PostScript

LETTERS

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,¹ 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.² Spectrophotometry to measure the A_{260}/A_{280} 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 from UV absorbance at 260 nm (A_{260}). To show that the DNA obtained could be analysed at the single base level, PCR was carried out with the primers AACACTGGTGG CGCAGAAAT (forward) and TGGGTGCACCT CTCACAGAA (reverse) to amplify exon 22 of the *human SCRIBBLE* gene. PCR products were viewed on a 1% agarose gel with a UV transilluminator, sequenced using BigDye v3.0 chemistry (PE Applied Biosystems, Warrington, UK) and run on an ABI 3100 Genetic Analyser. Sequence alignment with the BLAST program (<http://www.ncbi.nlm.nih.gov/BLAST/>) was used to confirm accurate sequence amplification.

Spectrophotometry confirmed successful DNA extraction from all samples with a mean DNA yield of 6.65 μ g (SD 4.07; range 1.65–16.40). This compares favourably with DNA yields obtained in similar studies with adult and child subjects where yields of 2–85 μ g have been described.^{3,4} Sequences matching exon 22 of *SCRIBBLE* were successfully obtained using both forward and reverse primers. The mean A_{260}/A_{280} ratio of samples in this work was 1.27 (SD 0.07; range 1.20–1.42). High quality DNA has an A_{260}/A_{280} ratio of about 1.80.

This preliminary study has shown that buccal cells can easily be obtained from term and preterm infants in sufficient quantity for DNA extraction, PCR, and sequencing. The technique is simple and non-invasive and we hope will facilitate neonatal research.

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References

- 1 Lench N, Stanier P, Williamson R. Simple non-invasive method to obtain DNA for gene analysis. *Lancet* 1988;1:1356–8.
- 2 Kunkel LM, Smith KD, Boyer SH, et al. Analysis of human Y-chromosome-specific reiterated DNA in chromosome variants. *Proc Natl Acad Sci USA* 1977;74:1245–9.
- 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.
- 4 Zheng S, Ma X, Buffler PA, et al. Whole genome amplification increases the efficiency and validity of buccal cell genotyping in pediatric populations. *Cancer Epidemiol Biomarkers Prev* 2001;10:697–700.

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,¹ the conjugate pneumococcal vaccine, Prevenar, has been shown to be protective.²

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.³ 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 cots, 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 immunised at 2, 4, and 6 months of age found Prevenar to be immunogenic, all of these infants had been discharged from the neonatal unit before vaccination, thus excluding the most preterm infants.⁴ A United Kingdom immunogenicity study of vaccination at 2, 3, and 4 months of age excluded preterm infants and did not include all vaccines in the current immunisation schedule.⁵ There is therefore no evidence to indicate that preterm infants vaccinated according to the current United Kingdom

Table 1 Patient groups receiving conjugate pneumococcal vaccine in neonatal units and reasons for not recommending its use

Indication for giving Prevenar	No of units	Reason for not recommending Prevenar	No of units
All infants <32 weeks gestation	1	Not sure of at risk criteria	1
All infants <30 weeks gestation	1	Too expensive	2
All infants with chronic lung diseases	19	Insufficient evidence of benefits of Prevenar in their patient group	14
Oxygen dependent infants	4	Currently reviewing guidelines	3
All infants who have respiratory difficulties while on the neonatal intensive care unit	1	Do not feel that recommendations apply to their patient group	10
Infants with congenital heart defects	2	Have prioritised other vaccines (BCG, hepatitis B)	1
Primary immunodeficiency	2	Would like more information	1
		GPs in area reluctant to administer Prevenar	3
		Previous adverse reaction in a patient	1

primary immunisation schedule benefit from the administration of conjugate pneumococcal vaccine, and further studies are required to address this.

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References

- 1 Koskela M, Leinonen M, Häivä V, *et al*. First and second dose antibody responses to pneumococcal polysaccharide vaccine in infants. *Pediatr Infect Dis* 1986;5:45–50.
- 2 Black S, Shinefield H, Fireman B, *et al*. Efficacy, safety and immunogenicity of heptavalent pneumococcal conjugate vaccine in children. Northern California Kaiser Permanente Vaccine Study Center Group. *Pediatr Infect Dis J* 2000;19:187–95.
- 3 Chief medical Officer. Pneumococcal vaccine for at-risk under 2 year olds. <http://www.doh.gov.uk/cmo/cmo0201.htm>.
- 4 Shinefield H, Black S, Ray P, *et al*. Efficacy, immunogenicity and safety of heptavalent pneumococcal conjugate vaccine in low birth weight and preterm infants. *Pediatr Infect Dis J* 2002;21:182–6.
- 5 Choo S, Zhang Q, Seymour L, *et al*. Primary and booster salivary antibody responses to a 7-valent pneumococcal conjugate vaccine in infants. *J Infect Dis* 2000;182:1260–3.

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 manual¹ 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 infant² 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.³

The APLS chapter on neonatal resuscitation refers to checking the heart rate rather than the pulse,¹ but it does not elucidate how this should be done. The European paediatric life support (EPLS) course and manual chapter on resuscitation of the newborn⁴ 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 manual⁵ 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.⁶ 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.

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References

- 1 Advanced Life Support Group. *Advanced paediatric life support: the practical approach*, 3rd ed. London: BMJ Publishing, 2001:59–67.
- 2 Kattwinkel J, Niermeyer S, Nadkarni V, *et al*. ILCOR advisory statement: resuscitation of the newly born infant; an advisory statement from the pediatric working group of the International Liaison Committee on Resuscitation. *Circulation* 1999;99:1927–38.
- 3 Owen CJ, Wyllie JP. Determination of heart rate in the baby at birth. *Resuscitation* 2004;60:213–17.
- 4 European Resuscitation Council. *European paediatric life support provider course manual*. London: Resuscitation Council (UK), 2003:77–87.
- 5 Resuscitation Council (UK). *Resuscitation at birth. The newborn life support provider course manual*. London: Resuscitation Council (UK), 2001:4–6.
- 6 Casalaz DM, Marlow N, Spiedel BD. Outcome of resuscitation following unexpected apparent stillbirth. *Arch Dis Child Fetal Neonatal* 1998;78:F112–15.