Phlebotomy in Blood Donors and Polycythemia Vera Patients– The Effect on Physiology: An Orienting Case-Cross Over Study

Short title

NOSFERATU

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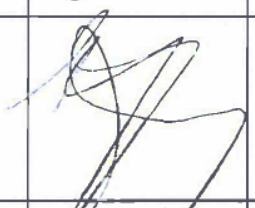
PROTOCOL SIGNATURE SHEET

Name	Signature	Date
Head of Department: **LUMC** Prof. dr. J.J. Zwaginga, Internist-hematologist		28-4-2022
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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	**General Assessment and Registration form (ABR form), the application form that is required for submission to the accredited Ethics Committee; in Dutch: Algemeen Beoordelings- en Registratieformulier (ABR-formulier)**
AE	**Adverse Event**
AR	**Adverse Reaction**
DSMB	**Data Safety Monitoring Board**
EQ5D	**EuroQol 5D**
GCP	**Good Clinical Practice**
GDPR	**General Data Protection Regulation; in Dutch: Algemene Verordening Gegevensbescherming (AVG)**
Hb	**Haemoglobin**
ICF	**Informed Consent Form**
MDS	**Myelodysplastic Syndrome**
METC	**Medical research ethics committee (MREC); in Dutch: medisch-ethische toetsingscommissie (METC)**
RBC	**Red Blood Cells**
(S)AE	**(Serious) Adverse Event**
SoC	**Standard of care**
Sponsor	**The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party**
UAVG	**Dutch Act on Implementation of the General Data Protection Regulation**
WMO	**Medical Research Involving Human Subjects Act**

SUMMARY

Rationale: Little is known about the effect of a hemoglobin-shift on patients, anaemic, non-anaemic or even polycythaemic. It has been established that severe anaemia has deteriorating effects on the patient, which can be (partially) reversed by treatments like transfusion, ESAs or iron/vitamin supplements, depending of the etiology of the anaemia. However, we have yet to determine the optimal haemoglobin target and threshold for such treatments. We therefore need to evaluate what the exact effect of various haemoglobin levels, and a shift therein, is on the physiology of patients. Only then can we properly weigh the benefits against the risk for individual patients when considering treatment for anaemia, safety of blood donation, or expected effect of phlebotomy for polycythaemia.

Primary Objective:

- Compare the per individual and per group effects of a reduction in Hb mass on physical functional outcomes (heart rate; activity parameters; QoL) in patients with polycythaemia vera.

Secondary Objectives:

- Compare the primary outcomes to data from the REMOTE-2 and FAINT-study (similar measurements in transfusion dependent patients and whole blood donors)

Study design: Case-cross over study. Patients will be remotely monitored one week before phlebotomy until one week after.

Study population: Adult patients with a polycythaemia requiring phlebotomies on a regular basis.

Main study parameters: heart rate, blood pressure, activity parameters and quality of life.

Nature and extent of the burden and risks associated with participation, benefit and group relatedness: No medical benefits, besides contributing to knowledge regarding their disease; No relevant risks. There is, however, the burden of 1) filling out the EQ5D forms (2 minutes a day); 2) measuring the bloodpressure (2 minutes a day) and wearing the smartwatch for two weeks per phlebotomy, three phlebotomies.

1. RATIONALE

We have very little knowledge of what effect a shift in haemoglobin (Hb) mass has in any person, anemic, polycythemic or normemic. Assessing functional outcomes at various Hb levels may help to better understand what effect changes in Hb*mass* have on the physiology. While a reduction in Hb level from 16 to 15 g/dL may not be clinically relevant for the subject itself, observing and properly documenting its effect will help put the puzzle of Hb optimization together. Because of the ready availability of patients with polycythaemia vera that undergo phlebotomies on a regular basis, we want to start by evaluating physiological parameters in these subjects. Parallel to this study, Sanquin will evaluate whole blood donors in a similar study (FAINT-study, https://www.sanquin.nl/over-sanquin/donorstudies) and myelodysplastic syndrome patients with chronic transfusion dependency receiving various amounts of red blood transfusions are monitored in the REMOTE 2 study (NL73847.58.20). Assessing the Hb*mass*-and-physiology question from different perspectives and comparing these outcomes will increase our understanding of the matter.

Our theoretic basis for this study starts with the function of haemoglobin, the main component of red blood cells (RBC), which transports oxygen. In healthy persons, the human body regulates the haemoglobin levels to maintain a level between 12.1 and 15.1 g/dL in females 13.8 and 17.2 g/dL in males. This Hb concentration, or level, is a result of the Hb*mass* (Total intravascular mass of Hb) and the plasma volume:

$$\mathrm{Hb}conc \approx \frac{\mathrm{Hb}mass}{\mathrm{Plasma\ volume} + \mathrm{Hb}mass}$$

Several diseases can cause this level of haemoglobin to decrease to levels below 8 g/dL, requiring patients to receive RBC transfusions. In these anaemic patients, the shortage of oxygen delivery in tissues causes symptoms like fatigue, dyspnea and lack of concentration, leading to a reduced quality of life.[1–3] On the other hand, altitude training and drugs like RhuEPO are used to increase the maximum oxygen transport capacity (VO_2max) in order to enhance athletic performance.[4–6] A somewhat linear association between Hb*mass* and absolute O_2 transport capability may be assumed. The less Hb, the less O_2 transport. In terms of VO_2max, which is the maximum amount of O_2 a subjects' body can transport, this will be limited for rheological reasons with high Hb levels. For instance, patients with polycythaemia vera (PV) suffer from hyperviscosity issues, which again may restrict oxygen transport capacity.

In daily life however, we are not that often dependent on our VO_2max, but more on our ''VO_2usual''. When our erythropoiesis for some reason is unable to maintain normal Hb levels, the human body has many compensation strategies to maintain basic O_2 delivery, like

increasing the cardiac output, vasoconstriction, and adjusting the plasma volume to increase the Hb concentration. A subject will probably not notice slight changes in Hb*mass*. At some point, however, the compensation mechanisms may be noticeable (higher heart rate/palpitations/fatigue).

We know that in patients with anaemia, red blood cell transfusions improve walk test distances and fatigue scores[7], and preliminary results show that heart rate is inversely correlated with changes in Hb level by transfusion. For these patients, it is clear that the change in Hb*mass* has an effect on their daily physiology. What we do not know, is the magnitude of this effect at different levels of Hb level. Do these effects correlate linearly to the change in Hb level? Or, is there a certain level above which an extra increase in Hb will not exert a beneficial effect on the patient?
Effects of changes in Hb*mass* on daily physiology in non-anaemic subjects have not yet been studied properly. In the past, studies showed a decrease in VO_2*max* following isovolemic Haemodilution and an increase in VO_2*max* following transfusions.[8] Studies evaluating donors have shown that donation increases resting heart rate 24 hours after donation,[9] and decreases VO_2*max.* A systematic review, however, shows no significant difference in VO2max, power or, heart rate, up until 48 hours after donation.[10] It also concludes that there are not enough studies regarding cardiorespiratory outcomes following blood donation. However, While the blood volume returns to normal within 24-48 hours after donation due to an increase in plasma volume, Hb restoration takes as much as up to 12 weeks. Therefore, to adequately measure the effect of the decrease in Hb level on physiological outcomes without the increased cardiac output due to the lower blood volume, evaluation of these outcomes should not start before 48 hours after donation.
The same goes for patients with PV that undergo phlebotomies: no proper physiological evaluation has been documented yet. This makes this study new and important for both the PV-patient group as well as for our general understanding of the effect of Hb-shifts on the physiology of the human body.

Tools for measuring the impact of change in Hb mass on the subjects well-being: vital signs, physical function.
We intent to evaluate vital signs and physical function of PV-patients with the Withings BPM connect and the Withings Steel HR. We have chosen to use the generic EQ5D for the PV-group and have also added a visual analog scale (VAS) 0-100 slider question: how active have you been today?‘.

2. OBJECTIVES

Primary Objective:

- Compare the per individual and per group effects of a reduction in Hb mass on physical functional outcomes (heart rate; activity parameters; QoL) in patients with polycythaemia.

Secondary Objectives:

- Compare the primary outcomes to data from the REMOTE-2 and FAINT-study (similar measurements in transfusion dependent patients and whole blood donors)

3. STUDY DESIGN

This an observational case-crossover study: Ten patients with polycythemia will be remotely monitored a week before until a week after they receive a phlebotomy. We will remotely monitor their 1) heart rate, 2) blood pressure 3) activity and 4) QoL of life. Participating subjects will be asked to measure their blood pressure and heart rate with the Withings BPM Connect on a daily basis during this study period. Furthermore, they will be asked to fill out an EQ5D questionnaire (5 questions) and a 0-100 visual analog scale question ‘how active they have been that day’, every day. Lastly, they will wear a Withings Steel HR smartwatch continuously, which will measure their heart rate and activity measures continuously.

The data in the week prior to the phlebotomy is meant to generate baseline data to which the data after the phlebotomy will be compared. Hospital staff will be provided access to a platform to monitor subject data. Withings data security is in accordance with the GDPR (https://www.withings.com/fr/en/legal/privacy-policy).

Both the questionnaires and vital parameters can be monitored remotely. Subjects missing a measurement will be sent a reminder to make up for a missed task. Detailed description of study procedures can be found in table 3.1. We will measure each patient during three phlebotomies.

Table 3.1. Study procedures

	Screening >7 days before phlebotomy	**Day -7→0 (+/-1)**	**Day 0**	**Day 0→7 (+/-1)**	**Day 7**
Obtain informed consent	X				
Eligibility criteria	X				
Baseline data collection	X				
Handout BPM Connect, Withings HR steel And deployment	X[1] →	→	→	→	→
Physiologic data training period	X First 48 hrs				
Phlebotomy/donation			X		
Hb measurement			X[2]		
EQ5D, daily		X		X	
Record AEs[3]			X		X

[1] *Data quality and continuity will be monitored.*

[2] *Hemoglobin measurement will be venepuncture for PV and POC measurement for donors*

[3] *Infusion reactions and AEs related to the Withings Steel HR will be collected.*

4. Study POPULATION

4.1 Population

- Ten patients with polycythaemia, requiring phlebotomy on a regular basis (at least every 4 months)

4.2 Inclusion criteria

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

- Subjects aged ≥18 years
- In possession of a smartphone
- Life expectancy ≥ 3 months

4.3 Exclusion criteria

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

- Poor performance/functional status (Eastern Cooperative Oncology Group system ECOG ≥3, see appendix)

- Participants with known arrhythmias or other significant cardiological conductivity disorders (Paroxysmal atrial fibrillation is allowed)
- Hospitalized subjects
- Subjects with a pacemaker.
- Subjects with a secondary polyglobulia due to eg smoking or other pulmonary issues.
- Scheduled oncological treatments or surgery during the study period.

Subjects currently participating in other clinical studies will be evaluated if those studies would interfere, which would make the subject ineligible.

4.4 Sample size calculation

The difference in heart rate after phlebotomy as primary outcome has not yet been studied at all. In respect to a change in Hb mass in the opposite direction: preliminary research showed a difference of 4 bpm before and after a transfusion. Because there is no other data, we anticipate the mean difference in heart rate to be 4 bpm with a standard deviation of 3.
Considering subjects will be compared to themselves as their own controls, with an α of 0.05 and a β of 0.20 this makes a sample size of 9 per group. With the explorative nature in mind, we chose to set the sample size for this initial case-cross over pilot at 10 subjects per group. We will measure each subject during three phlebotomies to reduce the intrapatient variability.

5. INVESTIGATIONAL PRODUCT

5.1 Phlebotomies

The investigational product in this trial is the phlebotomy. We will not change anything about the standard care.

6. METHODS

6.1 Study parameters/endpoints

6.1.1 Main study parameter/endpoint

- Data gathered by the Withings BPM Connect
  - Heart rate *resting*
  - Blood pressure *resting*

6.1.2 Secondary study parameters/endpoints

- Data gathered by the Withings Steel HR
  - Steps *daily total*

- Exercise time *daily total*
- Distance Walking or Running *daily total*
- Heart rate *median, resting*

- Hb-level pre-phlebotomy *(standard of care)* c*ontinuous*
- EQ5D *score*
- 0-100 VAS activity slider *score*

6.1.3 Other study parameters

- Co-morbidity (heart failure, kidney disease, diabetes, splenectomy) *yes/no, which*
- Beta-blockers *which*
- Adverse reactions *which, extent*

6.2 Study procedures

An overview of study procedures can be found in table 3.1.

Heart rate monitoring via the Withings BPM connect

The BPM connect is a clinically validated blood pressure and heart rate meter which patients will be using themselves at home. They will take their blood pressure and heart rate on a daily basis during the study. Patients will be trained in using the clinically validated BPM Connect. They will also be asked to measure their blood pressure, <2hr before and <2hr after phlebotomy.

Continuous monitoring via the Withings Steel HR

Patients will wear the CE marked Withings Steel HR continuously from one week before the phlebotomy until one week after the phlebotomy. Heart rate measurements of the Steel HR will be validated by comparing the Steel HR data to the heart rate data of the clinically validated BPM connect.

Quality of life measurements via EQ5D

Each day for the duration of the study, patients will fill out an EQ5D questionnaire (2min). The EQ5D is a generic 6-item quality of life questionnaire. The CASTOR-based questionnaire will be sent to the patient via an e-mail. Because of direct entry of the questionnaires in CASTOR by the patients, there will be no other source material.

Phlebotomy and donation

All phlebotomies will be scheduled and performed as usual. No interventions are used in this observative study.

6.3 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons.

6.3.1 Specific criteria for withdrawal

- Withdrawal of consent by patient
- Investigator decision
- Progression to MDS-EB2 or AML

6.4 Replacement of individual subjects after withdrawal

Drop-outs before three days after phlebotomy will be replaced.

6.5 Follow-up of subjects withdrawn from treatment

No follow-up is required as no substantial changes are initiated by the study.

7. SAFETY REPORTING

7.1 AEs, SAEs and SUSARs

7.1.1 Adverse events (AEs)

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to the investigational product. All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded.

7.1.2 Serious adverse events (SAEs)

A serious adverse event is any untoward medical occurrence or effect that

- results in death;
- is life threatening (at the time of the event);
- requires hospitalisation or prolongation of existing inpatients' hospitalisation;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect; or

- any other important medical event that did not result in any of the outcomes listed above due to medical or surgical intervention but could have been based upon appropriate judgement by the investigator.

An elective hospital admission will not be considered as a serious adverse event.

The investigator will report all SAEs to the sponsor without undue delay after obtaining knowledge of the events, except for the following SAEs:

- Elective hospitalisation to evaluate the treatment management plan for the patient's disorder, or for procedures;
- Elective hospitalisation for pre-existing conditions that, in the investigator's opinion, have not been exacerbated by trial treatment;
- Severe sepsis;
- Disease progression;
- Transient ischaemic attack, thromboembolic and ischaemic events (myocardial infarction, stroke, pulmonary embolus, DVT);
- Any other serious event related to the underlying disease or medication used to treat the disease.

7.2 Follow-up of adverse events

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.
SAEs need to be reported till end of study within the Netherlands, as defined in the protocol

7.3 Data Safety Monitoring

A DSMB is not deemed necessary as this is a low-risk study.

8. STATISTICAL ANALYSIS

Data will be presented descriptively, as continuous variables, e.g. mean heart rate and activity per day per patient group. Data will be analyzed according to the per protocol analysis. Patients dropping out earlier than 3 days after phlebotomy will be replaced and excluded from analysis. The data from this study will be combined/compared to data from a parallel similar study: the FAINT-study, executed by Sanquin, in which 30 whole blood donors will be monitored in the same way. This comparison will grant us more insight on what the effect of a shift in Hb-mass is on various types of patients/donors, with different Hb start-off levels.

8.1 Primary study parameters

- Data gathered by the Withings the clinically validated BPM connect
    - Heart rate

For the primary endpoints linear mixed model with random intercept analysis will be used for correlating repeated measures. Residuals will be checked for normality. If they are not normally distributed, we will proceed with a generalized linear mixed model. Data will first visually be reviewed, if the blood donors and phlebotomy subjects appear to have a similar effect from the change in Hb mass, they will be analyzed in the same model. If, for example due to hyperviscosity issues, the effect appears different or even reversed in one of the two groups, they will be analyzed separately.
Heart rate before phlebotomy/donation will be compared to the heart rate 1, 2, 3 and 6 days after the intervention.

Statistical analysis will be conducted using SPSS (version 25.0, SPSS Inc., Chicago, USA). A p-value <0.05 (two-sided) will be considered statistically significant.

8.2 Secondary study parameters

- Data gathered by the Withings Steel HR:
    - Heart rate (compared to the clinically validated BPM connect)
    - Activity parameters: steps, exercise time, distance running and/or walking
- Blood pressure as measured by the Withings BPM connect
- Quality of life parameters (EQ5D)
- Hb-level

Secondary endpoints will also be analysed with a linear mixed model, provided that residuals are normally distributed. If not so, a generalized linear mixed model will be used.

8.3 Other study parameters

Other study parameters will be used for confounding and baseline characteristics.

- Co-morbidity
- Medication
- Adverse reactions
- Age, sex of the recipient

9. ETHICAL CONSIDERATIONS

9.1 Regulation statement

This study will be conducted according to the principle of the 10th version of the Declaration of Helsinki (Oct 2013) and in accordance with the GDPR.

9.2 Recruitment and consent

Recruitment of patients will be done by the local investigator. Treating physicians will be asked by the local investigator to notify the local investigator of potential subjects for the study. The patient will be provided with a patient information letter and will receive further explanation by the coordinating investigator. Informed consent will be asked after patient has had enough time to think and talk about it with relatives. This form is attached to the information letter. The contact information of the coordinating investigator and the independent expert will be on the information letter for further questions. The form will be signed by the coordinating investigator and stored in the LUMC.

9.3 Benefits and risks assessment

Benefits

There are no medical benefits for the participating subjects, besides contributing to knowledge regarding their disease.

Risks

We believe there are no relevant risks. There is, however, the burden of 1) filling out the EQ5D forms (2 minutes for 14 times; 28 minutes in total); 2) measuring the bloodpressure (2 minutes, 16 times; 32 minutes in total) and wearing the smartwatch for two weeks.

9.4 Incentives

Participating subjects are not expected to travel more than usual, so travel expenses will not be compensated.

10. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

10.1 Handling and storage of data and documents

Biosensor derived data will be transmitted from the device to a smartphone to the Withings data platform, which is in accordance with the GDPR: (https://www.withings.com/fr/en/legal/privacy-policy). The LUMC will generate an unidentifiable 'Hart Long Centrum' (HLC)-account (e-mail and password example: box-3h8m@hlc.nl) per patient, which will be handed to the patient after inclusion to base the Withings account on. This way, Withings will not be able to identify the participating subjects.

Data can only be linked to the patient by the investigator and his research team. Secondary parameters will be stored in Castor (electronic data capture tool) and patient data will be coded so that data cannot be linked to the patient without the key. The key to link all patients to data will be kept on the departmental computer of the coordinating investigator (personal data I: in password secured files) in a separate file. Furthermore, handling and storage of personal data will comply to the Medical Treatment Agreement Act (MTAA) and the General Data Protection Regulation (GDPR). Coordination of the remote monitoring will be done in the LUMC. In order to send out the questionnaires, the patient's e-mail address is required. Therefore, the LUMC will gather personal data in the form of contact details in order to be able to contact the included patients. Patients will give their consent for sharing contact details in the ICF. The ICF's will be stored in the LUMC. All study documents will be stored for 20 years on the hospital computer of the coordinating investigator (departmental data I:) and will be deleted after 20 years.
If a subject decides to withdraw from the study before the end of the study, the collected data up to that point will be used in the analysis. Before start of the study, data flow and storage will be checked by the data protection functionary.

10.2 Monitoring and Quality Assurance

Monitoring will be done according to the protocol of the LUMC.

10.3 Amendments

Amendments will only be submitted to the METC if the changes possibly make the research subject to the WMO

10.4 Public disclosure and publication policy

No arrangements have been made concerning public disclosure. All relevant results will be submitted for publication in peer reviewed journals. Publication will come about conform the 'CCMO-notitie Publicatiebeleid'.

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