C-reactive protein for rapid diagnosis of infection in leukaemia

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SUMMARY C-reactive protein, measured in serum from 38 patients with leukaemia, was elevated to at least 100 mg/l at the beginning of 32 of 34 episodes of infection, and subsequently rose above 100 mg/l in all 34. Uninfected patients, whether in leukaemic remission or relapse and whether pyrexial or not, always had levels below 100 mg/l, with four exceptions out of 290 measurements. Estimation of two other acute-phase proteins, α_1 -antitrypsin and orosomucoid, was not of additional diagnostic value. Serial measurement of C-reactive protein may be important for the early detection of infection in the leukaemic patient with neutropenia.

Infection is a common cause of death in acute leukaemia. Its early detection is difficult, however, since clinical signs are often masked by neutropenia, and bacteriological culture usually requires 24 hours. Rapid screening tests for infection, such as neutrophil cytochemistry or the Limulus test for endotoxaemia, have not yet proved sufficiently reliable when applied to the individual patient (Martinez-G *et al.*, 1973, Steigbigel *et al.*, 1974; Mackie *et al.*, 1979).

The acute-phase protein response to infection is rapid enough to be of potential value as a screening test, since the level of C-reactive protein (C-RP) may rise some 6 hours after an acute stimulus (Kushner and Broder, 1972; Yen-Watson and Kushner, 1974). If this early rise occurs reliably in response to infection, then serial measurement would be of potentially greater clinical value than bacteriological culture for early diagnosis. The present study was therefore designed to assess the value of three acute-phase proteins (C-RP, α_1 -antitrypsin, and orosomucoid) as screening tests for infection in leukaemia.

Patients and methods

Thirty-eight patients with leukaemia (20 acute myeloblastic, five chronic granulocytic, six acute lymphoblastic, and seven chronic lymphocytic) were studied. Blood was taken at outpatient

attendances when the patients were also examined for clinical signs of infection and samples were taken for bacteriological culture when relevant. Blood samples from inpatients were taken at least three times a week with additional specimens during pyrexia. Inpatients were examined for infection at least once daily, and samples for culture were taken whenever infection was suspected; in addition, swabs from the nose, throat, axillae, and perineum were cultured at least weekly.

Patients with a clinical diagnosis of infection were divided into two groups: patients from whom pathogens could not be isolated on microbiological culture, and patients with microbiological confirmation of infection. Patients with neither clinical nor microbiological evidence of infection were grouped into those in leukaemic remission and those in relapse.

Blood samples were centrifuged, and the serum was stored at 4° in sterile containers to which molar sodium azide solution (0.05 ml per 5 ml serum) had been added. Levels of C-RP, α_1 -antitrypsin, and orosomucoid were subsequently measured by radial immunodiffusion (Mancini *et al.*, 1965) using commercial antisera (Seward Immunostics) for α_1 -antitrypsin and orosomucoid and an antiserum raised to purified human C-RP. The latter antigen was isolated from human ascitic fluid, by a modification of the method of Hokama and Riley (1963), followed by gel filtration on Sephadex G-200 and then concentrated to give a reference preparation. This material was used in rabbits to raise an anti-

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serum which was treated with solid-phase human serum immunoabsorbent to ensure monospecificity. A secondary human serum C-RP standard, equivalent to 62.5 mg/l, was calibrated against the reference preparation. In the radial immunodiffusion method, a calibration curve (range 12.5-62.5 mg/l) was prepared using 10 μ l aliquots of secondary standard.

Results

There were 17 episodes in which a diagnosis of infection was clear on clinical grounds but from which material for microbiological culture either could not be obtained or yielded no pathogens as a result of previous antibiotic therapy. These episodes included chest infection (7) and cellulitis and/or tissue necrosis (10). In a further 17 episodes of infection, positive cultures were obtained (Table). The level of acute-phase proteins in the first blood sample obtained during each infective episode, together with the levels found in uninfected patients, are shown in Figures 1-3.

Table 17 culture-positive episodes of infection

Site	Organism	No. of episodes
Blood	<i>Escherichia coli</i>	1
	<i>Bacteroides</i> sp.	1
	<i>Pseudomonas aeruginosa</i>	1
Upper respiratory tract	β -haemolytic streptococcus	3
	<i>Streptococcus viridans</i>	1
	<i>Streptococcus faecalis</i>	1
	<i>Klebsiella</i> sp.	1
	<i>Candida albicans</i>	1
Lower respiratory tract	<i>Streptococcus pneumoniae</i>	1
	<i>Klebsiella</i> sp.	1
	<i>Candida albicans</i>	2
	<i>Pseudomonas aeruginosa</i>	1
Urinary tract	<i>Staphylococcus aureus</i>	1
Thigh abscess	<i>Herpes simplex</i>	1
Facial vesicles		1
	Total	17

Levels of α_1 -antitrypsin at the beginning of the 34 episodes of infection, whether confirmed microbiologically or not, were significantly higher ($P < 0.001$, Kolmogorov-Smirnov two-sample test (Siegel, 1956)) than the levels for all uninfected patients whether in leukaemic remission or relapse (115 observations) (Fig. 1). There was considerable overlap, however, some levels at the beginning of infection falling within the normal range.

For orosomucoid (Fig. 2), levels at the beginning of infection were again significantly higher ($P < 0.001$) than in uninfected patients. The first value obtained in each infective episode was always, with one exception, elevated above the normal range, but there was still considerable overlap between the groups.

Fig. 1 Individual values for α_1 -antitrypsin in uninfected and infected patients. Number of estimations in parentheses. Normal range shown by hatching.

For C-RP, however, there was minimal overlap between infected and uninfected patients (Fig. 3). The latter, whether in leukaemic remission or relapse, had C-RP levels below 100 mg/l, with only four exceptions out of 290 measurements; the maximum C-RP level was 110 mg/l. C-RP levels showed no correlation with the degree of peripheral blood leucocytosis (in chronic leukaemia) or blast cell count (in acute leukaemia). C-RP was measured more frequently than the other two acute-phase proteins to confirm lack of overlap between infected and uninfected patients. Conversely, in infection, levels of 100 mg/l or above were obtained in the first blood specimen taken, with only two exceptions out of 34 infective episodes. In both of these cases C-RP levels exceeded 100 mg/l on the next day. Resolution of infection was accompanied by a fall in the C-RP level over three to four days.

Discussion

This study suggests that a C-RP level above 100 mg/l may be a useful early indicator of infection in leukaemic patients. There is an interval of about 6 hours between a stimulus, such as the onset of acute inflammation or infection, and elevation of C-RP

Fig. 2 Individual values for orosomucoid in uninfected and infected patients. Number of estimations in parentheses. Normal range shown by hatching.

Rapport *et al.*, 1957; Yen-Watson and Kushner, 1974). Nevertheless, in our study, the first serum sample taken in each infective episode had a C-RP level that was nearly always elevated above 100 mg/l. Presumably there is a latent period of several hours between the onset of infection and the development of clinical signs, C-RP levels increasing significantly during this interval. The exact time sequence for the development of pyrexia, clinical evidence of infection, and elevation of C-RP above 100 mg/l requires to be determined by prospective study. Peak levels of C-RP were not attained until at least 24 hours from the time of the initial blood specimen, and, during this period, other objective data supporting a diagnosis of infection, such as positive microbiological cultures or typical radiological features, usually became available.

C-RP levels are probably raised in any inflammatory process, whether infective or not. It is unlikely that the coexistence of a subclinical inflammatory disease would cause a diagnostically confusing elevation in C-RP level. In a study of 187 patients with rheumatoid arthritis, McConkey *et al.* (1972) showed that the level of C-RP exceeded 100 mg/l only in those patients with severe disease, which would be evident clinically. Many of our

Fig. 3 Mean values $\pm$ 2 SD for C-RP in uninfected patients and individual values in infected patients. Number of estimations in parentheses. Normal range 0-10 mg/l.

uninfected patients were receiving cytotoxic drugs, antibiotics, or blood products, which were followed in some cases by pyrexia and rigors. Nevertheless, with four exceptions out of 290 measurements, these patients had C-RP levels below 100 mg/l. The acute-phase response to infection, however, was quantitatively much greater and also occurred in those infected patients whose inflammatory response was clinically masked by severe neutropenia. Measurement of α_1 -antitrypsin and orosomucoid, which are not elevated until 24-48 hours after an acute stimulus (Crockson *et al.*, 1966; Werner, 1969), gave no additional information.

C-RP was measured by radial immunodiffusion with a between-batch variability of 10%; a level of 90-110 mg/l may therefore be regarded as equivocal evidence of infection. This protein may alternatively be measured within an hour by laser nephelometry, and a preliminary study by us on 87 sera has shown good correlation ($r = 0.964$) with the 18-hour radial immunodiffusion technique. Daily monitoring of C-RP by laser nephelometry may therefore provide a rapid test for infection in the neutropenic, leukaemic patient and thereby guide the use of antibiotics and granulocyte transfusions. Daily measurement of C-RP may also prove to be of diagnostic value in patients whose blood levels have

substantially increased but are still below the diagnostic level of 100 mg/l.

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