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Serum C-reactive protein and neopterin concentrations in patients with viral or bacterial infection

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Abstract

Serum C-reactive protein and neopterin concentrations were measured in samples taken at an early stage in different types of infection to see whether the combination of markers could contribute to the diagnosis of infection and help distinguish between bacterial and viral infections, tuberculosis, and infections due to "other" pathogens. Both markers were significantly raised in all categories of infection compared with controls, and there were significant differences between the means of both markers when comparing several of the categories of infection. Only C-reactive protein concentrations in bacteraemic patients, however, were both sensitive and specific at distinguishing the type of infection. The additional use of neopterin estimation played only a minor part in increasing the specificity of diagnosis in tuberculosis and in viral infections.

On the basis of this study it was not considered worth the time and expense of performing neopterin assays in addition to C-reactive protein estimations to differentiate viral from bacterial infection.

Serum C-reactive protein and neopterin concentrations have been used as non-specific indicators of infection, as well as of other conditions including various inflammatory and malignant disorders. C-reactive protein is produced by hepatocytes[1] and is normally present as a trace constituent of the plasma, the median value in healthy adults being 0·8 mg/l, with 90% having less than 3 mg/l and 99% less than 10 mg/l.[2] The rate of synthesis increases as part of the acute phase response within hours of acute injury, inflammation, or infection,[3] reaching peak concentrations within 24–48 hours.[4] C-reactive protein has been used to aid the diagnosis of infection and to distinguish bacterial from viral infections in various patient groups including children,[5–7] those with leukaemia,[8–10] and adults with bacteraemia.[11–12] Bacterial infections tend to be associated with high C-reactive protein concentrations, often more than 100 mg/l, and viral infections with lower concentrations, frequently less than 10 mg/l. Concentrations are usually less than 100 mg/l in tuberculosis but vary with severity of disease.[13]

Neopterin (6-D-erythro-hydroxy propyl pteridine) is synthesised by macrophages stimulated by γ interferon produced by T lymphocytes[14] and has thus been used as a measure of both macrophage function and cell medicated immunity.[15] Its upper limit of normal in healthy adults is 9–10 nmol/l. It is raised in viral infections,[16] fatal septicaemias,[17] and tuberculosis.[18] The rate of rise is slower than that of C-reactive protein, peaking at 9–12 days after contraction of viral infection or after vaccination with live virus.[19]

It was expected from these studies that the concentrations and ratios of the two markers would differ noticeably in bacterial, viral, and other infections, and that the combination could be more discriminatory than the use of C-reactive protein estimation alone. Such an indication of the type of infecting agent could be of value to clinicians by giving an additional guide to the early management of the patients, such as whether antibiotics need to be given.

Methods

Serum C-reactive protein and neopterin concentrations were measured in 88 patients with infections caused by a variety of different organisms, and in healthy age and sex matched controls. For comparison with concentrations in non-infective inflammatory conditions a random group of 14 patients with rheumatoid factor positive arthritis were also tested (table 1).

Table 1 Numbers of patients

Acute bacteraemias	(n = 30)	
Pneumococci		9
S aureus		5
Streptococci		3
Escherichia coli		7
Klebsiella sp		2
Pseudomonas sp		2
Salmonella sp		1
Meningococcus		1
Tuberculosis	(n = 13)	
Viral infections	(n = 32)	
Hepatitis A		16
Cytomegalovirus		2
Epstein-Barr virus		5
Rubella		4
Parvovirus B19		5
"Other" infections	(n = 13)	
Chlamydia psittaci		6
Mycoplasma pneumoniae		4
Coxiella burnetti		1
Toxoplasma gondii		2
Total numbers of infections	88	
Controls	88	
Rheumatoid arthritis	14	

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The infected patients were an unselected cohort all of whose samples had been processed by the microbiology laboratory. Only one of these (a leukaemic patient with bacteraemia) was known to be immunosuppressed. All the bacteraemic patients and 61·5% of the tuberculous patients were inpatients. All the patients with viral or other infections had been seen as outpatients or by general practitioners.

The 88 age and sex matched controls comprised 59 blood donors attending the North London Blood Transfusion Clinic, nine members of staff at the Central Middlesex Hospital from whom blood samples had been taken to test for rubella immunity, and 20 elderly patients from whom blood samples had been taken for syphilis screening (all negative) because of symptoms of deafness or dementia, but who were otherwise healthy. None of the controls had evidence of infection, malignancy, or inflammatory disease.

Most serum samples from bacteraemic and tuberculous patients had been sent to the microbiology laboratory for other tests, such as viral antibodies or gentamicin assays. The samples used were taken on the same day, or as soon as possible, as the specimen from which the diagnosis had been made (blood culture or sputum). The average time that samples were taken from bacteraemic patients was 2·4 days after the positive blood culture. For tuberculous patients the average number of days after the positive specimen was 0. For viral and "other" conditions serum samples from which the diagnosis had been made were used. Where both acute and convalescent samples had been sent, the acute sample was used.

Sera were stored at −40°C after being divided into two separate tubes for the two assays.

C-reactive protein was measured by rate nephelometry using a Beckman Auto ICS machine.[20] Neopterin was measured by radioimmunoassay using IMMUtest Neopterin (Henning, Berlin).[21] The coefficient of variation for C-reactive protein assay was 2·75% intrabatch, 3·69% inter-batch. For the neopterin assay the coefficient of variation was 12·5% and 8·6%, respectively.

Results

The C-reactive protein and neopterin concentrations in the different patient categories are shown in figs 1 and 2.

The means of both markers in all categories of infections were higher than the mean controls for each category. These differences were significant by matched Student's *t* tests, performed on the natural logarithms of the values ($p < 0·01$).

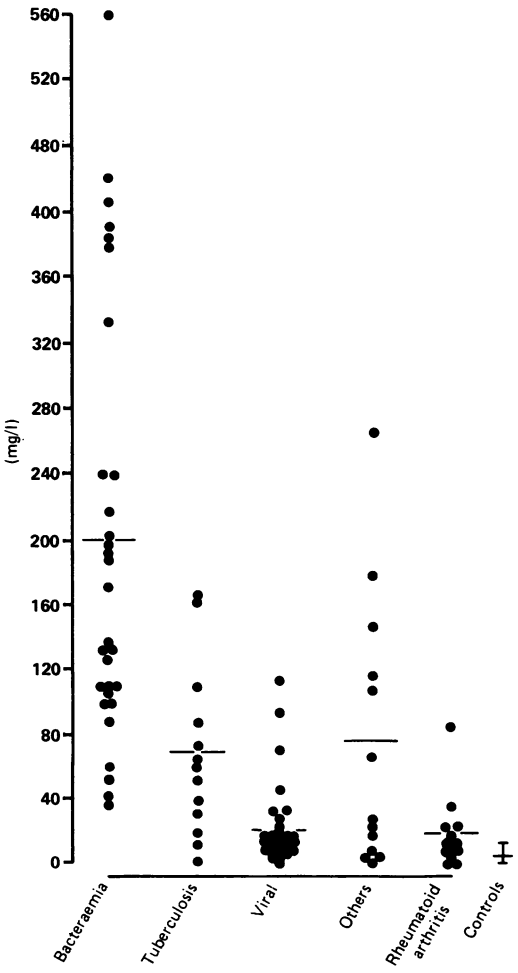

Figure 1 C-reactive protein concentrations.

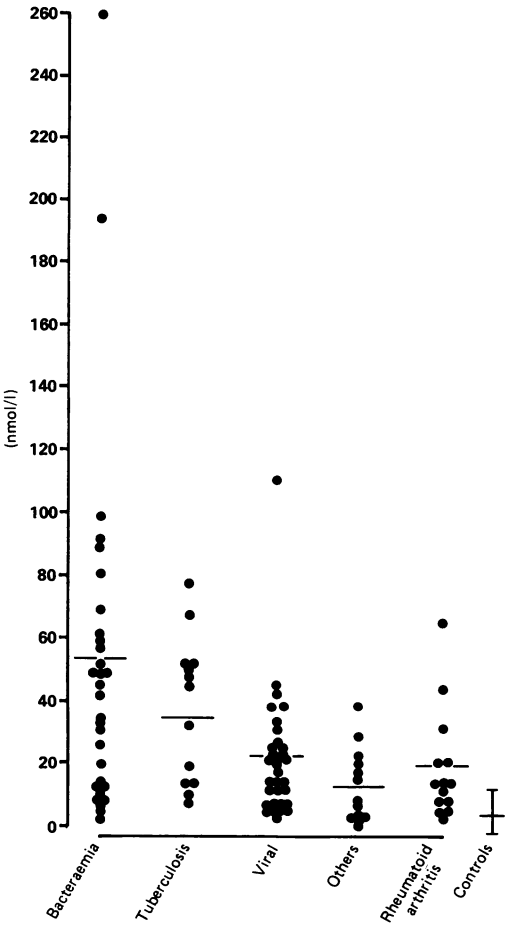

Figure 2 Neopterin concentrations.

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Table 2 Mean C-reactive protein and neopterin concentrations compared between different categories of patients by unmatched t tests

	Bacteraemic	*Tuberculous*	*Viral*	*"Others"*	*Rheumatoid arthritis*
C-reactive protein:					
Bacteraemic		$t = 4{\cdot}61$ $p < {\cdot}0005$	$t = 12{\cdot}02$ $p < {\cdot}0005$	$t = 5{\cdot}85$ $p < {\cdot}0005$	$t = 9{\cdot}58$ $p < {\cdot}00005$
Tuberculous			$t = 3{\cdot}78$ $p < {\cdot}0005$	$t = 1{\cdot}04$ NS	$t = 5{\cdot}56$ $p < {\cdot}0005$
Viral				$t = 1{\cdot}08$ NS	$t = 1{\cdot}89$ NS
"Others"					$t = 1{\cdot}98$ NS
Neopterin:					
Bacteraemic		$t = 0{\cdot}76$ NS	$t = 3{\cdot}6$ $p < {\cdot}0005$	$t = 3{\cdot}05$ $p < {\cdot}005$	$t = 2{\cdot}78$ $p < {\cdot}005$
Tuberculous			$t = 2{\cdot}16$ NS	$t = 3{\cdot}0$ $p < {\cdot}005$	$t = 5{\cdot}27$ $p < {\cdot}0005$
Viral				$t = 1{\cdot}6$ NS	$t = 0{\cdot}04$ NS
"Others"					$t = 1{\cdot}30$ NS

NS = Not significant at $p = 0{\cdot}01$.

When the means of logged C-reactive protein and neopterin concentrations for each category were compared with each other by unmatched *t* tests, there was a significant difference of $<0{\cdot}0005$ between the mean C-reactive protein of bacteraemic patients and all the other categories, and between the mean C-reactive protein of the tuberculous and viral categories (table 2). Differences in mean neopterin concentrations were significant ($p < 0{\cdot}005$) between bacteraemias and all other categories except tuberculous and between tuberculous and "others".

There was no significant difference at $p = 0{\cdot}01$ between either the mean logged C-reactive protein or neopterin concentrations in Gram negative compared with Gram positive bacteraemias.

The coefficient of correlation of all logged C-reactive protein and neopterin concentrations of infected patients was significant ($r = 0{\cdot}536$), suggesting that the C-reactive protein and neopterin values do not give completely independent information.

The ratios of C-reactive protein (mg/l) to neopterin (nmol/l) calculated from the logged data tended to be higher in bacteraemic than viral infections, a ratio of less than 1 being a contraindication for bacteraemia (table 3).

Using the values in the 88 infected patients and the 14 rheumatoid factor positive patients, a value of more than 100 mg/l C-reactive protein was associated with bacteraemia with a clinical sensitivity of 80% and a specificity of 87·5%. The predictive value of a C-reactive protein of more than 100 mg/l for bacteraemia was 73·5%.

Table 3 Ratios of natural logarithmic values of C-reactive protein (mg/l) and neopterin (nmol/l) in different categories of patients

	Mean ratio	*SD*	*No with ratio of less than 1*
Bacteraemias	5·23	4·72	0%
Tuberculosis	2·19	1·32	15·4%
Viral	2·19	3·3	48·5%
"Others"	5·9	5·8	23·1%
Rheumatoid	1·38	1·57	64%

A C-reactive protein of 40–100 mg/l was 46% sensitive and 88·8% specific for tuberculosis, with a predictive value of 40%, and a C-reactive protein of 10–40 mg/l was 68·7% sensitive and 84·3% specific for viral infections, predictive value 66·6% (81·5% if rheumatoid positive patients were excluded).

The neopterin concentrations did not contribute to the diagnosis of bacteraemia, but a combination of ranges of C-reactive protein and neopterin values could improve the specificity of diagnosis in tuberculous and viral infections. Thus a C-reactive protein of less than 100 mg/l with a neopterin concentration of more than 40 nmol/l was 95·7% specific for tuberculosis, although only 31% sensitive. The predictive value was 50% (66·6% if rheumatoid factor positive patients were excluded). A C-reactive protein of 10–40 mg/l with a neopterin concentration of 10–40 nmol/l was 92·5% specific for viral infections (100% if rheumatoid factor positive patients were excluded), but the sensitivity was only 43·8%. The predictive value was 73·7% (100% if rheumatoid factor positive patients were excluded). The concentrations in "other" infections were too diverse to have any typical combinations of concentrations.

Discussion

On the basis of previous studies it was anticipated that neopterin concentrations would be raised significantly more in patients with viral infections than in bacteraemic patients, as opposed to the tendency for C-reactive protein to be higher in bacteraemia. This did not prove to be the case, however, although there was a tendency for the ratio of C-reactive protein to neopterin concentrations to be higher in bacterial than in viral infections. This finding agrees with Myara's study,[22] which compared C-reactive protein and neopterin concentrations in postoperative infections in heart transplant recipients. The authors found that when serum C-reactive protein and/or neopterin concentrations were high, a ratio of C-reactive protein:neopterin of more than 1 was observed in all bacterial infections and a ratio of less than 1 during viral infections and rejection episodes. They did find that the neopterin concentration was much higher, however, in viral infections than in bacterial infections, unlike this present study. Their values were presumably peak concentrations, which could only be found by testing daily samples. This would be impractical except in specialist units, and waiting for the peak concentration to be reached before calculating the ratio could impose an unnecessary delay on diagnosis.

In our study samples taken soon after the onset of bacteraemia could readily be obtained, but less frequently so after viral or "other" infections, which tended to be investigated at a later stage in the illness. Although samples were taken soon after diagnosis in tuberculous patients, the patients may have been infected for some time before a diagnostic specimen was taken. These are the times, however, at which patients present with, or are investigated for,

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these illnesses, therefore it is practical to compare single samples which could be taken to aid diagnosis at this time.

Although C-reactive protein concentrations peak early in infection, neopterin concentrations peak later, so should still have been raised in most samples in the viral and "other" categories at the time they were taken, assuming the illness affects neopterin concentrations.

The wide range of concentrations measured in each category could not be explained by the timing of samples alone. There seemed to be little correlation between either the C-reactive protein or neopterin concentrations and the severity of bacteraemia, although unlike Strohmaier's study,[17] all the patients survived. Both markers, however, were related to the severity of illness in tuberculosis and rheumatoid arthritis, in accordance with findings from other studies.[13 18 23] The wide spread of concentrations found in viral infections and in the group affected by "other" pathogens might be expected to be partly related to the variety of different pathogens and resulting disease processes. In viral infections the differences between groups of organisms were not noticeable. For example, the mean C-reactive protein and neopterin concentrations for rubella and parvovirus infections together were 21·4 mg/l and 13·5 nmol/l, respectively; for Epstein-Barr virus and cytomegalovirus infections the means were 39·7 mg/l and 20·6 nmol/l. In the "other" category the resulting disease processes could have influenced the concentrations. For example, the mean C-reactive protein in the two toxoplasma infections was only 4·5 mg/l compared with a mean of 69·9 mg/l for the mycoplasma, psittacosis, and coxiella infections combined, these three organisms causing pneumonia.

All categories of infected patients had significantly raised C-reactive protein and neopterin concentrations compared with the age and sex matched controls, so they could both be used to ascertain whether an infective or inflammatory process is going on. It may not be possible, however, to distinguish, for example, a viral infection and rheumatoid arthritis using these markers.

Only C-reactive protein concentrations were both sensitive and specific at distinguishing bacteraemias from the other categories of infection. Mean neopterin concentrations alone showed a significant difference between bacterial and viral infections, but in the opposite way to that expected from other studies. The addition of neopterin to C-reactive protein concentrations contributed little to the diagnosis of bacteraemia, their only role being to increase the specificity and predictive value in the diagnosis of viral infections and tuberculosis, but at the expense of sensitivity.

In conclusion, it was not considered worth the expense and inconvenience of performing estimations of neopterin concentrations in addition to those of C-reactive protein to aid the diagnosis of infection, especially using radioimmunoassay kits with a short shelf-life, which would make routine testing impractical.

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