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The Limitations and Usefulness of C-Reactive Protein and Elastase-α_1-Proteinase Inhibitor Complexes as Analytes in the Diagnosis and Follow-up of Sepsis in Newborns and Adults

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Summary: C-reactive protein and elastase-α_1-proteinase inhibitor complexes were compared in the diagnosis of neonatal sepsis and bacterial infections in adults on the intensive care unit. Both analytes were measured in the same sample immediately after receipt.

EDTA-plasma samples (n = 115) from 28 neonates (gestational age 29–42 weeks) within the first 72 hours of life with suspected neonatal sepsis, 2 babies between 14 and 28 days old with B-*streptococcus* infections and 28 adults on the intensive care unit with positive bacterial cultures were analysed for both analytes. Two adults with long-term infections were followed up over a period of 28 and 65 days respectively.

The results showed that in 17 cases of confirmed neonatal sepsis within the first 24 hours of life, c-reactive protein levels were undetectable in 16 cases, one level of 13 mg/l being recorded. All had elevated elastase-α_1-proteinase inhibitor concentrations. Of the remaining 15 samples, 13 were normal and 2 were borderline for this analyte. C-reactive protein levels were between 5 and 10 mg/l in 5 cases and undectable in the remaining 10 samples. Those neonates with detectable c-reactive protein levels were between 20 and 72 hours old with a gestational age greater than 31 weeks. C-reactive protein was undetectable in samples taken at the same time interval after birth from full-terms babies with a gestational age of 41–42 weeks, even in confirmed cases of neonatal sepsis.

In adults, 64/68 samples showed both elevated c-reactive protein and elevated elastase-α_1-proteinase inhibitor, the remaining 4 samples being elevated for c-reactive protein and normal for elastase-α_1-proteinase inhibitor. Three of these four patients had a pronounced leukopenia. Although the diagnostic value of a single determination of either analyte was sufficient and similar in both cases, the time course of each was different during the course of illness and treatment, so that the correlation between c-reactive protein and elastase-α_1-proteinase inhibitor, although statistically significant ($p = 0.01$) was relatively low ($r = 0.312$, $n = 115$).

From the results it can be shown that the analyte of choice for neonatal sepsis within the first 3 days of life is elastase-α_1-proteinase inhibitor. In older infants and adults, c-reactive protein is preferable, as it is quicker to determine, can be performed in most clinical chemistry laboratories and is of similar diagnostic value.

Introduction

In addition to their widespread use in the diagnosis of bacterial infection, c-reactive protein (1–5) and elastase-α_1-proteinase inhibitor complexes (6–10) also have their place in the follow-up of patients.

C-reactive protein belongs to the pentraxines, a group of non-specific defence proteins found in all vertebrates. It consists of five identical non-glycosylated subunits of M_r 21 000 and binds to the galactan residues of microorganisms causing agglutination and precipitation, followed by classical activation of the complement system.

Increases in serum c-reactive protein are seen within a few hours after infection.

Elastase is a proteinase secreted from polymorphic neutrophils and is neutralised by complexing with α_1-proteinase inhibitor (α_1-antitrypsin), so that its activity is normally localised at the site of polymorphic neutrophil interaction with bacteria, usually, but not always, in the lung. Elastase also complexes with α_2-macroglobulin. In this case, the elastase retains its enzymatic activity, but is enclosed by the α_2-macroglobulin, thus restricting the active substrates to those small enough to penetrate into the complex.

Although many textbooks refer to the usefulness of c-reactive protein in diagnosing bacterial infection (11, 12), it is surprising to find reference values for newborns which are higher than those for adults (12). It is known, that c-reactive protein synthesis in newborn rabbits is not mature (13), and that serum c-reactive protein levels in preterm infants with (14) and without (15) infection increase over the first 48 hours after birth. Several groups have noted the absence of a c-reactive protein response in many cases of neonatal sepsis (16–17). Other groups report the usefulness of c-reactive protein in diagnosing neonatal sepsis (18–19) even on the first day after birth.

On the other hand, elastase-α_1-proteinase inhibitor complexes are suitable for the diagnosis and therapy control of sepsis at all ages, including neonates (20–22). Whether this is practicable is discussed below.

Materials and Methods

C-reactive protein was measured using the Behring Turbitimer nephelometric method, according to the manufacturer's instructions. The reference range for healthy adults was less than 6 mg/l. Values above 10 mg/l were taken as elevated.

Elastase-α_1-proteinase inhibitor concentrations were measured by an immunoluminometric assay as already published (20). The reference range for healthy adults was 250 µg/l, for neonates 200 µg/l. Values above 300 µg/l for adults and 250 µg/l for neonates were taken as elevated. The standard used was human PMN-elastase (Protogen, Läufelfingen, Switzerland) complexed with α_1-antitrypsin (Sigma, Deisenhofen, Germany).

Patients

The patients consisted of 28 neonates (gestational age 29–42 weeks) within the first 3 days of life, 2 babies between 14 and 24 days old and 28 adults from the intensive care unit. Two adults were studied over a period of one month, during the time they were in the intensive care unit. The relevant details are given below:

Case 1 (Figure 1a)

A 44 year old male was admitted on day 0 with fever and was treated with broad spectrum antibiotics. On day 11, *Candida* antibodies were detected in serum, which persisted throughout the observation period. Between day 14 and day 21, *Candida* antigen was positive in tracheal secretion cultures. Between day 18 and 20, *Enterobacter* cultures were positive in tracheal secretion. On day 27, *Citrobacter*, *Pseudomonas*, and *Escherichia coli* were found in tracheal secretion and pleura punctate. Selective therapy was commenced on the same day. On day 38, *Pseudomonas* was again found in tracheal secretion, a situation which persisted throughout the observation period. From day 42, a severe sepsis developed in which *Staphylococci*, *Enterococci*, *Xanthomonas* and *Enterobacter* persisted. Treatment was continued, the patient recovered slowly, and he was transferred to a peripheral ward on day 65.

Case 2 (Figure 1b)

A 34 year old woman was admitted to the intensive care unit with respiratory complications and had to be intubated. On day 5 and day 17, B-*streptococci* were found in lung aspirates. After antibiotic treatment no further microbiological problems arose, and the patient was transferred to a peripheral ward on day 28.

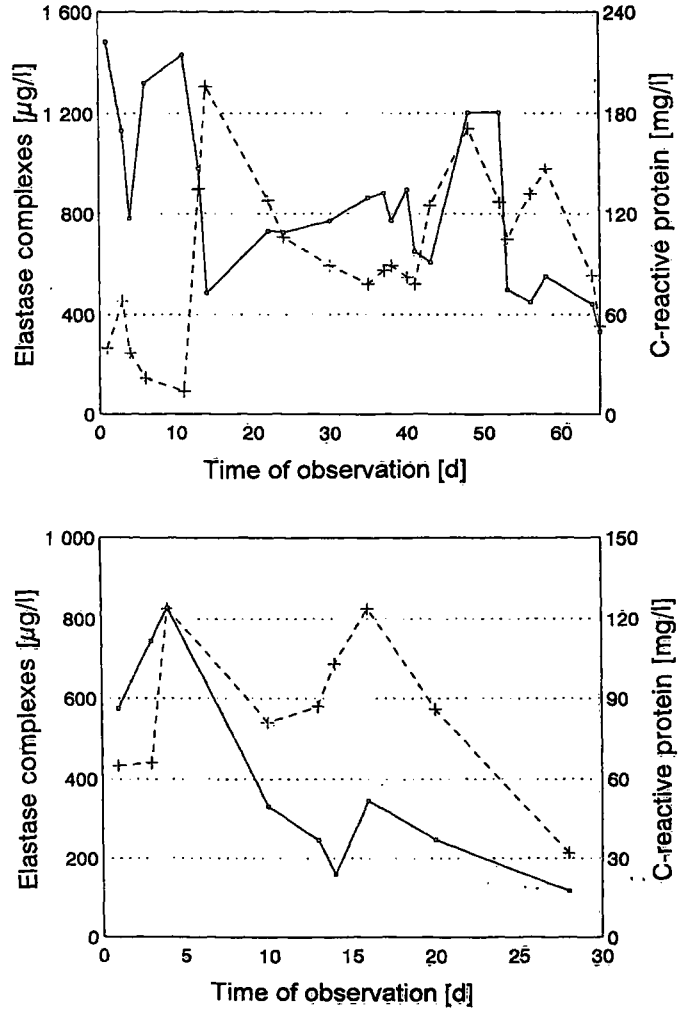

Fig. 1 C-reactive protein (+–+) and elastase-α_1-proteinase inhibitor (□–□) concentrations in patients with recurrent infections. The abscissa shows the observation time. Day 1 was the day on which the first plasma sample was taken. Bacterial cultures were set up from tracheal secretion and pleural punction. The details and course of disease are given in the text.
a) A 45 year old male patient with recurrent multiple infection.
b) A 34 year old female patient with recurrent B-*streptococcus* infection during intubation.

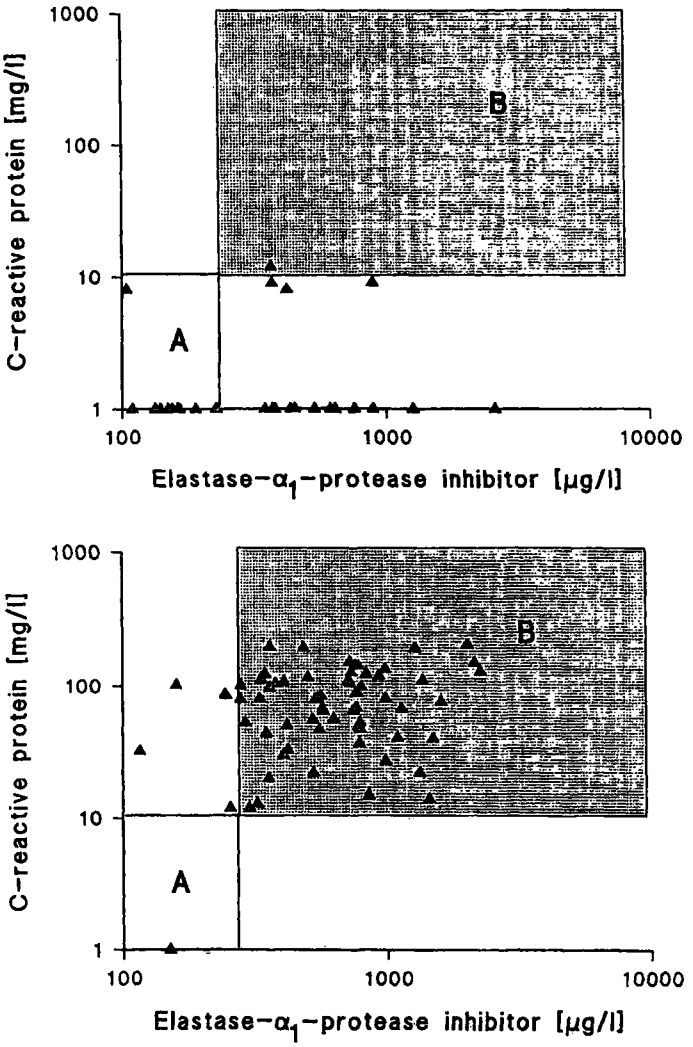

Fig. 2 Elastase-α_1-proteinase inhibitor and c-reactive protein levels in samples from adults and newborns.
a) In 69 samples from adults on the intensive care unit with known bacterial infections. The area denoted by A is the reference range for healthy adults for both analytes. Area B (hatched) shows the elevated values for both analytes.
b) In newborns with the tentative diagnosis of neonatal sepsis. The values in area A were normal in both assays and corresponded to those newborns without neonatal sepsis. All other cases had neonatal sepsis and, with one exception, were misclassified (false negative results) by the c-reactive protein assay. The hatched area (B) shows the elevated results for both analytes.
The upper limits of the reference range are shown for adults for c-reactive protein, and for newborns for elastase-α_1-proteinase inhibitor.

Results

Figures 1a and 1b show the two time courses of the cases described above. Figure 2a shows the distribution of c-reactive protein and elastase values in adults, and figure 2b in newborns, measured within the first 36 hours of life.

In the adults, elevated elastase-α_1-proteinase inhibitor levels were found in 52 cases, borderline concentrations in 10 cases and normal values in 5 cases. Elevated c-reactive protein levels were observed in 66 cases, normal levels being seen in a single case. Seventeen neonates showed elevated values for elastase-α_1-antiproteinase, 2 were borderline and 13 showed normal values. C-reactive protein levels were above 10 mg/l in one case (gestational age 40 weeks), between 5 and 10 mg/l in 5 cases (gestational age 31–40 weeks) and undetectable in 26 cases (gestational age 29–42 weeks).

Among the neonates, 17 cases of neonatal sepsis were confirmed, all having elevated elastase-α_1-antiproteinase levels. The c-reactive protein levels in these cases were all under 13 mg/l, 16 being under 5 mg/l. The values for the babies older than 14 days were between 514 and 2750 µg/l for elastase-α_1-proteinase inhibitor, and between 50 and 122 mg/l for c-reactive protein. Both babies were suffering from B-*streptococcus* infections.

Discussion

This article compares the merits and disadvantages of c-reactive protein and elastase-α_1-proteinase inhibitor levels in patients with bacterial infection. Whereas for adults, both markers were similar in their diagnostic value, c-reactive protein was not suitable as a monitor of neonatal sepsis.

The varying degrees of maturity of the c-reactive protein synthesis at birth make its diagnostic sensitivity and specificity so low as to render the analyte worthless within the first few days of life, with a real danger that "normal values" may be seen despite the presence of an infection. There appeared to be no correlation between the gestational age and time of appearance of c-reactive protein in serum after birth. The values from the two babies with pneumonia showed that c-reactive protein synthesis was active 14 days after birth. This is in accordance with literature reports that c-reactive protein synthesis increases steadily after the first 24 hours of life (15).

Three of the four discrepant cases in figure 2a (elastase-α_1-proteinase inhibitor normal, c-reactive protein elevated) the patients had a pronounced leukopenia, which could account for the low elastase-α_1-proteinase inhibitor levels. It is known that in prolonged bacterial infection, the granula secreting elastase become depleted, so that low concentrations are detected in plasma.

The results presented here indicate the c-reactive protein is the marker of choice in adults and children, as it is not only equal in diagnostic reliability, but also much cheaper and quicker to analyse. Elastase-α_1-proteinase inhibitor is the marker of choice for diagnosis of neonatal sepsis, especially when the samples are taken from preterm infants within the first days of life. The use of this marker for adults shows no advantages over c-reac-

tive protein and therefore can be replaced by the latter to confirm the diagnosis. The time course of both analytes can be quite different as shown in figures 1a (partly discordant) and 1b (full concordance) and is reflected by the low correlation coefficient between the two analytes of $r = 0.313$ ($n = 115$, $p = 0.01$).

The markers reflect two different responses, one by the liver, the other by the leukocytes. It may be interesting to study both markers during the course of the disease in children and adults. The main purpose of the present communication, however, was to consider the diagnostic reliability of single measurements of both markers.

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