inflammatory response. The sedimentation is accelerated by anaemia which may or may not be part of the inflammatory activity. ESR is also increased in patients with monoclonal proteins, renal failure, obesity and pregnancy. Postmenopausal women have higher ESR than women that still are menstruating and postmenopausal women taking oestrogen. Age and abnormal red cell morphology also influence the ESR. Thus, several factors other than the inflammatory response influence ESR.

We have compared ESR with P-fibrinogen and other acute phase markers to see how well they correlated. P-fibrinogen concentration has a large influence on the ESR during inflammation, but it is less influenced by factors in the sample other than the acute phase response. Thus, P-fibrinogen may be an interesting alternative to ESR. P-fibrinogen is also a risk factor for the development of arteriosclerosis and cardiovascular disease. Patients with inflammatory response and increased P-fibrinogen levels have an increased risk of cardiovascular disease.

MATERIALS AND METHODS

Patients

26 patients, who fulfilled the criteria of the American College of Rheumatology for rheumatoid arthritis (1), were included in the study. The patients were referred to the Department of Rheumatology, Uppsala University Hospital, due to active disease. All patients gave informed consent prior to inclusion in the study. The study was approved by the local ethics committee.

Clinical examination

Each patient was evaluated according to Ritchie index (17), Thompson index (20), Stoke index (6) and NSJ (number of swollen joints). Pain at rest was recorded using a 10 grade visual analogue scale (2). The duration of morning stiffness was also registered. All clinical

examinations were performed by the same doctor (NGA). Clinical examination and laboratory testing was performed independently.

Blood samples

Venous blood samples were collected at 7.30 am in 7 ml serum tubes (367609, Becton Dickinson, Rutherford, NJ) and in 4.5 ml EDTA-plasma tubes (367657, Becton Dickinson). Samples for IL-6 and SAA measurements were stored at -70^{0}C until analyzed.

Determination of acute phase proteins

Human haptoglobin and fibrinogen was analyzed in EDTA-plasma by rate nephelometry on a Beckman Array protein system (Beckman Instruments, Brea, CA), according to the recommendations of the manufacturer except that goat anti-human fibrinogen (Atlantic Antibodies, Stillwater, MN) was used for the fibrinogen analysis. The assay was calibrated against a human plasma standard (Behring, Marburg, Germany). Serum (S) amyloid protein A was analyzed by an ELISA (BioSource International, Inc., Camarillo, CA). CRP was measured in serum on a Hitachi 717 (Boehringer Mannheim, Mannheim Germany) according to the recommendations of the manufacturer. IL-6 was analyzed by an ELISA (R&D Systems Inc., Minneapolis, MN).

Statistical analysis

Statistical analysis was performed with Statistica 4.5 (Statsoft Inc., Tulsa, OK, USA). Spearman rank correlation was used as the material was not normal distributed. $P < 0.05$ was considered significant.

RESULTS

Interrelationships between acute phase markers

There was a strong correlation between all acute phase markers (Table 1). The strongest correlation was seen between S-CRP and SAA that both respond rapidly to inflammation. S-IL-6 is also a quick responder and correlates more closely with S-CRP and SAA than with the markers that respond more slowly.

There was a strong correlation between ESR and P-fibrinogen. This may be explained by

the influence of P-fibrinogen on ESR.

Correlation between acute phase markers and clinical score

There were no significant correlations between any of the acute phase markers and clinical score except Stoke (Table 2). The correlations with Stoke probably reflects that acute phase markers are included in Stoke.

Table 1 Correlation between different markers for acute phase response.

	ESR	S-CRP	SAA	P-Haptog	P-Fibrinog	S-IL-6
ESR	0	.000037	.000033	.008042	.000004	.007606
S-CRP	.000037	0	.000000	.028202	.000088	.000342
SAA	.000033	.000000	0	.005497	.003711	.004595
P-Haptoglobin	.008042	.028202	.005497	0	.029987	.202971
P-Fibrinogen	.000004	.000088	.003711	.029987	0	.065494
S-IL-6	.007606	.000342	.004595	.202971	.065494	0
Morning stiffness	.730848	.517469	.937602	.401542	.595845	.113735
Pain at rest	.851845	.173968	.499170	.237707	.201585	.293340
Thompson	.326779	.173700	.263025	.411270	.159329	.165778
Ritchie	.809151	.333009	.928466	.553687	.717302	.739281
Stoke	.000324	.000228	.000485	.013071	.007284	.009913
NSJ	.661948	.837882	.600724	.282984	.993534	.479587

Table 2 Correlation between different markers for acute phase response and clinical examination.

	Stiffness	Pain at rest	Thompson	Ritchie	Stoke	NSJ
ESR	.730848	.851845	.326779	.809151	.000324	.661948
S-CRP	.517469	.173968	.173700	.333009	.000228	.837882
SAA	.937602	.499170	.263025	.928466	.000485	.600724
P-Haptoglobin	.401542	.237707	.411270	.553687	.013071	.282984
P-Fibrinogen	.595845	.201585	.159329	.717302	.007284	.993534
S-IL-6	.113735	.293340	.165778	.739281	.009913	.479587
Morning stiffness	0	.244203	.601034	.136270	.247487	.833108
Pain at rest	.244203	0	.437857	.191557	.465575	.931093
Thompson	.601034	.437857	0	.036991	.007185	.000718
Ritchie	.136270	.191557	.036991	0	.076908	.000032
Stoke	.247487	.465575	.007185	.076908	0	.098694
NSJ	.833108	.931093	.000718	.000032	.098694	0

DISCUSSION

Current clinical practice relies heavily on serologic testing for accurate diagnosis of rheumatic disorders. The testing is used to support patient history and physical examination. Inflammatory markers are routinely used to monitor disease activity and response to disease-modifying drugs in rheumatoid arthritis patients. ESR and S-CRP are the most widely used assays to monitor the inflammatory state. However, which of these two tests that is recommended varies (3, 8). The ESR is influenced by several factors such as the plasma concentration of fibrinogen and other acute phase proteins, the immunoglobulin concentration, the number, size and shape of the erythrocytes and the patients age and sex. In an inflammatory reaction the ESR is mainly influenced by the P-fibrinogen concentration (18).

ESR requires a test time of at least 60 min which sometimes makes it less suited for diagnosis of inflammatory and infectious diseases. Assessment of ESR has thus often been replaced in primary care in Sweden by analysis of S-CRP which responds more rapidly to an inflammatory reaction. There are also several other acute phase proteins and cytokines that may be used to monitor inflammatory response. In the future some of these assays may replace ESR and CRP for monitoring disease activity but presently the cytokine assay is not well suited to provide rapid test results. Nephelometric or turbidimetric determination can be used to provide rapid test results at a low cost. The molecular size of fibrinogen makes it well suited for nephelometric determination, which is a rapid method suited for clinical use. P-fibrinogen has a half-life of approximately 3.5 days and the increase in P-fibrinogen concentration will remain after the inflammation has vanished. P-fibrinogen is thus less suited to follow rapid changes in the inflammatory response. On the other hand, the long half-life decreases the day to day variations making P-fibrinogen well suited to monitor chronic inflammation such as rheumatoid arthritis.

We have compared the correlation of ESR, P-haptoglobin, P-fibrinogen, S-CRP, SAA and S-IL-6 in rheumatoid arthritis patients. We found significant correlations between all the tests used. There was a very strong correlation between S-CRP and SAA ($p<.000001$). There was also a strong correlation between S-IL-6 and S-CRP. Thus SAA and S-IL-6 did not seem to add any information to S-CRP. All these assays respond rapidly to an inflammatory stimulus. There was also a good correlation between S-CRP and the acute phase proteins that respond less rapidly

(ESR, P-haptoglobin and P-fibrinogen). The strong correlation between ESR and P-fibrinogen (p=.000004) indicates that P-fibrinogen could be used instead of ESR.

Our data indicate that the different assays provide similar information. The use of several acute phase markers instead of a single assay seems not to be very cost effective. Possibly, it may be useful to have one rapid marker (S-CRP) and one that responds later. We would recommend P-fibrinogen instead of ESR as the later marker due to the higher specificity of P-fibrinogen. Every year, approximately 5,000 nephelometric fibrinogen tests in plasma are performed at the Department of Clinical Chemistry, University Hospital, Uppsala. The assay is inexpensive and can be performed rapidly. Our impression is that P-fibrinogen usually show a stronger response (in standard deviations) in patients with autoimmune disorders than P-haptoglobin, P-α_1-acid glycoprotein and P-α_1-antitrypsin.

Both S-CRP and P-fibrinogen may be of interest as cardiovascular risk markers. Ischaemic heart disease and stroke are the major causes of death in the western world. During the last decade several reports have shown a correlation between elevated levels of P-fibrinogen (4, 5, 12, 15, 21) and S-CRP (13, 16) and cardiovascular disorders. Platelet activation and thrombus formation play an important role in the development of ischemic heart disease. Today, aspirin is routinely given to patients with acute myocardial infarction and unstable angina pectoris to prevent thrombus formation. Fibrinogen may play a part in thrombus formation (7) via several mechanisms: 1. by promoting atherosclerosis, 2. as an essential component of platelet aggregation, 3. because the amount of fibrin deposited and the size of the clot are directly related to the plasma fibrinogen level and 4. because fibrinogen increases plasma viscosity (19) Patients with rheumatoid arthritis have increased serum levels of CRP and fibrinogen due to the inflammatory activity and they also have an increased risk of cardiovascular disease.
Conclusion: There are strong interrelationships between different acute phase markers in serum and plasma of rheumatoid arthritis patients. The correlations between all the acute phase markers indicate that it is usually sufficient to use one marker only.

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