Prevention of late-onset sepsis in the neonate

The number of infants at risk of late-onset sepsis (LOS) has increased due to the improved survival of very low birthweight infants and their need for invasive monitoring and support. LOS is a cause of significant mortality and morbidity that in turn results in life-long economic consequences for society. Effective strategies to prevent LOS and regular feedback of surveillance data are associated with a progressive decrease in incidence of infection. This literature review explores such preventative strategies and the evidence for NICU care bundles in preventing LOS.

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Keywords

newborn; intensive care; low birth weight; premature; late-onset sepsis; infection; hand hygiene; care bundle

Key points

Soni R., English S. Prevention of late-onset sepsis in the neonate. *Infant* 2015; 11(2): 42-46.

1. Late-onset sepsis poses a significant problem in the neonatal intensive care setting leading to significant morbidity and mortality.
2. Hand hygiene remains the most effective intervention for reducing healthcare-associated infection.
3. There are available guidelines to prevent catheter-related infections.
4. There is an increasing focus on NICU care bundles to reduce the incidence of hospital-acquired infection.

Neonatal sepsis is conventionally divided into early-onset (EOS) and late-onset sepsis (LOS). EOS is acquired intrapartum and occurs within the first 48 hours of life. LOS is usually acquired after birth, often nosocomial and occurs after 48 hours of life.[1]

Unlike EOS, for which an intervention (intrapartum antibiotic prophylaxis) has resulted in a marked decrease in the overall incidence, the number of infants at risk of LOS has increased due to the improved survival of very low birthweight (VLBW) infants and their need for invasive monitoring and support.[2,3] LOS is a cause of significant mortality and morbidity which in turn results in life-long economic consequences for society.[4]

Effective strategies to prevent LOS and regular feedback of surveillance data are associated with a progressive decrease in incidence of infection.[5-8] The incidence of LOS varies among neonatal intensive care units (NICUs) depending on environmental factors and on differences in clinical practice.[9-13]

This literature review intends to study LOS and identify effective preventative strategies. The evidence for NICU care bundles in preventing LOS has been looked at in detail.

The search strategy

Medline was searched via the OVID interface from 1996 to June (week 3) 2013 using the following keywords: nosocomial infection, late-onset sepsis, healthcare associated infection with neonate and neonatal intensive care. No limits were set.

A total of 291 articles were identified and their abstracts reviewed. Thirty-five articles were considered relevant. Some of the quoted references from these articles were also deemed suitable and included in this review.

Review of available literature

Incidence and risk factors for LOS

In developed countries, LOS occurs in approximately 0.8% of live births and in 3-28% of patients admitted to neonatal units.[14-16] The variable reported incidence in different neonatal units suggests that multiple factors are involved.[16] 'Suspected' sepsis is much more frequent than 'confirmed' sepsis.[15]

VLBW (<1,500g) infants are most susceptible to infection. This is due to:

- deficiencies in their immune system (including immunoglobulin production, opsonic and phagocytic functions and complement)
- a requirement for more invasive procedures, total parenteral nutrition (TPN) and mechanical ventilation
- a longer length of hospital stay.[14]

When VLBW neonates are considered alone, rates of LOS range between 10.6 and 31.7%.[14]

According to one study, mechanical ventilation (with mean days of 23.7 ± 0.5), a central venous catheter (CVC, with mean days of 16.4 ±0.4) and TPN (with mean days of 33.1 ± 0.5) were associated with increased risk of LOS.[15]

Other risk factors for LOS include intravenous cannulae and structural factors such as overcrowding and nurse-to-

patient ratio.[14] Studies have consistently demonstrated the relationship between central catheter use and an increased risk of infection.[17-19] Intravenous lipid administration may be an independent risk factor for bacterial or fungal sepsis.[20]

Definitions[19]

Late-onset sepsis

Various definitions of LOS are in use. LOS has been defined as sepsis occurring after 48 hours, 72 hours, after three or four days. Some definitions use specific clinical signs to define sepsis and others only require a blood culture to be positive.

Central line-associated bloodstream infection

The terms used to describe intravascular catheter-related infections can also be confusing because catheter-related bloodstream infection (CRBSI) and central line-associated bloodstream infection (CLABSI) are often used interchangeably even though their meanings differ.

CRBSI is a clinical and more thorough definition used when diagnosing and treating patients. It requires specific laboratory testing that more thoroughly identifies the catheter as the source of the bloodstream infection (BSI). Due to difficulties with precisely establishing if a BSI is a CRBSI, simpler definitions such as CLABSI are used for surveillance purposes. The definition of CLABSI must meet all of the following criteria:

1. The patient has one or more recognised pathogens cultured from one or more blood cultures
2. The presence of one or more CVCs at the time of the blood culture, or CVC removed within the preceding 48 hours
3. The signs and symptoms and positive laboratory results, including pathogen cultured from the blood, are not primarily related to an infection at another site.

Since some BSIs are secondary to sources other than the central line that may not be easily recognised, the CLABSI surveillance definition may overestimate the true incidence of CRBSI.

Outcome and long-term impact

Nearly 20% of deaths in VLBW infants are caused by sepsis. Infants with sepsis are three times as likely to die as those without sepsis, even after adjusting for gestational age, sex and other co-morbidities.[15,16]

In addition to incurring higher costs during hospitalisation in the NICU, surviving VLBW infants are at increased risk for developing morbidities, including bronchopulmonary dysplasia, neuro-developmental impairment and prolonged hospital stay.[15, 21-23] They frequently require additional educational resources, leading to life-long economic consequences for society.[24,25]

One study[26] reported outcomes of extremely low birthweight (ELBW) infants using the Bayley Scales of Infant Development (BSID)-II. Infants with LOS had an increased risk of:

- cerebral palsy
- mental developmental index <70
- psychomotor developmental index <70
- growth impairment at 18 months of age.

Infants with a history of both necrotising enterocolitis (NEC) and sepsis were at the highest risk.

The Swiss Neonatal Network reported neurodevelopment outcomes of preterm infants with a gestation of 24-27 weeks at two years of age using BSID-II.[27] Twenty-five percent of infants had culture-proven sepsis, which was independently associated with an increased risk of cerebral palsy (p=0.017).

Responsible organisms

LOS is most often caused by gram positive organisms, followed by gram negative organisms and *Candida.*[15,28]

Staphylococcus aureus may cause mild to severe disease depending on its virulence and host defences. The rapid recent increase of methicillin-resistant *Staphylococcus aureus* (MRSA) infection in the healthcare setting has raised great concern, with neonates being particularly vulnerable. MRSA may cause serious disease or asymptomatic colonisation. There are limited data on the impact of MRSA in neonates compared to that in adults. Studies have demonstrated an increase in length of stay following MRSA infection and subsequent increased healthcare cost, but no statistically significant effect on mortality.[29]

Coagulase-negative staphylococci (CoNS) are a common cause of healthcare-associated infection (HCAI) and the most common cause of BSI in the intensive care setting.[30] Thirty-eight species of CoNS have been recognised, of which 13 are known to colonise humans. CoNS are frequently reported in VLBW infants: those that are immunocompromised, burns patients and those with indwelling intravascular devices, ventricular shunts, peritoneal catheters or other implanted medical devices.[30]

CoNS are normal skin commensal flora and can contaminate cultures either at the time of blood sampling or during the blood culture process. Differentiating infection from contamination to establish a diagnosis of CoNS sepsis can therefore be difficult.

The clinical presentation of CoNS and *Candida* sepsis in the neonate is often slow and non-specific.[31] This is in contrast to the septic shock seen in infections caused by gram negative rods or virulent gram positive organisms. In addition, the sensitivity of blood cultures in diagnosing candidaemia is low (50-80%) compared to that in detecting bacteraemia.[32]

Although BSI is the most common presentation, *Candida* can cause meningitis, renal, splenic or liver abscesses, endophthalmitis, osteomyelitis or invasive dermatitis. The crude reported mortality caused by fungal infections is 25-50%.[19,33]

With regards to central catheters, extraluminal contamination of the intracutaneous tract, by CoNS or other organisms colonising the skin around the catheter exit site, is believed to be responsible for catheter-related infections that happen in the first week after insertion.[34]

After the first week of placement, intraluminal colonisation due to hub contamination is responsible for most CLABSI.[34] One study demonstrated that the frequency of catheter manipulations was directly related to the frequency of CLABSI.[35] Translocation across the gastrointestinal tract epithelium by *Candida* and other organisms can also cause catheter-related infections.[36]

Strategies for prevention of HCAI

Hand hygiene

Hand hygiene remains the most effective intervention for reducing HCAI.[37] Hospitals with higher rates of hand hygiene compliance have lower rates of central line BSI. However, compliance is problematic.[38]

The sixth edition of *Guidelines for Perinatal Care*[39] recommends use of an antiseptic soap or an alcohol-based gel or foam for routine hand sanitising if hands are not visibly soiled. When hands are visibly contaminated, they should first be washed with soap and water. Several studies have demonstrated the effectiveness of alcohol-based products. Compliance

may be enhanced by having alcohol-based products available at each infant's bedside.

Aseptic precautions

Evidence indicates that strict aseptic precautions during the maintenance and utilisation of CVCs can contribute to lowering the risk of catheter infection in critically ill neonates.[34]

Chlorhexidine gluconate skin cleanser rapidly and effectively kills most skin pathogens with residual effects for up to 24 hours.[40] It is well tolerated in paediatric and adult patients. Episodes of contact dermatitis are seen to occur in neonates but mostly so in those less than one week old and ≤28 weeks' gestation at birth.[40]

Techniques to reduce catheter contamination

Techniques to reduce the likelihood of extraluminal contamination of central catheters include proper hand hygiene, aseptic catheter insertion (including use of a maximal sterile barrier for catheter insertion and care), use of a topical antiseptic and use of sterile dressing. Although transparent dressings permit easier inspection of catheter sites, they have no proven benefit in reducing infection. Catheter sites must be monitored visually or by palpation on a daily basis.[34]

In order to minimise intraluminal contamination, tubing used to administer blood products or lipid emulsions should be changed daily. Tubing used to infuse dextrose and amino acids should be replaced every 4-7 days.[34] It is important to frequently review the need for all CVCs and remove them when no longer essential. Topical antibiotic agents or creams should not be used at the insertion site for catheters.[34] Guidelines for the prevention of intravascular catheter-related infections have been published by the Healthcare Infection Control Practices Advisory Committee,[20] which make specific recommendations for umbilical catheters (**TABLE 1**).

MRSA infection

There are different approaches to controlling MRSA colonisation and infection. There is no good evidence to support screening patients for MRSA but it may allow identification of distribution and targeting of control measures.[41,42] Screening of staff is controversial. There is no consensus on treatment of colonised neonates. Deep cleaning, restricting admissions to avoid overcrowding and having adequate members of staff and occasional closing of the unit may be required in an attempt to curb spread of infection.[43] There are reports of successful management of outbreaks by setting up an outbreak management team.[44]

NICU care bundles

There is now an increasing focus on NICU care bundles to reduce the incidence of hospital acquired infections. The Institute for Healthcare Improvement defines a care bundle as: 'A small set of evidence-based interventions for a defined patient population and care setting that, when implemented together, will result in significantly better outcomes than when implemented individually'. NICU care bundles have been extrapolated from studies in adults or recommendations from professional organisations. Several studies have demonstrated a reduction in the incidence of HCAI where NICU care bundles have been implemented (**TABLE 2**).[5,10-13]

Other strategies

Topical emollients have been used to decrease transepidermal water loss and have been suggested as a method to decrease HCAI. The use of expressed breast milk has been associated with a lower risk of sepsis and NEC in preterm infants.[45]

There has been consistent evidence on the use of prophylactic fluconazole for prevention of fungal LOS. More recently, bovine lactoferrin has been shown to be effective in prevention of both bacterial and fungal infections. This innate immune system glycoprotein plays an important role in host defence and has been shown to be effective in a multicentre randomised controlled trial on VLBW neonates.[46]

1. Remove and do not replace umbilical artery catheters if any signs of CLABSI, vascular insufficiency in the lower extremities or thrombosis are present.
2. Remove and do not replace umbilical venous catheters if any signs of CLABSI or thrombosis are present.
3. Cleanse the umbilical insertion site with an antiseptic before catheter insertion. Avoid tincture of iodine because of the potential effect on the neonatal thyroid.
4. Do not use topical antibiotic ointment or creams on catheter insertion sites because of the potential to promote fungal infections and antimicrobial resistance.
5. Add low doses of heparin (0.25-1.0 units/mL) to the fluid infused through umbilical arterial catheters.
6. Remove umbilical catheters as soon as possible when no longer needed or when any sign of vascular insufficiency to the lower extremities is observed. Optimally, umbilical artery catheters should not be left in place for more than five days.
7. Umbilical venous catheters should be removed as soon as possible when no longer needed but can be used for up to 14 days if managed aseptically.
8. An umbilical catheter may be replaced if it is malfunctioning and there is no other indication for catheter removal and the total duration of catheterisation has not exceeded five days for an umbilical artery catheter or 14 days for an umbilical vein catheter.

TABLE 1 Guidelines for the prevention of intravascular catheter-related infections.[20]
Key: CLABSI = central line-associated bloodstream infection.

Conclusions

LOS poses a significant problem in the neonatal intensive care setting. VLBW infants are the most susceptible. Surviving infants are at increased risk of morbidity. The immediate and long-term economic consequences add to the disease burden of LOS.

There is increasing evidence to demonstrate a progressive decrease in the incidence of infection following effective preventative strategies and regular feedback of surveillance data. However, implementation of these strategies can be challenging and requires a concerted team effort by all individuals involved in the care of infants.

References

1. **Garner J.S., Jarvis W.R., Emori T.G. et al.** CDC definitions for nosocomial infections, 1988. *Am J Infect Control* 1988;16:128-40.
2. **Centers for Disease Control and Prevention.** Early-onset group B streptococcal disease-United States, 1998-1999. *Morb Mortal Wkly Rep* 2000;49:793-96.
3. **Centers for Disease Control and Prevention.** Perinatal group B streptococcal disease after

universal screening recommendations-United States, 2003-2005. *Morb Mortal Wkly Rep* 2007;56:701-05.
4. **Bizzarro M.J.** Health care associated infections in the neonatal intensive care unit: barriers to continued success. *Semin Perinatol* 2012;36:437-44.
5. **Bizzarro M.J., Sabo B., Noonan M. et al.** A quality improvement initiative to reduce central line-associated bloodstream infections in a neonatal intensive care unit. *Infect Control Hosp Epidemiol* 2010;31:241-48.
6. **Ng S.P., Gomez J.M., Lim S.H., Ho N.K.** Reduction of nosocomial infection in a neonatal intensive care unit. *Singapore Med J* 1998;39:319-23.
7. **Curtis C., Shetty N.** Recent trends and prevention of infection in the neonatal intensive care unit. *Curr Opin Infect Dis* 2008 21:350-56.
8. **Graham P.L.** Simple strategies to reduce healthcare associated infections in the neonatal intensive care unit: line, tube, and hand hygiene. *Clin Perinatol* 2010;37:645-53.
9. **Borghesi A., Stronati M.** Strategies for the prevention of hospital-acquired infections in the neonatal intensive care unit. *J Hosp Infect* 2008;68:293-300.
10. **Aly H., Herson V., Duncan A. et al.** Is bloodstream infection preventable among premature infants? A tale of two cities. *Pediatrics* 2005;115:1513-18.
11. **Kilbride H.W., Wirtschafter D.D., Powers R.J., Sheehan M.B.** Implementation of evidence based potentially better practices to decrease nosocomial infections. *Pediatrics* 2012;129:e1085-93.
12. **Bloom B.T., Craddock A., Delmore P.M. et al.** Reducing acquired infections in the NICU: observing and implementing meaningful differences in process between high and low acquired infection rate centers. *J Perinatol* 2003;23:489-92.
13. **Andersen C., Hart J., Vemgal P., Harrison C.** Prospective evaluation of a multi-factorial prevention strategy on the impact of nosocomial infection in very-low-birth weight infants. *J Hosp Infect* 2005;61:162-67.
14. **Jones A.R., Kuschel C., Jacobs S., Doyle L.W.** Reduction in late-onset sepsis on relocating a neonatal intensive care nursery. *J Paediatr Child Health* 2012;48:891-95.
15. **Stoll B.J., Hansen N., Fanaroff A.A. et al.** Late-onset sepsis in very low birth weight neonates: the experience of the NICHD Neonatal Research Network. *Pediatrics* 2002;110:285-91.
16. **Stoll B.J., Hansen N., Fanaroff A.A. et al.** Changes in pathogens causing early-onset sepsis in very-low-birth-weight infants. *N Engl J Med* 2002;347:240-47.
17. **Clark R., Powers R., White R. et al.** Nosocomial infection in the NICU: A medical complication or unavoidable problem? *J Perinatol* 2004;24:382-88.
18. **Karlowicz M.G., Furigay P.J., Croitoru D.P. et al.** Central venous catheter removal versus *in situ* treatment in neonates with coagulase-negative staphylococcal bacteremia. *Pediatr Infect Dis J* 2002;21:22-27.
19. **Weese-Mayer D.E., Fondriest D.W., Brouillette R.T., Shulman S.T.** Risk factors associated with candidemia in the neonatal intensive care unit: a case-control study. *Pediatr Infect Dis J* 1987;6:190-96.
20. **O'Grady N.P., Alexander M., Burns L.A. et al.** Healthcare Infection Control Practices Advisory Committee (HICPAC). Guidelines for the prevention of intravascular catheter-related infections. *Clin Infect Dis* 2011;52:e162-93.
21. **Taylor H.G., Minich N., Bangert B. et al.** Long-term neuropsychological outcomes of very low birth weight: associations with early risks for periventricular brain insults. *J Int Neuropsychol Soc* 2004;10:987-1004.
22. **Polin R.A.** Systemic infection and brain injury in the preterm infant. *J Pediatr (Rio J)* 2008;84:188-91.
23. **Silveira R.C., Procianoy R.S., Dill J.C., da Costa C.S.** Periventricular leukomalacia in very low birth weight preterm neonates with high risk for neonatal sepsis. *J Pediatr (Rio J)* 2008;84:211-16.
24. **Shah D.K., Doyle L.W., Anderson P.J. et al.** Adverse neurodevelopment in preterm infants with postnatal sepsis or necrotizing enterocolitis is mediated by white matter abnormalities on magnetic resonance imaging at term. *J Pediatr* 2008;153:170-75.
25. **Johnson T.J., Patel A.L. et al.** Cost of morbidities in very low birth weight Infants. *J Pediatr* 2013;162:243-49.
26. **Stoll B.J., Hansen N.I., Adams-Chapman I. et al.** Neurodevelopmental and growth impairment

Citation	Study type/design	Outcomes	Results	Comments
Kilbride et al (2003)[11]	A prospective study. Six participating NICUs. Three areas addressed: hand hygiene, line management and accuracy of diagnosis	Incidence of CoNS bacteraemia	24.6% in 1997 decreased to 16.4% in 2000	Baseline assessment of compliance, education and feedback to staff
Bloom et al (2003)[12]	Implementation process differences between high and low infection centres were studied and meaningful differences shared. A case control study was conducted at a single site (the demonstration site)	Positive blood culture >3 days	Network infection rate = 3/1000 (from 3.8) patient days. Demonstration site infection rate = 4/1000 (from 7.4)	At the demonstration site, the average hospital charge reduced from $272,348 to $220,044 and the average total cost reduced from $60,826 to $48,916
Anderson et al (2005)[13]	Six months' surveillance followed by package of interventions	BSI, length of stay, length of ventilation, death	BSI decreased from 21% to 9% No difference in length of stay, length of ventilation and mortality	174 newborn infants required 1,359 intravascular devices, including peripheral vascular lines
Aly et al (2005)[10]	Infection data from 16 NICUs reviewed. Practices examined in the NICU with the lowest rate (eg closed medical system for line management) and implemented at another NICU	BSI rate	CRBSI reduced from 15.17/1000 line days to 2.1/1000	The study included 233 VLBW infants: 90 in the pre-intervention group, 143 in the post-intervention group
Bizzarro et al (2010)[5]	Quasi-experimental study of educational intervention for CVC placement and care	CLABSI rate pre- and post-intervention	CLABSI decreased from 8.4 to 1.28/1000 line days Late-onset sepsis reduced from 5.84 to 1.42/1000 patient days	All NICU patients with CVCs were included

TABLE 2 Effects of care bundles in reducing infection rates.
Key: CoNS = coagulase-negative staphylococci, BSI = bloodstream infection, CLABSI = central line-associated bloodstream infection, CRBSI = catheter-related bloodstream infection, VLBW = very low birthweight, CVC = central venous catheter.

among extremely low-birth-weight infants with neonatal infection. *JAMA* 2004;292:2357-65.
27. **Schlapbach L.J., Aebischer M., Adams M. et al.** Impact of sepsis on neurodevelopmental outcome in a Swiss national cohort of extremely premature infants. *Pediatrics* 2011;128:e348-57.
28. **Stoll B.J., Gordon T., Korones S.B. et al.** Late-onset sepsis in very low birth weight neonates: a report from the National Institute of Child Health and Human Development Neonatal Research Network. *J Pediatr* 1996;129:63-71.
29. **Song X., Perencevich E., Campos J. et al.** Clinical and economic impact of methicillin-resistant *Staphylococcus aureus* colonization or infection on neonates in intensive care units. *Infect Control Hosp Epidemiol* 2010;31:177-82.
30. **National Nosocomial Infections Surveillance (NNIS) System Report.** Data Summary from January 1992-June 2001. *Am J Infect Control* 2001;29:404-21.
31. **St Geme J.W.** Staphylococcus epidermidis and other coagulase-negative staphylococci. In: Long S.S. (ed.) *Principles and Practice of Pediatric Infectious Diseases*. New York: Churchill Livingstone; 1997:pp795.
32. **Pizzo P.A., Robichaud K.J., Gill F.A., Witebsky F.G.** Empiric antibiotic and antifungal therapy for cancer patients with prolonged fever and granulocytopenia. *Am J Med* 1982;72:101-10.
33. **Saxen H., Virtanen M., Carlson P. et al.** Neonatal *Candida* parapsilosis outbreak with a high case fatality rate. *Pediatr Infect Dis J* 1995;14:776-81.
34. **Polin R.A., Denson S., Brady M.T. et al.** Strategies for prevention of health care-associated infections in the NICU. *Pediatrics* 2012;129;e1085-93.
35. **Mahieu L.M., De Muynck A.O., Ieven M.M. et al.** Risk factors for central vascular catheter-associated bloodstream infections among patients in a neonatal intensive care unit. *J Hosp Infect* 2001;48:108-16.
36. **Saiman L., Ludington E., Pfaller M. et al.** Risk factors for candidaemia in neonatal intensive care unit patients: The National Epidemiology of Mycosis Survey study group. *Pediatr Infect Dis J* 2000;19:319-24.
37. **Larson E.** Skin hygiene and infection prevention: more of the same or different approaches? *Clin Infect Dis* 1999;29:1287-94.
38. **Harbarth S., Pittet D. et al.** Interventional study to evaluate the impact of an alcohol-based hand gel in improving hand hygiene compliance. *Pediatr Infect Dis J* 2002;21:489-95.
39. **Ong S.** Guidelines for Perinatal Care, 6th edition. *The Obstetrician & Gynaecologist* 2008;10:207 doi: 10.1576/toag.10.3.207.27429.
40. **Garland J.S., Alex C.P., Uhing M.R. et al.** Pilot trial to compare tolerance of chlorhexidine gluconate to povidone-iodine antisepsis for central venous catheter placement in neonates. *J Perinatol* 2009;29:808-13.
41. **Jeyeratnam D., Whitty C.J., Phillips K. et al.** Impact of rapid screening tests on acquisition of meticillin resistant *Staphylococcus aureus*: cluster randomised crossover trial. *BMJ* 2008;336:927-30.
42. **Melzer M., Bain L., Drabu Y.J.** Preventing infections from cannulas reduces MRSA. *BMJ* 2008;336: 1085-86.
43. **Laing I.A., Gibb A.P., McCallum A.** Controlling an outbreak of MRSA in the neonatal unit: a steep learning curve. *Arch Dis Child Fetal Neonatal Ed* 2009;94:F307-10. doi:10.1136/adc.2008.151290.
44. **Heinrich N., Mueller A., Bartmann P. et al.** Successful management of an MRSA outbreak in a neonatal intensive care unit. *Eur J Clin Microbiol Infect Dis* 2011;30:909-13.
45. **Schanler R.J.** Evaluation of the evidence to support current recommendations to meet the needs of premature infants: the role of human milk. *Am J Clin Nutr* 2007;85:625S-28S.
46. **Manzoni P., Tarnow-Mordi W., Franco C. et al.** Clinical use of lactoferrin in preterm neonates: an update. *Minerva Pediatr* 2010;62:101-04.