Complement activity in the cord blood of term neonates with the amniotic fluid infection syndrome

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Summary

Cord blood samples from 11 term neonates whose placentas showed histological changes typical of the amniotic fluid infection syndrome were analysed in order to determine haemolytic activity of the classic and alternative complement pathways and serum levels of complement proteins and immunoglobulins. Although the mean values of all these parameters were higher in this group than in an age-related control group, only classic haemolytic pathway activity was significantly elevated ($P < 0,025$).

S Afr Med J 1983; **63:** 86-87.

Decreased levels of complement activity have been implicated as a factor contributing to infection in the newborn infant.[1] The low incidence of clinical infection seen in neonates with the amniotic fluid infection syndrome (AFIS) may largely be due to the short duration of infection and relative avirulence of the organisms involved. In this paper the complement protein and immunoglobulin levels in cord blood samples from 11 neonates whose placentas showed histological features of the AFIS are reported.[2] Our data suggest that when exposed to this type of infection the neonates can respond by effectively increasing classic haemolytic pathway activity.

Material and methods

Cord blood samples and placentas from 22 term infants delivered in the maternity unit at Groote Schuur Hospital, Cape Town, were studied. Information concerning maternal well-being, duration of rupture of membranes, labour and fetal growth was recorded. The infants were examined fully and their gestational ages assessed by dates and by the Dubowitz criteria. A further clinical examination was carried out before discharge, usually at 3 days of age.

Histological examination of the placentas

Placentas were refrigerated at 4°C and processed within 24 hours. A transverse section of umbilical cord was sampled together with a block of placental tissue, consisting of an area of chorionic plate including fetal vessels and adjacent placental parenchyma.

Inflammatory changes were defined as maternal (subchorionic intervillositis), fetal (chorionic and/or umbilical vasculitis) or mixed. A more detailed description of the method of grading has been published elsewhere.[2]

The placentas of the 11 infants with the AFIS had grade 2-3 inflammatory changes, whereas those of the 11 infants in the control group showed neither maternal nor fetal inflammatory changes. Cord blood samples from the 22 infants were submitted for analysis as described below.

Complement and immunoglobulin assays

Samples of cord blood were obtained before delivery of the placenta. 'Milking' of the cord was avoided. The blood was allowed to clot at room temperature for 30 minutes. Aliquots were taken from the separated sera and stored at −80°C.

Classic haemolytic pathway activity (CH_{50}) and alternative haemolytic pathway activity (AP_{50}) titrations were performed as previously described.[3] A single volume of normal adult serum was frozen and stored in aliquots at −80°C. Each batch analysis of patient serum was done in parallel with this control and the values were expressed as a percentage of the normal adult control value. The coefficient of variation for the control serum was 6,5% for CH_{50} and 8,1% for AP_{50} titrations.

Serum C3, C4, factor B, IgG and IgM levels were measured using radial immunodiffusion plates (Behringwerke AG, Marburg, West Germany).

Statistical methods

The Student t test was used to identify significant differences between the study and control groups. Correlations between levels of individual complement components and haemolytic activity were calculated for both these groups.

Results

All 22 infants were born at term. Mean birth weights approximated 3 000 g in both groups, and 1 infant in each group was small for gestational age (below the 10th percentile for weight). No infants in the study group received prophylactic antibiotics and all appeared healthy at birth and at examination before discharge.

Table I shows the comparative results between the AFIS and control groups.

The haemolytic pathway activity (especially AP_{50}) and specific complement component values in the cord sera of both groups were below adult values. When the groups are compared the mean CH_{50} was significantly higher in the AFIS group ($P < 0,025$). Although mean AP_{50} and C3, C4 and factor B levels appeared to be higher in the AFIS group, no statistically significant difference between these groups could be shown. There was a significant correlation between CH_{50} and C3 levels within the

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Date received: 19 February 1982.

TABLE I. COMPARISON OF MEAN VALUES (± SD) FOR TERM INFANTS WITH AND WITHOUT AFIS

	AFIS	Controls	*P*	Adult reference range*
Birth weight (g)	**2 988 ± 597**	**2 925 ± 680**	**NS**	**—**
CH_{50} (% of adult control value)	**76 ± 14**	**63 ± 13**	**< 0,025**	**—**
AP_{50} (% of adult control value)	**55 ± 19**	**46 ± 13**	**NS**	**—**
C3 (mg/dl)	**72 ± 11**	**67 ± 15**	**NS**	**92 ± 16**
C4 (mg/dl)	**28 ± 12**	**25 ± 8**	**NS**	**46 ± 20**
Factor B (mg/dl)	**11 ± 3,7**	**10,2 ± 3,3**	**NS**	**16 ± 3,6**
IgG (mg/dl)	**1 610 ± 590**	**1 522 ± 490**	**NS**	**—**

*Mean values of normal adult sera.

AFIS group ($r = 0{,}80$; $P < 0{,}01$). Factor B correlated significantly with AP_{50} levels in both groups ($r = 0{,}864$; $P < 0{,}001$). No other significant correlations emerged ($P > 0{,}05$). Only 1 infant (in the control group) had a marginally elevated IgM value (35 mg/dl). No significant differences emerged between mean IgG levels ($P > 0{,}05$).

Discussion

In a recent collaborative study in the USA , Naeye and Peter[4] found illness resulting from infection of the amniotic fluid to be the commonest single cause of perinatal death.[4] This condition is even more common in developing countries, causing almost 22 deaths per 1 000 live births in Addis Ababa.[5]

The incidence of amniotic fluid infection in our hospital deliveries, comprising mothers drawn mainly from lower socio-economic groups, is extremely high. Based on histological evidence of placentitis, infection rates of 44% in term and 53% in preterm deliveries have been documented.[2,6] Surprisingly, most infants born from a contaminated intra-uterine environment show no clinical features of illness after delivery. The outcome of amniotic fluid infection in terms of fetal morbidity will depend on the virulence and concentration of the infecting agent, the duration of exposure to that agent and the efficiency of the fetomaternal immune response.

In the healthy neonate, most of the classic pathway components increase in concentration within the first few days of life,[7] although haemolytic activity only reaches adult levels by 3-6 months of age.[8] Of the alternative pathway components, low concentrations of factor B and properdin appear to be limiting factors in AP_{50} in neonatal serum.[9] Our results in the control patients reflect the observations described above.

The significant elevation of CH_{50} in the AFIS group suggests a positive response to infection. Increase in CH_{50} correlated strongly with C3 levels.

The failure to demonstrate a similar augmentation of AP_{50} in the AFIS group was unexpected. Unlike the classic pathway, this pathway does not depend on a specific antibody for activation. It might therefore be expected to feature prominently in the early response of the fetus and neonate to infection. In support of the contention that low levels of factor B are a major limiting factor of AP_{50} in neonatal serum, we found a significant correlation between AP_{50} and factor B levels in AFIS and control infants.

Other possible reasons for lack of a more substantial increase in AP_{50}, and to a lesser extent CH_{50}, include the nature of the responsible organisms and the duration of the infection. Naeye *et al.*[5] and Roos *et al.*[6] both isolated a significant number of anaerobes and other organisms of low virulence in their respective studies.

The duration of fetal exposure may also be limited in most cases, owing to the tendency for amniotic fluid infection to precipitate rupture of the membranes, and probably the initiation of labour.

We have demonstrated a moderate but significant increase in CH_{50} in the cord blood of term infants born with evidence of amniotic fluid infection. Lack of significant alternative pathway activity may be related to maturational deficiencies in factor B synthesis. This preliminary evidence suggests that the newborn infant can respond to an infectious challenge by effectively increasing complement activation.

This study was supported in part by a grant from the South African Medical Research Council.

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