STATISTICAL ANALYSIS PLAN
REducing STEroids in Relapsing Nephrotic syndrome: the RESTERN study

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INTRODUCTION

Background and rationale

The first-line treatment for idiopathic nephrotic syndrome in children is oral corticosteroids.[1] Most children experience several relapses of nephrotic syndrome, needing repeated courses of corticosteroid therapy or even more aggressive treatment with other immunosuppressive drugs. For most children, long-term prognosis is for complete resolution of their disease over time with maintenance of normal kidney function. Therefore, it is vital to focus on minimizing adverse events of the disease and its treatment. The aim of the RESTERN study (REducing STEroids in Relapsing Nephrotic syndrome) is to assess the safety and effectiveness of a reduced alternate day steroid schedule for treatment of a nephrotic syndrome relapse in comparison with the current standard therapy.

Objectives

Primary objective

To study the effectiveness of a reduced corticosteroid schedule for the treatment of a relapse in children with steroid sensitive nephrotic syndrome.

Secondary objectives

- To study the influence of maintenance immunosuppressive therapy on the effectiveness of a reduced steroid schedule for the treatment of a relapse in children with steroid sensitive nephrotic syndrome. Maintenance immunosuppressive therapies include levamisole, cyclosporine, tacrolimus, mycophenolate mofetil and mycophenolate sodium, and alternate day prednisolone with a maximum of 4 mg/m^2;
- To investigate the occurrence of relapses, frequency of relapses and progression to steroid dependent and frequent relapsing nephrotic syndrome in children with nephrotic syndrome under the standard treatment regimen;
- To study the influence of maintenance immunosuppressive therapy on the occurrence of subsequent relapses, frequency of subsequent relapses and progression to steroid dependent and frequent relapsing nephrotic syndrome under the standard regimen, and;
- To study the effectiveness of a reduced steroid schedule for the treatment of a relapse and occurrence and frequency of subsequent relapses in children with steroid dependent nephrotic syndrome.

STUDY METHODS

Trial design

National, single center, double-blind, randomised, placebo controlled, noninferiority intervention study.

Randomisation

After prednisolone 60 mg/m^2 daily in 1 dose until complete remission for 3 days, randomisation (allocation ratio 1:1) between:

- Standard treatment: 6 weeks prednisolone 40 mg/m^2 (max. 40 mg) every other day;

- Study treatment: 2 weeks prednisolone 40 mg/m^2 (max. 40 mg) every other day, then 4 weeks placebo every other day.

Blinding
All researchers involved in the preparation of the analysis plan don't have access to trial data broken down by treatment allocation. Once data quality checks are satisfactory and the database is locked, a blind review will be undertaken to quantify missing data of the entire dataset and allow for any final amendments to the statistical analysis plan. During interim analysis and interpretation, group allocation will be masked using dummy group names (for example, group A, group B). The true group allocation will be unmasked only if necessary and after the database is locked. In addition, due to a difference of 4 weeks between the standard treatment and placebo group regarding timing of final prednisolone dose, time to first relapse and thereby all final statistical analyses can only be determined after unblinding. The randomisation list remains preserved by the hospital trial pharmacy and will not be accessible to the investigators until the end of the follow-up of the last patient. An unblinding procedure at the hospital pharmacy department will be available at all times.

Sample size
From previous studies, we know the incidence of nephrotic syndrome in Dutch children to be 1.52/100,000 children/year.[2] Eighty percent of the pediatric patients develop at least 1 relapse of whom 50% continue towards frequent relapsing nephrotic syndrome. Based on data from previous studies, average time to relapse in the first year is approximately 185 days (standard deviation of 120 days).[3] Using the power calculation for a noninferiority trial with a continuous primary outcome, a power of 80% and a noninferiority limit of 50 days, 72 patients per group are required. Using a Cox Proportional Hazard time-to-relapse analysis (survival analysis)[4], similar numbers can be calculated.

Timing of interim analysis and stopping guidance
Interim analysis will be performed by the Data Safety and Monitoring Board (DSMB), three months after inclusion of the first 40 participants. The study will be terminated in a premature stage if the safety of participants is jeopardized. As the placebo group only receives less active medication (in comparison with the standard treatment group), the occurrence of any serious adverse event is considered highly unlikely. The only risk may be found in the primary outcome of the study, i.e. significant inferiority of the placebo-treatment schedule with regards to time to next nephrotic syndrome relapse.

Timing of final analysis
Final analysis will take place after completion of the 24 months follow-up period of the last study subject.

STATISTICAL PRINCIPLES

Level of confidence intervals and p-values
For this noninferiority trial, the upper bound of the 2-sided 95% confidence interval for the treatment effect has to be below the margin to declare that noninferiority has been shown. P-values <0.05 will be considered statistically significant.

Adherence and protocol deviations
All substantial protocol violations will be listed. Adherence to study treatment (Table 1) will be defined as having consumed 100% of the study medicine, measured by self-report in the medication diary. This will be supported by the participant's returned medication weight/volume.

Table 1: Adherence to study treatment

	Standard treatment (*n* = xxx)	**Placebo (*n* = xxx)**
Self-reported daily dose (mg/m^2/day)		
Week 1		
Week 2		
Week 3		
Week 4		
Participants returning study medication	n/N (%)	n/N (%)
Participants consuming 100% prescribed dose		
Medication diary	n/N (%)	n/N (%)
Returned medication count	n/N (%)	n/N (%)

Analysis populations
The main analysis will consist of an intention-to-treat analysis. Participants who are lost to follow-up or in whom trial medication is stopped prematurely will be analyzed according to their allocated groups. In addition, as intention-to-treat analysis may increase the risk of type 1 errors in a non-inferiority trial, a per-protocol analysis will also be conducted.[5]

STUDY POPULATION

Screening data
Pediatric patients with steroid sensitive nephrotic syndrome are eligible for enrolment.

Eligibility
- Age over 1 and less than 18 years;
- Steroid sensitive nephrotic syndrome with at least 1 episode of nephrotic syndrome in the preceding 24 months.

This will include the following groups:
- Subjects without maintenance immunosuppressive therapy;
- Subjects with maintenance immunosuppressive therapy:
    - Long term immunosuppressive therapies: levamisole, ciclosporine, tacrolimus, mycophenolate mofetil (Cellcept®), mycophenolate sodium (Myfortic®), prednisolone max. 4 mg/m^2 on alternate days
    - Cyclophosphamide (oral of intravenous), at least three months post completion of therapy
    - A single dose or course of intravenous rituximab, at least three months post completion of therapy
- The last prednisolone use (at a dose over 10 mg/m^2 on alternate days) for the treatment of a previous episode was at least 4 weeks ago.

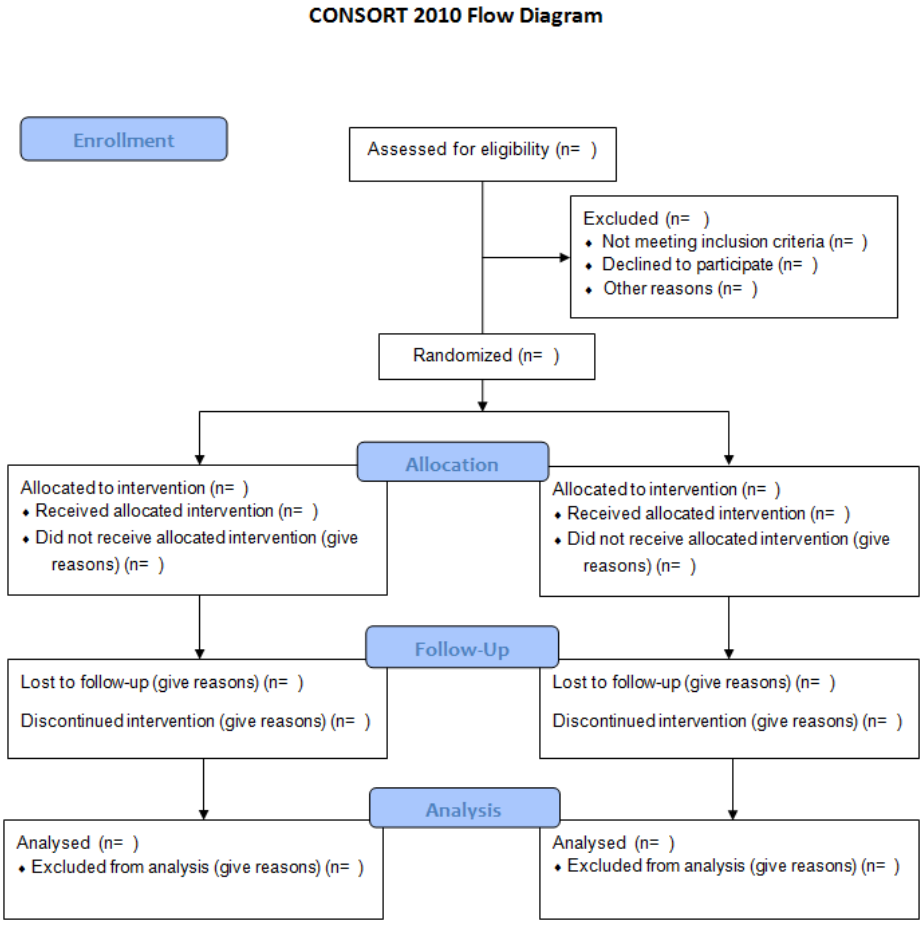
- Subjects experience a relapse nephrotic syndrome, defined as Albustix positive proteinuria (3+ or higher) for three consecutive days or the presence of generalized edema plus 3+ proteinuria on a single occasion.

Exclusion criteria
A potential subject who meets any of the following criteria will be excluded from participation in this study:

- Steroid resistant nephrotic syndrome;
- Receiving, or within 3 months after receiving, cyclophosphamide or rituximab;
- Daily prednisolone maintenance therapy at any dose;
- Alternate day prednisolone maintenance therapy at a dose over 4 mg/m^2;
- Documented or suspected significant non-compliance;
- Pregnancy;
- Stimulant drug use;
- Comorbidity:
    - Kidney transplant recipient
    - Any disease that requires the variation in oral prednisolone to be at the discretion of the treating physician(s)
- Concomitant use of moderate and strong CYP3A inducers;
- Concomitant use of moderate and strond CYP3A inhibitors, other than cyclosporine.

Recruitment
Subjects will be notified via their treating physician, via the patient association and/or the study website about the existence of the RESTERN study. Subjects will be recruited by the research team at the Radboudumc. The trial profile and inclusion will be shown in a Consolidated Standards of Reporting Trials (CONSORT) flow diagram (Figure 1), including the total number of randomised patients and per treatment group the numbers receiving allocated treatment, withdrawing consent, and lost to follow up.

Figure 1: CONSORT flow diagram



Withdrawal/follow-up

Patients randomised who did not take their allocated treatment will be considered as having deviated from the protocol. Their data will be included in both the intention-to-treat and per- protocol analyses. If a patient or their representative withdraws consent for data collection, only data up to the point of withdrawal will be used in the analysis.

Baseline patient characteristics

Discrete variables will be demonstrated by frequencies and percentages. Continuously distributed variables will be demonstrated using either mean ± standard deviation for data with normal distribution, or median and interquartile range for non-normally distributed data.

Table 2: Participant baseline characteristics

	Standard treatment (*n* = xxx)	**Placebo (*n* = xxx)**
Participant characteristics		
Male, n (%)	n/N (%)	n/N (%)
Age, years; median (IQR)	xx.x (xx.x to xx.x), n	xx.x (xx.x to xx.x), n
Age at onset, years; median (IQR)	xx.x (xx.x to xx.x), n	xx.x (xx.x to xx.x), n
Descent, n (%)	n/N (%)	n/N (%)

- Caucasian - Asian - African - Other, specify		
Maintenance therapy, n (%) - Levamisole - Cyclosporine - Tacrolimus - Mycophenolate mofetil - Mycophenolate sodium - Prednisolone	n/N (%)	n/N (%)
Total number of relapses prior to study participation	n/N (%)	n/N (%)
Number of relapses in preceding 24 months	n/N (%)	n/N (%)

ANALYSIS

Outcome definitions

Primary outcome

Time to first relapse

- Definition: time to first relapse (=first day of treatment of the next relapse) after the final prednisolone dose. The final prednisolone dose represents the beginning of study medication for the placebo group and the end of study medication for the prednisolone group.
- Timing: censored at 12 and 24 months
- Measurement value: days

Secondary outcomes

1. Number of relapses
    - Definition: number of relapses per patient after the final prednisolone dose
    - Timing: censored at 12 and 24 months
    - Measurement value: number of patients (%)

2. Progression to frequent relapsing nephrotic syndrome
    - Definition (according to KDIGO criteria): four or more relapses in any 12-month period
    - Timing: censored at 24 months
    - Measurement value: number of patients (%)

3. Progression to steroid dependent nephrotic syndrome
    - Definition (according to KDIGO criteria): two consecutive relapses during corticosteroid therapy, or within 14 days of ceasing therapy
    - Timing: censored at 24 months
    - Measurement value: number of patients (%)

4. Cumulative dosage of prednisolone
    - Definition: cumulative dosage of prednisolone during study period after the final prednisolone dose

- Timing: censored at 12 and 24 months
- Measurement value: mg/m^2

Analysis methods

Primary endpoint

Time to first relapse after the final prednisolone dose

- As normal distribution is not assumed, the median time to relapse will be compared between the groups using a Mann-Whitney test;
- In case patients did not experience any relapse after 24 months or patients are lost to follow up before they experienced a relapse, incidence rates or person-time rates will be used to compare the groups;
- If a normal distribution is shown in the Shapiro-Wilk W test, a *t* test will be used;
- The percentages of study subjects with a relapse at 12 and 24 months will be compared between the groups;
- The cumulative probability on the absence of a relapse will be estimated by survival analysis, according to the Kaplan-Meier method. Log-rank tests are used for the comparison between the standard treatment group and placebo group;
- To test for the influence of factors, such as the use of maintenance immunosuppressive therapy, logistic regression analysis for nephrotic syndrome relapses with covariate analysis will be performed;
- Within each subject time to relapse after study medication will be compared to time to relapse before entering the study using a paired sample *t* test. This test will exclusively be performed in patients without changes in maintenance therapy.

Secondary endpoints

Number of relapses after the final prednisolone dose

- Follow-up will be categorized into three periods (period 1, 0-12 months, period 2, 12-24 months, period 3, 0-24 months) and within each period, the number of relapses is counted;
- Poisson regression will be used to evaluate relapse rates in relation to treatment, maintenance immunosuppressive treatment, sex, age and period.

Development of frequent relapsing nephrotic syndrome (according to KDIGO criteria)

- This categorical outcome will be analyzed with either the Pearson chi-squared or Fisher exact test.

Development of steroid dependent nephrotic syndrome (according to KDIGO criteria)

- This categorical outcome will be analyzed with either the Pearson chi-squared or Fisher exact test.

Cumulative dosage of prednisolone during study period

- Depending on the distribution of the data, this will be analyzed with either the *t* test or the Mann-Whitney test.

Missing data

Missing baseline and outcome data will not be imputed. We will state which data are missing and calculate frequencies using the total number of patients with available data. When a patient is lost to follow-up or has withdrawn consent, we will use all available data up until

withdrawal of consent or loss to follow-up.

Interim analysis

The total inclusion of 144 patients is expected to be finalized in 1.5 years, thereby the DSMB meeting will take place approximately 8 months after the start of inclusion. The mean follow-up period of the 40 patients at that time will be 5.5 (range 3-8) months. Previous literature shows a cumulative incidence rate of a first relapse of 30% after 5.5 months.[3] As the placebo group will receive approximately 35% less prednisolone during the study period, we based the power calculation on a difference of maximum 1/3rd earlier until the first relapse after study medication. Therefore we would like to advise the DSMB to check whether the patients with a relapse are in group A or B (dummy groups) if over 8 patients have experienced a relapse at interim analysis. Aim is then to check for a significant difference in relapse rate between the two groups. If there seems to be a significant difference between the two groups and the percentage of patients with a relapse in either one or both groups exceeds 40% than they might consider to unblind the groups.

Harms

Information on adverse events will be collected by means of reported adverse events in the questionnaires and spontaneous reports from patients and caregivers. The number of adverse events, serious adverse events and suspected unexpected serious adverse reaction will be compared between the two arms using a chi-squared test (or Fisher's exact test), with risk ratios and 95% confidence intervals when these are computable. For all continuous variables either means with standard deviation or medians with interquartile range will be calculated where appropriate and testing for difference will be performed with either the *t* test or Mann-Whitney test where appropriate.

STATISTICAL SOFTWARE

All analyses will be performed with IBM SPSS Statistics.

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