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Variations in dried blood spot immunoreactive trypsin in relation to gestational age and during the first week of life

J.P. Bourguignon[1,2], G. Deby-Dupont[1], A. Reuter[3], J. Senterre[2], A. Gérard[1], and P. Franchimont[1]

[1] Laboratoire de Radioimmunologie, University of Liège, Sart Tilman, B 4000 Liège, Belgium
[2] Clinique Pédiatrique, University of Liège, Hôpital de Bavière Bd de la Constitution, 66, B 4020 Liège, Belgium
[3] Institut des Radioéléments, Fleurus, Belgium

Abstract. Dry blood spot immunoreactive trypsin was measured by radioimmunoassay in 84 preterm babies and in 65 full-term newborns studied daily from the first to the fifth day of life. In a control group of 3858 full-term newborns, trypsin concentrations at days 4–6 of life exhibited a log-normal pattern distribution, the geometric mean being 18 ng/ml serum. Immunoreactive trypsin concentrations did not change significantly between days 1, 2, 3, 4 and 5 after birth. Immunoreactive trypsin was found to be significantly lower (geometric mean 9 ng/ml, $P < 0.01$) in preterm newborns before 32 weeks of gestation. In hypotrophic newborns of 34 weeks gestational age, immunoreactive trypsin values were higher than those observed at 31 weeks of gestation in eutrophic newborns, the mean birth weight not being different between both groups. These data suggest that trypsin production by the pancreas is dependent on maturity but does not seem related to intrauterine nutritional status. Immunoreactive trypsin concentrations do not change after 32 weeks gestational age and during the first postnatal week.

Key words: Immunoreactive trypsin – Premature newborns

Introduction

In 1979 Crossley et al. [6] reported high immunoreactive trypsin (IRT) concentrations in the serum of newborns with cystic fibrosis (CF). This has further been confirmed by several authors [2, 5, 8–11, 14, 15]. In addition, these studies have shown that IRT can easily be measured by radioimmunoassay (RIA) using dried blood spots. The immunoreactive material detectable by RIA was shown to be heterogenous; it may consist of trypsinogen, trypsin-α_1-proteinase inhibitor complex and free trypsin [13]. In CF newborns, IRT values were found to be increased, although they return to the normal range within 1 or 2 years of age [12]. No data were available on the factors other than CF that may affect IRT values in newborns. This might be critical in the interpretation of screening data. Therefore, we investigated the influence of several factors on dry blood spot IRT concentrations: age during the first postnatal week, gestational age, weight for gestational age and birth circumstances.

Offprint request to: Jean-Pierre Bourguignon, M.D., Department of Pediatrics, Hôpital de Baviére, B 4020 Liège, Belgium

Abbreviations: IRT = immunoreactive trypsin; CF = cystic fibrosis; RIA = radioimmunoassay

Subjects and methods

a) Subjects. A longitudinal study was carried out in 65 full-term babies studied daily during the first week of life whereas single samples were obtained in 84 premature newborns. Their gestational ages ranged from 28–42 weeks as determined either by the time of the last menses or by an early ultrasonography. In five newborns, the existence of chronic fetal distress was established by prolonged phases of bradycardia during the labour and by passage of meconium before birth.

In 3858 full-term newborns, a single blood sample was obtained on the fourth or fifth day of life to establish the control IRT concentrations.

b) Blood sampling procedure. After a micropuncture of the heel, whole blood was collected on filter paper Macherey-Nagels no. 818. After drying at room temperature, the blood spots were stored at +4°C in the dark until assayed (maximal storage time: 8 days).

c) Radioimmunoassay of IRT. A duplicate assay was performed in the eluate of 8 mm diameter disks using reagents supplied in the Trypsik kit (Sorin, Saluggia, Italy) [13]: rabbit antiserum against human cathodic trypsin (initial dilution: 1/7500) and ^{125}I-human cathodic trypsin (80 ± 16 Ci/g).

The lyophilized standards were reconstituted with clarified bovine serum. Human erythrocytes, washed with isotonic saline, were added to obtain a 50% haematocrit; 50 µl aliquots were applied to filter papers to prepare IRT standards at concentrations ranging from 0–200 ng/ml serum. The spots were incubated for 4 h at room temperature, in the presence of 100 µl diluted antiserum and 100 µl buffer (phosphate saline 0.05 *M*; bovine serum albumin 0.05%; sodium azide 0.05%; pH 7.5). After addition of 100 µl of the tracer solution, an overnight incubation was performed at +4°C.

Separation of bound material was performed by precipitation in 1 ml of a 20% polyethylene glycol 6000 solution and centrifugation for 15 min at 3500 rpm.

The results are expressed as µg/ml serum. The limit of detection was 2.0 ng/ml. The intra-assay and inter-assay variation coefficients were 5% and 12%, respectively.

d) Statistical analysis. Since the distribution of IRT values in normal newborns showed a log-normal pattern, the geometric means and standard deviations were calculated after logarithmic transformation of the data. The significance of comparisons was determined using Student's *t*-test. A paired test was used for the data obtained longitudinally.

Results

1. Neonatal screening of 3858 newborns

As we reported previously [4], the geometric mean of serum IRT concentrations, in normal newborns was found to be 17.8 ng/ml, this value being equivalent to the 50th centile. The 97th and 99th centiles were found to be 60 and 87 ng/ml serum, respectively.

2. IRT during the first week of life

Figure 1 represents the correlation between IRT values determined on the first day of life and those observed later on, up to the fifth day. Highly significant linear correlations were obtained. According to the equations of the regression lines, it can be seen that time differences during the first week of life do not result in significant changes in IRT concentrations. However the slopes of the lines indicate that IRT levels are somewhat higher on day 1 than later on.

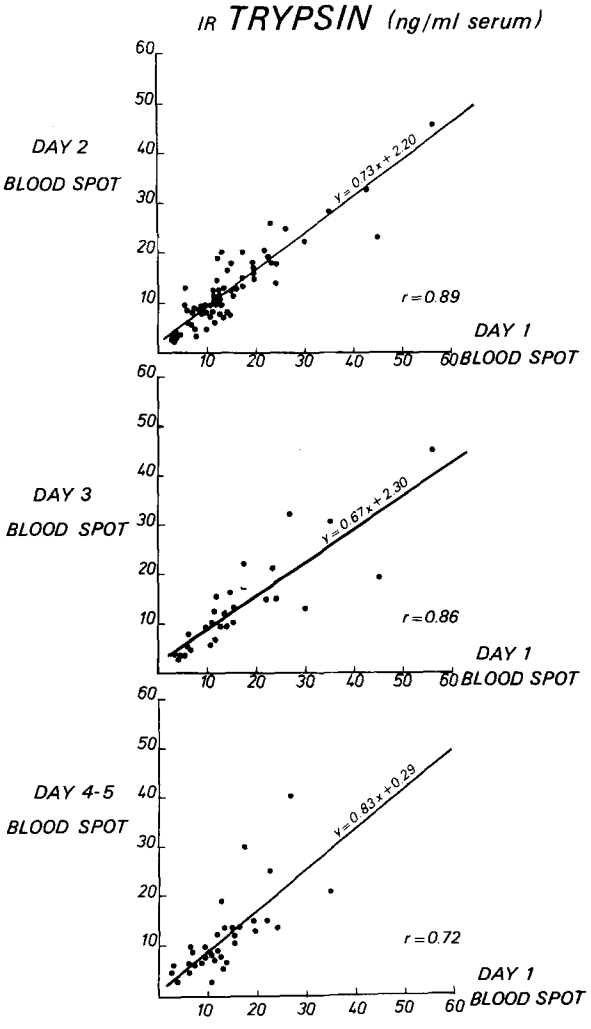

Fig. 1. Correlations between dried blood spot immunoreactive trypsin values measured on the first day of life and those obtained on days 2, 3 and 4–5 of life in full-term newborns studied longitudinally

3. IRT in premature children

Premature newborns were divided into three groups according to their gestational ages (Fig. 2A). In newborns with gestational ages ranging from 28–31 weeks, mean serum IRT was 9 ng/ml. In premature newborns aged 32–35 weeks or 36–38 weeks, as well as in full-term newborns, the mean serum IRT level was significantly higher, around 15 ng/ml.

In Fig. 2B, individual values of serum IRT and mean ± 1 SD are shown in two groups of premature newborns: 29 eutrophic newborns with a mean birth weight of 1512 g and 18 hypotrophic newborns with a mean birth weight of 1410 g. Although the mean birth weights, were similar in both groups, the newborns studied at a mean gestational age of 34.2 weeks showed a mean serum IRT level higher than those studied at a mean gestational age of 31.3 weeks.

4. IRT in relation to several perinatal conditions

In dried blood spots obtained during the first day of life, no significant variations in IRT levels were observed in relation to Apgar score and to the mode of delivery. In five newborns with a chronic fetal distress, significantly increased levels of IRT (geometric mean: 26.5 ng/ml, $P < 0.05$) were found to occur in the first day sample. However, this difference was no longer observed in samples obtained 2 days after birth (mean: 13.4 ng/ml).

Discussion

In this paper, we calculate the influence of gestational and postnatal ages on the levels of immunoreactive trypsin measured in the eluate of dried blood spots. We conclude that serum IRT concentrations are low in premature newborns of less than 32 weeks gestation whereas no other factors have been found to affect the serum levels of IRT in healthy newborns. In this work, the mean IRT value obtained in full-term newborns is in agreement with some reports in the literature, ranging from 16–20 ng/ml [9, 10].

We have also shown that no significant differences occur between IRT values measured on dried blood spots collected on days 1, 2, 3, 4 or 5 during the first week of life. For practical reasons, the fifth day of life seems to be the appropriate age to obtain blood for the screening of CF, since that is the right time for the screening for hypothyroidism and phenylketonuria. Furthermore, the increase in IRT levels found on the first day of life in newborns with chronic fetal distress, is an additional reason for that delay.

We have observed lower IRT values in newborns under 32 weeks gestational age than in older premature infants. This is in agreement with the results published in 1960 by Borgström et al. [3]. Using a bioassay, they found that premature infants weighing about 2000 g had a decreased trypsin concentration in the duodenum; those authors claimed that, in premature infants, the pancreatic function is not yet completely developed. More recently [1], Borgström and coworkers have described similar serum IRT values in preterm and full-term infants, which is not in accordance with the present data. However, Borgström et al. studied preterm babies of 34 weeks gesta-

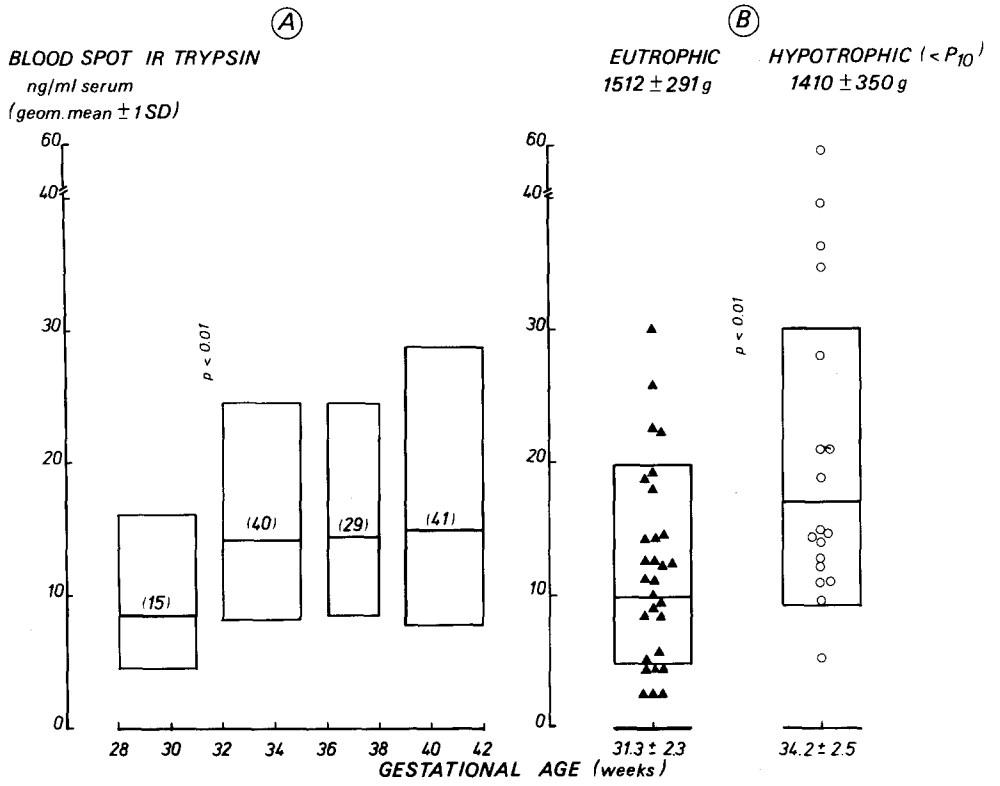

Fig. 2. Immunoreactive serum concentrations of trypsin in relation to gestational age (A). The number of infants in each group is given in brackets. Individual values and geometric means (± 1 S.D.) are compared in two groups of premature infants with similar birth weights but different gestational ages (B). Noteworthy, standard deviations were calculated after logarithmic transformations of all values. The results were retransformed to their antilogs to be shown on arithmetic coordinates

tional age whereas in this study, a low serum IRT level was only found between 28 and 31 weeks gestational age. Nevertheless, our observations are not in agreement with those recently reported by Dinwiddie et al. who found no differences in IRT concentrations between 24–34 weeks of gestational age [7].

Although no data are available on the serum IRT levels in CF premature infants born before 32 weeks of gestation, all values obtained at that period of life should be interpreted carefully. In those newborns, it would seem reasonable to delay the screening of CF up to the age of several weeks.

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