Capillary plasma elastase α_1 -proteinase inhibitor in infected and non-infected neonates

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Abstract

Capillary heel prick plasma elastase α_1 -proteinase inhibitor (E α_1 -PI) measured by an immunoassay (using commercially available reagents) was examined as an early indicator of neonatal sepsis. Fifty five infants were studied within 24 hours of birth; 60 (including 10 studied on the first day of life) were examined between two and 30 days after birth. Reference ranges for the neonatal period were developed. Raised E α_1 -PI concentrations (range 440–2600 μ g/l) were found at the outset of each of the 24 infectious episodes including five with concomitant neutropenia. On the first day of life, obstetric and neonatal complications were also associated with high concentrations (range 190–2400 μ g/ml). In infants who survived infection, E α_1 -PI normalised with antibiotic treatment. It is concluded that capillary heel prick plasma is suitable for E α_1 -PI testing and raised concentrations provide a sensitive but non-specific index of infection in the first 24 hours after birth. Sequential testing may provide early warning of infectious complications and serve as a guide to the cessation of antibiotic treatment.

Recent studies in both adults¹⁻³ and neonates⁴⁻⁶ indicate that raised venous plasma elastase α_1 -proteinase inhibitor (E α_1 -PI) complex concentrations provide a sensitive indicator of systemic bacterial infections. Furthermore, E α_1 -PI appears to correlate with both the severity of inflammation and the degree of illness.¹⁻² The E α_1 -PI complex appears in plasma after tissue injury: elastase is released into the circulation as activated polymorphonuclear leucocytes (PMN) degranulate.⁷ The elastase is subsequently inactivated and complexed predominantly by α_1 -proteinase inhibitor (α_1 -PI) and to a lesser degree by α_2 -macroglobulin.⁸

In many neonatal intensive care units, capillary blood samples obtained by heel prick puncture are used for serial monitoring of haematological and biochemical indices. In the present study we assessed the suitability of capillary heel prick plasma for E α_1 -PI testing and examined the role of E α_1 -PI in the early diagnosis and monitoring of neonatal infections.

Patients and methods

The infants studied were drawn from the neonatal population of the Mater Mothers' Hospital, South Brisbane, Australia. The study was approved by the Mater Mothers' Hospital

ethics committee. Informed parental consent was not considered necessary as testing was performed on residual plasma from blood samples submitted to the clinical laboratory for haematological studies. The study population included infants from two age groups. Group 1 comprised infants examined within 24 hours of birth who were undergoing a sepsis screen because of the presence of predisposing factors including prematurity, prolonged rupture of membranes, and maternal complications such as hypertension or instrumental birth. Group 2 comprised infants studied two to 30 days after birth in whom there was a clinical suspicion of infection or who were undergoing full blood count testing to monitor their clinical progress and as a screening test for sepsis. Group 1 (n=55) included 33 preterm infants with mean (SD) gestational age 30.2 (3.1) weeks (range 25–36 weeks) and 22 term infants with mean gestational age 39.4 (0.6) weeks (range 37–41); group 2 (n=60) included 10 group 1 infants. Infants were enrolled in the study if: (i) a capillary heel prick sample was submitted; (ii) there was sufficient residual plasma for E α_1 -PI testing; and (iii) separation of plasma was performed within two hours of collection.

Maternal and infant patient records and postmortem reports were reviewed and clinical, radiological, microbiological, and haematological data collected. Infants were classified as proved, probable, or possible infection and as non-infected. The non-infected group was subdivided into those with a non-eventful clinical course and those with obstetric or neonatal complications. Infants with positive blood cultures (performed according to standard techniques by the clinical laboratory) and a clinical course consistent with sepsis were classified as proved infection. The 'time of onset' of infection was designated as the earliest finding of either the clinical onset of infection or the time of collection of the blood culture which subsequently proved positive. Causative organisms in the group 1 infants (n=7) with proved infection were: group B streptococcus (n=2), *Escherichia coli* (n=3), *Bacteroides fragilis* (n=1), and *Serratia marcescens* (n=1). Causative organisms in four of the seven group 2 infants with nine infectious episodes were: *Staphylococcus epidermidis* (n=1), *Streptococcus viridans* (n=1), *Candida albicans* (n=1), and *S. marcescens* (n=1); there were five episodes of necrotising enterocolitis. Criteria for probable infection (n=9, group 1) were negative blood cultures but strong clinical (and in some cases radiological) evidence of infection associated with intrapartum antibiotic treatment. Criteria for possible infection (n=4, group 1)

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were: maternal clinical and histological chorioamnionitis, intrapartum antibiotic therapy, asymptomatic infant given antibiotic cover, negative blood cultures, and haematological changes suggestive of infection. All other infants were considered non-infected.

Blood samples (approximately 300 to 400 μ l) were collected by heel prick puncture into tubes anticoagulated with EDTA (Microtainer, Becton Dickinson). After haematological testing, the plasma was separated and stored at -70°C until testing. The time of collection of blood and separation of plasma was recorded. Specimens not separated within two hours of collection were discarded as $\text{E}\alpha_1\text{-PI}$ concentrations rise if separation is delayed beyond two hours.⁹

ASSAY¹⁰

Duplicate testing was performed (without knowledge of the infection status of the infants) using a commercially available solid phase enzyme linked immunoassay kit (E Merck). The two hour test procedure was modified slightly to provide a micromethod using only 10 μ l of plasma per test. According to the method principle, standard and plasma samples were exposed sequentially (with incubation and washing steps between) to an elastase antibody

and an alkaline phosphatase labelled antibody to $\text{E}\alpha_1\text{-PI}$ which attach to the elastase and $\text{E}\alpha_1\text{-PI}$ antigenic determinants respectively. To reduce the plasma volume required for the test, a Gilson Dilugil V dilutor (Gilson Medical Electronics) was used to dilute 10 μ l of the plasma samples directly with 490 μ l of diluent into the elastase antibody labelled tubes. Colour development of plasma samples from a 4-nitro phenylphosphate substrate was compared with results of a standard curve.

Results

Reference ranges for $\text{E}\alpha_1\text{-PI}$ for the first 30 days of life (fig 1) were determined from 20 group 1 non-infected infants with an uneventful perinatal and postnatal course and 102 determinations on 53 healthy group 2 infants. Because of the restricted number of estimations in some time frames, preliminary bounds were drawn by visual inspection using the non-parametric method employed by Speer *et al.*⁴ The maximum concentration on the first day of life was 265 $\mu\text{g/l}$ and there was a postnatal rise on day 2 to a maximum of 375 $\mu\text{g/l}$ which persisted till day 8. Thereafter, the maximum value decreased (342 $\mu\text{g/l}$ between days 9 and 20; 235 $\mu\text{g/l}$ between days 21 and 30) until 30 days when a stable value of 200 $\mu\text{g/l}$ was found (a further 40 estimations were performed on 25 infants between 31 and 60 days after birth who were less than 40 weeks' adjusted gestational age and the range of values was 75 to 225 $\mu\text{g/l}$ with 95% of values ≤ 200 $\mu\text{g/l}$; data not shown).

Figure 2 shows the distribution of $\text{E}\alpha_1\text{-PI}$ concentrations in 55 group 1 infants. All infants with proved ($n=7$) or probable ($n=9$) infection had $\text{E}\alpha_1\text{-PI}$ concentrations greater than 440 $\mu\text{g/l}$ including four infants with proved infection who were neutropenic (according to the reference ranges of Manroe *et al.*¹¹) at the time of testing. Two of the four neutropenic infants died: initial PMN count and $\text{E}\alpha_1\text{-PI}$ concentrations were $0.15 \times 10^9/\text{l}$ and 780 $\mu\text{g/l}$ respectively for one infant with *E coli* septicaemia and $1.9 \times 10^9/\text{l}$ and 580 $\mu\text{g/l}$ respectively for the other infant with group B streptococcal septicaemia. Corresponding values for PMN count and $\text{E}\alpha_1\text{-PI}$ concentrations for the two survivors were $0.8 \times 10^9/\text{l}$ and 600 $\mu\text{g/l}$ (*E coli* septicaemia) and $0.24 \times 10^9/\text{l}$ and 440 $\mu\text{g/l}$ (group B streptococcal septicaemia). Four infants with possible infection had values ranging from 360 to 750 $\mu\text{g/l}$. Both obstetric and neonatal complications were associated with increased $\text{E}\alpha_1\text{-PI}$ concentrations. Grossly raised concentrations were found for two of three non-infected infants with severe intrapartum hypoxia (1000 and 2400 $\mu\text{g/l}$), one infant with clinically and virologically proved cytomegalovirus embryopathy (1150 $\mu\text{g/l}$), and one infant with ABO haemolytic disease (1000 $\mu\text{g/l}$). Raised concentrations (range 305–700 $\mu\text{g/l}$) were also found for five of seven infants of mothers with pregnancy induced hypertension and two of three infants with retained fetal lung fluid.

In group 2 infants, clear delineation between infected (nine infectious episodes) and non-infected infants was obtained (fig 3) including

Figure 1 Reference ranges for capillary heel prick plasma concentrations of $\text{E}\alpha_1\text{-PI}$ in the first 30 days after birth.

Figure 2 Capillary heel prick plasma concentrations of $\text{E}\alpha_1\text{-PI}$ in the first 24 hours after birth in infants with proved ($n=7$), probable ($n=9$), or possible ($n=4$) infection, an uneventful clinical course ($n=22$), and obstetric and neonatal complications ($n=15$).

one infant with necrotising enterocolitis who was neutropenic at the time of testing. Another infant (with *S epidermidis* septicaemia) had a raised concentration (1000 µg/l) 24 hours before clinical onset of disease. Only one non-infected healthy infant had an unexplained raised Eα₁-PI concentration (1200 µg/l).

The results of sequential testing performed on four infected group 1 infants who survived infection is shown in fig 4. The concentrations of Eα₁-PI normalised with response to antibiotic treatment and recovery from infection. Case 1

Figure 3 Results of 102 determinations of capillary heel prick plasma Eα₁-PI concentrations in infected (nine infectious episodes) and non-infected infants between two and 30 days after birth.

Figure 4 Sequential capillary plasma Eα₁-PI concentrations in four infants with early onset sepsis who survived. Case 1 had probable infection with a leukaemoid blood picture; case 2 had *E coli* septicaemia and pneumonia and was neutropenic at birth; and cases 3 and 4 had uncomplicated septicaemia due to *S marcescens* and *B fragilis* respectively.

Figure 5 Serial Eα₁-PI data in an infant transferred to our unit eight days after birth with severe neutropenia and extensive necrotising enterocolitis (NEC) involving both small and large bowel and who required surgery for perforation. Subsequently, the infant had a complicated and protracted course with relapses of NEC 13 and 38 days after birth.

was an infant with probable intrauterine infection (radiological evidence of pneumonia) born after seven day rupture of maternal membranes who developed a leukaemoid blood picture (PMN count of $31.8 \times 10^9/l$ and Eα₁-PI value of 1500 µg/l at birth, rising to a zenith on the third day after birth of $65.8 \times 10^9/l$ (Eα₁-PI 4000 µg/l) with normalisation ($3.3 \times 10^9/l$ and 200 µg/l respectively) 27 days after birth. Case 2 had septicaemia and pneumonia due to *E coli* with severe neutropenia (PMN count $<0.1 \times 10^9/l$) at birth with an Eα₁-PI of 440 µg/l. Adjunctive treatment included a granulocyte transfusion eight hours after birth and intravenous immunoglobulin (Sandoglobulin) for five days. The infant developed a leukaemoid blood picture with the PMN count reaching a zenith of $58 \times 10^9/l$ eight days after birth (Eα₁-PI 600 µg/l); the Eα₁-PI normalised (125 µg/l) 10 days after birth with clinical recovery but the PMN count remained high until 20 days after birth. Cases 3 and 4 had uncomplicated septicaemia due to *S marcescens* and *B fragilis* respectively.

Figure 5 displays the results of sequential Eα₁-PI measurements in one infant who was tested longitudinally. The infant who was neutropenic at the time of initial testing was transferred to our unit eight days after birth with necrotising enterocolitis and a perforated bowel requiring resection. The infant had a protracted and complicated clinical course with two relapses of necrotising enterocolitis during the period of testing. The Eα₁-PI concentrations fluctuated with changes in the infant's clinical progress.

Discussion

Our findings indicate capillary heel prick plasma is suitable for Eα₁-PI testing with the present methodology and indicates this complex is a sensitive but non-specific indicator of infection. A microtechnique using capillary plasma is advantageous, particularly in the preterm infant in whom blood sampling should be minimised. With capillary plasma, falsely raised Eα₁-PI values (due to in vitro release of elastase from PMN) could be obtained if difficulty was experienced in collection of the sample or the plasma was contaminated with PMN at the time of separation. However, although no comparative data are available for reference ranges for capillary plasma, our results are in general agreement with the published reference range for venous plasma.⁴⁻⁶ Capillary plasma Eα₁-PI concentrations were only slightly higher at corresponding postnatal ages than venous plasma (maximum values on day 2 of 375 µg/l compared with 275 µg/l; maximum stable value at 4 weeks of 200 µg/l compared with 190 µg/l between 5 weeks and 14 years of age). The higher Eα₁-PI values in capillary samples may be due to the higher leucocyte counts in the latter samples when compared with venous and arterial samples¹² as Speer *et al* found Eα₁-PI values and the total white blood cell count are positively correlated.⁴

Examination of Eα₁-PI concentrations in the first 24 hours of life may provide a valuable adjunct to the early diagnosis of sepsis in at risk

infants. In the present study all infants with proved, probable, or possible infection had raised Ea_1 -PI values including four infants who were neutropenic at the time of testing. Raised Ea_1 -PI values may assist in earlier identification of the latter group in whom the mortality may be high¹³ and may also prove useful in the assessment of clinically well infants whose mothers received intrapartum antibiotic treatment. Our findings in neutropenic infected infants confirm previous reports in neonates and children.⁴⁻⁶ However, these data should be interpreted with caution in other clinical groups as normal Ea_1 -PI values have been reported in infected patients with neutropenia secondary to chemotherapy induced myelosuppression.¹⁴⁻¹⁵ A recent report indicates intracellular elastase concentrations decrease commensurate with chemotherapy.¹⁵ Thus the efficiency of Ea_1 -PI as an indicator of infection in neutropenic patients may depend on both an adequate intracellular elastase concentration and/or an adequate marrow myeloid pool. In the present study, both obstetric and neonatal complications including pregnancy induced hypertension, maternal chorioamnionitis, intrapartum hypoxia, retained fetal lung fluid, cytomegalovirus embryopathy, and ABO haemolytic disease were also associated with high Ea_1 -PI values. Our findings are in accord with previous reports that found raised Ea_1 -PI concentration with several non-infectious factors including meconium aspiration, severe prolonged acidosis, and persistent fetal circulation.⁴⁻⁶ To date, all tests used to screen for sepsis on the first day of life including gastric aspirate microscopy,¹⁶ neutrophil profiles,^{11 17 18} the haematological scoring system,¹⁸ erythrocyte sedimentation rate,¹⁹ and C reactive protein^{17 20} have been limited in their usefulness by their non-specificity.

After the first day of life clear delineation between infected and non-infected infants for Ea_1 -PI concentrations was obtained. In one infant, with *S. epidermidis* septicaemia, the Ea_1 -PI concentrations rose 24 hours before the clinical onset of disease. Sequential testing was performed on a small number of infected infants and appears of value in monitoring disease and response to treatment. In infants who survived infection the Ea_1 -PI concentrations normalised with antibiotic treatment, and in one infant with necrotising enterocolitis serial monitoring showed Ea_1 -PI values fluctuated in parallel with changes in the infant's clinical condition. These findings corroborate the studies by Speer *et al.*⁴⁻⁶

We conclude that capillary plasma Ea_1 -PI testing provides a valuable adjunct to the early diagnosis of neonatal sepsis. Sequential testing may provide early warning of infectious compli-

cations, serve as a guide to cessation of antibiotic treatment in infected infants, and prove useful in monitoring the clinical progress of infants with necrotising enterocolitis.

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