Neonatal Haemophilus influenzae infections

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Abstract
Nine cases of neonatal Haemophilus influenzae septicaemia were recorded in Finland during 1985–9; incidence was 2.8/100 000 live births, and 1.6% of all cases of neonatal septicaemia. The onset of the disease was early in all cases, ranging from 0–6 hours after delivery. Seven of the infants were preterm and three died (overall mortality 33%). H influenzae was isolated from blood in seven of the cases, and in two neonates with clinical signs of septicaemia it was found on several surface sites and the placenta. One of the eight strains of H influenzae was capsular type b and biotype I, the rest being non-typable—distribution similar to those previously reported. Four of the uncapsulated strains were of biotype III, and three of biotype II. None of the strains of H influenzae was of biotype IV, which has been reported to be characteristic of neonatal and genital isolates of H influenzae. All nine mothers had some sign of infection at the time of or shortly after delivery. H influenzae was isolated from the placenta of four mothers: from the blood (n=1) or from the placenta or cervix (n=4).

Neonatal Haemophilus influenzae infections were once thought to be rare, but during the past decades an apparent increase of such infections has been reported. H influenzae infections have been found mainly among preterm, low birthweight infants,2–6 and are characterised by the early onset of symptoms and the fulminant course of the disease.7 It has been suggested that these infections may have started during pregnancy and are the cause of the prematurity birth.7 The mortality associated with neonatal H influenzae septicaemia is high, up to 86% having been reported.3 6

Most (80–90%) of the strains of H influenzae that cause neonatal septicaemia are non-encapsulated,8 whereas 95% of strains that cause invasive disease among older children are capsular type b.7 The neonatal strains and the strains that colonise the female genital tract have been reported to be predominantly biotype IV, and seldom biotype II or III,8 10 whereas most of strains with the type b capsule are of biotypes I or II.11–13

A nationwide intensified surveillance of invasive infections in children (including neonates) was started in Finland in 198514 as a part of a trial of the efficacy of a vaccine against H influenzae capsular type b.15 We report the results of a five year prospective follow up of neonatal H influenzae infections in Finland and describe the clinical and bacteriological features of nine cases of neonatal H influenzae infection.

Patients and methods
In the nationwide surveillance all bacterial isolates from blood, cerebrospinal fluid, or other sterile body sites of children (0 to 15 years of age) with invasive disease were sent to the National Public Health Institute from the 25 microbiological laboratories in Finland. In addition, if a neonate (defined as an infant less than 28 days of age) was thought clinically to have septicaemia and the same bacterial species was isolated from many surface sites (for example, ear, umbilicus, or throat) and the placenta, this strain was included in the collection. The identification of strains was confirmed at the National Public Health Institute and samples were stored at –70°C. The laboratory records from the years 1985–9 in each of the laboratories were also examined retrospectively to find possible omissions from the collection. Detailed data about the patients and their mothers were collected from the hospital records.

The capsular types (a–f) of the strains of H influenzae were ascertained by a coagglutination technique.11 Strains were designated to biotypes I–VIII on the basis of their urease and ornithine decarboxylase activity and the production of indole.10 Production of β-lactamase was tested by the clover leaf method17 or the acridometric assay, or both.18

Results

Clinical features among the neonates
During the five year study period from January 1985 to December 1989, nine neonates with H influenzae septicaemia were recorded. During that time there were 317 673 live births in Finland, giving an annual incidence of 2.8/100 000 live births. All nine cases were identified by the prospective surveillance, and no further cases were found through the retrospective evaluation of laboratory records. During the same period the surveillance yielded a total of 547 cases of invasive neonatal disease, with positive bacterial cultures from blood, cerebrospinal fluid, or any other usually sterile body fluid. H influenzae comprised 1.6% of all neonatal infections.

In seven neonates H influenzae was isolated from the blood and in two cases from several surface sites as well as from the placenta. None
of the nine cases had signs of meningitis, and the cerebrospinal fluid was not cultured from any of the patients.

The onset of disease was early in all the cases, ranging from 0–6 hours after delivery (table 1). Seven patients were premature (gestational age range 25–35 weeks) and weighed less than 2500 g at birth. All three deaths occurred among the preterm neonates.

H influenzae septicaemia was accompanied by raised (>30×10⁹/l) white cell count in two, by leucopenia (<8×10⁹/l) in three, and by normal values in four of the patients. All six neonates from whom serum C reactive protein concentrations were available had increased values (≥20 mg/l). Respiratory distress syndrome was noted in five neonates, all of whom were preterm (gestational age 25–30 weeks) (table 1). Only one child had septicaemia caused by type b H influenzae (case 3, table 1). This was the mother’s third pregnancy and delivery. She had an intrauterine device in place and was not aware of the pregnancy until the delivery started at 30 weeks’ gestation. The child was born at home with a nurse in attendance. Immediately after birth (the labour lasted 1.5 hours) the child was transferred to hospital. On arrival he had breathing difficulties with hyperventilation, bradycardia, hypotonia, and hypothermia. After intubation, and correction of his haemodynamic state, his condition improved. After blood had been taken for culture, antimicrobial treatment with penicillin G and netilmicin was started. Mild respiratory distress syndrome developed and he required two days on the ventilator. Antibiotic treatment was stopped on day 3, before the results of the blood culture were available. As no further signs of septicaemia developed, and the white cell count and C reactive protein remained normal, however, the antibiotic treatment was not restarted again. The child recovered uneventfully and his development has been normal.

Table 1 Clinical features and bacteriological findings in nine infants with H influenzae septicaemia

<table>
<thead>
<tr>
<th>Case No</th>
<th>Sex</th>
<th>Gestation (weeks)</th>
<th>Birth weight (g)</th>
<th>Apgar score</th>
<th>Time from birth/ onset of symptoms (hours)</th>
<th>Outcome</th>
<th>Site of isolation</th>
<th>Isolates of H influenzae</th>
<th>Capsular type</th>
<th>Bio-type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Female</td>
<td>25</td>
<td>810</td>
<td>4</td>
<td>3</td>
<td>0</td>
<td>Died</td>
<td>Blood, Not typable</td>
<td>III</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Male</td>
<td>26</td>
<td>830</td>
<td>3</td>
<td>6</td>
<td>0</td>
<td>Recovered</td>
<td>Blood Not tested</td>
<td>II</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Female</td>
<td>28</td>
<td>1315</td>
<td>5</td>
<td>6</td>
<td>0</td>
<td>Died</td>
<td>Blood Not typable</td>
<td>III</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Male</td>
<td>29</td>
<td>1735</td>
<td>6</td>
<td>7</td>
<td>0</td>
<td>Died</td>
<td>Blood Not typable</td>
<td>III</td>
<td></td>
</tr>
<tr>
<td>5*</td>
<td>Male</td>
<td>30</td>
<td>1420</td>
<td>7</td>
<td>9</td>
<td>0</td>
<td>Recovered</td>
<td>Blood b</td>
<td>I</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Female</td>
<td>35</td>
<td>2410</td>
<td>1</td>
<td>4</td>
<td>0</td>
<td>Recovered</td>
<td>Ear, umbilicus, placenta</td>
<td>Not typable</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Male</td>
<td>35</td>
<td>1760</td>
<td>8</td>
<td>9</td>
<td>1</td>
<td>Recovered</td>
<td>Blood Not typable</td>
<td>II</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Male</td>
<td>35</td>
<td>3130</td>
<td>7</td>
<td>8</td>
<td>0</td>
<td>Recovered</td>
<td>Throat, ear, placenta</td>
<td>Not typable</td>
<td>III</td>
</tr>
<tr>
<td>9</td>
<td>Male</td>
<td>41</td>
<td>3730</td>
<td>9</td>
<td>9</td>
<td>0</td>
<td>Recovered</td>
<td>Blood Not typable</td>
<td>II</td>
<td></td>
</tr>
</tbody>
</table>

*Home delivery.

ISOLATES OF H INFLUENZAe FROM NEONATES

Isolates of H influenzae were available from eight neonates (table 1). In only the above mentioned case was the isolate of capsular type b. It was of biotype I and produced ß-lactamase. The rest of the isolates were non-typable. Four of these were biotype III and three biotype II. None of the non-typable isolates produced ß-lactamase. The antimicrobial treatment given was according to the regimens in the respective hospitals, and in most cases was a combination of netilmicin and ampicillin.

Table 2 Details of mothers of the nine infants with H influenzae septicaemia

<table>
<thead>
<tr>
<th>Case No</th>
<th>Age</th>
<th>Parity</th>
<th>Prematurity rupture of membranes (hours)</th>
<th>Delivery Mode</th>
<th>Length (hours)</th>
<th>Signs of infection</th>
<th>White cell count (×10⁹/l)</th>
<th>C reactive protein concentration mg/l</th>
<th>Site of isolation of H influenzae (strains not available for typing)</th>
<th>Used antimicrobial drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16</td>
<td>I</td>
<td>No</td>
<td>Vaginal</td>
<td>5-5</td>
<td>None</td>
<td>13-3, 11-1</td>
<td>Not recorded</td>
<td>Blood</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>27</td>
<td>II</td>
<td>No</td>
<td>Vaginal</td>
<td>4</td>
<td>Chorioamnionitis</td>
<td>11-9, 31-2</td>
<td>Not recorded</td>
<td>Cervix</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>38</td>
<td>III</td>
<td>No</td>
<td>Caesarean section</td>
<td>60</td>
<td>Septicaemia</td>
<td>19-7</td>
<td>Not recorded</td>
<td>Blood</td>
<td>Yes</td>
</tr>
<tr>
<td>4</td>
<td>30</td>
<td>II/III</td>
<td>No</td>
<td>Vaginal</td>
<td>0-5</td>
<td>None</td>
<td>34-1</td>
<td>Not measured</td>
<td>Cervix</td>
<td>No</td>
</tr>
<tr>
<td>5*</td>
<td>23</td>
<td>III</td>
<td>No</td>
<td>Vaginal</td>
<td>1</td>
<td>Chorioamnionitis</td>
<td>29-5</td>
<td>Not measured</td>
<td>Placenta</td>
<td>No</td>
</tr>
<tr>
<td>6</td>
<td>22</td>
<td>II</td>
<td>No</td>
<td>Vaginal</td>
<td>Not known</td>
<td>Chorioamnionitis, two episodes of meningitis before antibiotic treatment during delivery</td>
<td>Not measured</td>
<td>No</td>
<td>Placenta</td>
<td>No</td>
</tr>
<tr>
<td>7</td>
<td>19</td>
<td>II</td>
<td>No</td>
<td>Vaginal</td>
<td>4</td>
<td>Amnionitis</td>
<td>14-2, 19-8, 12-2</td>
<td>Not measured</td>
<td>Cervix</td>
<td>No</td>
</tr>
<tr>
<td>8</td>
<td>23</td>
<td>I/II</td>
<td>No</td>
<td>Vaginal</td>
<td>6-5</td>
<td>Amnionitis</td>
<td>Not measured</td>
<td>Not measured</td>
<td>Placenta</td>
<td>No</td>
</tr>
<tr>
<td>9</td>
<td>22</td>
<td>I</td>
<td>No</td>
<td>Caesarean section</td>
<td>Not applicable</td>
<td>Chorioamnionitis</td>
<td>11-4, 12-3</td>
<td>Not measured</td>
<td>Cervix</td>
<td>No</td>
</tr>
</tbody>
</table>

*Home delivery.
White cell count \(\times 10^9/l\) & Day of measurement & C reactive protein concentration mg/l & Day of measurement & Respiratory distress syndrome & No of days in respirator & Antimicrobial treatment \\
--- & --- & --- & --- & --- & --- & --- \\
3-2, 6-4 & 0, 1 & Not measured & --- & No & 2 & Not known \\
1-1, 24-4 & 0, 2 & Not measured & --- & Yes & 10 & Netilmicin, ampicillin \\
10-8 & 1 & Not measured & --- & Yes & 2 & Netilmicin, ampicillin \\
6-7, 0-8 & 0, 0 & 21, 51 & 0, 0 & Yes & 1 & Netilmicin, ampicillin \\
17-5 & 2 & 45, <10 & 0, 3 & Yes & 1 & Netilmicin, penicillin G \\
12-3, 33-1 & 0, 1 & 102 & 1 & Not known & 1 & Gentamicin, penicillin G \\
2-6, 13-3 & 0, 4 & <18, 140 & 0, 2 & Not known & 6 & Netilmicin, ampicillin \\
5-1, 14-1 & 0, 2 & 0, 171 & 0, 1 & Not known & Not known & Netilmicin, amoxyclillin \\
23-7, 11-8 & 1, 2 & 51 & 1 & Not known & Not known & Netilmicin, ampicillin \\

Intrauterine devices emerged as a possible risk factor in neonatal \(H\) influenzae infections, as two of the mothers had devices in place during their pregnancies.

Discussion

In the United States between 2-6% and 7-9% of all neonatal septicemias are caused by \(H\) influenzae and an apparent increase of these infections has been reported during the past decades. In addition, a report describing neonatal septicemia in seven centres in Finland during a 10 year period covering roughly 43% of all deliveries in Finland was published in 1989. In that study, a total of six cases of \(H\) influenzae septicemia were found, three during each five year period, 1976–80 and 1981–5.

The present study describes a nationwide study for the five year period 1985–9 in which nine cases of neonatal \(H\) influenzae infection were identified by intensified prospective surveillance. It seems therefore that all cases of neonatal \(H\) influenzae infection from that period are included. Compared with the previous report from Finland there seems to have been no increase in the incidence neonatal \(H\) influenzae infections during the past 15 years, and \(H\) influenzae infections account for only a minority, 1-6%, of all neonatal septicemias.

Our study includes two cases in which \(H\) influenzae was not isolated from blood, but from several surface sites as well as from the placenta. Both these neonates had clinical signs of septicemia, there was a raised white cell count (33-1 \(\times 10^9/l\)) in one case and raised concentrations of C reactive protein in both cases (171 mg/l and 102 mg/l, respectively). The value of culturing a \(H\) influenzae from surface sites is supported by the study in which 70% of neonates with blood cultures growing \(H\) influenzae also had positive cultures from surface sites.

All the six patients in whom C reactive proteins concentrations were assayed had increased values. In contrast to a previous report in which it was suggested that the measurement of C reactive protein is no use in the diagnosis of neonatal septicemia, our results suggest that in \(H\) influenzae septicemia high concentrations of C reactive protein do occur, and the measurement of the concentration might be useful.

The clinical course of neonatal \(H\) influenzae infection was characterized by prematurity, the presence of maternal complications, and high neonatal mortality, as has been reported previously. In the present series all the cases were of early— that is, at delivery (seven cases) or within a few hours after delivery. This compares with earlier reports in which 85% of cases were of early onset defined as less than 24 hours after delivery. All the mothers in the present study had some signs of infection at the time of, or shortly after, delivery and \(H\) influenzae was isolated from 56% of them. Premature rupture of membranes was not a serious predisposing factor to neonatal infection as previously suggested. Three findings support the hypothesis that neonatal \(H\) influenzae infections are transmitted from the mother, that the infection starts before birth, and that it could be the cause of premature birth. Two of the mothers had intrauterine devices in place, a possible predisposing factor for \(H\) influenzae infection. An association between intrauterine devices and neonatal \(H\) influenzae infection has previously not been reported, and there are only few case reports about other pathogens (Escherichia coli and \(C\) cidda albicans) associated with neonatal septicaemia and intrauterine devices.

Seven of the eight neonatal isolates of \(H\) influenzae in the present series were non-typable, and there was one isolate of capsular type b, a finding similar to previous reports. The relative absence of type b isolates causing neonatal \(H\) influenzae infections is thought to be the result of maternal anti-\(H\) influenzae type b antibodies, which last for roughly the first six months of life; after this age the incidence of type b \(H\) influenzae infections increases appreciably. In addition, the colonisation of the female genital tract with type b \(H\) influenzae is rare: carriage of \(H\) influenzae is about 1%, and of the isolates only a few are type b. Preliminary results indicate that serum antibodies against non-typable \(H\) influenzae are relatively high among female population of child bearing age (M Leinonen, personal communication). This may partly explain the rarity of neonatal \(H\) influenzae infections despite the 1% vaginal carriage rate of non-typable \(H\) influenzae.

None of the non-typable isolates of \(H\) influenzae were of biotype IV, which has been reported to account for up to 38% of neonatal isolates in the United States. Geographical differences have also been found in the comparison of isolates of type b \(H\) influenzae that cause invasive infections among older children, using several subtyping methods including biotyping. In these infections biotypes I and II both account...
for about half the isolates in Finland, while biotype I predominates (90%) in the United States and The Netherlands. Geographical differences have also been found among strains of the most common neonatal pathogen, group B streptococcus, with serotype III accounting for a third of cases of early onset in the United States compared with only 10% in Finland. It has been suggested that there could be a difference in the virulence of these strains, leading to a predominance of group B streptococcal disease of early onset and a relative absence of late onset in Finland, a similar difference to that found in the present study of neonatal H influenzae sepsicaemia. It might be that the emergence of biotype IV H influenzae as a genital pathogen in the United States is the cause for the increase of neonatal H influenzae infections.

In Finland these strains (or this clone) do not occur, and thus the incidence of neonatal H influenzae infections has been stable. This clone may also have special properties of virulence, as has been documented for H influenzae type b isolates of a certain clone characteristic to Finland.  
