

# Complement and contact activation in term neonates after fetal acidosis

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## Abstract

**Aims**—To evaluate complement and contact activation after fetal acidosis.

**Methods**—Fifteen term neonates with hypoxic-ischaemic encephalopathy after umbilical arterial pH < 7.10 were compared with 15 healthy neonates with umbilical arterial pH > 7.20. Determinations of the complement function and C1-inhibitor activity were performed as kinetic tests 22–28 hours after birth. C1q, C1-inhibitor, and factor B concentrations were determined by radial immunodiffusion and those of C3a, C5a, and factor XIIa by enzyme immunoabsorbent assay.

**Results**—Median complement function (46 vs 73 %), C1q (4.3 vs 9.1 mg/dl), and factor B (5.2 vs 7.7 mg/dl) decreased after fetal acidosis. The activated split products C3a (260 vs 185 µg/l), C5a (5.0 vs 0.6 µg/l), and factor XIIa (3.2 vs 1.3 µg/l) increased in the neonates after fetal acidosis. No differences were found in the concentration and activity of C1-inhibitor.

**Conclusions**—Complement and contact activation occurred in the newborns with hypoxic-ischaemic encephalopathy. Activation of these systems generates mediators which can trigger inflammation and tissue injury.

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Keywords: birth asphyxia; complement activation; contact activation; fetal acidosis

The complement and contact systems are activated after ischaemia and reperfusion injury, for example, myocardial infarction,<sup>1,2</sup> cardiac surgery with cardiopulmonary bypass,<sup>3,4</sup> or liver transplantation.<sup>5,6</sup> Fetal acidosis, as a marker for uteroplacental insufficiency, is associated with hypoxia and reperfusion injury in neonates.<sup>7</sup> Complement and contact components in these babies have not been systematically investigated.

This study aimed to assess complement and contact system activation in term neonates with hypoxic-ischaemic encephalopathy after fetal acidosis by examining several complement components and factor XIIa (Hageman factor) (figs 1 and 2).

## Methods

The study was approved by the local ethics committee and written parental consent obtained. Inclusion criteria for the study group were: an umbilical arterial pH < 7.10 and hypoxic-ischaemic encephalopathy 20 hours after birth.<sup>8</sup> Grade of hypoxic-ischaemic en-

cephalopathy, renal function (diuresis by weighing the nappies and serum creatinine 24 hours after birth), coagulation disorders (prolonged clinical bleeding time, decrease in platelets, evidence of fibrin split products) and duration of mechanical ventilation were recorded. Seventeen neonates, admitted to our neonatal intensive care unit between July 1995 and March 1996, fulfilled the inclusion criteria. Because two sets of parents did not give their consent, the study involved 15 infants with a median gestational age of 39 weeks (range 37–41). None of patients had evidence of infection (C-reactive protein > 0.5 mg/dl 24 hours after birth or positive blood culture). Fifteen healthy term neonates without perinatal complications, with the same gestational age, and an umbilical arterial pH > 7.20 were enrolled in the control group at the same time.

The study group were monitored to maintain mean arterial blood pressure, body temperature, normoglycaemia, normocalcaemia, normoxaemia, and normocapnia. Therapeutic interventions included infusion of fluids, mechanical ventilation, and administration of glucose and calcium. Five infants with convulsions were treated with phenobarbital, three received erythrocyte transfusion because of anaemia within the first 24 hours. Eleven infants were given pasteurised plasma solutions (Biseko, Biotest, Dreieich, Germany) for volume expansion. None of the patients received dexamethasone.

Blood samples were taken 22–28 hours after birth. Samples of blood (0.4 ml) were collected in two tubes containing either disodium-ethylene diamine tetra acetic acid (EDTA; Kabi-Laborstechnik, Germany) or 0.07 ml sodium citrate (Fa; Saarlstedt, Germany) and within 20 minutes centrifuged for 5 minutes at 3000 rpm. The plasma was immediately

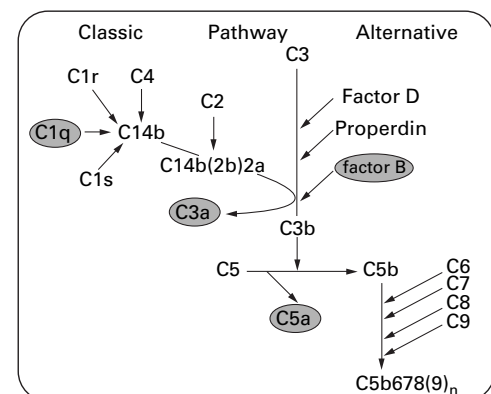


Figure 1 Complement system activation.

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detect those babies likely to be at risk. Low umbilical arterial pH and Apgar score are the traditionally used definitions of fetal acidosis, but they are poor predictors of latent disability in surviving infants.<sup>15 16</sup> The best predictors of death or handicap are neurological symptoms in the early neonatal period referred to as hypoxic-ischaemic encephalopathy.<sup>17 18</sup> Therefore, in this study we investigated only those neonates with both fetal acidosis and development of hypoxic-ischaemic encephalopathy within 20 hours of birth.

In the study group concentrations of C1q and factor B in plasma as well as haemolytic activity of the complement system were lower than in the control group. The reasons for the diminished values may be the consumption of the native complement proteins following complement activation, or reduced protein synthesis due to transient liver failure or a combination of both. To verify the complement activation we determined the activated split products acting as anaphylatoxins. C3a and C5a both increased after fetal acidosis. Schrod *et al*<sup>19</sup> reported increased C3a concentrations in preterm neonates with adult respiratory distress syndrome due to the surfactant inactivation after shock. We found increased C3a concentrations in acidotic neonates with (n=2) and without (n=13) respiratory distress syndrome. The increased C5a after fetal acidosis indicates an enhanced production, because C5a first binds to granulocyte receptors and only the free anaphylatoxin molecules were found in plasma.<sup>4</sup> Considering the increased anaphylatoxin concentrations, as well as decreased function and concentration of native proteins, we assumed that the complement system is activated after birth acidosis. Because the complement activation was evident 22 to 28 hours after birth, the effect of therapeutic interventions on the complement system activation within this time frame cannot be excluded. However, we found no differences between the infants who were treated with phenobarbital, transfusion, or pasteurised plasma solutions and those who were not in the study group. None of the study infants received drugs or acute interventions that are reported to influence the immune system. Thus the main reason for the complement activation is probably cell disintegration. Ischaemia releases subcellular constituents—mostly mitochondrial proteins—which bind to C1q and activate the complement cascade *in vitro* and *in vivo*.<sup>20 21</sup> Another reason for complement activation is the loss of protective membrane proteins on injured cells, which may be due to the activation of complement cascade in the ischaemic area.<sup>22 23</sup>

The complement system has a major role in initiating some of the inflammatory events occurring in ischaemia and reperfusion after myocardial infarction, cardiopulmonary bypass surgery, and liver transplantation.<sup>2-4 6 24</sup> Anaphylatoxins contribute to an increased permeability of small blood vessels, the contraction of smooth muscles, the release of histamine, the secretion of lysosomal enzymes and cytokines as well as granulocyte migration and adher-

ence. Another aspect of the complement activation is the direct cytotoxic effect of the membrane attack complex on endothelial cells.<sup>2</sup> All these mechanisms may enhance tissue injury following ischaemia and reperfusion.<sup>22 25 26</sup>

The increased concentrations of factor XIIa in the study group may be explained by contact activation after fetal acidosis. The reason for this activation likely is the contact of factor XII with negatively charged surfaces or cell constituents after cell destruction. Additionally, the contact system is activated by hypoxanthine,<sup>27</sup> which increases after birth asphyxia.<sup>28</sup> The activated contact or kinin system is involved in inflammatory tissue injury through bradykinin and kallikrein release with increased vascular permeability, leucocyte accumulation, and arterial hypotension. The contact system is closely related to the complement system, and mutual activation is possible.<sup>27</sup> Additionally, factor XIIa influences coagulation and fibrinolysis,<sup>27</sup> which act simultaneously after birth asphyxia.<sup>29</sup> Thus increased values of factor XIIa can contribute to the development of disseminated intravascular coagulation disorders in acidotic neonates.

Activation of the complement and contact system is controlled by rapid binding of C1-inhibitor to factor C1 and factor XIIa.<sup>30</sup> We therefore expected decreased concentration and activity in the study group after complement and contact activation. But the values were no different from those of the control group despite complement activation. This may be due to increased synthesis of this acute phase protein after asphyxia.<sup>30</sup> However, the additional amount of C1-inhibitor produced could not prevent the activation of both systems.

Complement and contact activation occur in neonates with hypoxic-ischaemic encephalopathy after fetal acidosis. Whether such activation causes tissue damage is not proved by our data, but it generates mediators which can promote inflammation and may contribute to the pathogenesis of reperfusion injury.

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