Neonatal buccal cell collection for DNA analysis

It is considered undesirable to take blood from an infant as a means of obtaining DNA for research purposes. Sufficient DNA for direct gene analysis can be obtained from adult buccal epithelial cells, but there is no evidence that neonatal buccal epithelial cells can similarly be used. We have carried out a preliminary study, approved by the Riverside Research Ethics Committee, to determine the ease of isolating neonatal buccal cells for DNA extraction followed by polymerase chain reaction (PCR) and sequence analysis.

Buccal cells were obtained from eight term and four preterm infants after informed parental consent. A standard microbiological cotton swab or cotton dental roll was rubbed on the inner cheek, and the baby was allowed to suck on it for 30 seconds. The infants were unperturbed by the procedure and mothers found it very acceptable.

Swabs/dental rolls were placed in 0.9% sodium chloride and centrifuged to concentrate the cells into a pellet. The cells were lysed with a standard lysis buffer, and DNA was extracted using a simple phenol/chloroform technique. Spectrophotometry to measure the A260/A280 ratio in order to assess purity of the extracted DNA was carried out using an Eppendorf Biophotometer V1.20. DNA concentration and yield were calculated using an Eppendorf Biophotometer V1.20. DNA concentration and yield were calculated from UV absorbance at 260 nm (A260).

To show that the DNA obtained could be analysed at the single base level, PCR was carried out with the primers AACACTGGTGG CGACAGAA (forward) and TGGGTGCACCT CGCAGAAAT (reverse) to amplify exon 22 of human SCRIBBLE. PCR products were viewed on a 1% agarose gel with a UV transilluminator, sequenced using BigDye and run on an ABI 3100 transilluminator, sequenced using BigDye and run on an ABI 3100 transilluminator, sequenced using BigDye and run on an ABI 3100 transilluminator, sequenced using BigDye and run on an ABI 3100 transilluminator.

Use of conjugate pneumococcal vaccine by United Kingdom neonatal intensive care units

Although the polysaccharide pneumococcal vaccine is ineffective in children less than 2 years of age, the conjugate pneumococcal vaccine, Prevenar, has been shown to be protective.

In 2002, the Chief Medical Officer advised that children under 2 years of age at risk of invasive pneumococcal disease should receive three doses of Prevenar with their primary immunisations followed by a booster in the second year of life. Many of these infants start their immunisations on neonatal units.

In our centre, infants complete their primary course, but not the booster dose. We surveyed 73 United Kingdom neonatal intensive care units to determine Prevenar usage.

Of the 58 (79%) units that replied, 26 (45%) routinely recommend Prevenar vaccination to some or all of their “at risk” patients (table 1). These units immunise a median of 15 infants per year (interquartile range 5–20).

There was no significant difference in size, by number of intensive care beds, between neonatal units that recommend Prevenar and those that do not.

Seventeen units recommend a primary course of three immunisations. The other units recommend one, two, or five doses. Twelve recommend a booster in the second year of life; no units thought that all of their patients were receiving that dose.

Of the units reporting problems with immunisation administration, four found that general practitioners were reluctant to administer Prevenar; the remaining unit had observed adverse effects in one patient. No units were checking vaccine responses.

Our survey indicates that many infants in the United Kingdom at risk of invasive pneumococcal disease are not adequately immunised despite recent recommendations. Infants with chronic lung disease are immunised in only 23% (40%) of the responding neonatal intensive care units.

The most common reason for not recommending conjugate pneumococcal vaccine to patients is the lack of evidence of benefit in a neonatal intensive care population (table 1). Although the Kaiser Permanente subanalysis of preterm and low birthweight infants observed adverse effects in one patient. No units were checking vaccine responses.

Table 1

<table>
<thead>
<tr>
<th>Category</th>
<th>No of units</th>
<th>Reason for not recommending Prevenar</th>
</tr>
</thead>
<tbody>
<tr>
<td>All infants &lt;32 weeks gestation</td>
<td>1</td>
<td>Not sure of at risk criteria</td>
</tr>
<tr>
<td>All infants &lt;30 weeks gestation</td>
<td>1</td>
<td>Too expensive</td>
</tr>
<tr>
<td>All infants with chronic lung diseases</td>
<td>19</td>
<td>Insufficient evidence of benefits of Prevenar in their patient group</td>
</tr>
<tr>
<td>Oxygen dependent infants</td>
<td>4</td>
<td>Currently reviewing guidelines</td>
</tr>
<tr>
<td>All infants with respiratory difficulties</td>
<td>1</td>
<td>Do not feel that recommendations apply to their patient group</td>
</tr>
<tr>
<td>All infants with congenital heart defects</td>
<td>2</td>
<td>Have prioritised other vaccines (BCG, hepatitis B)</td>
</tr>
<tr>
<td>Primary immunodeficiency</td>
<td>2</td>
<td>Would like more information</td>
</tr>
<tr>
<td>GPs in areas reluctant to administer Prevenar</td>
<td>3</td>
<td>Prevenar</td>
</tr>
<tr>
<td>Previous adverse reaction in a patient</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

G Gavriel, N Modi
Faculty of Medicine, Division of Paediatrics, Obstetrics and Gynaecology, Imperial College London, Chelsea & Westminster Hospital, 369 Fulham Road, London SW10 9NH, UK

P Stanier, G E Moore
Faculty of Medicine, Division of Paediatrics, Obstetrics and Gynaecology, Institute of Reproductive and Developmental Biology, Imperial College London, Du Cane Road, London W12 0NN, UK

Correspondence to: Dr Modi; n.modi@imperial.ac.uk
doi: 10.1136/adc.2004.062661

Competing interests: none declared

References
3 Freeman B, Smith N, Curtis C, et al. DNA from buccal swabs recruited by mail: evaluation of storage effects on long-term stability and suitability for multiplex polymerase chain reaction genotyping. Behav Genet 2003;33:67–72
primary immunisation schedule benefit from the administration of conjugate pneumococcal vaccine, and further studies are required to address this.

S J Moss, A C Fenton
Ward 35, Royal Victoria Infirmary, Queen Victoria Road, Newcastle upon Tyne, NE1 4LP, UK

A R Gennery
School of Clinical Medical sciences (Child Health), Newcastle General Hospital, West Road, Newcastle upon Tyne NE4 6BE, UK

Correspondence to: Dr Moss, Neonatal Research Fellow, Ward 35, Royal Victoria Infirmary, Queen Victoria Road, Newcastle upon Tyne NE1 4LP; samantha.f.moss@nuthsc.org.uk

doi: 10.1136/archdischild-2004.061911

Competing interests: none declared

References


Neonatal resuscitation and assessment of cardiovascular status

Following a recent case involving a neonatal resuscitation performed by general paediatricians, it has become apparent that the instructions for assessing circulation in neonates during resuscitation at birth contained within the Advanced paediatric life support (APLS) course and manual are not explicit, and do not highlight the differences between neonatal and paediatric resuscitation practices.

Assessment of circulation in paediatric life support involves checking the pulse, usually at the brachial or femoral artery. The International Liaison Committee on Resuscitation (ILCOR) advisory statement on resuscitation of the newly born infant states that heart rate should be determined by listening to the precordium with a stethoscope, feeling for pulsations at the base of the umbilical cord, or feeling the brachial or femoral pulse. It also points out that central and peripheral pulses are often difficult to feel in infants and should not be relied on independently, if they are absent. This has recently been confirmed by findings in healthy term neonates with heart rates of >100 beats/min on auscultation at 5 minutes of age of impalpable pulses in 20–60%, dependent on the artery palpated.

The APLS chapter on neonatal resuscitation refers to checking the heart rate rather than the pulse,’ but it does not elucidate how this should be done. The European paediatric life support (EPLS) course and manual chapter on resuscitation of the newborn does explain how the circulatory status may be assessed, references the ILCOR advisory statement, but errs towards palpation of the umbilical artery rather than an audible heart rate, and does not add the proviso contained within the ILCOR advisory statement regarding absence of a palpable pulse. Only within the Newborn life support (NLS) course and manual do the guidelines on assessment of heart rate echo the advisory statement from ILCOR, particularly with reference to weak or absent pulses.

Termination of neonatal resuscitation efforts can be difficult to manage for neonatologists, as even apparently stillborn neonates may respond to prolonged and vigorous resuscitation efforts with relatively good outcomes. Among the vast majority of non-neonatologists in the United Kingdom who are very occasionally called upon in this difficult situation, the APLS or EPLS course may constitute the limit of their neonatal resuscitation training, potentially making these decisions even more difficult, because of the lack of specific clarification on how to assess circulatory status and heart rate in neonates. We would urge all those who may at any time undertake neonatal resuscitation to follow the ILCOR advisory statement, and encourage all courses and manuals that include neonatal resuscitation to make their recommendations explicit and concordant with that statement. The Advanced Life Support Group and Resuscitation Council (UK) have both been made aware of our concerns.

P J Davis
Paediatric Intensive Care Unit, Bristol Royal Hospital for Children, Bristol, UK

P A Cairns
Peter Dunn Intensive Care Nursery, St Michael’s Hospital, Bristol, UK

Correspondence to: Dr Davis, Consultant Paediatric Intensive Care Unit, Bristol Royal Hospital for Children, Upper Maudlin Street, Bristol BS2 8BJ, UK; peter.davis@ubht.swest.nhs.uk

doi: 10.1136/archdischild-2004.067173

Competing interests: none declared

References

Neonatal buccal cell collection for DNA analysis

G Gavriel, N Modi, P Stanier and G E Moore

Arch Dis Child Fetal Neonatal Ed 2005 90: F187
doi: 10.1136/adc.2004.062661

Updated information and services can be found at:
http://fn.bmj.com/content/90/2/F187.1

These include:

References
This article cites 4 articles, 2 of which you can access for free at:
http://fn.bmj.com/content/90/2/F187.1#BIBL

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/