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## Diagnostic potential of neutrophil elastase inhibitor complex in the routine care of critically ill newborn infants

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**Abstract** It has been suggested that determination of the neutrophil elastase  $\alpha$ 1-proteinase inhibitor complex (E- $\alpha$ 1PI) improves the diagnosis of bacterial infection in newborns. We evaluated the use of E- $\alpha$ 1PI measurements in 143 newborns, consecutively admitted to a tertiary intensive care unit, employing a new random access assay and a sampling procedure that minimises post-collection artefacts. The 95% range for non-infected newborns was 20–110  $\mu$ g/l up to the 5th day of life and 20–85  $\mu$ g/l thereafter. The sensitivity as to the diagnosis of culture-proven bloodstream infection was 80% for E- $\alpha$ 1PI, 86% for the immature to total neutrophil ratio, 64% for C-reactive protein and 37% for the total white blood cell count. The corresponding specificity amounted to 97%, 85%, 85% and 86%, respectively. E- $\alpha$ 1PI increases preceded elevations of C-reactive protein by 18 h. Like C-reactive protein, E- $\alpha$ 1PI levels did not distinguish between bloodstream infection and non-bacterial inflammatory responses. Results of E- $\alpha$ 1PI became available within 1 h of collection and usually 2–3 h before manual leucocyte counts.

**Conclusion** Determination of neutrophil elastase  $\alpha$ 1-proteinase inhibitor levels yields diagnostic advantages comparable to those of manual differential counts but provide faster turnaround times.

**Key words** Infant · Newborn · Sepsis · Diagnosis · Neutrophil-elastase · C-reactive protein

**Abbreviations** *AUC* area under the receiver operating characteristic curve · *CRP* C-reactive protein · *E- $\alpha$ 1PI* neutrophil elastase  $\alpha$ 1-proteinase inhibitor complex

### Introduction

Among the many parameters which have been suggested to improve the diagnosis of bacterial infection in newborn infants is the determination of circulating complexes of neutrophil elastase and the  $\alpha$ 1-proteinase

inhibitor (E- $\alpha$ 1PI) [7, 8]. A turbidimetric immunoassay is now being marketed which is adaptable to most laboratory analysers used in clinical chemistry [2]. When performed in addition to other analyses, this assay requires only 4  $\mu$ l of plasma. Unfortunately, routine E- $\alpha$ 1PI measurements are hampered by the sampling

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procedures available as these result in unpredictable post-collection artefacts [1]. Recently, we identified a collection method that minimises the risk of false-positive E- $\alpha$ 1PI results [1]. Here we present data on the diagnostic performance of E- $\alpha$ 1PI, applying the new assay and sampling procedure to a heterogeneous cohort of critically ill newborn infants.

## Patients and methods

This prospective cohort study comprised 143 critically ill newborn infants (62% males), who were consecutively admitted to the multidisciplinary neonatal and paediatric intensive care unit of a tertiary referral hospital. The study population included 66 premature infants. Major surgical procedures had been performed in 35 patients. In order to model routine care, an automatic request for E- $\alpha$ 1PI was generated whenever physicians ordered the determination of C-reactive protein (CRP). In this unit, CRP is one of the standard parameters for routine surveillance of patients and for suspected infection. In addition WBC count and differential count were determined. The study was approved by the Institutional Review Board.

Blood was collected into lithium-heparin tubes containing an inert cell-plasma separation gel (Capiject T-MLHG, Terumo, Elkton, Md., USA). Samples were centrifuged using a bedside microcentrifuge (Microcentrifuge, Denver Instrument Company, Arvada, Colo., USA) within 15 min of collection at 1550 g for 3 min. E- $\alpha$ 1PI levels were measured by a turbidimetric assay (Ecoline PMN Elastase, Merck KG, Darmstadt, Germany) adapted to a Beckman Synchron CX5 automated analyser (Beckman Instruments, Fullerton, Calif., USA). The detection range of the assay extends from 12 to 480 ng/ml.

Samples from non-infected critically ill newborn infants were regarded as control data [6], provided the sample had been collected more than 48 h prior to the onset of suspected infection. Symptoms suggesting infection were a priori defined using published criteria [6]. In detail, systemic infection was suspected provided the patient presented with at least two otherwise unexplained clinical signs (hypothermia  $< 36.0$  °C or fever  $> 38.5$  °C, respiratory distress, hypotension, lethargy/irritability, otherwise unexplained feeding intolerance/vomiting/abdominal distension) and two laboratory signs (CRP  $> 20$  mg/l, total WBC count  $< 4 \times 10^9$  cells/l, immature to total neutrophil ratio  $> 0.3$ , thrombocytopenia  $< 100 \times 10^9$  cells/l, pH  $< 7.2$ ). Symptoms suggesting localised infection comprised: new consolidation on chest X-rays and respiratory deterioration (pneumonia), urine leucocytosis  $> 5$  granulocytes per field (urinary tract infection), diarrhoea and blood in the stool (intestinal infection), positive CSF biochemistry or microscopy plus one of the above clinical signs (meningitis).

The diagnostic performance of E- $\alpha$ 1PI was determined using the results from samples collected at clinical suspicion of infection. Additional samples were collected during the course of each episode to determine the kinetics of E- $\alpha$ 1PI in comparison to that of CRP or of changes in the WBC count. Patients were able to contribute samples both to the control data and to episodes of illness. For every sample, data for E- $\alpha$ 1PI, CRP and WBC count was available. Manual differential counts were performed in all samples collected at suspicion of infection and during the episode. Two investigators independently classified each episode of suspected infection after all clinical data, laboratory findings and microbiological results had become available. Classification as culture-proven bacterial bloodstream infection required satisfaction of the definition of bloodstream infection by the Center for Disease Control and a positive blood culture drawn within 24 h before or 48 h after the onset of the episode. Patients in whom history, clinical course, and routine laboratory data suggested bloodstream infection but whose blood cultures remained negative were classified as clinically suspected sepsis [5]. The diagnosis of localised bacterial infection required clinical signs of infection plus micro-

biological evidence of a local focus (e.g. a patient with fever, raised WBC count and  $> 10^6$ /ml pathogens in a urine specimen). Episodes were classified as non-bacterial inflammatory reaction when all cultures remained negative and both investigators agreed on a plausible alternative explanation of the patients condition (e.g. post-operative fever). Episodes with conflicting classification were regarded as unclassifiable.

Statistical analysis was carried out using non-parametric tests (Kruskal-Wallis test) to compare E- $\alpha$ 1PI levels between the groups. The diagnostic accuracy was evaluated by logistic regression analysis and compared between parameters by the area under the receiver operating characteristic curve (AUC) [3]. Episodes of culture-proven bloodstream infection served as cases, results obtained from non-infected infants served as controls. Times from suspicion of infection to abnormal test results were compared by the Kaplan-Meier method  $\mu$ log-rank test). The program SAS (Version 6.12, SAS Inc, Cary, N.C., USA) was used for statistical calculations.

## Results

The study population comprised 154 newborn infants from whom 652 samples were collected. Some 20 samples from 11 newborns were excluded because of delayed centrifugation (5 samples) and erroneous requests from capillary blood (15 samples); thus 632 samples were used in the analysis. The median post-gestational age of infants at sample collection was 33.4 weeks (range 25–44 weeks). A total of 249 samples from 105 newborns were collected from infants free of symptoms suggestive of infection. These samples were regarded as control data and served to establish the proposed reference range. In these infants, plasma levels were higher during the first 5 post-natal days ( $n = 114$  samples, 65 patients, median = 65  $\mu$ g/l, 95% range 18–110  $\mu$ g/l) than later ( $n = 135$  samples, 75 patients, median = 52  $\mu$ g/l, 95% range 20–85  $\mu$ g/l).

During their hospitalisation, 95 infants became symptomatic giving rise to 133 episodes of suspected infection (383 samples). Table 1 presents the descriptive statistics for plasma levels of E- $\alpha$ 1PI across the subgroups arising from the outcome adjudication for samples collected at suspicion of infection. E- $\alpha$ 1PI levels yielded a sensitivity of 80% and a specificity of 97.5% in distinguishing culture-proven bloodstream infection from critically ill newborn infants without symptoms of infection. The AUC amounted to 0.95 which was similar to that of the ratio of immature to total neutrophils (AUC = 0.93, sensitivity 86%, specificity 85%). The diagnostic performance was superior to that of CRP (AUC = 0.78,  $P < 0.05$ ; sensitivity 64%, specificity 85%). This difference was attributable to the delayed rise of CRP in comparison to E- $\alpha$ 1PI (median delay 18 h, log rank test  $P < 0.05$ ). Total WBC counts were of poor diagnostic value (AUC = 0.61, sensitivity 37%, specificity 86%). In routine care, E- $\alpha$ 1PI results became available at the same time as CRP results (usually within 1 h of collection) and always before data from manual differential counts.

It is important to note that none of the parameters showed good ability to discriminate between symptomatic patients who were subsequently classified as having

**Table 1** Descriptive statistics for plasma levels of E- $\alpha$ 1PI

| Parameter   | Classification category                        |  |                            |                                     |                         |  |
|---|--|--|----------------------------|-------------------------------------|-------------------------|--|
|   | Culture-proven bacterial bloodstream infection | Clinically suspected bacterial bloodstream infection | Proven localised infection | Non-bacterial inflammatory reaction | Unclassifiable episodes | Control data from non-infected newborn infants |
| Number of values used in analysis <sup>a</sup> ( <i>n</i> ) | 15   | 11   | 41                         | 51                                  | 15                      | 249  |
| Mean ( $\mu\text{g/l}$ )                                    | 168  | 129  | 129                        | 120                                 | 151                     | 45   |
| Standard error of the mean ( $\mu\text{g/l}$ )              | 28   | 30   | 16                         | 17                                  | 27                      | 1.4  |
| Median ( $\mu\text{g/l}$ )                                  | 144  | 104  | 98                         | 81                                  | 109                     | 41   |
| Interquartile range ( $\mu\text{g/l}$ )                     | 104–431  | 61–358   | 64–146                     | 62–140                              | 66–227                  | 30–53  |
| Maximum ( $\mu\text{g/l}$ )                                 | 431.0  | 358.0  | 466.0                      | 795.0                               | 359.0                   | 137.0  |
| Comparison to control data                                  | $P < 0.001$                                    | $P < 0.001$  | $P < 0.001$                | $P < 0.001$                         | $P < 0.001$             | –  |

<sup>a</sup>The result of each episode which was obtained from the sample collected at clinical suspicion was entered into the analysis

an episode of non-bacterial inflammatory response (e.g. following surgery) and those with culture-proven bloodstream infection. The results for E- $\alpha$ 1PI, using a discrimination criterion of 120  $\mu\text{g/l}$  were: AUC = 0.68, sensitivity 60%, specificity 71% (other parameters: data not shown).

## Discussion

We introduced determination of plasma levels of E- $\alpha$ 1PI into the daily care of critically ill newborn infants using a new assay requiring minute amounts of sample and a collection method that is compatible with clinical routine. In this heterogeneous cohort of patients, the 95% interval containing plasma levels of non-infected patients extended from 20–110  $\mu\text{g/l}$  during the first 5 days of life and from 20–85  $\mu\text{g/l}$  thereafter. The latter range is equivalent to the suggested reference range in healthy adults [2].

The main finding of the study was that at clinical suspicion, plasma levels of E- $\alpha$ 1PI discriminated well between patients with serious bacterial infection and non-infected patients. The diagnostic accuracy was similar to that of manual differential leucocyte counts (the calculated immature to total neutrophil ratio) and superior to that of CRP or total WBC count. The diagnostic advantage over CRP is attributable to the earlier rise of E- $\alpha$ 1PI in relation to onset of symptoms. The major clinical advantage of E- $\alpha$ 1PI measurements resulted from the small sampling volume and the rapid turnaround times. Because E- $\alpha$ 1PI determinations can be performed on automated random access analysers, the analysis requires much less of the technician's time than manual differential counts. The difference in turnaround times became even more apparent during periods of reduced laboratory staffing (night hours and weekends).

Two important restrictions of the method were observed. First, we found unpredictable elevations after capillary blood collection (data not shown) as reported by Jensen et al. [4]. Thus, E- $\alpha$ 1PI determinations should

be restricted to samples obtained from free-flowing blood from venous or arterial access. Second, in this heterogeneous cohort of newborn infants with a relevant caseload of surgical patients, we observed limited diagnostic ability to discriminate between infection and non-bacterial causes of inflammatory reactions (e.g. fever after surgery). It is conceivable that E- $\alpha$ 1PI measurements may provide greater diagnostic benefits in settings with a higher proportion of premature infants [6] or where non-bacterial inflammatory reactions occur less frequently [7].

We conclude that non-infected critically ill newborn infants yield similar plasma E- $\alpha$ 1PI levels to those observed in healthy adults except for the first 5 days of life. The diagnostic power of E- $\alpha$ 1PI in the early identification of patients with culture-proven bloodstream infection is equal to that of the immature to total neutrophil ratio calculated from manual differential leucocyte counts and superior to that of CRP measurements. E- $\alpha$ 1PI measurements provide fast turnaround times and require the smallest sampling volume of all currently available parameters for the routine diagnosis of infection.

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