



Grazielle S. Verzosa, MD\*  
Mary Mae Catherine N. Yu, MD\*  
Kathlyne Anne Abat-Senen, MD\*  
Maria Isabel O. Quilendrin, MD\*

\*Philippine General Hospital, University of the Philippines  
Manila

Correspondence:

Dr. Grazielle S. Verzosa  
Email: z.verzosa@gmail.com

The authors declare that the data presented are original material and has not been previously published, accepted or considered for publication elsewhere; that the manuscript has been approved by all authors, and all authors have met the requirements for authorship.

**3<sup>RD</sup> PRIZE 2018 PIDSP RESEARCH CONTEST**

**ORIGINAL ARTICLE**

**GRANULOCYTE COLONY STIMULATING FACTOR IN IMPROVING OUTCOMES OF NEONATAL SEPSIS: A META-ANALYSIS**

**ABSTRACT**

**Background:** Neonatal sepsis complicated with neutropenia increases risk of mortality by 50%. The immature neutrophil production of neonates is often overwhelmed by severe infection. Granulocyte colony stimulating factor (G-CSF), a naturally occurring cytokine used to support neutrophil recovery during chemotherapy, is a possible treatment that can improve outcomes of neonatal sepsis.

**Objectives:** To determine the efficacy of G-CSF in decreasing mortality and morbidity in septic neonates.

**Methodology:** Electronic searches were conducted on online journal databases. Unpublished or ongoing studies were sought in training institutions accredited by the Philippine Pediatric Society. The investigators included randomized control trials using G-CSF on septic neonates.

**Results:** Twenty-two trials were identified and thirteen were assessed to be eligible for review. The studies had a total of 530 participants, with the largest having 78 subjects. Relative risks (RR), mean differences (MD) and standard mean differences (SMD) with 95% confidence intervals (CI) using the fixed effect model and random effects model were reported in the results. There was a significant decrease in mortality (RR 0.69, 95% CI 0.48 to 0.99) with a greater reduction for preterm neonates, low birth weight neonates and neutropenic neonates. There was no significant reduction in morbidities caused by neonatal sepsis.

**Conclusions:** There is moderate quality evidence that suggests that G-CSF as an adjunct treatment for neonatal sepsis significantly decreases mortality with greater benefit to preterm neonates, low birth weight neonates and those with baseline neutropenia. The studies did not show any benefit in reducing sepsis-related morbidity.

**KEYWORDS:** *granulocyte colony stimulating factor, neonatal sepsis, neutropenia*

## INTRODUCTION

Neonatal sepsis is one of the principal causes of morbidity and mortality worldwide. More than one-third of the 2.7 million deaths in the neonatal period is attributed to severe infection<sup>1</sup>. It also leads to permanent disability such as cerebral palsy and chronic lung disease<sup>2,3</sup>. Incidence is much higher in developing countries; but even in more sophisticated settings, neonatal sepsis still proves to be difficult to manage<sup>4</sup>. This is attributed to the immature immune system of neonates that is more profound in the susceptible groups of preterm neonates and those with low birth weights. When sepsis is accompanied with severe neutropenia, risk of mortality increases to more than 50%<sup>5</sup>. Neutropenia occurs when the immature neutrophil production of neonates is overwhelmed by severe infection. Another factor that contributes to increased mortality and morbidity is the functionally immature neonatal neutrophils<sup>6</sup>. Even with advancements in antibiotic and adjunctive therapies, the presence of neutropenia in the context of neonatal sepsis poses a difficult challenge to the clinician. These coupled with the alarming rise of antibiotic resistance stresses the need to explore alternative or adjunct therapies for neonatal sepsis<sup>7</sup>.

Granulocyte colony stimulating factor (G-CSF) is a naturally occurring cytokine often used in cancer patients after chemotherapy to hasten neutrophil recovery<sup>8</sup>. Use of G-CSF is relatively safe with common side effects including headache, loss of appetite, bone pain, diarrhea, constipation and mild liver changes<sup>9</sup>. In previous studies, there was no associated increase in mortality or morbidity among neonates administered with G-CSF<sup>10,11</sup>.

It is hypothesized that use of G-CSF in neutropenic neonates will increase numbers of circulating neutrophils as well as improve their phagocytic function. In a previous meta-analysis done by Carr et al. in 2003, it was concluded that there was insufficient evidence supporting the use of G-CSF in decreasing mortality in septic neonates.

The studies that were reviewed included 257 neonates with suspected bacterial infection. However, each study had small sample sizes with 60 subjects at the most<sup>10</sup>. Since 2003, more studies have been conducted with larger populations recruited from multiple centers. This additional data could help determine if the administration of G-CSF will improve outcomes of neonatal sepsis. Hence, this study aimed to determine the efficacy of G-CSF in decreasing mortality and morbidity from neonatal sepsis as well as in increasing absolute neutrophil count (ANC) in septic neonates.

## METHODS

### Criteria for Considering Studies for Review

The following criteria were used to identify studies for inclusion:

#### Types of Studies

- randomized control trials
- with or without blinding
- with or without placebo control

#### Types of Participants

Newborn infants (0-28 days old) with culture proven or suspected sepsis fulfilling one or more of the following criteria:

- admitted to a neonatal intensive care unit or hospital ward
- with neutropenia (ANC < 1,500)
- at high risk for developing sepsis (i.e. preterm, low birth weight, small for gestational age)

#### Types of Interventions

Administration of G-CSF in any dose alongside conventional medical treatment compared with standard care with or without placebo.

#### Types of Outcome Measures

##### Primary Outcomes

1. mortality
2. morbidities caused by neonatal sepsis (i.e. chronic lung disease, necrotizing enterocolitis, cerebral palsy, etc.)

## Secondary Outcomes

1. absolute neutrophil count
2. leukocyte count
3. immature: total neutrophil ratio (I:T Ratio)
4. duration of hospital stay
5. duration of ventilatory support
6. adverse effects that can be attributed to the administration of G-CSF

## Search Methods for Identification of Studies

Electronic searches were conducted on online medical journal databases (Cochrane Library, PubMed, MEDLINE, Embase, WHO International Clinical Trials Registry, Herdin) as well as on other online journal databases (Google Scholar, Jstor, Directory of Open Access Journals, Science Direct) to identify relevant studies. The following search strategy was utilized: (“G-CSF” OR “rhG-CSF” OR “granulocyte colony stimulating factor”) AND “neonatal sepsis”. There was no limitation in terms of language or publication period. In the articles retrieved, the reference lists were searched for other relevant trials.

Local pediatric training institutions accredited by the Philippine Pediatric Society were also contacted to inquire about any unpublished or ongoing studies that fulfill the inclusion criteria.

## Data Collection and Analysis

### Selection of Studies

Two review authors separately searched for all available articles that meet the inclusion criteria. Meta-analyses and systematic reviews were also scanned for eligible studies. Full-text articles of all potentially eligible studies were obtained and reviewed. Studies that were published multiple times only had one final report included in the review. For articles with data that are either insufficient or unclear, authors were contacted for clarification. Such articles whose authors could not be contacted were excluded from the review. For articles that are written in languages other than English, a translated paper was searched for or

requested from the author/s. If there was none available, then the study was excluded. In case of disagreements between the authors, issues were resolved through discussion.

### Data Extraction and Management

From each of the eligible articles, data was extracted independently by the reviewers and organized into a standard database. A modified data collection form based on the one published by The Cochrane Collaboration was used to organize the extracted data that included<sup>12</sup>:

1. General Information: title, primary investigator, year of publication
2. Population: age (in weeks), birth weight, sex
3. Sample Size
4. Characteristics of Intervention: dose, route
5. Characteristics of Control: standard care, placebo
6. Blinding: treatment allocation, intervention, outcome measure assessment

The data collected was summarized and entered into the Review Manager ver. 5.3 program (Cochrane Collaboration software)<sup>13</sup>.

### Assessment of Risk of Bias in Included Studies

The reviewers independently evaluated the overall risk of bias and assessed the quality of evidence based on indicators of internal validity. The criteria for evaluating the articles were based on the Cochrane Handbook for Systematic Reviews of Interventions<sup>14</sup>. The articles were assessed as low risk, high risk or unclear based on the following indicators:

1. Sequence Generation (screening for selection bias)

For each eligible study, the method used to generate treatment allocation was described. The methods were assessed as:

- Low Risk - any form of randomization (ex. random number table, computer generated)

- High Risk - non-randomized processes (ex. alternating case numbers, odd or even date of birth)
  - Unclear - insufficient information
2. Allocation Concealment (screening for selection bias)

For each eligible study, the method used to conceal treatment allocation was described. The methods were assessed as:

- Low Risk - opaque and sealed envelopes, sequentially numbered drug containers, central randomization
  - High Risk - open label trials, predictable allocation (ex. alternation, rotation), clear or unsealed envelopes
  - Unclear - insufficient information
3. Blinding (screening for performance bias and detection bias)

For each eligible study, the blinding of patients, personnel and/or outcome assessors were described. Whether or not placebo was used in the study was also indicated. The methods were assessed as:

- Low Risk - no/incomplete blinding but the outcome is not likely to be influenced by the lack of blinding, blinding was ensured and was unlikely to be broken
  - High Risk - no/incomplete blinding and the outcome is likely to be influenced by the lack of blinding, blinding was done but was likely to be broken
  - Unclear - insufficient information
4. Incomplete Outcome Data (screening for attrition bias)

For each eligible study, the completeness of data including reasons for exclusions, withdrawals, dropouts and protocol deviations was evaluated. The final number of included participants in the analysis was also compared to the initial randomized participants. The studies were assessed as:

- Low Risk - complete outcome data, reasons for missing outcome data are

unlikely to be related to the true outcome, missing outcome data balanced in numbers across intervention groups, with similar reasons for missing data across groups

- High Risk - reasons for missing outcome data are likely to be related to the true outcome, missing outcome data are not balanced in numbers across intervention groups
  - Unclear - insufficient information
5. Selective Outcome Reporting (screening for reporting bias)

For each eligible study, the presence of possible selective reporting bias was evaluated. The studies were assessed as:

- Low Risk - published reports include all expected outcomes that have been pre-specified
  - High Risk - one or more primary outcomes were not pre-specified, outcomes of interest are not reported completely, does not include results of a key outcome which was expected of the study
  - Unclear - insufficient information
6. Other sources of bias

For each eligible study, the presence of other sources of bias that were not clearly stated above was assessed. The studies were assessed as:

- Low Risk - appears to be free of other sources of bias
- High Risk - has at least one important risk of bias
- Unclear - insufficient information

#### Data Synthesis and Measures of Treatment Effect

The collected data from all articles that are included in the final data analysis were run through the Review Manager ver. 5.3 software. Dichotomous data included incidence of mortality, morbidity and adverse events while continuous data included absolute neutrophil count, leukocyte

count, immature: total neutrophil ratio, duration of hospital stay and duration of ventilatory support. For continuous data, the mean difference was used if outcomes were measured using the same method and unit of measure. Otherwise, the standardized mean difference was used to combine trials using different methods and units to measure the same outcome.

### Dealing with Missing Data

It was anticipated that some trials did not report all relevant figures to the study. Where there is significant loss of data, trial authors were contacted. In cases where missing data could not be retrieved, imputation methods were utilized and such studies underwent sensitivity analysis.

### Assessment of Heterogeneity

The heterogeneity of data included in the analysis was measured through the chi-square test, the I<sup>2</sup> statistic and visual inspection of forest plots. Significant heterogeneity is defined as P-value of < 0.10 in the chi-squared test and an I<sup>2</sup> value > 50%.

### Assessment of Reporting Biases

For eligible studies included in the study, funnel plots were drawn to investigate if there is an association between the sample size and the effect estimates. When an association was detected, the studies were further examined and possible reasons for such an association were reported.

### Subgroup Analysis and Investigation of Heterogeneity

The study aimed to do the following subgroup analyses should there be enough data for these to be conducted:

1. preterm infants (gestational age less than 37 weeks) versus term (gestational age less than 37 weeks)
2. birth weight (low - < 2500g, very low - < 1500g, extremely low - <1000g)
3. initial Absolute Neutrophil Count (ANC) = (%Neutrophils + %Bands) x WBC/100
4. culture-positive sepsis versus suspected sepsis dose and duration of G-CSF treatment

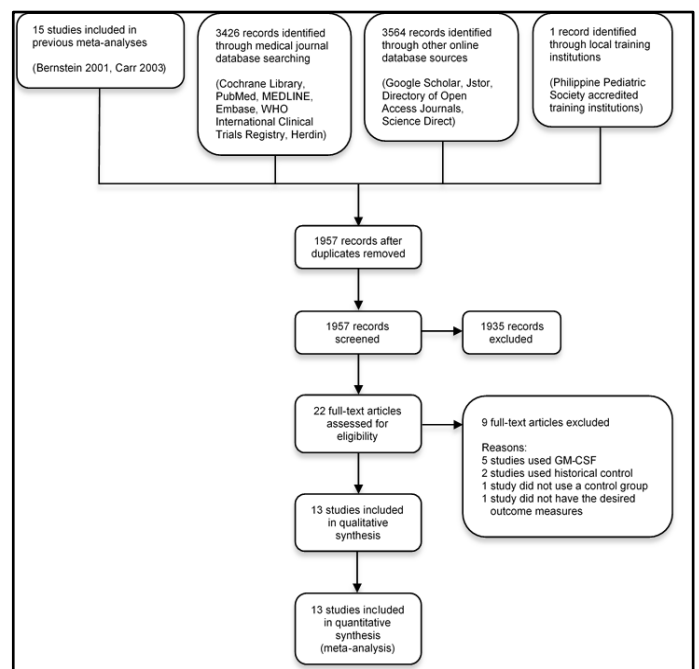
### Sensitivity Analysis

To assess the impact of the quality of the studies included in the study as well as the imputation of missing data on the results of the meta-analysis, sensitivity analysis was conducted. The presence or absence of significant association was included in the report.

## RESULTS

### Results of the Search

The search identified 22 trials, 13 of which were included in the review while 9 were excluded.



**Figure 1.** The PRISMA flow diagram showing the process of study selection

### Included Studies

Fifteen studies from previous meta-analyses (Bernstein 2001, Carr 2003) were originally planned to be included; however, 6 were excluded due to reasons enumerated in the next segment. There was a total of 530 participants in all 13 studies included for review, the largest having 78 participants (Chaudhuri 2012). Three were conducted in multiple centers (Aktas 2013, Gillan 1994, Schibler 1998) while the rest were conducted in single institutions. Eight trials specified that they were conducted in

neonatal intensive care units (Ahmad 2002, Bedford Russell 2001, Chaudhuri 2012, El-Ganzoury 2012, Gathwala 2011, Miura 2001, Schibler 1998, Sezer 2002). All studies recruited neonates with either proven or suspected sepsis. Certain studies had the following common characteristics in their inclusion criteria: neutropenia in 10 studies (Ahmad 2002, Aktas 2013, Bedford Russell 2001, Borjanyazdi 2013, Chaudhuri 2012, Drossou-Agakidou 1998, Drossou-Agakidou 2002, Gathwala 2011, Miura 2001, Schibler 1998), prematurity in 9 studies (Ahmad 2002, Aktas 2013, Bedford Russell 2001, Borjanyazdi 2013, Chaudhuri 2012, Drossou-Agakidou 1998, Gathwala 2011, Miura 2001, Sezer 2002), low birthweight in 5 studies (Bedford Russell 2001, Chaudhuri 2012, Gathwala 2011, Miura 2001, Sezer 2002). Nine studies used a placebo of equal volumes of either normal saline or the same diluent used for administering G-CSF (Ahmad 2002, Bedford Russell 2001, Borjanyazdi 2013, Chaudhuri 2012, Drossou-Agakidou 2002, Gillan 1994, Miura 2001, Schibler 1998, Sezer 2002). The rest of the studies compared the treatment group with standard medical care. (Aktas 2013, Drossou-Agakidou 1998, El-Ganzoury 2012, Gathwala 2011).

**Excluded Studies**

There were 9 excluded studies. Reasons for exclusion were:

1. Five studies used granulocyte-macrophage colony stimulating factor instead of granulocyte colony stimulating factor.
2. Two studies used historic control subjects instead of randomizing patients into treatment and control groups.
3. One study compared the effects of two different doses of G-CSF. There was no control group to which the treatment groups were compared to
4. One article discusses the neuropsychological development and anthropometrics of children included in the PROGRAMS trial conducted in the

UK. The article did not include the necessary outcome measurements.

**Risk of Bias in Included Studies**

Randomization

Eight studies (62%) specified the method of randomization used for treatment allocation (Bedford Russell 2001, Borjanyazdi 2013, Chaudhuri 2012, El-Ganzoury 2012, Gathwala 2011, Miura 2001, Schibler 1998, Sezer 2002). The studies used either a computer-generated randomization or a table of random numbers. The rest of the studies (38%) stated that the patients included in their trials were randomized; however, the method used was not specified.

Allocation Concealment

Among the included studies, only 3 studies (23%) adequately described how treatment allocation was concealed (Chaudhuri 2012, El-Ganzoury 2012, Gathwala 2011). The studies used either opaque sequentially numbered sealed envelopes or allocation numbers concealed in the cover of each medication/placebo. The other 10 studies (77%) did not mention allocation concealment methods in their articles.

	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and personnel (performance bias)	Blinding of outcome assessment (detection bias)	Incomplete outcome data (attrition bias)	Selective reporting (reporting bias)	Other bias
Ahmad 2002	?	?	+	+	+	+	?
Aktas 2013	?	?	+	+	+	+	+
Bedford Russell 2001	+	?	+	+	+	+	+
Borjanyazdi 2013	+	?	+	+	+	+	+
Chaudhuri 2012	+	+	+	+	+	+	+
Drossou-Agakidou 1998	?	?	+	+	+	+	?
Drossou-Agakidou 2002	?	?	?	?	+	+	?
El-Ganzoury 2012	+	+	+	+	+	+	+
Gathwala 2011	+	+	+	+	+	+	+
Gillan 1994	?	?	?	?	+	+	?
Miura 2001	+	?	+	+	+	+	?
Schibler 1998	+	?	+	+	+	+	?
Sezer 2002	+	?	+	+	+	+	?

**Figure 2.** Risk of Bias Summary: review authors' judgements about each risk of bias item for each included study

### Blinding

Ten studies (77%) followed a double-blinded study design (Ahmad 2002, Bedford Russell 2001, Borjanyazdi 2013, Chaudhuri 2012, Drossou-Agakidou 1998, El-Ganzoury 2012, Gathwala 2011, Miura 2001, Schibler 1998, Sezer 2002) while 3 studies (23%) had insufficient information regarding blinding (Aktas 2013, Drossou-Agakidou 2002, Gillan 1994).

### Incomplete Outcome Data

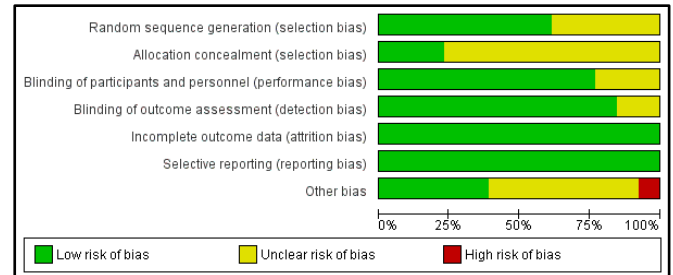
Most of the studies included all enrolled participants in the final analyses. In cases where there are withdrawals or exclusions in analyses, there was sufficient information explaining why certain participants were not included in the final analyses. The most common reason for exclusion was mortality. Patients who died during the trial were excluded from the analysis of the duration of hospital stay as well as the duration of ventilatory support.

### Selective Reporting

It is difficult to assess true selective reporting bias since the protocols for the included studies could not be retrieved. Instead, judgment was based on the outcomes mentioned in the methods section compared with the final results reported in the article

### Other Potential Sources of Bias

The authors of one article (8%) stated that their study was funded by the pharmaceutical company that manufactured the G-CSF used in the trial (Bedford Russell 2001). Seven studies (54%) mentioned the pharmaceutical companies who manufactured the G-CSF used in the trial; however, they did not declare any connections to those companies mentioned (Ahmad 2002, Drossou-Agakidou 1998, Drossou-Agakidou 2002, Gillan 1994, Miura 2001, Schibler 1998, Sezer 2002). One study (8%) did not mention any pharmaceutical company at all. Four studies (31%) explicitly mentioned that they do not have any conflicts of interest to declare.



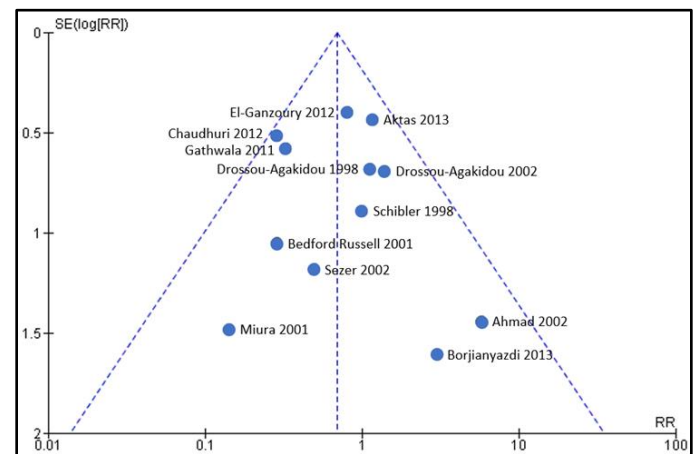
**Figure 3.** Risk of Bias Graph: review authors' judgements about each risk of bias item presented as percentages across all included studies

## **Effects of Interventions**

### Primary Outcomes

#### Mortality

All thirteen included studies reported data on all-cause mortality. The studies exhibited homogenous results, as shown in Figure 4.



**Figure 4.** Funnel Plot of Comparison: Mortality Rate

Five hundred thirty participants were included in the analysis. There were 41 deaths from the 282 participants in the treatment group while there were 54 deaths from the 248 participants from the control group. There was a significant decrease in mortality in the treatment group [RR 0.69 (0.48, 0.99)] (Figure 5).

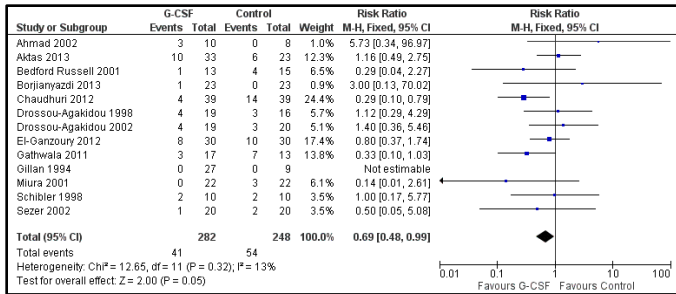


Figure 5. Forest Plot: Mortality Rate

There was a greater reduction of mortality rate for preterm neonates [RR 0.60 (0.39, 0.94)] and neonates with low birthweight [RR 0.29 (0.15, 0.57)]. However, there was no significant reduction of mortality in neonates who had baseline neutropenia [RR 0.68 (0.45, 1.02)].

### Morbidity

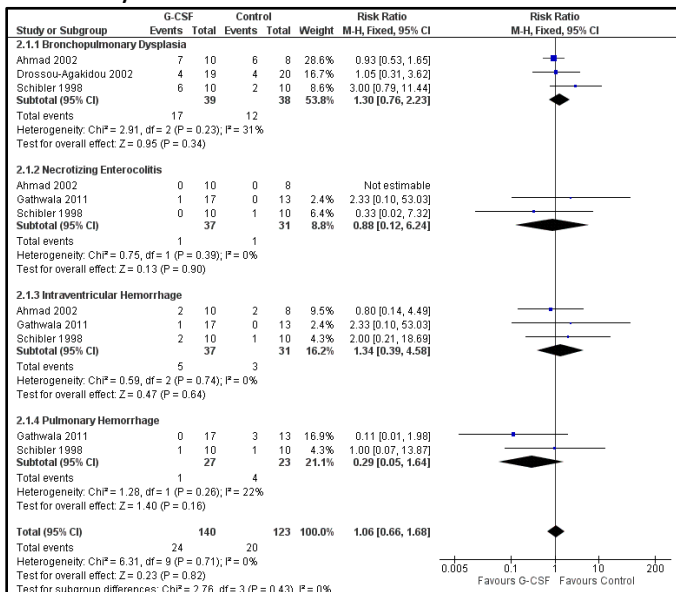


Figure 6. Forest Plot: Morbidities (Bronchopulmonary Dysplasia, Necrotizing Enterocolitis, Intraventricular Hemorrhage, Pulmonary Hemorrhage)

Four studies reported morbidities related to neonatal sepsis (Ahmad 2002, Drossou-Agakidou 2002, Gathwala 2011, Schibler 1998). There was no significant reduction of morbidities reported: Bronchopulmonary Dysplasia [RR 1.30 (0.76, 2.23)], Necrotizing Enterocolitis [RR 0.88 (0.12, 6.24)],

Intraventricular Hemorrhage [RR 1.34 (0.39, 4.58)], Pulmonary Hemorrhage [RR 0.29 (0.05, 1.64)], Overall Morbidity [1.06 (0.66, 1.68)] (Figure 6).

### Secondary Outcomes

#### Duration of Hospital Stay

There were six studies that reported the duration of hospital stay (Ahmad 2002, Bedford Russell 2001, Borjanyazdi 2013, Drossou-Agakidou 2002, El-Ganzoury 2012, Gathwala 2011). There was a reduction in the duration of hospital stay for the treatment group [MD -4.91 (-6.92, -2.90)]. However, the studies analyzed showed heterogenous results with a P-value of 0.0004 and an I<sup>2</sup> value of 78% (Figure 7).

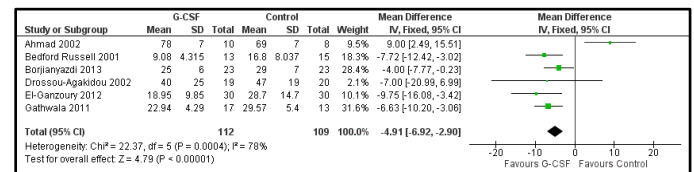


Figure 7. Forest Plot: Duration of Hospital Stay in Days

#### Duration of Ventilatory Support

Three studies reported the duration of ventilatory support (Bedford Russell 2001, Drossou-Agakidou 2002, El-Ganzoury 2012). There was a reduction in the duration of ventilatory support for the treatment group [MD -3.72 (-6.94, -0.50)]. The studies analyzed also showed heterogenous results with a P-value of 0.003 and an I<sup>2</sup> value of 83% (Figure 8).

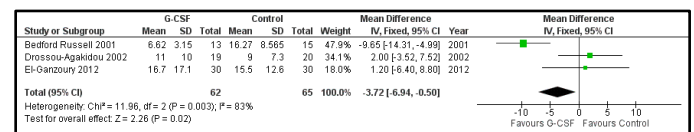


Figure 8. Forest Plot: Duration of Ventilatory Support in Days

#### Increase in Absolute Neutrophil Count

There were nine studies that reported ANC; however, they did not report the means and standard deviations for change from the baseline counts (Ahmad 2002, Aktas 2013, Borjanyazdi 2013,



Chaudhuri 2012, El-Ganzoury 2012, Gathwala 2011, Miura 2001, Schibler 1998, Sezer 2002). To derive this data from the results, the following formula from the Cochrane Handbook for Systematic Reviews of Interventions was used:  $SDE\text{-}change = \sqrt{[SDE\text{-}baseline2 + SDE\text{-}final2 - (2 \times 0.5 \times SDE\text{-}baseline \times SDE\text{-}final)]^{14}}$ . One study reported the medians and range of values in their results (Miura 2001). The authors were contacted to retrieve the values of the means and standard deviations; however, the investigators did not receive a reply. Instead, the formulas developed by Hoze et al. were used to derive the necessary data<sup>15</sup>. Sensitivity analysis was done which showed that the imputation of missing data did not have a significant effect on the final analysis.

In the analyzed studies, ANC was usually measured during the 1st, 2nd and 3rd day of treatment. There was an increase in ANC for the G-CSF treated group on the first three days of treatment: 1st day [SMD 0.99 (0.70, 1.28)], 2nd day [SMD 1.11 (0.74, 1.49)], 3rd day [SMD 2.05 (1.71, 2.38)]. There were different treatment periods between the studies ranging from 3-14 days with the most common treatment period lasting 3 days. There was an increase in ANC in the G-CSF group after treatment regardless of duration [SMD 0.73 (0.52, 0.95)]. There was a smaller effect for those who already had baseline neutropenia [SMD 0.71 (0.46, 0.95)]. However, the studies showed significant heterogeneity with P-values < 0.10 (0.003, 0.003, < 0.00001, < 0.00001) and I2 values > 50% (75%, 78%, 91%, 89%) (Figure 9).

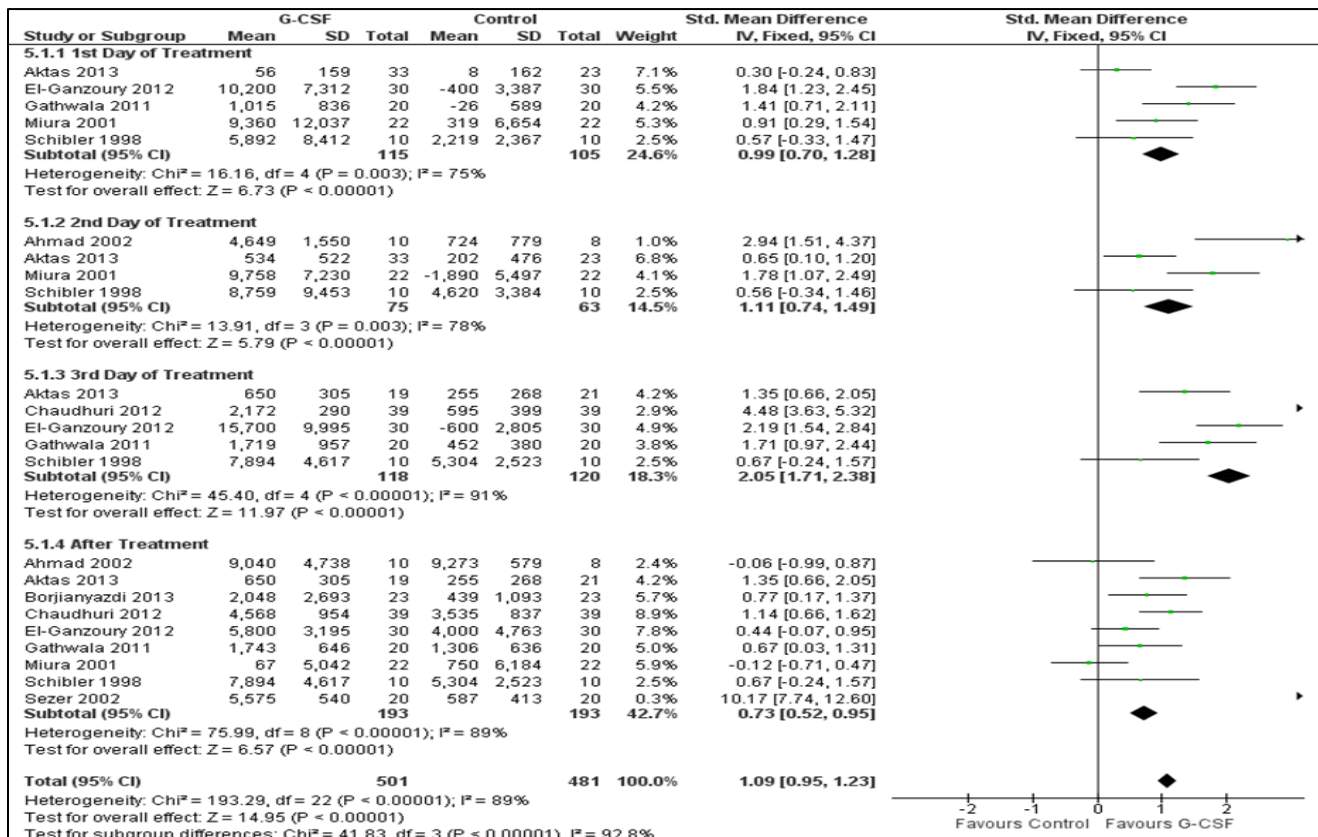


Figure 9. Forest Plot: Increase in Absolute Neutrophil Count

### Increase in Leukocyte Count

Three studies reported leukocyte counts of the participants but did not report the means and standard deviations for change from the baseline counts (El-Ganzoury 2012, Gathwala 2011, Miura 2001). The same imputation methods mentioned above were used to derive the necessary data. The studies show that there was a significant increase in leukocyte counts in the treatment group [SMD 0.41 (0.08, 0.74)] (Figure 10).

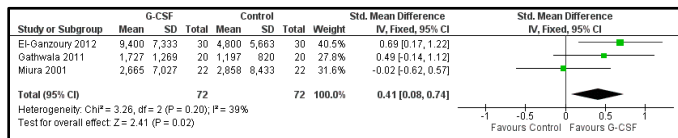


Figure 10. Forest Plot: Increase in Leukocyte Count

### Decrease in Immature:Total Neutrophil Ratio

There were three studies that reported I:T ratios of the participants (Aktas 2013, El-Ganzoury 2012, Sezer 2002). However, they did not include the means and standard deviations for change from the baseline ratios. The same formula mentioned above was used to derive the necessary data. The studies show that there is no significant difference in I:T ratios between the treatment group and the control group [SMD 0.23 (-0.10, 0.57)] (Figure 11).

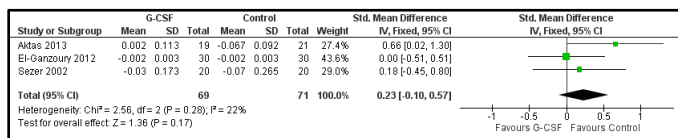


Figure 11. Forest Plot: Decrease in Immature: Total Neutrophil Ratio

### Adverse Effects Caused by G-CSF

Twelve studies mentioned in their methods that they monitored for toxicity or adverse effects caused by G-CSF administration (Ahmad 2002, Aktas 2013, Bedford Russell 2001, Borjanyazdi 2013, Chaudhuri 2012, Drossou-Agakidou 1998, Drossou-Agakidou 2002, El-Ganzoury 2012, Gillan 1994, Miura 2001, Schibler 1998, Sezer 2002). The drug was found to be well tolerated by the participants in the treatment group as no toxicity or adverse effects were noted. However, there was no data regarding

long-term adverse effects in any of the included studies.

### DISCUSSION

The purpose of this meta-analysis was to integrate and examine the data collected from published reports on the effect of G-CSF on the outcome of neonatal sepsis. There were 13 randomized control trials included with a total of 530 neonates. The studies reviewed had relatively small population sizes with the largest having only 78 participants (Chaudhuri 2012). This greatly affected the quality of data as 9 out of the 17 outcomes analyzed only had low to very low quality of evidence partly due to small population size.

The studies had participants with different ranges of age of gestation, birthweight, severity of neutropenia, as well as severity of sepsis. The age of gestation of the neonates ranged from 24-40 weeks while their birth weights ranged from 530-3667g. The definition of neutropenia also varied greatly between studies. The inclusion criteria used in the studies usually had ANC < 1000 cells/μL, < 1500 cells/μL or < 5000 cells/μL while one study had an inclusion criterion with ANC < 20000 cells/μL (Drossou-Agakidou 2002). Only three studies used the Score for Neonatal Acute Physiology (SNAP) in order to score the severity of sepsis (El-Ganzoury 2012, Miura 2001, Schibler 1998). The dose, frequency and duration of G-CSF administration were also different between studies and some compared these in their results. The most common dose across the studies was 10 μg/kg/day given either q12h or OD for 3-5 days. These may be significant confounding factors to the final analysis.

There was a significant reduction in all-cause mortality. There was a greater benefit for participants who were preterm and had low birth weight as compared to those who had baseline neutropenia. This is contrary to the conclusion of the previous meta-analyses<sup>10,14</sup>. This may be because some of the excluded studies from the previous meta-analyses showed a greater reduction

in mortality in neutropenic infants. Another likely source of bias would be the different severity of neutropenia between the studies as mentioned above.

There was no significant reduction in sepsis-related morbidities. For bronchopulmonary dysplasia and necrotizing enterocolitis, this may be due to the inflammatory role of neutrophils in the pathogenesis of these diseases themselves<sup>16,17</sup>. It is notable that the data collected for morbidity had low quality of evidence due to unclear risk for selection bias, sparse data and lack of agreement between studies.

There was a reduction in the duration of hospital stay and the duration of ventilatory support; however, the studies analyzed showed heterogeneous results. A possible confounding factor may be different nosocomial infection rates in different hospitals that may prolong hospitalization as well as ventilatory support.

As was expected, there was an increase in the ANC for the treatment group from the first three days of G-CSF administration up to the end of the studies. A majority of the studies analyzed showed results favoring the administration of G-CSF; yet, there was a smaller increase in those who had baseline neutropenia. This may have a significant correlation with the minimal effect of G-CSF on the mortality rate of neutropenic patients. However, the studies had significant statistical heterogeneity, hence there is only low to very low-quality evidence supporting this. One possible cause for this heterogeneity may be the different baseline ANC of the participants in the different studies. Another would be the different treatment durations in the studies analyzed.

There was a significant increase in total leukocyte count that could be attributed to the increase in neutrophil production. As for the I:T ratio, there was no significant difference between the groups.

No toxicity or adverse effects attributed to G-CSF administration were reported in any of the

studies included in the review. However, there were no studies which followed through long enough to report long term effects of G-CSF.

## CONCLUSIONS

Current evidence shows that administering G-CSF to septic neonates could possibly reduce mortality rates. Preterms and low birthweight newborns are shown to be the populations that could benefit the most from this treatment. However, there was minimal improvement of outcomes for septic neonates with concomitant neutropenia. Nevertheless, treatment for this population warrants further study.

Further investigation on the effects of G-CSF on septic neonates should be done with standardized protocols and larger populations. Future trials should have a consistent set of inclusion criteria with the same definition of terms (i.e. neutropenia) across all studies. Stratification of patients according to gestational age, birth weight, baseline ANC and severity of sepsis may help pinpoint the populations that will most likely benefit from the treatment. The effects of G-CSF on culture-positive sepsis may also be compared to its effects on suspected sepsis. Different doses, frequencies and treatment durations may also be compared in future trials. Sepsis-related morbidities should be correlated with the ANC of the patient at the time of onset. This could determine the effect of increase in ANC on morbidities linked to neutrophilia (i.e. bronchopulmonary dysplasia, necrotizing enterocolitis). A more extensive follow-up should be done to elicit possible long-term adverse effects of the drug.

## REFERENCES

- 1 Liu L, Oza S, Hogan D, Perin J, Rudan I, Lawn JE et al. Global, regional, and national causes of child mortality in 2000-13, with projections to inform post-2015 priorities: an updated systematic analysis. *Lancet* 385:430-440, 2015.
- 2 Jenster, Meike, Bonifacio, Ruel, Rogers, Tam, Partridge, Barkovich, Ferriero, and Glass. "Maternal or neonatal infection: association with neonatal

- encephalopathy outcomes." *Pediatric Research* 76:1 93-99, 2014.
- 3 Lahra MM, Beeby PJ, and Jeffery HE. "Intrauterine Inflammation, Neonatal Sepsis, and Chronic Lung Disease: A 13-Year Hospital Cohort Study." *Pediatrics* 123:5 1314-319, 2009.
  - 4 "Neonatal sepsis - a major killer to be tackled in communities." WHO. World Health Organization [Internet]. 19 January 2009 [cited 2017 June] Available from [http://www.who.int/maternal\\_child\\_adolescent/news\\_events/news/2009/19\\_01/en/](http://www.who.int/maternal_child_adolescent/news_events/news/2009/19_01/en/)
  - 5 Rodwell, Robyn ., Kerry Mcd. Taylor, Tudehope DI, and Gray PH. "Hematologic scoring system in early diagnosis of sepsis in neutropenic newborns." *The Pediatric Infectious Disease Journal* 12:5 372-76, 1993.
  - 6 Carr, Robert. "Low soluble FcRIII receptor demonstrates reduced neutrophil reserves in preterm neonates." *Archives of Disease in Childhood - Fetal and Neonatal Edition* 83:2 F160-F164, 2000.
  - 7 Shah, Arpitajigar, Summaiya Mulla, and Sangitab Revdiwala. "Neonatal sepsis: High antibiotic resistance of the bacterial pathogens in a neonatal intensive care unit of a tertiary Care hospital." *Journal of Clinical Neonatology* 1:2 72-75, 2012.
  - 8 Krishnankutty, Binny, Advani, Achreckar S, and Thomas D. "Granulocyte colony-stimulating factor (filgrastim) in chemotherapy-induced febrile neutropenia." *Indian Journal of Medical and Paediatric Oncology* 31:3 125-128, 2010.
  - 9 Gunduz, Eren, and Zafer Gulbas. "Supportive Care In Hemato-Oncology: Reviewing In The Light Of Latest Guidelines." *Turkish Journal of Hematology* 29:1 1-9, 2012.
  - 10 Carr, Modi, and Doré. "G-CSF and GM-CSF for treating or preventing neonatal infections." *Cochrane Database of Systematic Reviews* 3:CD003066 1-25, 2003.
  - 11 Bernstein, Hilton M., et al. "Administration of recombinant granulocyte colony-stimulating factor to neonates with septicemia: A meta-Analysis." *The Journal of Pediatrics* 138:6 917-920, 2001.
  - 12 Effective Practice and Organisation of Care (EPOC). Data collection form. EPOC Resources for review authors. Oslo: Norwegian Knowledge Centre for the Health Services; 2013. pp. 1-11
  - 13 Review Manager (RevMan) [Computer Program]. Version 5.3. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014.
  - 14 Higgins, Julian P.T., and Sally Green. *Cochrane handbook for systematic reviews of interventions*. Chisester: Wiley-Blackwell; 2017. p. 488
  - 15 Hozo, Stela Pudar, et al. "Estimating the mean and variance from the median, range, and the size of a sample." *BMC Medical Research Methodology, BioMed Central* 5:13 1-10, 2005.
  - 16 Speer, CP. "Pulmonary inflammation and bronchopulmonary dysplasia." *Journal of Perinatology* 26:1 S57-S62, 2006.
  - 17 Precioso, Alexander, Roberto, and Renata Suman Mascaretti Proença. "Necrotizing enterocolitis, pathogenesis and the protector effect of prenatal corticosteroids." *Revista do Hospital das Clínicas* 57:5 243-248, 2002.
- Included Studies
- Ahmad 2002
- Ahmad A, Laborada G, Bussel J, and Nesin M. Comparison of recombinant granulocyte colony-stimulating factor, recombinant human granulocyte-macrophage colony-stimulating factor and placebo for treatment of septic preterm infants. *The Pediatric Infectious Disease Journal* 21:11 1061-1065, 2002.
- Aktas 2013
- Aktas D, Demirel B, Gursoy T, and Ovali F. A Randomized Case-Controlled Study of Recombinant Human Granulocyte Colony Stimulating Factor for the Treatment of Sepsis in Preterm Neutropenic Infants. *Pediatrics & Neonatology* 56:3 171-175, 2014.
- Bedford Russell 2001
- Bedford Russell AR, Emmerson AJB, Wilkinson N, Chant T, Sweet DG, Halliday HL, Holland B, and Davies EG. A trial of recombinant human granulocyte colony stimulating factor for the treatment of very low birthweight infants with presumed sepsis and neutropenia. *Archives of Disease in Childhood. Fetal and Neonatal Edition* 84:3 F172-F176, 2000.
- Borjanyazdi 2013
- Borjanyazdi L, Froomandi M, Noori Shadkam M, Hashemi A, and Fallah R. The Effect of Granulocyte Colony Stimulating Factor Administration on Preterm Infant with Neutropenia and Clinical Sepsis: A Randomized Clinical Trial. *Iranian Journal of Pediatric Hematology Oncology* 3:2 64-68, 2013.
- Chaudhuri 2012
- Chaudhuri J, Mitra S, Mukhopadhyay D, Chakraborty S, and Chatterjee S. Granulocyte Colony-stimulating Factor for Preterms with Sepsis and Neutropenia: A Randomized Controlled Trial. *Journal of Clinical Neonatology* 1:4 202-206, 2012.
- Drossou-Agakidou 1998
- Drossou-Agakidou V, Kanakoudi-Tsakalidou V, Sarafidis K, Taparkou A, Tzimouli V, Tsandali H, and

Kremenopoulos G. Administration of recombinant human granulocyte colony stimulating factor to septic neonates induces neutrophilia and enhances the neutrophil respiratory burst and  $\beta$ 2 integrin expression. *European Journal of Pediatrics* 157:7 583-588, 1998.

Drossou-Agakidou 2002

Drossou-Agakidou V, Kanakoudi-Tsakalidou F, Sarafidis K, Tzimouli V, Taparkou A, Kremenopoulos G, and Germenis A. In Vivo Effect of rhGM-CSF and rhG-CSF on Monocyte HLA-DR Expression of Septic Neonates. *Cytokine* 18:5 260-265, 2002.

El-Ganzoury 2012

El-Ganzoury MM, El-Farrash RA, Saad AA, Mohamed AG, and El-Sherbini IG. In Vivo Effect of Recombinant Human Granulocyte Colony-Stimulating Factor on Neutrophilic Expression of CD11b in Septic Neonates: A Randomized Controlled Trial. *Pediatric Hematology and Oncology* 29:3 272-284, 2012.

Gathwala 2011

Gathwala G, Walia M, Bala H, and Singh S. Recombinant Human Granulocyte Colony-Stimulating Factor in Preterm Neonates with Sepsis and Relative Neutropenia: A Randomized, Single-Blind, Non-Placebo-Controlled Trial. *Journal of Tropical Pediatrics* 58:1 12-18, 2011.

Gillan 1994

Gillan ER, Christensen RD, Suen Y, Ellis R, van de Ven C, and Cairo MS. A Randomized, Placebo-Controlled Trial of Recombinant Human Granulocyte Colony-Stimulating Factor Administration in Newborn Infants with Presumed Sepsis: Significant Induction of Peripheral and Bone Marrow Neutrophilia. *Blood* 84:51427-1433, 1994.

Miura 2001

Miura E, Procianoy RS, Bittar C, Miura CS, Miura MS, Mello C, and Christensen RD. A Randomized, Double-Masked, Placebo-Controlled Trial of Recombinant Granulocyte Colony-Stimulating Factor Administration to Preterm Infants With the Clinical Diagnosis of Early-Onset Sepsis. *Pediatrics* 107:1 30-35, 2001.

Schibler 1998

Schibler KR, Osborne KA, Leung LY, Le TV, Baker SI, and Thompson DD. A Randomized, Placebo-Controlled Trial of Granulocyte Colony-stimulating Factor Administration to Newborn Infants with Neutropenia and Clinical Signs of Early-onset Sepsis. *Pediatrics* 102:1 6-13, 1998.

Sezer 2002

Sezer T, Yildiran A, Albayrak D, and Küçüködük S. Randomized, Double-blinded, Placebo controlled Trial of Early Administration of Recombinant Human Granulocyte Colony stimulating Factor to Non-

Neutropenic Preterm Newborns Between 33 and 36 Weeks with Presumed Sepsis. *Scandinavian Journal of Infectious Diseases* 34:12 893-897, 2002.

Excluded Studies

Barak 1997

Barak Y, Leibovitz E, Mogilner B, Juster-Reicher A, Amitay M, Ballin A, Koren A, and Goebel M. The in vivo effect of recombinant human granulocyte-colony stimulating factor in neutropenic neonates with sepsis. *European Journal of Pediatrics* 156:643-646, 1997.

Bilgin 2001

Bilgin K, Yaramisi A, Haspolat K, Ali Tas MD, Gunbey S, and Derman O. A Randomized Trial of Granulocyte-Macrophage Colony-Stimulating Factor in Neonates With Sepsis and Neutropenia. *Pediatrics* 107:1 36-41, 2000.

Cairo 1995

Cairo MS, Christensen R, Sender LS, Ellis R, Rosenthal J, van de Ven C, Worcester C, and Agosti JM. Results of a Phase I/II Trial of Recombinant Human Granulocyte-Macrophage Colony-Stimulating Factor in Very Low Birthweight Neonates: Significant Induction of Circulatory Neutrophils, Monocytes, Platelets, and Bone Marrow Neutrophils. *Blood* 86:7 2509-2515, 1995.

Cairo 1999

Cairo MS, Agosti J, Ellis R, Laver J, Puppala B, deLemos R, Givner L, Nesin M, Wheeler JG, Seth T, van de Ven C, and Fanaroff A. A randomized, double-blind, placebo-controlled trial of prophylactic recombinant human granulocyte-macrophage colony-stimulating factor to reduce nosocomial infections in very low birth weight neonates. *The Journal of Pediatrics* 134:1 64-70, 1999.

Carr 1999

Carr R, Modi N, Doré CJ, El-Rifai R, and Lindo D. A Randomized, Controlled Trial of Prophylactic Granulocyte-Macrophage Colony-Stimulating Factor in Human Newborns Less Than 32 Weeks Gestation. *Pediatrics* 103:4 796-802, 1999.

Carr 2009

Carr R, Brocklehurst R, Doré CJ, and Modi N. Granulocyte-macrophage colony stimulating factor administered as prophylaxis for reduction of sepsis in extremely preterm, small for gestational age neonates (the PROGRAMS trial): a single-blind, multicentre, randomised controlled trial. *Lancet* 373:226-233, 2009.

Kocherlakota 1997

Kocherlakota P and La Gamma EF. Human Granulocyte Colony-stimulating Factor May Improve Outcome Attributable to Neonatal Sepsis Complicated by Neutropenia. *Pediatrics* 100:1 1-6, 1997.



**Marlow 2015**

Marlow N, Morris T, Brocklehurst P, Carr R, Cowan F, Patel N, Petrou S, Redshaw M, Modi N, and Doré C. A randomised trial of granulocyte-macrophage colony-stimulating factor for neonatal sepsis: childhood outcomes at 5 years. *Archives of Disease in Childhood: Fetal and Neonatal Edition* 100:4 F320-F326, 2015.

**Nayeri 2011**

Nayeri F, Soheili H, Kaveh M, Yazdi ZO, Shariat M, and Dalili H. Comparison of Two Regimens of RhG-CSF in Neutropenic Neonatal Septicemia: A Randomized Clinical Trial. *Acta Medica Iranica* 49:9 575-578, 2011.