International Neonatal Immunotherapy Study

Non-specific intravenous immunoglobulin therapy for suspected or proven neonatal sepsis: an international, placebo controlled, multicentre randomised trial

June 2001 - UK Edition

ISRCTN 94984750

Summary

Background

Background

Sepsis is an important, but sometimes undiagnosed, cause of perinatal brain damage and mortality. In term infants, blood concentrations of inflammatory cytokines are elevated in those later diagnosed with cerebral palsy. In preterm infants, infection remote from the brain may predispose to cerebral white matter damage. While effective antibiotic treatment is essential, resistance to antibiotics is increasing. Adjuvant therapies, such as intravenous immunoglobulin (IVIG), therefore offer an important additional strategy. Three recent Cochrane systematic reviews of randomised controlled trials in nearly 6,000 patients suggest that non-specific, polyclonal IVIG is safe, reduces sepsis by 3-4% in prophylaxis and may reduce mortality by 50% in treatment of neonatal sepsis. However, the trials of treatment were small and lacked follow up data. This protocol is for a large, simple, international trial, to assess reliably whether treatment of neonatal sepsis with IVIG reduces mortality and adverse neuro-developmental outcome. It needs no special expertise and can be conducted simultaneously with other studies.

Costs

Costs

IVIG, placebo and trial materials will be imported in full compliance with the requirements of national regulatory authorities and supplied free of charge throughout the study.

Safety

Safety

IVIG is one of the safest blood products available. Heat treatment and alcohol fractionation, precautions not available for fresh frozen plasma or cryoprecipitate, increase the safety of IVIG. The process also removes IgM, the main source of anti T antibody linked with haemolysis in infections such as *Clostridium difficile*. There are no reports of significant neonatal haemolysis with IVIG.

Support

Support

Resources are available to train and support part time local research nurses to facilitate recruitment and data collection.

Follow up

Follow up

Neuro-developmental status will be assessed by appropriate methods in all survivors at 2 years.

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Participating centres:
150 neonatal intensive care units around the world.

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PROTOCOL SUMMARY

? Sepsis - consider

Eligibility

Babies are eligible if:

1. they are receiving antibiotics with clinical evidence of severe or life-threatening infection

   AND

2. they have at least one of the following:

   - birthweight is less than 1500g
     OR
   - evidence of infection in blood culture, CSF or usually sterile body fluid
     OR
   - respiratory support via an endotracheal tube

   AND

3. there is substantial uncertainty that IVIG is indicated

EXCLUSIONS:
- IVIG has already been given
- IVIG is thought to be needed or contra-indicated

Consent
- On or before admission, all parents receive an Information Leaflet from the nursing staff, outlining the study.
- If a baby becomes eligible, the parents are asked, in person or by telephone, for consent to participate in the study and later follow up.
- Parents who participate will receive a leaflet thanking them, with the name of a senior doctor they can contact about the study. Parents are also encouraged to ask the nurses or doctors any further questions at any stage.

Randomisation

(study entry)
- By sequential Study Drug Pack, so it is not necessary to make a phone call.
- Short **Entry Form** to complete.

Treatment

- 500 mg/kg (10 ml/kg) of IVIG or identical placebo solution over 4-6 hours, repeated 48 hours later. No more IVIG or placebo can be given.

After discharge

- Short **Discharge Form** completed after baby leaves unit.

CONTENTS

INTRODUCTION

Introduction

This protocol is for a large, simple-in-design, double blind, placebo controlled pragmatic multicentre randomised trial.

HYPOTHESIS TO BE TESTED

Hypothesis

That, in infants receiving antibiotics for clinical sepsis, the addition of non-specific, polyclonal intravenous immunoglobulin IgG (IVIG) therapy reduces mortality and major morbidity compared with antibiotics alone.

BACKGROUND

Background

Neonatal sepsis is a major cause of mortality and morbidity and has been implicated in the causation of perinatal brain damage and cerebral palsy, both in term and preterm infants[1 2]. Although antibiotics are the mainstay of therapy, increasing numbers of bacteria are resistant to them[3 4]. Effective adjunctive strategies are therefore needed.

Incidence, potential impact on mortality and problems in diagnosis

Incidence, potential impact on mortality and problems in diagnosis

In a prospective study in seven Australian neonatal intensive care units (NICUs), Isaacs and colleagues reported an annual incidence of sepsis of 6.6 per 1000 live births, of which 75% were late onset (more than 48 hours after birth). Overall hospital mortality for sepsis was 10%[5]. In a cohort of 54 UK neonatal units in 1998 (www.child-health.dundee.ac.uk/research/ukneonatal-staffing/)[6], 204 (5%) of 3,963 consecutive admissions to neonatal units had a positive blood culture. Of these, 16 (8%) died. Of 3,759 (95%) babies with negative blood cultures, 95 babies died (2.5%). For very low birthweight (VLBW) infants with positive blood cultures, mortality was 14% (see table 1, p 10). In a recent North American cohort, mortality in VLBW infants with septicaemia was 21%[7]. However, these figures may underestimate the true incidence of neonatal sepsis. Blood cultures may often be negative if less than 1 ml of blood is sampled[8]. Furthermore, while sepsis was the primary cause of death in most infants under 1000g at autopsy, it was clinically undiagnosed in 61% of cases[9]. Sepsis-specific mortality rates should therefore be interpreted with caution, as the diagnosis may often be inaccurate. More reliable evidence would be provided by randomised comparisons of the effects of specific interventions on mortality from all causes.

Potential impact of sepsis on the perinatal brain

Potential impact of sepsis on the perinatal brain

Recent evidence suggests that sepsis is also important in the pathogenesis of neuro-developmental impairment of perinatal origin. In a case-control study of 424 births, Grether and Nelson found that infants exposed in utero to maternal infection at term were 9 times more likely to have cerebral palsy than controls[1]. In another case-control study of 96 term infants, levels of cytokines in neonatal blood spots were consistently higher in children diagnosed with cerebral palsy at 3 years of age than in controls, suggesting that an inflammatory response may be important in the aetiology of cerebral impairment[10]. In preterm infants, sepsis is also associated with subsequent adverse neuro-developmental outcome[2]. Dammann and Leviton have suggested that infection remote from

the preterm brain may predispose to cerebral white matter damage[11] with disruption of oligodendroglial myelination and disordered migration of precursors. The damage could result partly from inadequate endogenous protection from developmentally regulated factors such as oligotrophins[12]. As antenatal and postnatal sepsis may predispose to neuro-developmental impairment and disability in term and preterm infants, these are essential measures of outcome.

Possible adjunctive treatments:

Immunoglobulin

Immunoglobulin

Newborn infants, particularly those who are very low birthweight or preterm[13], are deficient in IgG, which binds to cell surface receptors, provides opsonic activity, activates fixation of complement, promotes antibody dependent cytotoxicity, improves neutrophil chemiluminescence[13] and phagocytosis[14] and can improve neutropenia by enhancing the release of stored neutrophils[15 16]. Intravenous immunoglobulin (IVIG) is therefore a theoretically attractive strategy, with multiple mechanisms of action. Its potential clinical relevance is confirmed by recent evidence from randomised controlled trials (see below).

Pentoxifylline

Pentoxifylline

In animal models of sepsis, pentoxifylline, a methylxanthine derivative, inhibits production of Tumour Necrosis Factor (TNF), preserves micro-vascular blood flow, prevents circulatory failure and intestinal vaso-constriction and improves survival[17 18]. It is well tolerated and decreases TNF production in adults and preterm infants with sepsis[19-21]. Two randomised controlled trials (RCTs) of pentoxifylline[21 22] recruited 140 preterm infants with clinical sepsis. Among the 107 with positive blood cultures, pentoxifylline was associated with 86% reduction in risk of mortality (RR 0.14, 95% CI 0.03 to 0.76). Outcomes for the other 33 infants have been requested from the authors. Pentoxifylline may be a promising therapy in neonatal sepsis.

Cytokines

Cytokines

Other adjunctive strategies for prophylaxis or treatment of neonatal sepsis are also attractive, such as use of the recombinant cytokines Granulocyte Colony Stimulating Factor (G-CSF) or Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF) to prevent neutropenia[23]. However, no systematic reviews of RCTs of these agents are yet available. In four RCTs of G-CSF therapy which recruited 125 infants with neonatal sepsis, there was a trend to reduced mortality which was not statistically significant (OR 0.6, 95% CI 0.2 to 1.8)[24-27]. Two recent RCTs of GM-CSF prophylaxis in a total of 339 high risk infants showed no reduction in sepsis or mortality[28 29]. However, these findings do not rule out a moderate benefit[30].

Blood products other than immunoglobulin

Blood products other than immunoglobulin

White cell (granulocyte) transfusions are also a logical approach. Although preliminary clinical evidence is encouraging[31], there are potential risks from transmission of infection (e.g. HIV or hepatitis) or from graft-versus-host disease, and the technology is not widely available. Exchange transfusion with fresh whole adult blood appeared effective in one RCT of 22 septicaemic

infants[32], but may also transmit infection. In another RCT, in 776 infants of less than 32 weeks gestation, there was no evidence that prophylactic fresh frozen plasma reduced the risks of mortality from all causes or of disability in survivors at 2 years[33].

Overall, therefore, the evidence suggests that IVIG therapy is one of the most promising strategies in neonatal sepsis and should be assessed in a definitive RCT.

Non-specific versus specific immunoglobulin

Non-specific versus specific immunoglobulin

This trial will use non-specific, polyclonal IVIG (normal human IgG immunoglobulin) produced from plasma from non-UK donors. It was decided that specific IVIG would not be used and that there was no necessity to characterise the specific antibacterial profile of the non-specific IVIG for the following reasons:

1) Previous RCTs of nonspecific, polyclonal IVIG in neonates and adults did not characterise any specific aspects of antibacterial function in the products used. There is therefore no reference laboratory data against which to judge the possible antibacterial efficacy of polyclonal IVIG.

2) As the mechanism of action of IVIG is likely to be multifactorial, the precise aspects of antibacterial function which should be assessed are speculative.

3) Despite the production of monoclonal antibodies with demonstrable in vitro and in vivo antibacterial function in laboratory studies, they have not been associated with reductions in mortality in RCTs (see p 11). There is therefore no evidence that laboratory studies which attempted to characterise specific aspects of antibacterial function in IVIG products would be more predictive of clinical efficacy than the existing clinical evidence from RCTs in support of nonspecific, polyclonal IVIG therapy.

Results of previous randomised controlled trials

Results of previous randomised controlled trials

A Cochrane systematic review of the *prophylactic* use of non-specific IVIG in 15 RCTs with a total of 5,054 preterm or low birthweight infants has demonstrated that prophylactic, non-specific IVIG reduced sepsis (RR 0.83, 95% CI 0.72 to 0.97) and was safe, with no major adverse effects, but showed no reduction in mortality[34].

A Cochrane systematic review[35] of reports of RCTs of IVIG therapy for *proven or suspected* neonatal sepsis identified four studies[15 36-38] with a total of 208 infants. IVIG therapy also appeared to be safe and was associated with a 50% reduction in the relative risk of mortality but the confidence interval was wide (RR 0.52, 95% CI 0.28 to 0.98) (Table 1).

Table 1

Table 1

Review: IVIG in neonatal infection
Comparison: IVIG vs placebo or no intervention for suspected infection
Outcome: Mortality from any cause

Study	Exptl n/N	Ctrl n/N	Relative Risk (95% CI Fixed)	Weight
Christensen 1991	0/11	0/11	not estimable	0
Erdem 1993	6/20	9/24	0.80 [0.34, 1.86]	36.9%
Haque 1988	1/30	6/30	0.17 [0.02, 1.30]	27.0%
Sidiropoulos 1981	4/41	8/41	0.50 [0.16, 1.53]	36.1%
Total (95% CI)	11/102	23/106	0.52 [0.28, 0.98]	100%

When, in the sensitivity analysis, a quasi randomised trial of 82 patients was excluded[38] the point estimate of the relative risk remained similar but the confidence interval was wider and included 1 (RR 0.53, 95% CI 0.25 to 1.15) (Table 2).

Table 2

Table 2

Review: IVIG in neonatal infection
Comparison: IVIG vs placebo or no intervention for suspected infection
Outcome: Mortality from any cause (excluding quasi randomized trials)

Study	Exptl n/N	Ctrl n/N	Relative Risk (95% CI Fixed)	Weight
Christensen 1991	0/11	0/11	not estimable	0
Erdem 1993	6/20	9/24	0.80 [0.34, 1.86]	57.7%
Haque 1988	1/30	6/30	0.17 [0.02, 1.30]	42.3%
Total (95% CI)	7/61	15/65	0.53 [0.25, 1.15]	100%

The authors concluded that '*the imprecise estimate of the effect size to prevent one death (number needed to treat 10, 95% confidence interval 5 – 200) and the lack of statistical significance in secondary and sensitivity analyses justify further research. Researchers should be encouraged to undertake well designed trials to confirm or refute the effectiveness of IVIG'*.[35]

Using slightly different selection criteria and methods for analysis, Jenson and Pollock have published a systematic review of three RCTs of IVIG in neonatal sepsis in which 55 infants received IVIG and 55 received placebo or no infusion[39]. The odds ratio for mortality in treated versus control infants was 0.173 (95% CI 0.031 to 0.753) indicating that IVIG was associated with 83% lower (95% CI 25% to 97%) odds of mortality. These authors reached a conclusion which many would consider premature, namely that *'IVIG should be considered as part of the routine therapy of neonatal sepsis'*. However, it remains true that, among all the interventions currently reviewed in the Cochrane Library, IVIG therapy in neonatal sepsis is associated with one of the largest reductions in the odds of death. A further RCT of IVIG in neonatal sepsis in Brazilian neonatal units, of which one of the INIS investigators (K Haque) is an investigator, is expected to report in 2001. Its results will be incorporated into the current meta-analysis as soon as they are available.

Another recent Cochrane systematic review and meta-analysis of intravenous immunoglobulin used for treating sepsis and septic-shock in all patients (adults, children and neonates) suggested a beneficial effect of non-specific IVIG on all cause mortality (RR 0.58, 95% CI 0.45 to 0.74)[40] (Table 3).

Table 3

Table 3

Review: **Intravenous immunoglobulin for treating sepsis and septic shock**
Comparison: **Polyclonal IVIG vs placebo or no intervention**
Outcome: **All-cause mortality (ACM)**

Study	Exptl n/N	Ctrl n/N	Relative Risk (95% CI Fixed)	Weight
Standard IVIG vs placebo or no intervention, ACM				
Chen 1996	2/28	1/28	2.00 [0.19, 20.82]	1.2%
De Simone 1988	7/12	9/12	0.78 [0.44, 1.39]	10.8%
Dominioni 1991	11/29	22/33	0.57 [0.34, 0.96]	24.8%
Grundmann 1988	15/24	19/22	0.72 [0.51, 1.03]	23.8%
Weisman 1992	2/14	5/17	0.49 [0.11, 2.13]	5.4%
Subtotal (95% CI)	37/107	56/112	0.68 [0.51, 0.89]	66.1%
IgM-enriched IVIG vs placebo or no intervention, ACM				
Haque 1988	1/30	6/30	0.17 [0.02, 1.30]	7.2%
Schedel 1991	2/27	9/28	0.23 [0.05, 0.97]	10.6%
Wesoly 1990	8/18	13/17	0.58 [0.33, 1.04]	16.1%
Subtotal (95% CI)	11/75	28/75	0.38 [0.22, 0.67]	33.9%
Total (95% CI)	48/182	84/187	0.58 [0.45, 0.74]	100.0%

There was significant heterogeneity between studies, which makes this summary measure difficult to interpret. A sensitivity analysis including only the trials of good quality, however, did not detect any heterogeneity and also suggested a decreased risk of mortality (RR 0.52, 95% CI 0.36 to 0.76).

The same Cochrane review also explored the effect of monoclonal antibodies and failed to demonstrate evidence of a decrease in the risk of mortality. The authors concluded that although there was evidence that non-specific IVIG appears to be beneficial '*large, multi-centre studies are needed to confirm the effectiveness of polyclonal IVIGs in reducing mortality in patients with sepsis. These are particularly indicated for neonatal sepsis, where evidence for benefit is still conflicting*'.

Safety: No evidence of transmission of blood borne viruses or prion disease

Safety

The risk of transmissible infection by blood products remains a potent source of anxiety for many clinicians and patients. However, IVIG produced to modern standards of quality control is one of the safest blood products available. There have been no reports of transmission of viruses or prions by the IVIG to be used in this study. Methods of production including alcohol fractionation, partitioning, microfiltration and heat treatment, and use of plasma originating from non-UK donors, have reduced the risk of transmission of infection to an absolute minimum[41 42].

In particular, for prion disease, leucocytes represent the main source of infectivity in Creutzfeld-Jacob disease. Owing to the physico-chemical characteristics of the abnormal prion protein, the process of partitioning and filtration during fractionation further reduces the risk of transmission in IVIG[43]. This theoretical risk must be considered in the context of the significantly increased risk of mortality and morbidity in infants eligible for the study.

As a further safeguard, fractionation pools of IVIG are tested with PCR (polymerase chain reaction) for known blood borne viruses.

Safety

Safety: No evidence of haemolysis related to T activation of red cells

Bacteria such as Clostridia can strip neuraminic acid residues from the red cell membrane, exposing the T antigen (T activation). Adult plasma contains anti T antibodies, so transfusing newborn infants whose red cells are T activated with whole blood, unwashed red cells or unselected plasma may lead to polyagglutination and haemolysis[44]. However, anti T antibodies are predominantly IgM immunoglobulins[45] a fraction which is removed from the IVIG used in this study. T activation is not a contra-indication to its use in neonatal sepsis. Although neonatal haemolysis has been noted in association with IVIG, it was not clinically significant[46]. The UK Committee on Safety of Medicines has received no reports of neonatal haemolysis or other adverse reactions in association with IVIG over a 30 year period until the present (personal communication, September 1999).

Current practice

Current practice

IVIG is not currently widely used for prophylaxis or treatment of neonatal sepsis in UK NICUs. In 1997, a postal survey of all paediatricians who were members of the British Association of Perinatal Medicine was undertaken into practice in the investigation and treatment of neonatal sepsis[47]. Of the 181 (66%) who responded, only 13 (7%) used IVIG routinely as adjuvant therapy alongside antibiotics.

Summary

Summary

There is good preliminary evidence that IVIG therapy may reduce mortality in severe neonatal sepsis. However, there is no information on longer term quality of survival, the number of babies included in the existing systematic reviews is small and the effect size seems larger than would be anticipated. As a consequence a reliable multicentre trial is needed to provide definitive evidence that IVIG therapy for severe neonatal sepsis is or is not of benefit, with mortality or major morbidity as the outcome. IVIG is not yet widely used as routine therapy. There remains, therefore, a window of opportunity to perform such a trial before an intervention which has been inadequately assessed begins to be incorporated into routine practice.

TRIAL ELIGIBILITY

Eligibility

Hospitals will be eligible for entry if they can provide neonatal intensive or special care, can achieve satisfactory rates of follow up at two years and would be able to institute the routine use of adjuvant IVIG for babies with sepsis if the trial demonstrates evidence of benefit.

Babies are eligible if:

1. they are receiving antibiotics with clinical evidence of severe or life-threatening infection

   AND

2. they have at least one of following:
   - birthweight less than 1500g

     OR
   - evidence of infection in blood culture, CSF or usually sterile body fluid

     OR
   - respiratory support via an endotracheal tube

   AND

3. there is substantial uncertainty that IVIG is indicated

Exclusion criteria are:

1. IVIG already given
2. IVIG thought to be needed or contra-indicated (e.g. because of severe congenital abnormality or contra-indications in the manufacturer's licensed product information sheet).

RECRUITMENT AND TRIAL ENTRY

Recruitment and trial entry

Recruitment will depend on good teamwork, knowledge and confidence among all clinicians, particularly front line nursing and medical staff, so that parents receive appropriate information about the study before entry and throughout their baby's stay. The ORACLE study[48 49] has recruited over 11,000 infants in 161 centres. Experience from that trial suggests that it is helpful if nurses and doctors understand the study background, see clinical research as an integral part of neonatal care contributing to future quality of care, and if a named nurse is appointed and trained as a local trial co-ordinator. If those caring for the baby are well informed about the study, they can discuss it without transmitting anxiety. Indeed, parents are likely to feel less anxious if given the opportunity to discuss the options for their baby's treatment in the context of the study with knowledgeable staff.

The named nursing and medical representative in each unit will therefore receive opportunities for training, regular information and support to enable them to orientate and update new and established nursing and medical staff. The protocol, printed materials and relevant new research will be widely available and staff will be kept informed by newsletters, personal visits and the worldwide web (as in the UK Neonatal Staffing Study; www.child-health.dundee.ac.uk/research/ukneonatal-staffing/).

More than half of all babies receive antibiotics for sepsis during their stay in a neonatal unit. Neonatal sepsis may present with subtle changes and clinicians normally have a low threshold for antibiotic treatment which should begin quickly, as infected infants can deteriorate rapidly. **The threshold for IVIG therapy in this study will be greater than for antibiotics, and infants must be considered at increased risk of mortality to be eligible for IVIG. As there is evidence that nursing staff can estimate the risk of mortality as or more accurately than medical staff, it would be valuable to consult them.**[50] Once an infant is considered sufficiently ill to be eligible, it is important that enrolment takes place as soon as practically possible.

All parents should routinely be given an information leaflet about INIS by the nursing staff when their baby is admitted to the neonatal unit. This will include details of their local medical and nursing contact[51] who they can discuss the study with. If their baby becomes eligible they will be asked for consent to participate in the study and later follow up, by the most appropriate member of staff available, in person or by telephone. If they consent in person a copy of the signed consent form will be give to the parent(s). If telephone consent is considered necessary and appropriate by the recruiting clinician, a 'Telephone consent' form will be completed. This form should then be read and signed by the parent(s) at their next visit to the hospital. Once this has happened, a copy of the consent form will be given to the parent(s).

TREATMENT ALLOCATION

Treatment allocation

The practical arrangements for random allocation to trial groups will be as simple as possible, based on that used in the MRC ORACLE study[48 49]. Staff will open the next sequentially numbered study pack kept in the neonatal unit, which contains all the materials necessary to give a course of study drug.

CLINICAL MANAGEMENT

Clinical management

IVIG group:	an intravenous infusion of IVIG of 500 mg (10 ml) per kg, repeated after 48 hours.
Control group:	an intravenous infusion of 10ml per kg of 0.2% albumin solution in normal saline (placebo) repeated after 48 hours.

Both infusions are of identical appearance: they are colourless and froth on agitation.

Administration of treatment

Administration of treatment

The IVIG or placebo infusion will be given according to the manufacturer's instructions, over about 4-6 hours. A second, similar, dose will be given at or around 48 hours after the first dose. No further IVIG or placebo should be given, in this or any subsequent episodes of sepsis.

Neonatal management

Neonatal management

All other aspects of neonatal management will be left to the discretion of the paediatrician responsible for care. No special investigations and no delays of discharge will be required.

MEASUREMENT OF OUTCOME

Measurement of outcome

Primary outcome measure:

1. mortality or major disability at two years, corrected for gestational age at birth

Secondary short term outcomes:

2. mortality, chronic lung disease or major cerebral abnormality before hospital discharge

Health service utilisation:

3. length of hospital stay

DATA COLLECTION

Data collection

Hospital mortality, chronic lung disease, major cerebral abnormality, level of care and length of stay will be assessed from case notes. Major disability at two years will be assessed by questionnaires sent to the child's parents and health care professionals. Major disability will be defined according to the criteria set out in the National Perinatal Epidemiology Unit (NPEU) and Oxford Regional Health Authority document [52 53] and will include any major disability in the following domains: neuromotor function, seizures, auditory function, communication, visual function, cognitive function and other physical disability. The instruments to be used for measuring disability at 2 years are not currently specified. Some instruments do exist, such as the Vineland Adaptive Behaviour Scales. It is likely, however, that additional scales will be validated by the time follow-up of children in this trial is undertaken. Various instruments are under development in the UK, USA and Australia and are being evaluated in the context of large scale trials or observational studies and which may be able to provide accurate and reliable measurement of disability at minimal cost.

Experience of other trials in this area at the NPEU, Oxford and elsewhere, suggests that it is possible to determine early neonatal events for all babies recruited. Loss to follow-up after hospital discharge of the child is more problematic. There are likely to be few children who cannot be traced in the UK either through the hospital of recruitment or the NHS Central Register. Similar high rates of follow up will be expected in countries participating outside the UK.

ANALYSIS

Analysis

An intention to treat analysis will be performed comparing the outcome of all children allocated IVIG with all those allocated placebo, regardless of what treatment was received, or how complete that treatment was. Statistical analysis will use standard methods to calculate the relative risk of an outcome in the IVIG group compared with the placebo group along with a 95% confidence interval. For secondary analyses, 99% confidence intervals will be calculated to take account of the number of comparisons. Where appropriate c^2 tests of significance will be performed and presented as p-values.

Sub-group analyses

Sub-group analyses:

The primary analyses will also be undertaken for

(a) infants of very low birth weight (VLBW: less than 1500g)

(b) larger infants

Clinicians may also want to know if IVIG is effective in infants presenting at different levels of risk, based on data available to them at entry into the study[54-60]. Therefore, secondary analyses will be performed for subgroups presenting with:

(i) <u>clinical evidence of high mortality risk:</u>

i.e. looking seriously ill and inactive and has

(a) capillary refill time > 3 seconds OR

(b) bowel perforation or definite necrotising enterocolitis OR

(c) prolonged bleeding from puncture sites OR

(d) ventilated, SaO_2/ FiO_2 ratio or PaO_2/FiO_2 ratio consistent with >15% mortality risk for gestation* OR

(e) pH consistent with >15% mortality risk for gestation*

* Stratification by mortality risk will be extrapolated from oxygenation and pH data in a prospective cohort of 14,000 infants (UK Neonatal Staffing Study: www.child-health.dundee.ac.uk/research/) by methodology similar to that used in the development of the MRC funded CRIB score.

(ii) <u>does not satisfy above criteria, but</u>

(a) total white cell count < 5 x 10^9/l OR

(b) CRP above 15 mg/l OR

(c) platelet count < 50 x 10^9/l OR

(d) organism(s) isolated in blood or usually sterile site OR

(e) pneumonia on chest X-ray OR

(f) CSF consistent with bacterial meningitis

(iii) <u>does not satisfy criteria for (i) or (ii)</u>

Finally, clinicians may wish to know about the effectiveness of IVIG in subgroups of infants classified by data available after results of investigations are known. Secondary analyses will therefore also be performed for infants with:

(i) Early onset infection (non contaminant organisms isolated from culture sent <48 hours)
   (a) Group B streptococcal disease
   (b) Other pathogens
   (c) Indeterminate aetiology

(ii) Late onset infection
   (a) Gram positive organisms except *Staphylococcus epidermidis*
   (b) *Staphylococcus epidermidis*
   (c) Other pathogens
   (d) Indeterminate aetiology

(iii) Post surgery

Interim analyses

Interim analyses: the Data Monitoring and Ethics Committee

For the trial a Data Monitoring and Ethics Committee (DMEC) will be established. This will be independent of the trial organisers and will meet at least once per year. During the period of recruitment to the trial, interim analyses will be supplied, in strict confidence, to the DMEC, together with any other analyses the DMEC may request. In the light of interim data, and other evidence from relevant studies (including updated overviews of the relevant randomised controlled trials), the DMEC will inform the Steering Group, if in their view: i) there is proof beyond reasonable doubt that the data indicate that any part of the protocol under investigation is either clearly indicated or contra-indicated, either for all infants or for a particular subgroup of trial participants, or ii) it is evident that no clear outcome will be obtained. Decision to inform the Steering Group in either of these circumstances will in part be based on statistical considerations. Appropriate criteria for proof beyond reasonable doubt cannot be specified precisely. A difference of at least 3 standard deviations in the interim analysis of a major endpoint may be needed to justify halting, or modifying, such a study prematurely. If this criterion were to be adopted, it would have the practical advantage that the exact number of interim analyses would be of little importance, and so no fixed schedule is proposed[61]. Unless modification or cessation of the protocol is recommended by the DMEC, the Steering Group, collaborators and administrative staff (except those who supply the confidential information) will remain ignorant of the results of the interim analysis. Collaborators and all others associated with the study, may write through the trial office to the DMEC, to draw attention to any concern they may have about the possibility of harm arising from the treatment under study, or about any other matters that may be relevant.

The membership of the DMEC is:

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SAMPLE SIZE AND FEASIBILITY

Sample size and feasiblity

Table 4 shows positive blood culture rates (including probable contaminants) in 3,963 consecutive infants of all birthweights admitted to a randomly selected, nationally representative cohort of 54 UK neonatal units between 1st March 1998 and 4th September 1998 in a study of organisation and outcomes of neonatal care funded by the NHS Executive[6].

Table 4

Table 4

Number with positive cultures	Mortality (all causes)	Number with negative cultures	Mortality (all causes)
204 / 3,963 (5%)	16 / 204 (8%)	3,759 / 3,963 (95%)	95 / 3,759 (2.5%)

Among VLBW infants with positive blood cultures, mortality was 14%. In a recent North American cohort, mortality in VLBW infants with septicaemia was 21%[7]. Assuming combined rates of mortality and major morbidity of 10-20% for all infants and 20-30% for VLBW infants, Table 5 outlines estimated sample sizes.

Table 5

Table 5

Mortality or major morbidity in control group	Mortality or major morbidity in IVIG group	Relative risk reduction	Total sample size required to detect difference with 95% confidence	
			80% power	90% power
30%	26%	13%	4,052	5,392
30%	25%	17%	2,580	3,428
30%	20%	33%	626	824
25%	21%	16%	3,572	4,748
25%	20%	20%	2,266	3,006
25%	15%	40%	540	708
20%	16%	20%	2,994	3,972
20%	15%	25%	1,890	2,502
20%	12.5%	37.5%	810	1,066
15%	12%	20%	4,204	5,582
15%	10%	33%	1,450	1,914
12%	9%	25%	3,408	4,516
10%	7.5%	25%	4,166	5,524

Feasibility

About 5,000 infants will be needed to demonstrate moderate reductions in mortality or survival with major developmental delay with adequate power. Over a three year recruitment period, assuming that 7-10% of all admissions are diagnosed with clinical sepsis and considered eligible for recruitment, 150 NICUs with an average of 300 admissions per year will be required to achieve the recruitment target, assuming a 40-50% rate of recruitment of eligible infants. Neonatal units will initially be recruited in the UK, Australia and New Zealand. A census of all 186 UK neonatal intensive care units (NICUs) and 60 special care baby units in 1996[62] (which is a 100% response rate) found that the median number of admissions per year per NICU was 317. If a broadly representative sample of about half all UK NICUs and SCBUs participate, then over 50% of the projected recruitment rate for the trial will be possible within the UK, leaving the additional 50% to be recruited from the rest of the world. This study reflects the philosophy that the only practicable way to achieve comparisons which are sufficiently large to minimise the risk of being seriously misled by the play of chance is to design trials that are extremely simple and flexible[63]. Experience in the OSIRIS[64] and MRC ORACLE study[48 49] suggest that a large, simple trial of this scale of a potentially important intervention supplied free of charge to participating centres is feasible. Furthermore, systematic reviews of RCTs of IVIG therapy in neonatal sepsis suggest a substantial reduction in mortality. This contrasts with the systematic reviews of RCTs of antibiotics in threatened preterm birth which led to the ORACLE study, as these showed no evidence of a difference in neonatal mortality. This preliminary evidence that IVIG may reduce mortality may further enhance the appeal of the study.

The estimate of the incidence of the outcome (the event rate) for the trial is imprecise, particularly as the threshold at which clinicians will enter patients cannot be estimated. If clinicians enter babies where the likelihood of serious sepsis is lower then the event rate will also be lower. If clinicians restrict entry to only those babies who are very sick, then the event rate will be high. Either of these two scenarios is reasonable because it will define a population to which the trial result can be generalised. However, it does mean that until the trial has recruited sufficient numbers of babies it will not be possible to determine the optimum trial sample size with any certainty. As a consequence the trial sample size currently represents the minimum size desirable. Assuming the trial recruits for three years, the maximum number of babies which can be recruited during this time will be recruited and it is possible that this number may exceed 5,000. During recruitment to the trial the accumulating data will be seen by an independent Data Monitoring and Ethics Committee at least once per year (see above) and they will advise the Trial Steering Committee whether the trial has answered the clinical question being addressed. If not, the trial will continue to recruit until 5,000 babies have been recruited, or until funding is exhausted.

PUBLICATION POLICY

Publication policy

To safeguard the scientific integrity of the trial, data from this study should not be presented in public or submitted for publication without requesting consent from the Trial Steering Committee (see Organisation below). The success of the trial depends on the collaboration of a large number of doctors and nurses. For this reason, chief credit for the results will be given not to the committees or central organisers but to all who have wholeheartedly collaborated in the study.

ORGANISATION

Trial Steering Committee

Trial steering committee

The Trial Steering Committee (TSC) provides overall supervision of the trial on behalf of the Medical Research Council. Its terms of reference are:

1. To monitor and supervise the progress of the trial towards its interim and overall objectives.
2. To review at regular intervals relevant information from other sources (e.g. related trials).
3. To consider the recommendations of the Data Monitoring and Ethics Committee.
4. In the light of 1, 2 and 3 above, to inform the MRC Council and relevant MRC Research Boards on the progress of the trial.
5. To advise the MRC Council on publicity and the presentation of all aspects of the trial.

The membership of the TSC is:

Professor Richard Cooke, Professor of Neonatal Medicine, Neonatal Intensive Care Unit, Liverpool Womens Hospital, Crown Street, Liverpool L8 7SS. Email: mcl19@liverpool.ac.uk

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Meetings of the TSC will take place at least once per year.

Investigators' Group

Investigators' group

The Investigators' Group will consist of the trial investigators, representatives of specific groups whose expertise is necessary for the trial, and investigators of any ancillary studies. This group will supervise the practical aspects of the trial's conduct. It will resolve problems brought to it by the Project Management Group (see below) and will be responsible for organising reporting and dissemination of the trial's results.

Project Management Group

Project management group

The Project Management Group (PMG) will oversee the day-to-day running of the trial. The responsibilities of the Project Management Group include:

i) recruitment of participating centres
ii) distribution and supply of data collection forms and other appropriate documentation for the trial
iii) data collection and management
iv) organisation of the distribution system for the treatment packs
v) organisation of the follow-up of children at 2 years if age, including the distribution of questionnaires, follow-up of non-responders and liaison with local 'follow-up' personnel
vi) data entry and cleaning
vii) data analysis
viii) collection of adverse event data
ix) organising and servicing the Data Monitoring and Ethics Committee.

Local Co-ordination

Local co-ordination

Each participating centre will identify a local medical co-ordinator and a local neonatal nurse co-ordinator (as necessary). The responsibility of the local co-ordinators will be to:

i) be familiar with the trial
ii) liaise with the INIS Co-ordinating centre in Oxford
iii) ensure that all staff involved in the care of babies on the neonatal unit are informed about the trial
iv) ensure that mechanisms for recruitment of eligible babies (including information material) are in place, monitor their effectiveness, and discuss reasons for the non-recruitment of any eligible babies with relevant staff
v) ensure that supplies of data collection forms are always available, that they are completed and returned to the INIS Co-ordinating centre promptly, and to deal with any queries arising
vi) notify the trial co-ordinating centres of any serious adverse events
vii) facilitate other aspects of local collaboration as appropriate
viii) make all data available for verification, audit and inspection purposes as necessary
ix) ensure that the confidentiality of all information about trial participants is respected by all persons

Frequently asked questions

FREQUENTLY ASKED QUESTIONS

Is IVIG safe?

No blood product (and no treatment of any kind) is 100% safe, but IVIG is one of the safest blood products there is. Given prophylactically in randomised controlled trials with over 5,000 very low birth weight infants, IVIG was reported as safe, with no serious adverse reactions[23]. The IVIG for this study is IgG produced by alcohol fractionation, heat treatment and filtration from plasma imported from non UK donors and is screened for blood borne viruses by PCR, so the risk of transmission of infection is extremely low. It should be given according to the manufacturers' product information.

What about T activation, for example in infants with necrotising enterocolitis?

Infants with T activated red cells, as may occur with Clostridial infections and in necrotising enterocolitis, may suffer haemolysis unless they receive washed red cells or fresh frozen plasma with low titres of anti T antibody. Infants with T activated red cells can receive the non-specific IVIG to be used in this study, however, as it has a negligible concentration of anti T antibody (which is mainly IgM). There have been no reports of neonatal haemolysis in association with any type of IVIG.

Will IVIG be expensive?

Non-specific IVIG will be supplied free of charge during the study. It currently costs about £10 sterling per gram to produce. If this study shows that it is effective in reducing mortality, and between 5 - 200 infants needed to be treated to save an extra life, the cost (of IVIG alone) might vary, very approximately, between £50 - 6000 per life saved. This compares favourably with many other interventions - but does not take into account any additional hospital costs. Non-specific IVIG products made by non-commercial agencies, such as the manufacturers for this study, are likely to be less expensive than commercial products in the longer term.

Should all infants on antibiotics or with respiratory distress be recruited into the study?

No. About two thirds of infants admitted to neonatal units get antibiotics, and their overall risk of mortality or morbidity is very low. It would be reasonable to recruit any infant who makes a poor response to surfactant, or who is ventilated with suspected pneumonia. Also, all infants under 1000g or <28 weeks gestation who are ventilated for lung disease in the first 48 hours could be recruited, as a significant proportion will have early onset sepsis. Otherwise, infants with respiratory distress syndrome who respond well to surfactant with no further evidence of sepsis should not be recruited.

APPENDIX 1

CONTENTS OF IVIG AND PLACEBO UPON RECONSTITUTION

WITH 60ml WATER FOR INJECTION

	IVIG*	Placebo
Total Protein (g/L)	44.5±0.8	1.93
Sodium (mMol)	44±2	48
Potassium (mMol)	0.15	0.17
pH	6.9±0.1	6.65
Osmolality (mOsm/kg)	403±11	413
Ethanol (ml/L)	<0.1	<0.1
Citrate (mMol)	<0.1	<0.1
Albumin (g/L)	<0.1	2.4
Sucrose (%)	9.0±0.3	10.5
HPLC[#] (% Aggregate)	2.3±0.3	4.3
PKA^ (iu/ml)	<2.0	<2.0

* mean ± SD of 20 batches of 3g IVIG

[#] high purity liquid chromatography

^ pre-kallirien activator

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