Systemic Hydrocortisone To Prevent Bronchopulmonary Dysplasia in preterm infants (the SToP-BPD study): statistical analysis plan

Trial registration number: NTR2768, The Netherlands National Trial Register, registered on 17th February 2011, http://www.trialregister.nl/trialreg/admin/rctview.asp?TC=2768.

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Introduction SAP

The Systemic Hydrocortisone To Prevent Bronchopulmonary Dysplasia in preterm infants (the SToP-BPD) study is a multicenter, phase III, placebo-controlled, double-blind superiority randomized controlled trial (RCT) investigating the efficacy and safety of systemic hydrocortisone (HC) initiated between 7 and 14 days in reducing the incidence of the composite outcome of death or BPD at 36 weeks' postmenstrual age (PMA) after birth in mechanically ventilated preterm infants, as compared to a placebo (primary endpoint). The study protocol has been published previously.[1] This document describes the statistical analysis plan (SAP) in detail. This SAP is written without knowledge of the unblinded data.

Study protocol approval and registration

The SToP-BPD study is registered at

- the Netherlands Trial Register (NTR2768): date of registration February 17th 2011
- the European Clinical Trial Register (EudraCT number 2010-023777-19): date of registration November 2nd 2010.

The Ethics Committee of the Academic Medical Center in Amsterdam (The Netherlands), approved the study protocol on January 28th 2011. The local Ethics Committee of each participating hospital approved the local feasibility of the study protocol. Two amendments regarding changes of the inclusion criteria were approved. During the first months of the trial, the participating centers experienced that the arbitrarily chosen cutoff of 3.5 was too high. The centers noted that many infants ventilated between 7-14 days PNA with a respiratory index (RI) less than 3.5 were still at a high risk of developing BPD as indicated by the fact that most of these infants were treated with corticosteroids outside the trial. Therefore, the RI threshold in the inclusion criteria was reduced stepwise, first to 3.0 (May 21st 2012), and

finally to 2.5 (December 3rd 2012). Both RI adjustments were submitted as protocol amendments to the accredited ethics committee and approved.

All study sites were monitored by an independent clinical research associate of the AMC Clinical Research Unit. An independent data safety monitoring board (DSMB) monitored the study progress, with a special focus on safety.

Primary outcome

The primary outcome is the dichotomous composite outcome of death or BPD at 36 weeks PMA (BPD-free survival). Patients were categorized as having BPD when needing supplemental oxygen and/or positive pressure support at 36 weeks PMA, assessed according to the National Institutes for Child Health and Human Development (NICHD) Consensus Statement using the classification of severity as proposed by Jobe et.al.[2] and, if indicated, the oxygen reduction test as described by Walsh et al.[3, 4]

Secondary outcomes

Study phase I: Short term outcomes before initial hospital discharge

Secondary efficacy outcomes assessed during the study phase before initial hospital discharge include mortality (at 28 days PNA, 36 weeks PMA, and hospital discharge), BPD (at 28 days PNA and 36 weeks PMA, including the severity grade), failure to extubate (at 3, 7, 14, and 21 days after initiating study medication), total duration of mechanical ventilation, total time on supplemental oxygen, use of open label HC treatment, length of hospital stay, necrotising enterocolitis (NEC) with Bell stage II or more[5], gastrointestinal bleeding, spontaneous intestinal perforation (SIP), intraventricular haemorrhage (IVH) and/or periventricular leucomalacia (PVL)[6], retinopathy of prematurity (ROP)[7], hypertension, hyperglycemia requiring the use of insulin therapy, nosocomial infection including clinical or culture proven

sepsis, meningitis and pneumonia, patent ductus arteriosus for which medical intervention or surgical ligation is needed, and growth (weight, head circumference, and length at 36 weeks PMA).

Study phase II: Long-term follow-up outcomes at 2 years CA

Neurodevelopmental impairment at 2 years CA will be assessed according to the cognitive and motor composite scores of the Bayley III Scales of Infant and Toddler Development (BSID).[8] Norm value of BSID scores is 100 (SD 15), with higher values indicating better function. Other secondary outcomes are: mortality, number of hospital readmissions since first discharge home, cerebral palsy and its severity assessed by the gross motor function classification system[9], hearing loss requiring hearing aids, blindness, behavioral problems (Child Behavior Checklist[10]), weight, length and head circumference.

Safety

Safety outcomes were classified as:

- Complications of HC treatment (see short term secondary outcomes);
- Suspected unexpected serious adverse drug reactions (SUSARs);
- Other (serious) adverse events (SAEs).

Statistical analysis plan

Overall principles

The first phase of data-analysis and reporting will include all outcome data up to discharge home. Analysis will start once all data to discharge of the last included patient have been obtained, the database has been cleaned and locked, and the SAP has been submitted for publication. The analyses will be performed by a statistician and methodologist, neither of

whom are involved in the assessment of trial outcomes.

Analyses will be performed according to the intention to treat principle. Given the modulating effects of open label corticosteroids [11], analyses will also be done in an as treated, and a per protocol population (see below) to check the robustness of the main analyses. For all relevant parameters, 95% confidence intervals (CI) will be presented. P-values less than 0.05 will be regarded as statistically significant. For statistical programming and analysis, we will use the statistical package IBM SPSS statistics version 24 (IBM Corp., Armonk. NY, USA) and the R environment for statistical computing (R Foundation for Statistical Computing, Vienna, Austria).

The statistician and methodologist will perform the analysis blinded for the allocated study treatment and using a fictive randomization code. After the data analyses have been completed, they will be repeated using the true treatment allocation. These analyses will be performed and published before the assessment of long-term outcomes at 2 years CA of all included patients have been completed. Like other trials in neonatology, it is deemed unethical to withhold the results of the primary outcome analyses from the international community for another two years.

This first phase of data analysis and reporting will be followed by a second phase, analyzing and reporting the long-term neurodevelopmental outcomes after two years. Clinicians involved in care during the initial hospitalization, outcome assessors of the neurodevelopmental outcome at two years CA, and parents will be kept blinded to the treatment allocation of all included patients until this second report has been published.

Handling of missing data

In case of missing data, every attempt will be undertaken to retrieve the data. We will contact

both level III and II (referral) hospitals because many infants will be transferred back to referral hospitals once clinically stable.[12, 13] Since the primary outcome will be assessed before hospital discharge, we anticipate no or minimal missing values. In case an oxygen reduction test is indicated but has not been performed, a committee of three independent experts will assess the severity of the BPD diagnosis. These experts will kept blinded to the allocation arm during this assessment. Due to frequent standard care follow up visits and strong relationships between physicians and the families of the preterm infants, we anticipate little missing follow up data. All lost to follow-up cases will be recorded, including their available key characteristics and trial results and reason for the loss to follow up. Missing data will not be imputed with the exception of the long term neurodevelopmental outcomes (see Long-term outcomes at 2 years corrected age (Study phase II)).

Definition of analysis sets

Intention-to-treat (ITT) population

The ITT population includes all randomized infants irrespective of protocol deviations (see below) or use of open label corticosteroids (Table 1). This includes patients with a signed informed consent for the study, who were randomized but died before receiving the first dose of study medication.

Table 1: Definition of population analysis sets

Analysis population	HC treatment group	Placebo treatment group
Intention to treat 'as randomised'	*HC randomisation*: • including all protocol deviations (e.g. in eligibility criteria, cotreatment)	*Placebo randomisation*: • including all protocol deviations (e.g. in eligibility criteria, cotreatment)
As treated 'actual treatment'	*Patient received HC (at least 1 dose), irrespectively of allocated treatment at randomisation:* • including those infants who received rescue open label corticosteroids as described according to protocol • including those infants who received rescue open label corticosteroids not following protocol (protocol deviation) • including those infants with other protocol deviations (e.g. in eligibility criteria, cotreatment)	*Patient received placebo (at least one dose), irrespectively of allocated treatment at randomisation:* • including those infants not receiving any corticosteroid dose for pulmonary reasons • excluding those infants who received rescue open label corticosteroids following study protocol • excluding those infants who received rescue open label corticosteroids not following study protocol • including those infants with other protocol deviations (e.g. in eligibility criteria, cotreatment)
Per protocol	*HC randomisation and HC treated according to study protocol*: • including those infants receiving rescue open label corticosteroids as described by the study protocol • excluding those infants receiving rescue open label corticosteroids not according to study protocol (protocol deviation) • excluding other protocol deviations (e.g. in eligibility criteria, cotreatment)	*Placebo randomisation and placebo treated according to study protocol*: • including those infants receiving rescue open label corticosteroids following study protocol • excluding those infants receiving rescue open label corticosteroids not following study protocol (protocol deviation) • excluding other protocol deviations (e.g. In eligibility criteria, cotreatment)

HC: hydrocortisone.

As treated (AT) population.

Infants will be analysed in groups according to the actual treatment received, i.e. yes or no corticosteroids, irrespective of allocated treatment at randomisation (Table 1). Infants will still

be included in the AT population if there was a protocol deviation (see below).

Per protocol (PP) population

In the PP population, all patients included and treated in accordance with the study protocol will be included (Table 1). Patients allocated to the placebo group, but treated with open label corticosteroid treatment in compliance to the study protocol, will be included in the PP placebo group. Infants of both treatment groups treated with open label corticosteroids outside the study protocol or in whom other protocol deviations (see below) occurred, will be excluded from the PP population.

Statistical analyses

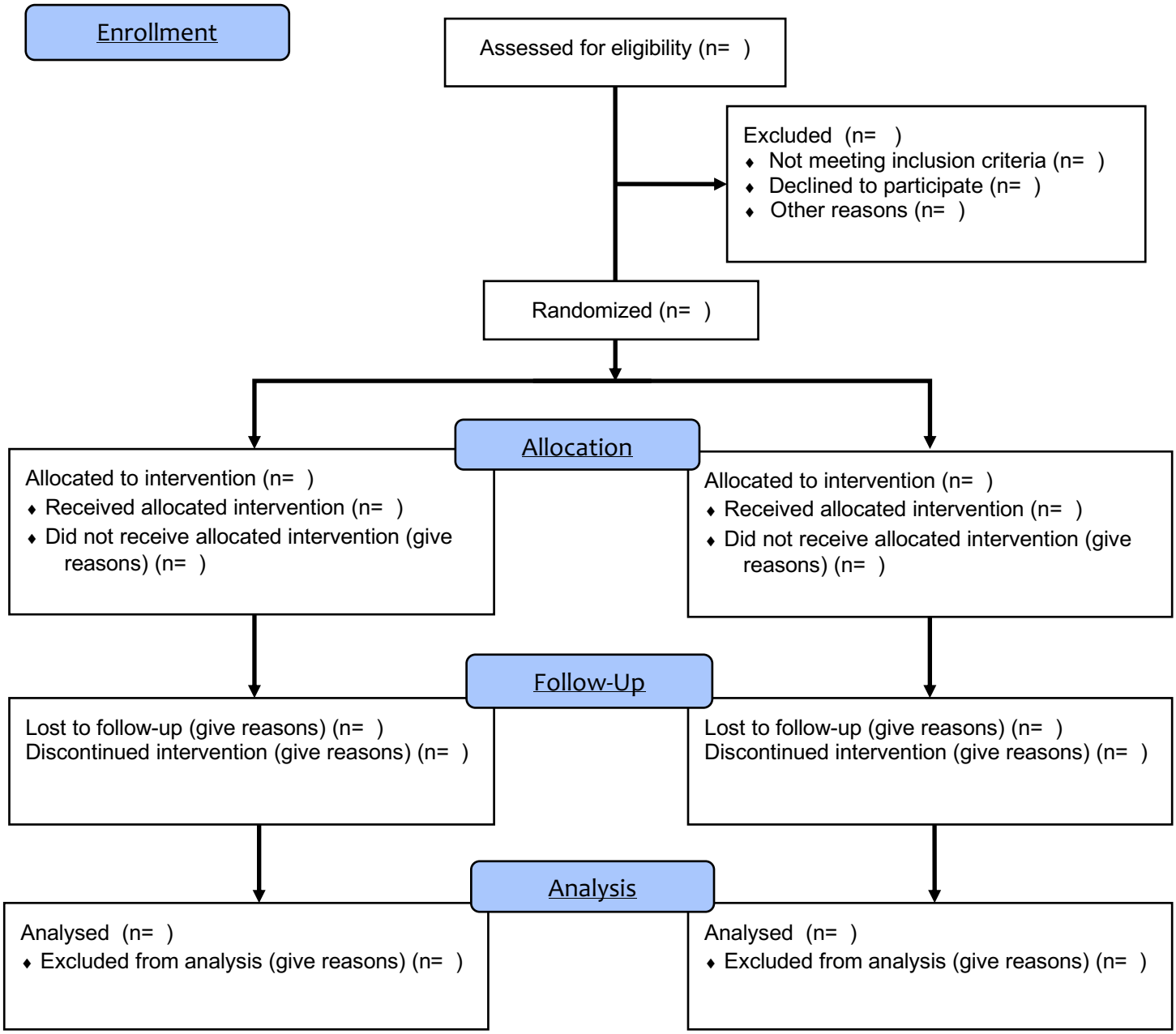
Patient flow

The flow of participants will be displayed in the Consolidated Standards of Reporting Trials (CONSORT) Flow diagram (Figure 1). Reasons that eligible patients were not included, such as parents declined consent or the medical team did not ask the parents, will be summarized.

Figure 1.

CONSORT
TRANSPARENT REPORTING of TRIALS

CONSORT 2010 Flow Diagram



Protocol deviations

Protocol deviations are defined as deviations in eligibility criteria, open label corticosteroid treatment not conform conditions set in the study protocol, such as deblinding. All protocol deviations will be line-listed.

Baseline characteristics

We will present the following maternal characteristics: age; ethnic origin; highest completed education; mode of delivery; antenatal corticosteroids; chorioamnionitis; pre-eclampsia; and multiple pregnancy. We will also present the following baseline characteristics of patients: gestational age; gender; birth weight; small for gestational age; Apgar score at 5 minutes; surfactant therapy; pulmonary air leak syndrome; patent ductus arteriosus; clinical and culture proven sepsis before randomization; inhalation of nitric oxide and hypotension, NEC, SIP and/or IVH before randomization; age at randomization; fraction of inspired oxygen, mean airway pressure at start study medication. All variables will be presented as summary statistics according to allocation arm of the trial.

Continuous, normally distributed variables will be summarized using the mean and standard deviation. Continuous, non-normally distributed variables will be summarized using the median and interquartile range. Categorical variables will be summarized using counts and percentages. No formal testing will be performed on the baseline characteristics.

Short -term outcomes until initial hospital discharge (Study phase I)

Primary outcome

In Table 2 an overview is given of all study outcomes with their planned analysis methods. Crude estimates of the relative risk and absolute risk reduction of the primary outcome in the HC group compared to the placebo group will be calculated. The main analysis of the effect

of HC on the primary outcome will be performed using a logistic regression model correcting for the stratification factors gestational age (less than or greater and equal than 27 weeks) and centre. Adjusting for stratification by centre can lead to problems with the convergence or stability of estimates of statistical parameters if some or all centers are small.[14] To ensure that the estimates of the regression parameters converge, hospitals with fewer than 10 subjects without and/or fewer than 10 subjects with the composite endpoint BPD free survival at 36 weeks PMA will be combined in this analysis [15]. The effects sizes from this model will be expressed as an adjusted odds ratio.

Table 2. The variables/outcomes to be presented and planned statistical analysis methods.

Variable/outcome	Type of outcome	Statistical analysis
Study phase I - Short -term outcomes until initial hospital discharge		
BPD free survival at 36 weeks PMA	Primary	Logistic regression with correction for stratification factors plus sensitivity and subgroup analyses
Death at 28 days PNA, at 36 weeks PMA and hospital discharge BPD at 36 weeks PMA Failure to extubate 3, 7, 14 and 21 days after start trial medication Time to extubation, censored at death Time to supplemental oxygen independence Time to hospital discharge Necrotising enterocolitis Gastrointestinal bleeding Spontaneous intestinal perforation Intraventricular haemorrhage and/or periventricular leucomalacia Retinopathy of prematurity Hypertension Hyperglycemia requiring insulin therapy Nosocomial infection including clinical or culture proven sepsis, meningitis, pneumonia Patent ductus arteriosus needing medical intervention or surgical ligation Weight, head circumference, length at 36 weeks PMA Use of open label HC treatment	Short term secondary	Linear, logistic or Cox regression or competing risk model, as appropriate
SUSARs, SAEs	Short term secondary	Descriptive statistics
Study phase II - Long-term follow up at 2 years CA		

Survival without neurodevelopmental impairment	Key long term secondary	Logistic regression with correction for stratification factors, sensitivity and subgroup analyses
Mortality Number of hospital readmissions since first discharge home Cerebral palsy and its severity Hearing loss requiring hearing aids Blindness Behavioral problems (Child behavior Checklist) Growth (weight, length, head circumference)	Long term secondary	Linear, logistic or Cox regression or competing risk model, as appropriate

In an additional analysis, the effect of HC on the primary outcome will be evaluated using a multivariate logistic regression model including five important biologically plausible baseline risk factors for BPD, preselected based on the literature and our clinical experience: (a) gestational age (less than or greater and equal than 27 weeks) [16]; (b) the presence of chorioamnionitis[17]; (c) respiratory index at randomization[18]; (d) gender[19]; (e) multiple pregnancy[20].

Subgroup analyses

We will perform pre-specified exploratory subgroup analyses for the primary outcome by examining treatment-subgroup interaction effects in logistic regression models. We will perform six analyses each examining one subgroup: gestational age groups (less than or greater and equal than 27 weeks); presence of chorioamnionitis (yes/no), respiratory index at randomization, gender (male/female), multiple pregnancy (yes/no), and center preference for type of steroid treatment prior to the study ('HC' versus 'dexamethasone'). Each of these analyses will require four parameters to be estimated in the logistic regression. If there are fewer than 40 patients with and/or fewer than 40 patients without the composite endpoint BPD free survival at 36 weeks PMA, these analyses will not be performed.

Sensitivity analyses

The international accepted definition of the primary outcome BPD is under debate given the uncertainty on how to rate continuous positive airway pressure (CPAP) or high flow nasal cannula (HFNC) with more than 2 liter/min flow and with low or no supplemental oxygen.[21] According to the international criteria used, infants who need this respiratory support should be rated as having severe BPD.[16] However, the reason for this support in these very premature infants could be an impaired control of breathing instead of chronic parenchymal lung damage. Therefore, an auxiliary analysis will be performed in which infants on CPAP and HFNC in room air will be classified as having mild BPD.

If possible, we will perform sensitivity analyses on the primary outcome to investigate the impact of correlation between infant outcomes within twin or higher order multiple births using generalized estimating equations with a logit link function.

Short-term secondary outcomes

The effect of HC on short-term secondary outcomes will be analyzed using linear regression, logistic regression, competing risk models (that consider death before the event of interest as a competing risk) or Cox regression models, as appropriate. The time to event will be calculated as the time between randomization and the event, death, or discharge home, whichever occurs first. If required, non-normally distributed continuous variables will be appropriately transformed. Only a small number of SUSARs and SAEs are expected. Hence, these will be evaluated by tabulations of counts and percentages for each treatment group, respectively and presented as absolute risk differences. These outcomes, although all pre-specified, should be considered exploratory, yielding hypothesis-generating findings, and so no formal adjustments for stratification or multiple comparisons will be made.

Long-term outcomes at 2 years corrected age (Study phase II)

Neurodevelopmental impairment

The key long-term outcome is survival without neurodevelopmental impairment at 2-year CA. Participants, who are alive and have a BSID III cognitive and motor composite score at 2-year CA of 85 or greater will be defined as having survived without neurodevelopmental impairment. If no BSID test could be done because of impairment, the attending paediatrician was asked to fill in an estimate of cognitive delay in three categories: no delay; 3 to 6 months delay; more than 6 months delay. A delay of three or more months is considered neurodevelopmental impairment and equivalent to a BSID III cognitive and motor composite score of less than 85. Parents of participants, who do not attend the 2-year assessment at the outpatient clinic, will be invited by telephone once more. If they refuse to attend, the reason for refusal will be documented. Based on available information about their neurological and developmental (ab)normality, participants will be classified as having neurodevelopmental impairment or not, if reasonably possible. If there is insufficient information to classify the neurodevelopmental outcome in more than 5% of the participants, missing continuous BSID scores will be imputed using multiple imputation using baseline characteristics. As previously recommended [38], we will obtain a number of imputed data sets equal to the percentage of missing data. These datasets will be combined using Rubin's rules [22, 23]. Inspection and imputation of missing data will be performed during the blinded review of the data. This strategy will be updated if during the blinded data review unexpected patterns are detected, requiring an appropriately adapted handling procedure. In that case, relevant deviations will be clearly documented and justified. Statistical analysis will be performed in the same way as for the short term primary outcome. If multiple imputation is performed, three additional sensitivity analyses will be performed to test the robustness of the results obtained. These sensitivity analyses will be performed 1) using complete cases only and by applying 2) best

and 3) worst case scenarios for the unobserved neurodevelopmental impairment outcome data.

Other long-term secondary outcomes

The effect of HC on the other long-term secondary outcomes will be analyzed using linear, logistic or Cox regression or competing risks models, as appropriate. The time to event will be calculated as the time between randomization and the event, death, or 2-year CA, whichever occurs first. If required, non-normally distributed continuous variables will be appropriately transformed. These outcomes, although all pre-specified, should be considered exploratory, yielding hypothesis-generating findings, and so no formal adjustments for stratification or multiple comparisons will be made.

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