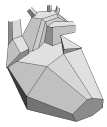
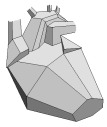


Intravenous Iron supplement for Iron deficiency in Cardiac transplant recipients (IronIC): A randomized clinical trial

NCT03662789

Date 29.02.2020



Statistical Analysis Plan

TRIAL FULL TITLE	Intravenous Iron supplement for Iron deficiency in Cardiac transplant recipients (IronIC)
EUDRACT NUMBER	2017-004871-30
SAP VERSION	1.0
SAP VERSION DATE	29 Feb, 2020
TRIAL STATISTICIAN	Kristine Victoria Brautaset Englund
TRIAL CHIEF INVESTIGATOR	Kaspar Broch
SAP AUTHOR	Kaspar Broch

1 SAP Signatures

I give my approval for the attached SAP entitled IronIC dated 29 Feb, 2020

Chief Investigator

Name: Kaspar Broch

Signature: _____

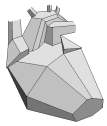
Date:

Statistician

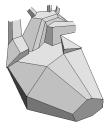
Name: Kristina Victoria Brautaset

Signature: _____

Date:



2 Contents



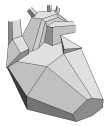
3 Introduction

3.1 Preface

Iron deficiency is associated with poor exercise capacity, lethargy and reduced quality of life. Our results show that iron deficiency is prevalent in heart transplant recipients. Cardiac allograft recipients have reduced exercise capacity and quality of life compared with the age and gender matched general population. We assume that some of these symptoms are due to iron deficiency, and hypothesise that intravenous iron supplement will improve peak oxygen consumption, muscle strength, functional capacity, cognition, and health-related quality of life in heart transplant recipients with iron deficiency.

3.2 Purpose of the analyses

These analyses will assess the efficacy and safety of iron isomaltoside in comparison with placebo and will be included in the clinical study report



4 Study Objectives and Endpoints

4.1 Study Objectives

The main goal of this study is to evaluate the ability of a single administration of intravenous iron isomaltoside to increase peak oxygen consumption in cardiac allograft recipients who have iron deficiency defined as serum ferritin < 100 µg/l or ferritin between 100 and 300 µg/l in combination with a transferrin saturation < 20 %.

Secondary objectives are to assess the impact of treatment on: (i) iron stores, (ii) muscle strength, (iii) body composition, (iv) cognitive function, (v) quality of life, (vi) markers of myocardial disease and inflammation, and (vii) safety and tolerability.

4.2 Endpoints

The primary endpoint is the baseline-adjusted peak oxygen consumption as measured on a treadmill exercise test 6 months after study drug administration.

Secondary endpoints:

- The number of patients with absolute or functional iron deficiency
- Muscle strength as measured by a dynamometer hand-grip device
- Body composition
- Cognitive function as assessed by the Cambridge Neuropsychological Test Automated Battery
- Quality of life as assessed by the SF-36 and 5D EuroQoL questionnaires
- N-terminal pro-B-type natriuretic peptide (NT-proBNP)
- Cardiac troponin T (TnT)
- C-reactive protein (CRP)
- Inflammatory and vasoactive peptides.

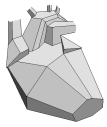
5 Study Methods

5.1 General Study Design and Plan

This is a balanced, randomised, placebo controlled, double blind, parallel group trial. Eligible patients are randomised 1:1 and allocated to intravenous iron supplement or matching placebo at baseline, after providing written informed consent and performing baseline exams. The patients receive the study intervention immediately after randomisation, and no more than 3 days after performing the baseline tests. The patients then return for efficacy tests 6 months ± 2 weeks after the study intervention.

5.2 Study plan and assessments

Phases	Screening	Baseline		Follow-up	
				3 months	6 months
Informed consent	x		Study drug infusion		
Confirmation of eligibility	x				
ECG		x			x
Echocardiography		x			
Safety samples[1]		x		x	x
Serum hCG[2]	x				
Randomisation		x			
Biobank samples[3]		x			x
Measurement of peak oxygen consumption		x			x
Hand grip strength		x			x
Body mass composition		x			x
Cognitive function		x			x
Quality of life		x			x
Registration of adverse events		x	x	x	x



5.3 Inclusion-Exclusion Criteria

Patients will be screened for eligibility upon admittance for routine follow-up at least one year after heart transplantation.

5.3.1 Inclusion criteria

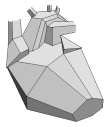
The following conditions must apply prior to administering the investigational medicinal product:

- Cardiac allograft.
- Presentation at least one year after heart transplantation.
- Iron deficiency defined as serum ferritin < 100 µg/l or ferritin between 100 and 300 µg/l in combination with a transferrin saturation < 20 %.
- Age between 18 and 80 years.
- Informed consent obtained and documented according to Good Clinical Practice (GCP), and national/regional regulations.

5.3.2 Exclusion criteria

Patients will be excluded from the study if they meet any of the following criteria:

- Anaemia (Haemoglobin < 100 mg/l)
- Haemochromatosis
- Haemosiderosis
- Porphyria cutanea tarda
- Blood dyscrasias or any disorders causing haemolysis or unstable red blood cells
- Decompensated liver disease (Child-Pugh score 7 or higher)
- End-stage renal failure, i.e. eGFR < 15 ml/min or on renal replacement therapy
- Planned cardiac surgery or angioplasty within 6 months
- Planned major surgery within 6 months
- Medical history of unresolved cancer (except for basal cell carcinoma)
- Treatment with systemic steroids more than the equivalent of 10 mg Prednisone/day at the time of informed consent or change in dosage of thyroid hormones within 6 weeks prior to informed consent
- Any uncontrolled endocrine disorder except type 2 diabetes
- Pregnancy
- On erythropoietin analogues
- Known sensitivity or intolerance to iron isomaltoside or other parenteral iron preparations
- Intravenous iron supplement within 6 months prior to inclusion
- On oral iron substitution (unless the subject agrees to stop treatment prior to randomisation)
- Ongoing rejections or infections
- Alcohol or drug abuse within 3 months of informed consent that would interfere with trial participation or any ongoing condition leading to decreased compliance with study procedures or study drug intake
- Intake of an investigational drug in another trial within 30 days prior to intake of study medication in this trial or participating in another trial involving an investigational drug and/or follow-up



5.4 Randomisation and Blinding

Balanced, permuted block randomisation (in a 1:1: ratio for the two study arms) using random block sizes was computed by the Research Support Unit at Oslo University Hospital. A complete, sealed randomisation list containing details of all patient numbers and study group is stored as essential documentation within the Trial Master File in a locked office. Treatment allocation is performed by random draw of sealed, opaque envelopes containing cards marked “iron isomaltoside 20 mg/kg” or “placebo”. A nurse who does not participate in the trial or has competing interest regarding study outcomes prepared the envelopes and has exclusive access to the list linking study drug numbers, and batch numbers and expiry dates. The administration of the study drug is performed by nurses who are not part of the study team. The study participants (patients) and all study personnel, including investigators, personnel assessing outcomes, study nurses, data analysts and treating physicians and nurses, are blinded to allocation to study drug.

5.5 Study Variables

Primary endpoint: The primary endpoint, the peak oxygen consumption, will be measured as the three highest ten-second averaged measurements before the termination of exercise. The oxygen consumption is measured in ml/kg/min, meaning that the absolute oxygen uptake (in ml/minute) divided by the patient’s weight (in kg) as measured on the day of the test procedure.

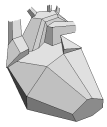
Secondary endpoints:

The muscle strength is measured in KG. The baseline-adjusted between-group values will be analysed by ANCOVA, if necessary after log.-transformation of the data.

Quality of life as assessed by the EuroQoL 5D 3L will be assessed as the sum of scores (1-3) for the five questions. The between-group difference will be assessed with the Mann Whitney U-test. Forms with more than one missing value will be discarded, whereas an average value will be imputed for forms with one missing variable. The between-group results of visual analogue scale data, on a scale from 1-100, will be assessed with baseline-adjusted ANCOVA. The scores for the SF-36v2 will be summed up and transformed to norm a based score of (mean ± standard deviation) 50 ± 10 as recommended for this version. The between-group difference in the physical and mental score components, as well as the sum of the components, will be analysed with baseline-adjusted ANCOVA analyses. If more than 20 % of values are missing, the forms will be regarded as invalid, and the patients with these forms will be excluded from the intention to treat analysis. If 20 % or fewer variables are missing, we will impute mean values derived from the existing values in the same subscore (mental of physical)

6 Sample Size

This trial is designed to assess the effect of intravenous iron on peak oxygen consumption in heart transplant recipients with iron deficiency. We consider an increase in peak oxygen consumption of 1.5 ml/kg/min, equivalent to approximately half a Metabolic Equivalent of Task (MET), to represent a clinically meaningful improvement. An increase in O2 consumption of 1.5 ml/kg/min is approximately 6 % of the baseline maximal oxygen consumption in our heart transplant recipients.



Forty-four patients in each group provides the trial with an 80 % power at an α of 5 % to show a difference between the groups of 1.5 ml/kg/min at an expected repeat-measurement standard deviation of 2.5 ml/kg/min. To allow for drop-out and to improve the chances of obtaining significance for secondary endpoints, we aim to include 100 patients.

7 General Considerations

7.1 Timing of Analyses

The final analysis will be performed after the last patient has completed the last (6-month) follow-up visit, and all data have been transferred to a separate file on having been documented as meeting the cleaning and approval requirements of the primary investigator and after the finalisation and approval of this Statistical Analysis Plan. Only when these requirements have been met, will database lock occur, and the randomisation code be opened.

7.2 Analysis Populations

7.2.1 Intention-to-treat Population (Primary analyses)

All subjects who were randomised. For the primary endpoint, only patients who (irrespective of receipt of actual treatment) performed an adequate cardiopulmonary exercise test at baseline and follow-up can be analysed. The adequacy of the cardiopulmonary exercise test must be determined before database lock and the randomisation code is opened.

7.2.2 Per Protocol Population

All subjects actually received the study drug and who performed baseline and follow-up tests.

7.2.3 Safety Population

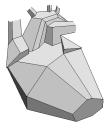
All subjects who received any study treatment (including control) but excluding subjects who drop out prior to receiving any treatment.

Each subject's inclusion or exclusion status regarding each analysis population must be determined prior to breaking the blind. The statuses must be documented in the final database prior to breaking the blind.

7.3 Covariates and Subgroups

The primary analyses (intention-to-treat population) will not be adjusted for baseline demographics. However, the primary endpoint and key secondary endpoints are calculated as the baseline-adjusted values (i.e. the follow-up result is adjusted for the baseline result of the same assessment in ANCOVA analyses) as specified for each endpoint.

We will perform binary subgroup analyses of the primary endpoint stratified by gender; age (median); whether the baseline transferrin saturation is < or ≥ 20 %; by whether or not the patient has anaemia (defined according to World Health Organization criteria of < 12 g/l for women and < 13 g/l for men); C-reactive protein (median) and whether or not iron parameters have normalised (to values no longer defined as iron deficiency as defined in "study objectives". A forest plot will be used



to communicate the results. The subgroup analyses are exploratory only, as the trial is not powered to show differences across subgroups.

7.4 Missing Data

Because the efficacy data are collected twice only, and the endpoint analyses will be baseline adjusted ANCOVA or single point assessments only, there will be no imputation of missing data. However, to elucidate possible biases this method entails, baseline data for patients for whom endpoint data exist will be compared with baseline data for patients who for some reason drop out or are unable to complete both sets of tests. We will also explore if the dropout rate differs between the treatment arms. The extent of missing data will be quantified for each endpoint variable.

We expect few missing baseline covariates and will not employ a strategy to impute missing baseline covariates.

8 Summary of Study Data

The baseline, intention-to-treat population demographics will be presented by columns for each treatment (Placebo, Intravenous iron isomaltoside). All continuous variables will be summarised using the following descriptive statistics: mean ± standard deviation; median (interquartile range). The frequency and percentages (based on the non-missing sample size) of observed levels will be reported for all categorical measures. All summary tables will be structured with a column for each treatment in the order (Control, Experimental) and will be annotated with the total population size relevant to that table/treatment, including any missing observations.

The sample size of non-missing values for univariable summary statistics may be larger than the sample size of non-missing values in a complete-case analysis used in the primary regression (ANCOVA) analysis.

Table 1

Demographics	**Placebo**	**Intravenous iron**
Age – years		
Male gender – no (%)		
Time since HTx – years (IQR)		
Reason for HTx, no (%)		
Ischemic		
DCMP		
GUCH no		
Other no		
Left ventricular ejection fraction		
NYHA functional class, no (%)		
I		
II		
III		
IV		
Body mass index – kg/m^2		

Systolic blood pressure – mmHg
Heart rate – beats per minute

Treatment

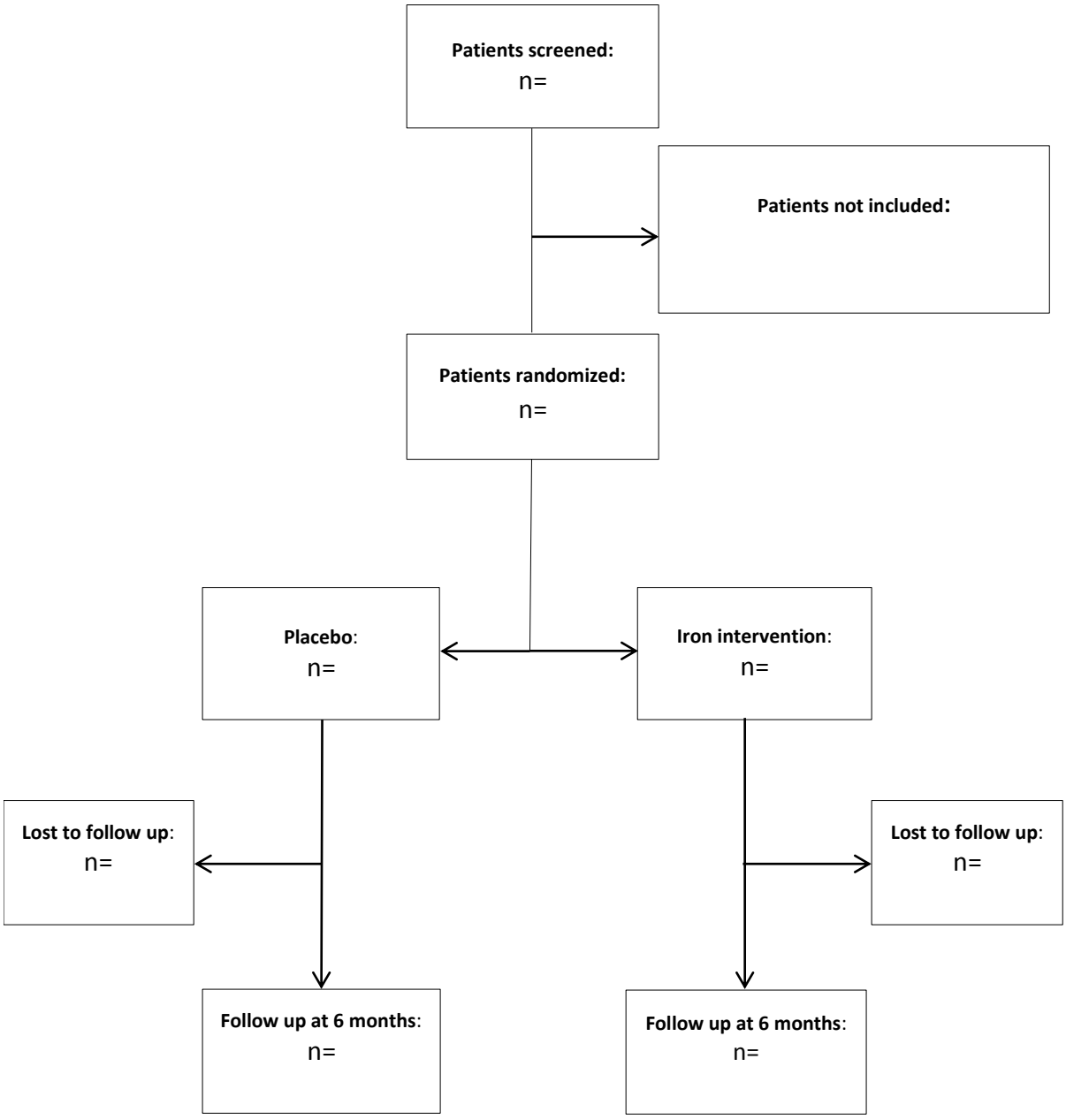
Cyclosporine/Tacrolimus
Everolimus
Mycophenolic acid/Azathioprine
Prednisolone
Angiotensin converting enzyme inhibitor
Angiotensin II-receptor antagonist
Loop diuretic
Thiazide
Beta-blocker
Calcium antagonist
Platelet inhibitors
Oral anticoagulants
Proton pump inhibitors

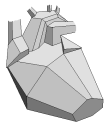
Biochemistry

Haemoglobin – g/l
Creatinine - µmol/L
Estimated glomerular filtration rate – ml/min/1.73m2
Ferritin – µg/l (IQR)
Transferrin saturation – % (IQR)
Transferrin receptor (mg/l) % (IQR)
Sedimentation Rate – mm (IQR)
C-reactive protein – µg/l (IQR)
Total plasma protein – g/l
Troponin T – ng/l (IQR)
N-terminal pro B-type natriuretic peptide-ng/l (IQR)
Ferritin < 30 µg/ - no (%)
Transferrin saturation < 20% - no (%)
Ferritin 100-300 µg/l +Transferrin sat. <20% -no (%)

8.1 Subject Disposition

We will document how many subjects reached the various stages of the trial (the number screened, randomised, reached the 6-month follow-up visit and could be analysed for the primary endpoint), and how many dropped out and for what reasons (death, withdrew consent, failed to show up) according to the following CONSORT diagram:





8.2 Demographic and Baseline Variables

The following baseline variables will be presented:

The summary statistics will be produced in accordance with section 8.

8.3 Treatment Compliance

Because the study intervention consists of a single, intravenous infusion, there is no issue with treatment compliance. The intention-to-treat primary analyses will be performed regardless of whether or not the study drug infusion was completed, but the number of patients who did not receive the intervention will be reported for each group.

9 Efficacy Analyses

9.1 Primary Efficacy Analysis

The primary endpoint, the baseline-adjusted peak oxygen consumption 6 months after the intervention, will be calculated by ANCOVA with treatment allocation and the baseline peak oxygen consumption as covariates according to the intention-to-treat principle, the statistical null-hypothesis being that the baseline-adjusted oxygen uptake does not differ between the two treatment arms. If the primary endpoint is met at a significance level of 0.05, the result will be considered confirmatory of the main research hypothesis.

9.2 Secondary Efficacy Analyses

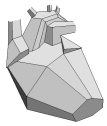
Secondary, per protocol analysis will be performed for the primary endpoint. Exploratory subgroup analyses will be performed for the six subgroup categories mentioned in the section “Covariates and subgroups. The latter results will be communicated as a forest plot, and the interaction value of the covariates “treatment allocation” and the binary subgroup identifier in question will be reported at a significance level of 0.05.

9.2.1 Secondary endpoints

The baseline-adjusted between-group difference in the change in i) hand grip strength, ii) normally distributed body composition data, and iii) the SF36 score and the visual analogue scale data from the EuroQoL 5D 3L form will be calculated by ANCOVA. The statistical null-hypotheses are that the changes in these characteristics do not differ between patients allocated to iron isomaltoside and patients allocated to placebo.

NT-proBNP, TnT and CRP at six months will be analysed using Mann Whitney U-tests, the statistical null-hypothesis being that the levels of these biomarkers do not differ between the treatment arms. The between-group difference in the proportion of patients with a minimum clinically important improvement, defined as an improvement of 4 points, and the between-group difference in the proportion of patients with a minimum clinically important deterioration, defined as a decrease of 4 points, will be calculated by Chi square tests.

Secondary per protocol analyses will be performed using the same methods as for the intention-to-treat analyses.



9.3 Exploratory Efficacy Analyses

Exploratory analyses of between-group differences in inflammatory parameters and parameters pertaining to iron metabolism, such as Hepcidin, Interleukin 6 and the ferritin index will be performed by ANCOVA or repeated test t-tests, if necessary after log-transformation to meet the requirements of the test.

10 Safety Analyses

Safety analyses will include tabulation of type and frequency of all adverse events. Any value of safety laboratory parameters outside normal ranges will be identified. The final report will include the absolute number of adverse and severe adverse events in each group. The results will be reported for the Safety Population only. The summary statistics will be produced in accordance with section 8.

10.1 Adverse Events

Serious adverse events and adverse events of special interest; pregnancies and adverse reactions to the investigational medicinal products will be reported with comprehensive narratives.

11 Technical Details

Statistical analyses will be performed in IBM SPSS Statistics version 21 or later.

12 Summary of Changes to the Protocol

This Statistical Analysis Plan differs from the protocol (Version 1.4) only with regard to the detail with which the statistical methods and plans for how results are communicated are presented, and the specification of exploratory subgroup analyses of the primary endpoint.