Journal of the Formosan Medical Association (2015) 114, 174–179

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ORIGINAL ARTICLE

Efficacy of recombinant human granulocyte colony stimulating factor in very-low-birth-weight infants with early neutropenia

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Received 17 August 2012; received in revised form 11 October 2012; accepted 11 October 2012

KEYWORDS
granulocyte-colony-stimulating-factor;
neutropenia;
nosocomial infection;
very-low-birth-weight

Background/Purpose: Neutropenia is a risk factor for nosocomial infections (NI) in very-low-birth-weight (VLBW) infants. Although recombinant human granulocyte colony stimulating factor (rhG-CSF) increases the neutrophil counts in neutropenic VLBW infants, its long-term efficacy for early neutropenia (EN) remains unknown.
Methods: In this case-controlled study, charts of VLBW recipients of rhG-CSF for EN (total neutrophil count $<1.5 \times 10^9$/L during first 7 days) were reviewed and compared to gestational age, total neutrophil count, and birth weight matched infants unexposed to rhG-CSF.
Results: Twenty-seven infants were identified in each group. Mortality and morbidity did not differ between the two groups. Rate of NI (16/27 vs. 4/27, $p = 0.002$, odds ratio = 8.36) as well as the total number of episodes of NI (22 vs. 4, $p = 0.007$) were higher in rhG-CSF (+) group than in the rhG-CSF (−) group.
Conclusion: Our experience does not show benefit in empirical use of rhG-CSF in preventing NI in VLBW infants with EN.

Introduction

Neutrophils play an important role in host defense against infections. Neutropenia is common in premature infants and neutrophil function is impaired[1] in this group. Neutropenia usually develops in the first week of life, affects 5–68% of premature infants, and is normally transient.[2–4] Manroe et al

Conflicts of interest: The authors have no conflicts of interest relevant to this article.

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http://dx.doi.org/10.1016/j.jfma.2012.10.009

first identified neutropenia as an important predictor of infection in newborn infants.[5] Subsequent studies supported the contention that early neutropenia (EN) in premature infants may increase the incidence of nosocomial infection (NI) especially when it is associated with maternal hypertension.[6,7] It has been proposed that increasing the neutrophil count in neutropenic premature infants by either recombinant human granulocyte-colony-stimulating factor (rhG-CSF),[8–10] or granulocyte-macrophage-colony-stimulating factor (rhGM-CSF)[11] may prevent or decrease NI. There is one randomized controlled trial published in neonatal neutropenia, developed within 21 days (mean >4 days) of life, to study prophylactic rhG-CSF use in decreasing NI in premature infants which demonstrates efficacy for only 2 weeks.[12] The *Cochrane Review* finds no sufficient evidence to support the introduction of either G-CSF or GM-CSF into neonatal practice, either as treatment of established systemic infection to reduce mortality, or as prophylaxis to prevent systemic infection in high risk neonates.[13] Nonetheless, some neonatologists empirically administer rhG-CSF to premature infants with EN on the assumption that it reduces the risk of NI. This practice prompted us to review our own experience. We previously reported that EN, developed in the 1st week of life, is not associated with increased incidence of NI in very-low-birth-weight (VLBW) infants.[14] We now examine the efficacy of empirical use of rhG-CSF for EN in this group of infants.

Methods

Records of VLBW infants admitted to the neonatal intensive care unit of University of Florida Health Science Center at Jacksonville, between January 2002 and July 2004, were reviewed with the approval of the Institutional Review Board (UF-IRB3). There were totally 338 VLBW infants with gestational age <34 weeks that were admitted during this period. EN was defined as a total neutrophil count (TNC) $<1.5 \times 10^9$/L within the 1st week of life. Infants who developed neutropenia within 24 hours of a positive culture, or those who received rhG-CSF within 24 hours of a positive culture, were excluded. Empirical rhG-CSF (5–10 μg/kg/day for 3–5 days) for EN, once daily via intravenous lines, was prescribed at the discretion of the attending neonatologist(s). Our group determined as the unit policy to give rhG-CSF via the intravenous route after discussion with our pharmacists.

Total white cell counts were obtained using an automatic cell counter after correction for nucleated red blood cells. TNC were obtained by multiplying total white cell counts by the sum of percentages of segment, band, and metamyelocyte. NI was defined as a positive culture of a body fluid after 3 days of life. Two positive cultures obtained from two different sites on the same day were required to categorize coagulase-negative *Staphylococcus* as a true NI. The time of positive culture was defined as the time when it was drawn. Microbiology reports were obtained from the hospital mainframe computer system and cross-referenced with both medical record number and date of birth of the patient. The computerized database of hospital pharmacy was used to identify the dates and times of administration of all prescriptions of rhG-CSF.

VLBW infants who received empirical rhG-CSF for EN and fulfilled our predetermined criteria were included in rhG-CSF (+) group. During the same period, VLBW infants with EN but unexposed to rhG-CSF and matched for gestational age (±1 week), birth weight (±150 g), and the lowest TNC in the 1st week of life ($\pm 250 \times 10^6$/L) comprised the rhG-CSF (−) group.

Gestational age (GA) was determined either by the last menstrual period or prenatal ultrasound before 20 weeks' gestation. Birth weight below the 10th percentile of the corresponding gestational age was defined as small for dates. Maternal hypertension included pre-eclampsia/eclampsia, chronic hypertension, and HELLP syndrome. Central line was placed by a team of health professionals, used only for parenteral nutrition, and was considered as positive when NI occurred in the presence of central line(s). Oxygen requirement at 28 days was defined as bronchopulmonary dysplasia, whereas oxygen usage after post-conceptional age of 36 weeks was defined as chronic lung disease (CLD). Necrotizing enterocolitis (NEC) was defined using modified Bell's staging criteria[15] and included infants with Stage II or higher NEC. An outcome of patent *ductus arteriosus* was assigned if an infant was treated either with indomethacin or ibuprofen or by surgical ligation. Papile's classification[16] was used to grade intraventricular hemorrhage. Age of NI was determined by the date of positive culture(s).

Statistical analysis

Data were analyzed after de-identification. All continuous variables were analyzed by nonparametric test (paired and nonpaired) and expressed as median (interquartile range, IQR). Odds ratios (OR) with 95% confidence intervals (CI) were obtained for Fisher's exact test. In order to avoid inappropriate matching, data of all infants with EN were entered into multivariate logistic regression using NI as the dependent variable with GA, birth weight, sex, premature rupture of membranes, maternal hypertension, central line usage, total days on parenteral nutrition, small for date status, lowest TNC within the first 7 days, and prophylactic use of rhG-CSF for EN as independent variables. Log rank test was used to compare the probability of NI between rhG-CSF (+) and rhG-CSF (−) groups and hazard ratio (with 95% CI) was obtained. A p-value <0.05 for any independent variable was interpreted as significant. MedCalc version 12.2 was used for statistical analyses.

Results

Of 338 VLBW infants, 332 had hemogram data within the 1st week of life. Among these 332 infants, 113 (34.0%) developed EN and 31 received rhG-CSF. Eleven infants were excluded due to early infection (8 infants) or dying within 72 hours after birth (3 infants; Fig. 1). Of the remaining 102 VLBW infants with EN not associated with infection, 27 (26.5%) received empirical rhG-CSF within 5 days after birth (median = 1 days, IQR: 0–2 days). These infants comprised the rhG-CSF (+) group. We selected 27 infants in the rhG-CSF (−) group who matched infants in the rhG-CSF (+) group. These two groups were compatible with the

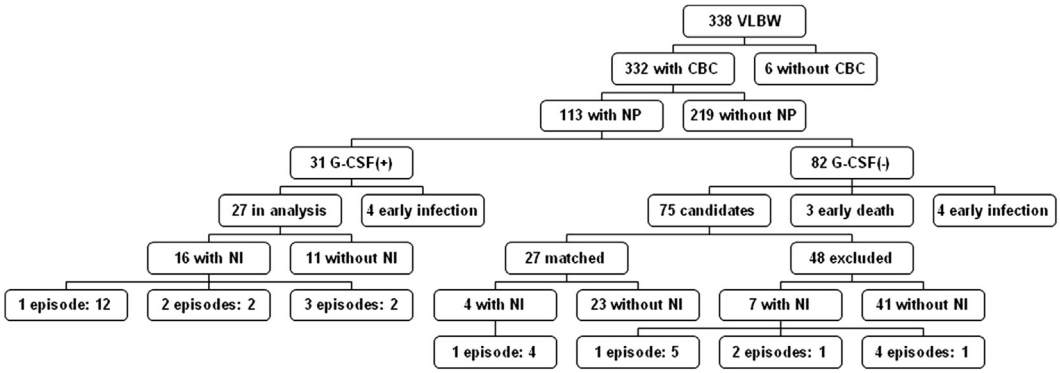

Figure 1 The flowchart of patient distribution. CBC = complete blood count; G-CSF = granulocyte colony stimulating factor; NI = nosocomial infection; NP = neutropenia.

exception that the percentage of small for date infants was lower in the rhG-CSF (+) group (4/27 vs. 12/27, $p = 0.035$; Table 1). The incidence of bronchopulmonary dysplasia, CLD, NEC, and intraventricular hemorrhage did not differ between the two groups. We did not observe any platelet count $<10.0 \times 10^9$/L after rhG-CSF or neutrophilia (TNC $> 14.5 \times 10^9$/L). No other episode of noninfection-related neutropenia was identified after rhG-CSF treatment. Nursing records did not identify any suggestive adverse effect during rhG-CSF treatment.

Infants in the rhG-CSF (+) group experienced a higher incidence of NI (16/27 vs. 4/27; OR = 8.36, 95% CI: 2.26–31.00, $p = 0.002$) than the infants in the rhG-CSF (−) group. There were 22 total episodes of NI for infants in the rhG-CSF (+) group compared to only four total episodes in the rhG-CSF (−) group ($p = 0.007$, $\tau = 0.466$). In the rhG-CSF (+) group, four infants experienced multiple episodes of NI. Logrank test showed a higher hazard ratio (5.50; 95% CI: 2.1–14.4, $p = 0.0005$, Fig. 2) for the rhG-CSF (+) infants to have NI. Multivariate logistic regression analysis showed that only prophylactic use of rhG-CSF for EN is associated with the development of NI ($r^2 = 0.34$). In the rhG-CSF (+) group, one infant experienced an episode of NI with polymicrobial pathogens and another infant experienced one episode of NI with positive cultures from both blood and catheterized urine with the same pathogen. The majority of the pathogens were Gram-positive cocci (12/26) followed by Gram-negative rods (11/26), and fungal pathogens accounting for the remaining infections. The median age of NI was 16.5 days for infants in the rhG-CSF (+) group, which was not different from infants in the rhG-CSF (−) group (median of 17.5 days, $p = 0.887$) (Fig. 3).

The TNC (9–1088 $\times 10^6$/L; median: 480 $\times 10^6$/L; IQR: 188–861 $\times 10^6$/L) among all 27 infants before administration of rhG-CSF increased (25–8462 $\times 10^6$/L; median: 1596 $\times 10^6$/L; IQR: 821–2034 $\times 10^6$/L) after the first dose of rhG-CSF ($p < 0.0001$). Among all infants in rhG-CSF (+) group, there was no difference ($p = 0.39$) in TNC prior to rhG-CSF administration between those who developed NI (32–1088 $\times 10^6$/L) and those who did not (9–1080 $\times 10^6$/L ; $p = 0.39$). There was also no significant difference in the change in TNC following the first dose of rhG-CSF between the NI and no-NI sub-groups (range: 288–8466 $\times 10^6$/L vs. 25–1836 $\times 10^6$/L, $p = 0.25$), which suggests that the difference in rate of NI was not due to a lack of response to rhG-CSF.

A full analysis of all 102 infants with EN showed empirical rhG-CSF use (OR = 8.64; 95% CI: 2.78–26.81; $p < 0.001$), total days on parenteral nutrition (OR = 1.05; 95% CI: 1.01–1.10; $p = 0.026$), and GA (OR = 0.77; 95% CI: 0.59–1.00; $p = 0.053$) were significantly associated with NI (area under receiver operating characteristic curve = 0.865 with 95% CI: 0.783–0.925; $r^2 = 0.462$, $p < 0.001$). The findings suggested that empirical use of rhG-CSF for EN does not prevent NI in premature infants.

Discussion

Neutropenia can be due to decreased production or increased consumption of neutrophils, excessive neutrophil margination, or some combination of these three mechanisms.[17] The most commonly encountered causes of neutropenia in VLBW infants are related to maternal hypertension and sepsis.[17] In this investigation, 34% of enrolled VLBW infants experienced EN which is similar to previous reports.[2–4] Others[6,7] reported EN in about 25–50% of the infants born to mothers with hypertension. This variety of neutropenia is often associated with a lower blood concentration of G-CSF[18] and is believed to be due to the release of an inhibitory factor from the placenta that reduces neutrophil production. Previous studies have suggested that neutropenia associated with maternal hypertension places these premature infants at a higher risk of NI[6,7] but our own experience refuted such association.[14]

NI is an important contributor to mortality and morbidity in premature infants. It is associated with a longer duration of hospitalization and increased hospital costs.[19–23] About 20% of all VLBW infants develop at least one episode of NI during their hospitalization.[24–26] One report found a threefold higher mortality rate among infants who experienced at least one episode of NI compared to infants who did not.[26] Prolonged parenteral nutrition, invasive line placement, prolonged mechanical ventilation, and neutropenia have all been suggested to increase the risk for NI. During the last 2 decades, prophylactic interventions to reduce NI have included use of intravenous immunoglobulin,[21,24] prophylactic antibiotics,[27] and other biological compounds,[28] but none of these treatments has been

Table 1 Demographic data, morbidity, mortality, and infectious pathogen(s) of very-low-birth-weight infants with early neutropenia according to the empirical rhG-CSF treatment.

	rhG-CSF(+)	rhG-CSF(−)	*p*
Gestational age (wk)	23.3−34.3	23.1−33.6	0.603
Birth weight (g)	325−1168	520−1281	0.098
Central line (d)	4−44	6−47	0.654
Central line	26/27	27/27	1.000
Lowest TNC ($\times 10^6$/L)	9−1088	240−1222	0.052
Sex (M/F)	20/7	18/9	0.766
Small for date*	12/27	4/27	0.035
Maternal hypertension	15/27	10/27	0.275
1-min Apgar score	0−9	1−8	0.207
5-min Apgar score	2−9	1−10	0.261
Nosocomial infection*	16/27	4/27	0.002
Episode(s) of NI*			
1	12	4	0.007
≥2	4	0	
Death	9/27	4/27	0.202
NEC	1/27	1/27	1.000
CLD	9/17	13/23	1.000
PDA	7/27	7/27	1.000
IVH			
I	5	3	0.669
II	1	3	
≥III	7	10	
Pathogen(s)			
Gram-positive	11	2	
CONS	5	1	
Enterococcus	3	1	
MRSA	2		
Staphylococcus aureus	1		
Gram-negative	9	1	
Escherichia coli	1	1	
Klebsiella pneumoniae	4		
Enterobacter	3		
Pseudomonas aeruginosa	1		
Candida sp.	4	1	

*$p < 0.05$.
CLD = chronic lung disease; CONS = coagulase negative *Staphylococcus aureus*; IVH = intraventricular hemorrhage; MRSA = methicillin-resistant *Staphylococcus aureus*; NEC = necrotizing enterocolitis; NI = nosocomial infection; PDA = patent *ductus arteriosus*; rhG-CSF = recombinant human granulocyte-colony-stimulating factor; TNC = total neutrophil count.

proven to be effective. Use of rhG-CSF in premature infants has been shown to improve the phagocytic function and oxidative burst activity of the neutrophils, especially in the presence of sepsis.[10] However, despite the efficacy of rhG-CSF in ameliorating neutropenia,[8] this effect has not been translated into a reduction of NI.[12]

We have previously demonstrated that irrespective of the status of maternal blood pressure, EN by itself is not associated with a higher incidence of NI.[14] This led us to question if empirical rhG-CSF use would be efficacious in preventing NI in VLBW infants with EN. In the present study we did not attempt to separate infants according to maternal blood pressure status during pregnancy for analysis due to the limited numbers of infants. The finding that empirical rhG-CSF for EN had increased the likelihood for NI was unexpected. We cannot account for this finding based upon neutropenia alone because all infants in rhG-CSF (+) group showed an increase of TNC after the first dose of rhG-CSF. Furthermore, the increase in TNC did not differ between those who later developed NI versus those who did not within the subgroup of rhG-CSF (+) infants. Although the rhG-CSF (+) group had a higher proportion of small for date infants, this factor was not predictive in the multivariate logistic regression analysis of a higher risk for NI. It is possible that other factors associated with EN contributed to the increased incidence of NI in this group.

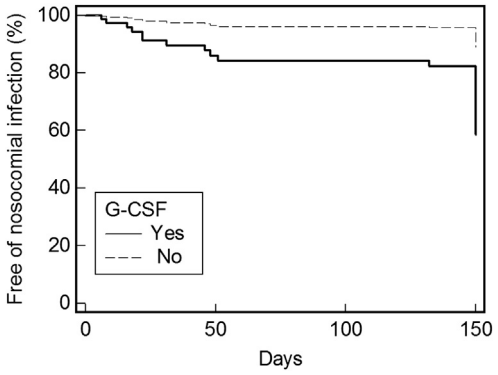

Figure 2 Log-rank test for the probability of NI between two groups. The data were censored with fatal cases. The rhG-CSF (+) group has significantly higher hazard ratio for having NI.

Compared to the study conducted by Kuhn et al,[12] the age of rhG-CSF treatment is less in our group (mean 1.3 days vs. 4.3 days) and we included all NI during the hospitalization instead of 4 weeks after rhG-CSF treatment. We chose neutropenia developed within the first 7 days since we observed that most VLBW infants with this kind of EN recovered spontaneously without treatment and it is not associated with increased risk of NI.[14] Another reason for us to choose the first 7 days to define our EN was the fact that most VLBW will still have their central lines and/or are endotracheally intubated, which could lead to higher chance of NI. Both studies fail to show long-term efficacy of prophylactic rhG-CSF for neonatal neutropenia in preventing NI.

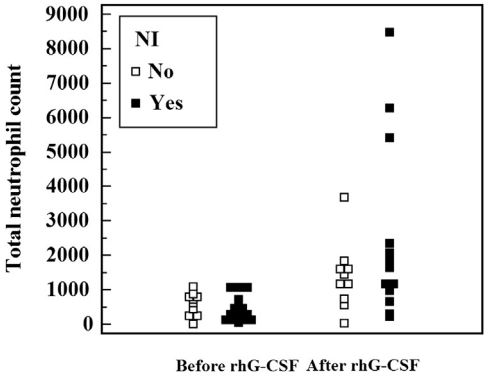

Figure 3 Changes in total neutrophil count after rhG-CSF treatment for early neutropenia.

There are several weaknesses in our study. First, because of the nonrandomized nature of this investigation, we might have missed identification of other factors that contribute to the risk of NI. Second, our study was limited to infants with EN. Third, due to limited case numbers, we cannot analyze the efficacy of rhG-CSF in premature infants with EN due to early infections. Most infants were clinically stable so that the TNC after one week of life was not available in most cases. Fourth, and more importantly, there were more hypertensive mothers in the rhG-CSF (+) group, which might have influenced the increased number of small for date infants in this group and thus increased the likelihood of NI.[6,7] However, our statistical analysis did not identify maternal hypertension or small for dates as risk factors for NI.

Despite these methodological weaknesses, we believe that it is important for the practicing neonatologist to be cautious in treating VLBW infants with EN by empirical rhG-CSF (+) in the hope of preventing the development of NI. Our study underscores the importance of the judicious use of, rather than routine prophylaxis with, rhG-CSF in VLBW infants with EN. There are several potential adverse effects of rhG-CSF administration such as injection pain, local tenderness after injection, bone pain, fever, hemogram derangement (thrombocytopenia and neutrophilia),[29,30] NEC and CLD.[31] Most of these adverse effects are very difficult to identify in VLBW infants by health professionals and documented relatively subjective. Parenteral administration potentially increases the possibility of infection but we did not identify any NI secondary to the intravenous rhG-CSF infusion. Long-term rhG-CSF use in severe congenital neutropenia may increase the chance of leukemia[32] but should not be a concern in short-term use in our study. Our study findings support the need for a randomized controlled trial designed to establish whether administration of rhG-CSF to premature infants with neutropenia will reduce the risk of NI or not.

Conclusion

Our data suggest that empirical rhG-CSF does not associate with a decreased rate NI in VLBW infants with EN. However, we cannot conclude that the use of rhG-CSF is the direct cause for the increase in NI due to the retrospective nonrandomized nature of our study. A randomized clinical trial is needed to determine whether rhG-CSF treatment of EN will reduce the incidence of NI. Our findings do not support the empirical administration of rhG-CSF for the prevention of NI in VLBW infants with EN and each institute should establish a strict guideline about when to prescribe this cytokine to prevent NI.

References

1. Yang KD, Chen MZ, Teng RJ, Yang MY, Liu HC, Chen RF, et al. A model to study antioxidant regulation of endotoxemia-modulated neonatal granulopoiesis and granulocyte apoptosis. *Pediatr Res* 2000;**48**:829—34.
2. Engle WD, Rosenfeld CR. Neutropenia in high-risk neonates. *J Pediatr* 1984;**105**:982—6.
3. Baley JE, Stork EK, Warkentin PI, Shurin SB. Neonatal neutropenia: clinical manifestations, cause and outcome. *Am J Dis Child* 1988;**142**:1161—5.
4. Gessler P, Lüders R, König S, Haas N, Lasch P, Kachel W. Neonatal neutropenia in low birth weight premature infants. *Am J Perinatol* 1995;**12**:34—8.
5. Manroe BL, Weinberg AG, Rosenfeld CR, Browne R. The neonatal blood count in health and disease. I. Reference values for neutrophilic cells. *J Pediatr* 1979;**95**:89—98.
6. Koenig JM, Christensen RD. Incidence, neutrophil kinetics, and natural history of neonatal neutropenia associated with maternal hypertension. *N Engl J Med* 1989;**321**:557—62.
7. Cadnapaphornchai M, Faix RG. Increased incidence of nosocomial infection in neutropenic low birth weight (2000 g or less) infants of hypertensive mothers. *J Pediatr* 1992;**121**: 956—61.
8. Kocherlakota P, La Gamma EF. Preliminary report: rhG-CSF may reduce the incidence of neonatal sepsis in prolonged preeclampsia-associated neutropenia. *Pediatrics* 1998;**102**: 1107—11.
9. Funke A, Berner R, Traichel B, Schmeisser D, Leititis JU, Niemeyer CM. Frequency, natural course, and outcome of neonatal neutropenia. *Pediatrics* 2000;**106**:45—51.
10. Ahmad M, Fleit HB, Golightly MG, La Gamma EF. *In vivo* effect of recombinant human granulocyte colony-stimulating factor on phagocytic function and oxidative burst activity in septic neutropenic neonates. *Biol Neonate* 2004;**86**:48—54.
11. Carr R, Modi N, Doré CJ, El-Rifai R, Lindo D. A randomized, controlled trial of prophylactic granulocyte-macrophage colony-stimulating factor in human newborns less than 32 weeks gestation. *Pediatrics* 1999;**103**:796—802.
12. Kuhn P, Messer J, Paupe A, Espagne S, Kacet N, Mouchnino G, et al. A multicenter, randomized, placebo-controlled trial of prophylactic recombinant granulocyte-colony stimulating factor in preterm neonates with neutropenia. *J Pediatr* 2009; **155**:324—30.
13. Carr R, Modi N, Doré C. G-CSF and GM-CSF for treating or preventing neonatal infections. *Cochrane Database Syst Rev* 2003;**3**:CD003066.
14. Teng RJ, Wu TJ, Garrison RD, Sharma R, Hudak ML. Early neutropenia is not associated with an increased rate of nosocomial infection in very low birth weight infants. *J Perinatol* 2009;**29**:219—24.
15. Walsh MC, Kliegman RM. Necrotizing enterocolitis: treatment based on staging criteria. *Pediatr Clin North Am* 1986;**33**: 179—201.
16. Papile LA, Burstein J, Burstein R, Koffler H. Incidence and evolution of subependymal and intraventricular hemorrhage: a study of infants with birth weights less than 1,500 gm. *J Pediatr* 1978;**92**:529—34.
17. Christensen RD, Calhoun DA, Rimsza LM. A practical approach to evaluating and treating neutropenia in the neonatal intensive care unit. *Clin Perinatol* 2000;**27**:577—601.
18. Tsao PN, Teng RJ, Tang JR, Yau KI. Granulocyte colony-stimulating factor in the cord blood of premature neonates born to mothers with pregnancy-induced hypertension. *J Pediatr* 1999;**135**:56—9.
19. Girard R, Fabry J, Meynet R, Lambert DC, Sepetjan M. Costs of nosocomial infection in a neonatal unit. *J Hosp Infect* 1983;**4**: 361—6.
20. Freeman J, Epstein MF, Smith NE, Platt R, Sidebottom DG, Goldmann DA. Extra hospital stay and antibiotic usage with nosocomial coagulase-negative staphylococcal bacteremia in two neonatal intensive care unit populations. *Am J Dis Child* 1990;**144**:324—9.
21. Baker CJ, Melish ME, Hall RT, Casto DT, Vasan U, Givner LB. Intravenous immune globulin for the prevention of nosocomial infection

in low-birth-weight neonates. The multicenter Group for the Study of Immune Globulin in Neonates. *N Engl J Med* 1992;**327**:213—9.
22. Gray JE, Richardson DK, McCormick MC, Goldmann D. Coagulase-negative staphylococcal bacteremia among very low birth weight infants: relation to admission illness severity. *Pediatrics* 1995;**95**:225—30.
23. Sohn AH, Garrett DO, Sinkowitz-Cochran RL, Grohskopf LA, Levine GL, Stover BH, et al. Prevalence of nosocomial infections in neonatal intensive care units: results from the first national point-prevalence survey. *J Pediatr* 2001;**139**:821—7.
24. Fanaroff AA, Korones SB, Wright LL, Wright EC, Poland RL, Bauer CB, et al. A controlled trial of intravenous immune globulin to reduce nosocomial infections in very-low-birth-weight infants. *N Engl J Med* 1994;**330**:1107—13.
25. Stoll BJ, Hansen N, Fanaroff AA, Wright LL, Carlo WA, Ehrenkranz RA, et al. Late-onset sepsis in very low birth weight neonates: the experience of the NICHD Neonatal Research Network. *Pediatrics* 2002;**110**:285—91.
26. Kaufman D, Fairchild KD. Clinical microbiology of bacterial and fungal sepsis in very-low-birth-weight infants. *Clin Microbiol Rev* 2004;**17**:638—80.
27. Jardine LA, Inglis GD, Davies MW. Prophylactic systemic antibiotics to reduce morbidity and mortality in neonates with central venous catheters. *Cochrane Database Syst Rev* 2008;**1**: CD006179.
28. Tubman TR, Thompson SW, McGuire W. Glutamine supplementation to prevent morbidity and mortality in preterm infants. *Cochrane Database Syst Rev* 2008;**1**:CD001457.
29. Russell ARB, Davies EG, Ball SE, Gordon-Smith E. Granulocyte colony stimulating factor treatment for neonatal neutropenia. *Arch Dis Child* 1995;**72**:F53—4.
30. Wiedl C, Walter AW. Granulocyte colony stimulating factor in neonatal alloimmune neutropenia: a possible association with induced thrombocytopenia. *Pediatr Blood Cancer* 2010;**54**: 1014—6.
31. Papoff P. Infection, neutrophils, and hematopoietic growth factors in the pathogenesis of neonatal chronic lung disease. *Clin Perinatol* 2000;**27**:717—31.
32. Freedman MH, Bonilla MA, Fier C, Boylard AA, Scarlata D, Boxer LA, et al. Myelodysplasia syndrome and acute myeloid leukemia in patients with congenital neutropenia receiving G-CSF therapy. *Blood* 2000;**96**:429—36.