

Serum levels of acute phase and cardiac proteins after myocardial infarction, surgery, and infection

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SUMMARY C-reactive protein and four other acute phase reactant proteins of non-cardiac origin (orosomuroid, α_1 -antitrypsin, haptoglobin, and α_2 -macroglobulin) were studied serially by laser immunonephelometric assay in sera from 17 patients with myocardial infarction. A similar comparison was made in 57 patients undergoing surgery and 72 patients with acute infection. C-reactive protein was consistently the most sensitive acute phase reactant in all three conditions. After myocardial infarction, a raised serum C-reactive protein level was found on admission in four patients before a rise in creatine kinase MB isoenzyme (CK MB). The peak C-reactive protein level was reached on the third post-infarct day and it then declined over seven days with a half-life similar to myocardial tropomyosin. Serial monitoring of serum C-reactive protein, in parallel with cardiac proteins of short half-life (CK MB) and long half-life (tropomyosin), provides maximal information for diagnosis and for detecting post-infarct complications.

Acute phase reactant proteins are synthesised by the liver in response to acute tissue damage. Serial estimation of these proteins, C-reactive protein in particular,¹ has been used to monitor the progress of a variety of clinical disorders and the recent introduction of rapid assays, using laser immunonephelometric,² rate immunonephelometric,³ and immunoradiometric⁴ techniques, will increase their clinical application.

The original observation⁵ that C-reactive protein could be detected in serum after myocardial infarction was followed by a number of studies using semi-quantitative immunoprecipitation techniques⁶⁻⁹ and, later, electroimmunoprecipitation¹⁰ and radial immunodiffusion¹¹ assays. These studies, and a more recent immunoradiometric assay,⁴ indicate the potential clinical value of measuring acute phase reactants in serum after myocardial infarction. There is, however, a need to determine whether C-reactive protein estimation has advantages over other acute phase proteins for cardiac studies and to investigate the time course of its rise and fall in serum after myocardial infarction in comparison with proteins of cardiac origin. Laser immunonephelometric assays of C-reactive protein and

four other acute phase proteins (orosomuroid, α_1 -antitrypsin, haptoglobin, and α_2 -macroglobulin) were therefore studied serially after myocardial infarction in comparison with a soluble, cytosolic myocardial enzyme (CK MB) and a structural protein of the cardiac myofibril (tropomyosin).¹²

Patients and methods

Sixty healthy adults, to give reference control values, and three groups of hospital in-patients were studied.

MYOCARDIAL INFARCTION

Seventeen patients were admitted to a coronary care unit with a typical history, and electrocardiographic and serum enzyme confirmation, of myocardial infarction. Patients were studied daily for 10 days using an indwelling forearm venous catheter, the time of onset of chest pain (day 1) being clearly established. Seven of the patients were studied every two hours for the first 24 hours.

SURGERY

Fifty-seven patients (33 undergoing hip replacement and 24 requiring herniorrhaphy) were admitted for elective surgery and studied on days 1 (day of operation), 4, and 8.

INFECTION

Seventy-two infected adult patients requiring admission to an infectious diseases unit were studied on days 1 to 3 of admission: all had laboratory confirmation of bacterial, viral, or protozoal (*P. vivax* malaria) infection.

ACUTE PHASE REACTANT PROTEINS

Serum specimens were deep frozen (-20°C) and assayed in batches using a Hyland Laser Nephelometer PDQTM (Travenol Laboratories, Thetford).² Polyethylene glycol of molecular weight 4000 was used for assay of C-reactive protein and haptoglobin, and of molecular weight 6000 for orosomuroid, α_1 -antitrypsin, and α_2 -macroglobulin. The following modifications of the C-reactive protein method² were required for the four other reactants.

Antiserum

Nephelometric grade goat antisera to orosomuroid, α_1 -antitrypsin, and α_2 -macroglobulin (Seward Immunostics, London), and rabbit antiserum to human haptoglobin (Dako Immunoglobulins, Copenhagen), were diluted respectively 1/100, 1/40, 1/50, and 1/100 in phosphate buffer and filtered through a $0.4\ \mu\text{m}$ Nuclepore (Pleasanton, California) polycarbonate membrane immediately before use.

Standard curves

The haptoglobin standard curve was prepared by double diluting a normal control plasma (Behringwerke, Marburg) from 1/50 to 1/1600 in 0.9% w/v sodium chloride. A normal serum pool (Seward Immunostics) was similarly diluted from 1/25 to 1/800 for orosomuroid, from 1/100 to 1/3200 for α_1 -antitrypsin, and from 1/50 to 1/1600 for α_2 -macroglobulin.

Test sera

These were diluted, in 0.9% w/v sodium chloride, 1/300 for haptoglobin and α_1 -antitrypsin, and 1/200 for orosomuroid and α_2 -macroglobulin.

Aliquots (0.1 ml) of test sera or diluted standard were added to 1 ml diluted antiserum or buffer (blank), mixed thoroughly by inversion, and incubated for one hour at room temperature before reading in the nephelometer according to the manufacturer's instructions.

CREATINE KINASE MB ISOENZYME

Creatine kinase MB isoenzyme (CK MB) was measured at 25°C using N-acetyl cysteine-activated, immunological assay kits from Boehringer Corporation (London) Ltd. Precipath E control sera (Boehringer, Mannheim) containing known levels of CK MB isoenzyme were used to standardise the assay and the upper limit of normal was taken as 8 IU/l.¹³

HUMAN CARDIAC TROPOMYOSIN

Serum cardiac tropomyosin was measured in triplicate by radioimmunoassay¹² with an upper limit of normal of 5 ng/ml.

Results

The mean (\pm SD) values for 60 healthy adults for the five acute phase reactant proteins were as follows: C-reactive protein all values below 7 mg/l, orosomuroid $0.8 (\pm 0.3)$ g/l, α_1 -antitrypsin $2.9 (\pm 0.6)$ g/l, haptoglobin $2.0 (\pm 1.1)$ g/l, and α_2 -macroglobulin $1.7 (\pm 1.0)$ g/l. The daily median serum levels for the 10 days after myocardial infarction in 17 patients are shown in Fig. 1 and 2. Haptoglobin and α_1 -antitrypsin levels rose to a peak (Fig. 1) at days 5 to 6, α_2 -macroglobulin did not reach a peak until day 8, and orosomuroid showed little change. On day 4 (Table), these four proteins showed a rise in mean value after myocardial infarction of 33 to 127% above the mean level (expressed as 100%) for healthy adults. In contrast, C-reactive protein reached a peak level (for both mean and median) on day 3 (Fig. 2), the mean level being 1570% above the upper limit of the controls (7 mg/l); the corresponding day 4 mean value (Table) was increased by 1357%.

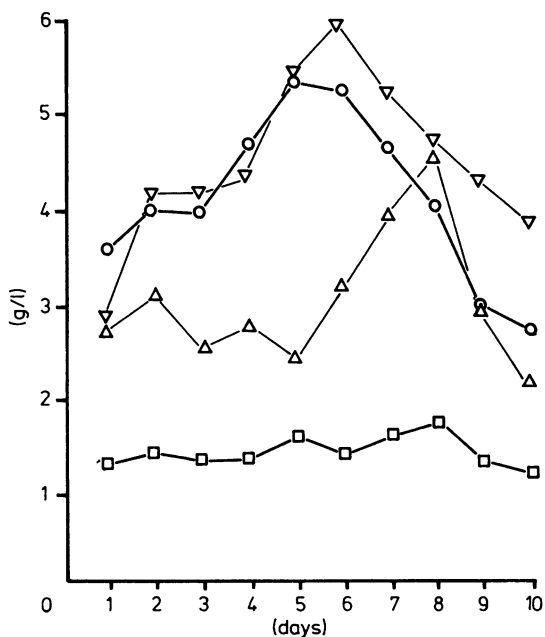


Fig. 1 Daily median values for four acute phase reactants (orosomuroid - □; α_1 -antitrypsin - ▽; haptoglobin - ○; α_2 -macroglobulin - △) during the 10 days after myocardial infarction in 17 patients.

Table Percentage change in mean serum level of five acute phase reactants after myocardial infarction (day 4), surgery (day 4), and infection (peak of days 1 to 3) in relation to mean (expressed as 100%) for 60 healthy controls: number of patients studied in parentheses

	Myocardial infarction (17)	Hip replacement (33)	Hernia repair (24)	Infection		
				Bacterial (31)	Viral (27)	Malaria (14)
C-reactive protein	+1357	+2771	+1001	+2069	+854	+1580
Orosomucoid	+ 101	+ 122	+ 90	+ 248	+225	+ 188
Alpha ₁ -antitrypsin	+ 33	+ 126	+ 34	+ 78	+ .18	+ 31
Haptoglobin	+ 127	+ 179	+ 72	+ 168	+ 96	- 18
Alpha ₂ -macroglobulin	+ 77	+ 146	+ 34	+ 126	+116	+ 132

This large differential between the magnitude of the C-reactive protein response and those of the four other reactants was also found after hip replacement, herniorrhaphy, and infection (Table), with viral infection showing the smallest C-reactive protein response relative to the others. Hip replacement caused the largest response for each of the five reactants. Haptoglobin, in patients with malaria, was the only reactant to fall below the mean normal value, presumably a consequence of haemolysis.

The time-scale and magnitude of the C-reactive protein response after myocardial infarction was compared with those of CK MB and tropomyosin (Fig. 2). Raised serum levels of CK MB and tropomyosin are usually first detectable seven to eight hours after the onset of symptoms.¹² Raised C-reactive protein levels of 25, 110, and 130 mg/l were detected in the first blood sample tested on admission (four hours from pain

onset), when CK MB was still normal, in three of the seven patients studied every two hours. There was no obvious explanation for this presumed pre-admission rise in serum C-reactive protein. Two of the remaining four patients, however, showed little or no increase in C-reactive protein during the first 24 hours when the CK MB level was already significantly raised. The median values for all 17 patients, however, showed that CK MB and tropomyosin reached their peak serum levels earlier than C-reactive protein (Fig. 2). The median serum CK MB level fell to normal within three days while tropomyosin fell to the upper limit of normal on day 8, when the C-reactive protein level was still raised.

Discussion

Of the five acute phase reactant proteins, C-reactive protein was clearly the most responsive, and therefore sensitive, to tissue damage caused by myocardial infarction. A similar situation was found within the four day period after operation and acute infection, with the increase in mean C-reactive protein level averaging 1605% for all 146 patients studied (Table) compared with average increases of 162% for orosomucoid, 53% for alpha₁-antitrypsin, 104% for haptoglobin, and 105% for alpha₂-macroglobulin. The observed late increase (day 8) in serum level of alpha₂-macroglobulin is in contrast to earlier studies.^{14 15} Apart from the smaller (854%) C-reactive protein increase in viral infection and the fall (-18%) in haptoglobin in *P. vivax* malaria, there was little evidence that the different types of tissue damage produced significantly different patterns of acute phase response. The extent of tissue damage was probably the main determinant of the peak serum level since the peak for each of the five reactants was between 1.4 to 4.3 times greater after hip replacement than herniorrhaphy.

Earlier studies showed that C-reactive protein was first detectable in serum one to two days after myocardial infarction but an increase in serum level one to two hours after chest pain has been found more recently by radial immunodiffusion assay.¹¹ We found a pre-

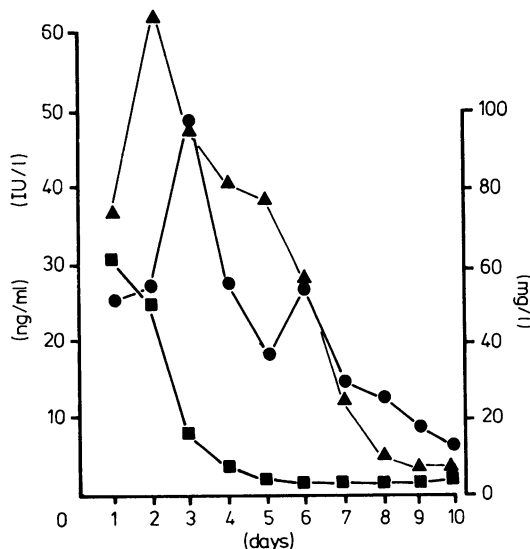


Fig. 2 Daily median values for C-reactive protein (●) in mg/l, creatine kinase MB (■) in IU/l, and cardiac tropomyosin (▲) in ng/ml after myocardial infarction in the same 17 patients as in Fig. 1.

existing increase at four hours in three of seven patients and this occurred before any rise in CK MB. Study of a larger number of patients in the immediate post-infarction period is therefore indicated. Thereafter, the mean C-reactive protein level rose in all 17 patients to a peak on day 3. This compares with a peak level at 50.5 hours in a recent study of 33 patients in whom the peak CK MB level occurred at 14.8 hours.⁴

A rise in serum C-reactive protein appears to be a sensitive test for myocardial necrosis and has been detected in 49 of 50 patients with acute infarction,⁹ in 100 patients with infarction whenever there were significant Q wave electrocardiographic changes,⁸ and in all 33 patients with myocardial infarction studied serially.⁴ While these results indicate good sensitivity, it is necessary to exclude obvious alternative causes of a raised serum C-reactive protein level (for example surgery, infection, arthritis) if the test is to have acceptable specificity; if this is not done, specificity will be no better than that of the erythrocyte sedimentation rate.¹⁶ Specificity for myocardial necrosis does require to be investigated further since a few patients with congestive cardiac failure¹⁷ and unstable angina,¹¹ but not angina associated with exercise testing,⁴ have shown small rises in serum level. The evidence that venous thrombosis causes a false positive rise in serum C-reactive protein is, however, anecdotal.^{6 8 17 18} Out of 29 hip replacement patients, 25 had venogram confirmed leg vein thrombosis by day 4; with the exception of three patients with extensive thrombosis and very high C-reactive protein values (275, 310, and 350 mg/l), all the others had C-reactive protein values between 125 to 258 mg/l irrespective of the extent, or absence, of thrombosis. The C-reactive protein level on day 8 also failed to differentiate patients according to extent or absence of thrombosis.

There is considerable potential value, for the assessment of myocardial necrosis, in studying proteins released from different tissue sites and with a different time scale of rise and fall in the plasma. The three proteins studied (CK MB, tropomyosin, and C-reactive protein) reached a peak level on days 1 to 3 and, while CK MB fell to normal within three days, both tropomyosin and C-reactive protein showed a more protracted fall. Myosin, the major contractile protein, has been shown to have a similar time course of release, in patients with myocardial infarction, to that of CK MB,¹⁹ though, in experimental infarction in the dog, the myosin level remains high for at least seven days.²⁰ Tropomyosin, the regulatory myofibril protein, shows a slower rate of fall which may be affected by recurrent infarction.¹² Parallel study of the similar half-lives of tropomyosin and C-reactive protein in complicated and uncomplicated myocardial infarction would therefore be of particular value, in view of their different tissues of origin, in differentiating between cardiac and non-

cardiac complications. As previously shown,⁴ a raised C-reactive protein level but normal CK MB in patients with chest pain of less than three days' duration indicates a pathological process other than myocardial infarction. Similarly, after the third day, when the CK MB level is no longer of diagnostic value, a raised C-reactive protein level but normal serum tropomyosin indicates non-cardiac tissue damage. After uncomplicated myocardial infarction, both C-reactive protein and tropomyosin should decline after the third day with similar half-lives, and failure of the fall in C-reactive protein to match that of tropomyosin therefore indicates an intercurrent non-cardiac complication which requires investigation. Serial monitoring of all three proteins thus provides maximal information for diagnostic purposes and in confirming post-infarct recovery; this approach is recommended for both clinical trial purposes and for individual patient care.

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