

Promising stratagems for reducing the burden of neonatal sepsis

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Two years ago we reviewed¹ the rationale for the use of haemopoietic colony stimulating factors (CSFs) in preterm neonates. Two small pilot studies had shown that treatment with either granulocyte colony stimulating factor (G-CSF) or granulocyte-macrophage colony stimulating factor (GM-CSF) could increase the neutrophil count without apparent short term toxicity.²⁻³ Follow up at 2 years of age of the small cohort treated with G-CSF found no longer term adverse effects.⁴ However, there were no data on clinical efficacy. Whether CSFs could reduce morbidity and mortality from sepsis, and how they might be used to greatest effect, remained unaddressed. A number of studies have since been completed which point the way for future investigation.

All preterm neonates are at high risk of bacterial sepsis.⁵⁻⁷ Acute mortality from sepsis has remained constant at about 15% for two decades⁸ and increases to over 50% when associated with severe neutropenia.⁹ Of possibly greater importance is the fact that sepsis interacts with other pathologies, increasing the overall risk of disability. The preterm brain is believed to be developmentally vulnerable to damage as a consequence of infection remote from the brain.¹⁰ Sepsis, by initiating endothelial injury, endotoxaemia, and uncontrolled inflammation/coagulation cascades is a prime candidate to cause white matter and other brain injury, including cerebral palsy. Infection provoked inflammation is also an important contributor to the multifactorial aetiology of chronic lung disease. These conditions are powerful determinants of outcome after preterm birth.

The high infection rates in preterm neonates are related to immaturity of both humoral and phagocyte immunity. Infants born before 30 weeks gestation are severely hypogammaglobulinaemic.¹¹ The high incidence of postnatal neutropenia in both well and septic neonates¹²⁻¹³ is a consequence of the reduced total body neutrophil mass in infants born before 32 weeks gestation¹⁴ and is clinical evidence of immature granulopoiesis.¹⁵ Even when peripheral blood neutrophil counts are normal, the organisms causing bacterial infections⁵ are similar to those seen in older children and adults with profound neutropenia, and this is clinical evidence of neutrophil functional immaturity.¹⁶ The haemopoietic CSFs, through their stimulation of granulopoiesis and phagocyte function, have

the potential to enhance these cellular defences against bacterial and fungal infection.

CSFs as treatment

Two strategies have been adopted for exploring whether the CSFs can provide clinical benefit. The first has investigated them as intervention treatment to increase circulating neutrophils in established sepsis complicated by a low neutrophil count. G-CSF has been used for this because of its powerful ability to mobilise preformed neutrophils from the marrow into the circulation and its effect on neutrophil precursor proliferation. A number of small studies have been undertaken with the primary aim of assessing early toxicity and effects on neutrophil number and function.²⁻¹⁷⁻²¹ Gillan *et al*² randomised 42 newborn infants with presumed sepsis to receive either placebo or various doses of G-CSF ranging from 1.0 µg/kg/day to 10.0 µg/kg every 12 hours. There were no deaths in the 33 treated infants or in the nine receiving placebo. Tibial marrow aspirate assessment of the neutrophil storage pool showed a dose dependent increase after G-CSF treatment. Schibler¹⁷ randomised 20 infants with neutropenia and clinical signs of early onset sepsis (less than three days after birth) to receive G-CSF 10 µg/kg/day for three days or placebo. In this study, mortality was similar in the two groups (two of 10 in the group receiving G-CSF *v* three of 10 in the group receiving placebo), and there was no difference in the severity of illness, as assessed by the score for neonatal acute physiology (SNAP) during the seven days of the study. A recent similar placebo controlled study of 22 neutropenic septic infants, using the same G-CSF regimen, also showed no difference in SNAP score between the treated and control infants during the period directly related to the specific infectious episode.¹⁸ There were no deaths in this study. Kocherlakota and La Gamma¹⁹ administered G-CSF 10 µg/kg/day for three days to 14 neutropenic (<1.5 × 10⁹/l) infants with clinical signs of sepsis and compared the outcome with 11 concurrent but retrospectively selected controls. There were fewer deaths in the G-CSF treated group (1/14) than in the control group (6/11), the greatest benefit being seen in the subgroup with early onset sepsis (≤ 24 hours from birth): death rate in the G-CSF treated group 0/7 *v* 4/5 in the control group. A small study in the United Kingdom,²⁰ in which 28 infants with suspected sepsis and a neutrophil count of less than 5.0 × 10⁹/l were randomised to receive G-CSF 10 µg/kg/day for up to 14 days or placebo, reported

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Accepted 5 January 2000

a mortality of 1/13 in the G-CSF treated arm and 4/15 in the control arm. As the temporal relation between the sepsis episode and deaths has not been reported, the extent to which mortality was related to infection is unclear. Finally, Drossou-Agakidou *et al*²¹ randomised 35 pre-term infants with proven sepsis and neutrophils $< 5.0 \times 10^9/l$, and found no difference in mortality (G-CSF 4/19; control 3/16).

Overall, the randomised trials to date do not provide evidence of reduced mortality following G-CSF as intervention rescue treatment in septic neutropenic neonates (odds ratio for mortality 0.6, 95% confidence interval (CI) 0.2 to 1.8; $p = 0.5$). However, these small studies, in which infants were selected and treated according to differing protocols, were primarily designed to assess early toxicity and whether G-CSF could correct neutropenia in the face of sepsis. These aims were achieved, in that all but one of the studies¹⁷ showed neutrophil counts to rise more rapidly with G-CSF treatment and no acute toxicities were identified. Regrettably, the largest study assessing clinical outcomes in septic neonates treated with G-CSF, a company sponsored American multicentre trial, was drawn to a close prematurely after interim analysis of about 100 subjects. Although closed almost two years ago, the results have yet to be published.

CSFs as prophylaxis

The alternative strategy has been to use CSFs prophylactically, to prevent sepsis by prospectively stimulating neutrophil production and enhancing phagocytic bactericidal function. We have recently published the results of a randomised controlled trial of prophylactic GM-CSF in 75 neonates of less than 32 weeks gestation.²² We elected to use GM-CSF because of its broader stimulation of phagocyte proliferation and function than G-CSF. The study showed that prophylactic GM-CSF, 10 $\mu\text{g}/\text{kg}/\text{day}$, begun within 72 hours of birth and administered subcutaneously for five days, completely prevented the development of neutropenia, including sepsis induced neutropenia, during the subsequent four weeks. In contrast, 71% of small for gestation (SGA) infants and 24% of appropriately sized (AGA) infants in the control arm developed neutropenia ($< 1.7 \times 10^9/l$) during the same period. Although not designed to address clinical benefit, there were fewer infants who developed one or more episodes of acute symptomatic blood culture positive sepsis during 14 days from study entry (treated 31%; control 46%), and this reduction in sepsis incidence was most pronounced in the subgroup of 25 SGA infants (treated 18%; control 50%).

Kocherlakota and La Gamma²³ administered G-CSF (10 $\mu\text{g}/\text{kg}/\text{day}$) prophylactically for three days to 15 non-infected neonates with pre-eclampsia associated neutropenia, and compared clinical outcome with 13 concurrent case matched controls. This study also showed a pronounced reduction in the number of infants developing sepsis. As in our study, sepsis was defined as the acute onset of clinical signs in association with a positive blood culture. In this study, 13% of treated infants developed sepsis, compared with 54% of controls.

The effect of longer term prophylactic CSF treatment in AGA ($> 10\text{th}$ centile) very low birthweight neonates was examined in a multicentre placebo controlled study. GM-CSF 8 $\mu\text{g}/\text{kg}/\text{day}$ was administered by intravenous infusion, daily for seven days and then on alternate days to 28 days from study entry.²⁴ The incidence of “nosocomial sepsis” during the 28 days of treatment in the 264 infants recruited was the same in the two study groups, 40% *v* 39%. Although these results would, at first glance, appear to contradict the benefit shown by the two previous studies, there are a number of reasons to question this conclusion. Firstly, the study specifically excluded SGA infants. This is the group at greatest risk of neutropenia and greatest risk of infection and the group in whom the greatest benefit has been shown in other prophylactic studies. The definition of sepsis used appears to be based on either positive cultures without supportive clinical signs of infection or a diagnosis of necrotising enterocolitis without positive blood cultures. The primary outcome, sepsis, may therefore have included clinically insignificant bacterial colonisation in addition to significant bacterial invasion, as well as focal bowel ischaemia. This study also had inadequate power to detect a realistic treatment effect in AGA infants, in whom the magnitude of sepsis reduction is likely to be substantially smaller than in SGA infants, although still clinically important. The rejection of promising interventions on the basis of inadequately sized trials that are then misinterpreted is a common problem that has led to several instances of delayed introduction of beneficial treatments.²⁵

The prophylactic studies carried out so far have shown a promising reduction in systemic infection in SGA infants (32% sepsis reduction) and in neonates with established neutropenia (41% sepsis reduction). The odds ratio for the effect of prophylactic CSF treatment on the prevention of sepsis in both of these studies combined is 0.2 (95% CI 0.05 to 0.84; $p = 0.03$). Whether this benefit is generalisable to AGA preterm infants, who are also at high risk of sepsis although with a lower incidence of neutropenia, remains unknown.

Safety of CSF treatment

Perhaps the most important information to emerge from these studies is the safety of both CSFs in preterm infants. None of the reported studies have identified any toxicity attributable to G-CSF or GM-CSF, either during treatment or during short term follow up. The greatest concern has been the theoretical risk that inappropriate activation of neutrophils may lead to tissue damage and so increase the incidence of chronic lung disease or necrotising enterocolitis. This has not been born out in practice in any of the published studies.

Potential haematological toxicity alluded to in our earlier review¹ has not only failed to be substantiated, but in addition, new knowledge has emerged that makes these theoretical adverse effects unlikely. Anxieties arose from the experience of children with Kostmann’s syndrome (severe chronic neutropenia) a proportion of whom have developed acute

myeloid leukaemia following long term G-CSF treatment. At the time of our previous review, it was believed to be the consequence of longer survival in an inherently premalignant condition. Since then this has been confirmed.²⁶ The development of leukaemia in these patients has been found to be associated with a pre-existing abnormality of the G-CSF receptor, which is unique to patients with Kostmann's syndrome.^{27, 28} Additional reassurance comes from observation of 229 children with other congenital neutropenic disorders, idiopathic and cyclical neutropenia, who similarly have been treated with long term G-CSF. None have developed leukaemia or other blood dyscrasias.²⁹

Concern about anaemia or thrombocytopenia, arising as a result of excess myeloid lineage stimulation due to a "lineage steal" effect, arose from some neonatal erythropoietin studies in which treatment was associated with neutropenia,^{30, 31} and from an early non-randomised G-CSF study in septic neonates, in which treatment appeared to be associated with thrombocytopenia.³² In practice, no other study has shown excess thrombocytopenia in the treatment arm, although late anaemia has yet to be assessed. Further insights into haemopoiesis substantiate the view that the effect of the haemopoietic CSFs is "permissive" rather than "instructive"; in other words, they expand lineage committed progenitors without influencing the lineage to which stem cell progeny become committed.³³ A lasting influence on the future balance of haemopoiesis thus appears unlikely.

Which CSF?

There has been a continuing debate about which cytokine, G-CSF or GM-CSF, is the more appropriate for prospectively improving phagocyte number and function in neonates. Both are in use in adults to stimulate granulopoiesis. G-CSF is used primarily to correct neutropenia, and GM-CSF where functional enhancement is also important, even in the presence of normal neutrophil counts.^{34, 35} The greater ability of GM-CSF to functionally prime both neutrophils and monocytes³⁶ would seem to make it the more appropriate agent for prophylactic use in neonates, in whom immaturity of phagocyte function contributes to the high incidence of bacterial and fungal infection. There are additional theoretical reasons for using GM-CSF. There is evidence that neonatal neutrophil dysfunction is at least in part secondary to reduced secretion of interferon γ and interleukin 12 by neonatal mononuclear cells.³⁷⁻³⁹ Incubation with interferon γ in vitro completely corrects the chemotactic defect of neonatal neutrophils.⁴⁰ GM-CSF increases interferon γ and interleukin 12 production in vivo⁴¹ and thus works to correct the neonatal neutrophil functional defect. G-CSF, in contrast, is an anti-inflammatory cytokine,⁴² and, by downregulating interferon γ , may further depress the neonate's neutrophil immunity. The converse argument that GM-CSF may promote inappropriate tissue damage, through its greater ability to enhance neutrophil and monocyte function, has not been born out in 185 GM-CSF treated infants reported in published trials.^{3, 22, 24} Thus for prophylactic use in neonates, the dual

proliferative and functional activity of GM-CSF gives it the greater potential to reduce sepsis in both infants with neutropenia and the majority who are not neutropenic at birth.

Other approaches

Before discussing how CSF treatment should be taken forward, it is important to look briefly at other potentially promising treatments, aimed at enhancing neonatal humoral immunity. The use of intravenous immunoglobulin (IVIG) in newborn infants has recently been examined in three systematic reviews, two for the Cochrane Library. The Cochrane meta-analysis⁴³ of 15 studies using IVIG prophylactically showed a small reduction in serious infection (n = 5054; risk difference -0.032, 95% CI -0.010 to -0.054) but with no reduction in mortality or serious morbidity. A meta-analysis of 12 IVIG prophylaxis studies, including 4933 evaluable infants, but using stricter criteria for the diagnosis of sepsis—that is, positive blood culture with clinical signs of systemic infection⁴⁴—similarly found IVIG to be of minimal although statistically significant benefit in preventing sepsis. It was concluded that, although prophylactic IVIG is safe and effective, it does not produce sufficient benefit to warrant routine use.

A small number of studies have examined the use of IVIG as an adjunct to antibiotic treatment in infants with suspected or proven sepsis. The quality of these IVIG treatment studies was poor. The Cochrane meta-analysis⁴⁵ showed a 10% reduction in mortality (n = 208; risk difference -0.102, 95% CI -0.005 to -0.199). However, the 95% CI for number needed to treat was wide (number needed to treat 10, 95% CI 5 to 200), and there was no statistically significant reduction in mortality after treatment with IVIG in cases where infection was proven. The meta-analysis of Jenson and Pollock⁴⁴ also found support for the use of IVIG as treatment for established sepsis, within the limitations of the small number of infants included in the review (n = 110; odds ratio 0.17, 95% CI 0.03 to 0.74). Both reviews conclude that IVIG as an adjunct to antibiotic treatment in neonatal sepsis is a promising strategy that needs to be tested in a large multicentre study.

The way forward

Where should further efforts be focused in the light of data from these preliminary studies and now that the risk of CSF related toxicity seems small and potential benefit large? Overall, the adverse long term consequences of infection make the prevention of sepsis the preferable aim. Here, the prophylactic studies using GM-CSF or G-CSF, which showed a promising reduction in sepsis in SGA infants and in those with pre-eclampsia associated neutropenia, suggest that this may be an effective strategy for reducing sepsis as well as sepsis related morbidities. For treating established sepsis, the evidence to date suggests that priority rests with a definitive evaluation of the role of IVIG. These strategies urgently need to be tested in appropriately powered trials in which the definition of sepsis clearly differentiates invasive systemic infection

from bacterial colonisation, and in which long term outcomes are assessed.

Two proposed randomised controlled trials, one evaluating the early use of CSF as prophylaxis against neonatal sepsis (PROGRAMS; www.npeu.ox.ac.uk) and the other a trial of non-specific IVIG treatment in established neonatal sepsis (NIS; www.npeu.ox.ac.uk) would address these issues. The trials should be conducted concurrently, allowing clinical strategies that would be implemented together to be assessed together, a scientifically valid and desirable approach in that it mimics real clinical practice.⁴⁶ Both trials could be conducted under the umbrella of a Perinatal Clinical Trials Network which would facilitate the rapid recruitment of the large numbers necessary to minimise the risk of being misled by the play of chance.²⁵ Given the size of the burden of neonatal sepsis, there is no time to lose.

We are grateful to Irene Roberts and William Tarnow-Mordi for reviewing and contributing to the paper.

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