

# BMJ Open

BMJ Open is committed to open peer review. As part of this commitment we make the peer review history of every article we publish publicly available.

When an article is published we post the peer reviewers' comments and the authors' responses online. We also post the versions of the paper that were used during peer review. These are the versions that the peer review comments apply to.

The versions of the paper that follow are the versions that were submitted during the peer review process. They are not the versions of record or the final published versions. They should not be cited or distributed as the published version of this manuscript.

BMJ Open is an open access journal and the full, final, typeset and author-corrected version of record of the manuscript is available on our site with no access controls, subscription charges or pay-per-view fees (<http://bmjopen.bmj.com>).

If you have any questions on BMJ Open's open peer review process please email [info.bmjopen@bmj.com](mailto:info.bmjopen@bmj.com)

# BMJ Open

## Ferritin-guided iron supplementation in whole blood donors: Optimal dosage, donor Response, return, and Efficacy (FORTE) – a randomized controlled trial protocol

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2021-056316
Article Type:	Protocol
Date Submitted by the Author:	10-Aug-2021
Complete List of Authors:	Karregat, Jan; Sanquin Research, Department of Donor Medicine Research Sweegers, Maike G; Sanquin Research, Department of Donor Medicine Research Quee, Franke A; Sanquin Research, Department of Donor Medicine Research Weekamp, Henriëtte H; Sanquin Blood Supply Foundation, Medical Donor Affairs Swinkels, Dorine W; Radboud University Nijmegen, Department of Laboratory Medicine; Sanquin Blood Supply Foundation, Center for Iron Disorders Sanquin Novotny, Věra M J; Sanquin Blood Supply Foundation, Department of Transfusion Medicine Zaaijer, Hans L; Sanquin Research, Department of Donor Medicine Research; Amsterdam UMC Location AMC, Department of Clinical Virology van den Hurk, Katja; Sanquin Research, Department of Donor Medicine Research
Keywords:	Blood bank & transfusion medicine < HAEMATOLOGY, Anaemia < HAEMATOLOGY, Clinical trials < THERAPEUTICS

SCHOLARONE™  
Manuscripts

1  
2  
3 1 **Ferritin-guided iron supplementation in whole blood donors: Optimal dosage, donor Response,**  
4 **return, and Efficacy (FORTE) – a randomized controlled trial protocol**

5  
6  
7 3 Jan H M Karregat<sup>1</sup>, [j.karregat@sanquin.nl](mailto:j.karregat@sanquin.nl)

8  
9 4 Maïke G Sweegers<sup>1</sup>, [m.sweegers@sanquin.nl](mailto:m.sweegers@sanquin.nl)

10  
11 5 Franke A Quee<sup>1</sup>, [f.quee@sanquin.nl](mailto:f.quee@sanquin.nl)

12  
13 6 Henriëtte H Weekamp<sup>2</sup>, [h.weekamp@sanquin.nl](mailto:h.weekamp@sanquin.nl)

14  
15  
16 7 Dorine W Swinkels<sup>3,4</sup>, [Dorine.Swinkels@Radboudumc.nl](mailto:Dorine.Swinkels@Radboudumc.nl)

17  
18  
19 8 Věra M J Novotny<sup>5</sup>, [v.novotny@sanquin.nl](mailto:v.novotny@sanquin.nl)

20  
21  
22 9 Hans L. Zaaijer<sup>1,6</sup>, [h.zaaijer@sanquin.nl](mailto:h.zaaijer@sanquin.nl)

23  
24  
25 10 Katja van den Hurk<sup>1</sup>, [k.vandenhurk@sanquin.nl](mailto:k.vandenhurk@sanquin.nl)

26  
27  
28 11 1. Department of Donor Medicine Research, Sanquin Research, Amsterdam, The  
29 Netherlands

30 12 2. Medical Donor Affairs, Sanquin, Zwolle, The Netherlands

31 13 3. Translational Metabolic Laboratory, Department of Laboratory Medicine, Radboud  
32 university medical center, Nijmegen, The Netherlands

33 14 4. Center for Iron Disorders, Sanquin, Amsterdam, The Netherlands

34 15 5. Department of Transfusion Medicine, Sanquin Blood Supply, Amsterdam, The  
35 Netherlands

36 16 6. Department of Clinical Virology, Amsterdam UMC, location AMC, Amsterdam, The  
37 Netherlands

38 17  
39 20  
40 21 Protocol version 1, 10-08-2021

41 22 Word count: 3760

42  
43  
44  
45 23  
46  
47 24 **Corresponding author**

48 25 Jan Karregat, Donor Medicine Research, Sanquin Research, Plesmanlaan 125, 1066 CX  
49 26 Amsterdam, the Netherlands

50 27 E-mail: [j.karregat@sanquin.nl](mailto:j.karregat@sanquin.nl)

51 28 Telephone: (+31) 0650093376

1  
2  
3 **29 Abstract**  
4

5 30 *Background:* Frequent whole blood donors have an increased risk of developing iron  
6  
7  
8 31 deficiency. Iron deficiency can have detrimental health effects when left untreated. Donation  
9  
10 32 intervals are commonly too short to replenish iron stores, and extending these reduces donor  
11  
12 33 availability. Oral iron supplementation is known to shorten iron store recovery time but may  
13  
14 34 also induce gastrointestinal complaints. We aim to optimize the effectiveness of iron  
15  
16 35 supplements while minimizing the risks of side effects. Therefore, we will evaluate the impact  
17  
18 36 of different iron supplementation protocols in terms of dosage and frequency on ferritin and  
19  
20 37 hemoglobin levels, gastrointestinal side-effects, iron deficiency-related symptoms, and donor  
21  
22 38 return compared to placebo supplementation.  
23

24  
25 39 *Methods:* Twelve hundred whole blood donors with ferritin levels  $\leq 30$   $\mu\text{g/L}$  are included into a  
26  
27 40 double-blind, randomized controlled trial. Participants are randomly allocated to one of six  
28  
29 41 arms, administering capsules containing 0, 30, or 60 mg of iron, either on alternate days or  
30  
31 42 daily for 56 days. At baseline and 56, 122, and 182 days of follow-up, ferritin and hemoglobin  
32  
33 43 levels are measured, and compliance, donor return, dietary iron intake, gastrointestinal, iron  
34  
35 44 deficiency-related symptoms, and general health are assessed by questionnaire.  
36  
37

38  
39 45 *Significance and outlook:* This study will provide a comprehensive overview of the effects of  
40  
41 46 different frequencies and dosages of administration of iron supplements on iron status and  
42  
43 47 health effects, thereby considering individual differences in treatment adherence and lifestyle.  
44  
45 48 The outcome will provide scientific evidence to guide the debate if and how oral iron  
46  
47 49 supplements may support the recovery of whole blood donors with low ferritin levels.  
48  
49

50 50

51 51

52 52 *Trial registration:* The Dutch trial registry NL8590.  
53

54  
55  
56 53 *Keywords:* Blood donation; Ferritin; Iron Deficiency; Iron Supplementation; Donor Health  
57  
58  
59  
60

1  
2  
3 54 **Strengths and limitations of this study**  
4  
5

- 6 55 • This is a large (n = 1200), double-blind, randomized controlled trial to determine the  
7  
8 56 optimal iron supplementation protocol in terms of both intake frequency and dosage.  
9  
10  
11 57 • Outcome variables include ferritin and hemoglobin levels, complete blood counts, iron  
12  
13 58 deficiency-related symptoms, and gastrointestinal side effects.  
14  
15 59 • Participants are thoroughly characterized at baseline regarding social-economic  
16  
17 60 status, medical background, physical fitness, dietary intake, and smoking status.  
18  
19  
20 61 • A limitation is the limited number of follow-up visits; however, these time points are  
21  
22 62 the most relevant, and temporal patterns are modeled based on earlier studies.  
23  
24  
25  
26  
27 64  
28  
29 65  
30  
31 66  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

## 67 Introduction

68 Due to the hemoglobin (Hb)-bound iron loss during donation, regular whole blood donors are  
69 prone to developing iron deficiency, often in the absence of anemia<sup>1,2</sup>. Iron deficiency without  
70 anemia is characterized by reduced serum ferritin levels. It is highly prevalent amongst  
71 frequent whole blood donors, with 15.0% of the female and 9.4% of male Dutch donors having  
72 ferritin levels <15 µg/L<sup>3</sup>. Reduced ferritin levels increase the risk of developing anemia and are  
73 associated with iron deficiency-related symptoms, including fatigue, reduced exercise  
74 endurance, restless legs, PICA (appetite for non-nutritious substances), and reduced  
75 neurocognitive functioning<sup>4-8</sup>.

76 At most international blood banks, including the Netherlands, Hb levels are measured before  
77 donation to safeguard donor health and blood product quality<sup>9</sup>. However, Hb levels do not  
78 reflect the amount of stored iron, which is significantly impacted by whole blood donations<sup>10</sup>.  
79 Therefore, ferritin measurements have become more common amongst blood banks to assess  
80 the donor's iron storage. Sanquin incorporated ferritin-guided donation intervals, deferring  
81 donors for 6 or 12 months when ferritin levels are ≥15 and ≤30 µg/L or <15 µg/L, respectively.  
82 These cut-off values are based on WHO standards, as described previously<sup>3</sup>. However, donor  
83 deferral has been shown to demoralize donors and reduce donor return rates<sup>3,11,12</sup>.

84 Several studies have shown that oral iron supplementation reduces the post-donation  
85 recovery time of ferritin and Hb levels to pre-donation levels<sup>13,14</sup>. While donors in the countries  
86 like the United States, Finland, and Denmark are already advised about iron supplementation  
87 for iron storage recovery after donation, other blood services are hesitant, often due to ethical  
88 concerns<sup>15-17</sup>. Kiss *et al.* showed in a randomized controlled trial that post-donation iron  
89 supplementation led to full recovery of ferritin levels within 56 days, whereas the non-iron  
90 supplementing group did not reach full recovery after 160 days<sup>18</sup>.

1  
2  
3 91 In several clinical trials, the iron supplement ferrous bisglycinate has been shown to result in a  
4  
5 92 relatively high fractional iron uptake. This has been attributed to its iron-bound chelates that  
6  
7 93 prevent the iron from binding to other dietary compounds (e.g., tannins, catechols, and  
8  
9  
10 94 phytates) that inhibit iron absorption<sup>19-22</sup>. Furthermore, supplementation with ferrous  
11  
12 95 bisglycinate has been shown to lower the risk of gastrointestinal discomfort compared to other  
13  
14 96 iron formulations, possibly due to the increased uptake by intestinal mucosal cells, leading to  
15  
16 97 less iron entering the colon<sup>19,23,24</sup>. Therefore, ferrous bisglycinate is already used by the Danish  
17  
18 98 blood bank for donors who suffer from gastrointestinal side-effects<sup>25,26</sup>.

19  
20  
21 99 Iron absorption in the duodenum and its entry into the plasma compartment is regulated by  
22  
23  
24 100 the peptide hormone hepcidin<sup>27</sup>. It has been shown that iron supplementation leads to an  
25  
26 101 increased hepatic production of hepcidin in iron-depleted women, causing a reduced intestinal  
27  
28 102 iron uptake up to 24 hours post-supplementation<sup>28,29</sup>. While the dosages of elemental iron  
29  
30 103 used in previous studies range from 19 to 240 mg/day, lower dosed iron supplements are  
31  
32  
33 104 shown effective in recovering the iron stores post-donation<sup>28,30</sup>. These findings suggest that  
34  
35 105 alternate-day supplementation with low-dose ferrous bisglycinate capsules may lead to higher  
36  
37 106 fractional iron uptake and fewer side effects compared to high-dose iron supplements taken  
38  
39 107 daily or twice daily.

40  
41  
42 108 The effects of oral iron supplementation on iron deficiency-related symptoms in donors with  
43  
44 109 low ferritin levels have currently only been studied in one non-blinded, non-randomized pilot  
45  
46  
47 110 study<sup>31</sup>. While many studies have shown beneficial effects of iron supplementation on iron  
48  
49 111 store recovery time after whole blood donation, the optimal intake frequency and dosages are  
50  
51 112 still unknown<sup>13,32-34</sup>. Similarly, the influence of dietary status and treatment adherence on  
52  
53 113 post-donation iron store recovery using iron supplements has not been thoroughly assessed.

1  
2  
3 114 This study aims to determine the effect of iron supplementation on hemoglobin and ferritin  
4  
5 115 levels, side effects, donor return, and iron deficiency-related symptoms in whole blood donors  
6  
7 116 with low ferritin levels, thereby comparing varying intake frequencies and iron dosages.  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

For peer review only



## 117 **Methods and analysis**

### 118 ***Setting***

119 Sanquin Blood Bank is a non-profit organization with a legal duty to collect, process, and  
120 provide blood products throughout the Netherlands<sup>35</sup>. Before every blood donation, the  
121 donor's eligibility to donate is assessed using a donor health questionnaire (DHQ)<sup>36</sup>. Donors  
122 should be in good health, aged between 18 and 79, and not at risk for any blood-borne  
123 infections. In accordance with European and international legislation, male and female whole  
124 blood donors with Hb  $\leq$  13.5 g/dL and Hb  $\leq$  12.5 g/dL (measured with the HemoCue 201,  
125 Angelholm, Sweden), respectively, are not eligible to donate for three months. Furthermore,  
126 routine ferritin measurements have been introduced since November 2017 for newly  
127 registered donors and at every 5<sup>th</sup> whole blood donation. Donors with ferritin levels  $\geq$ 15 and  
128  $\leq$ 30  $\mu$ g/L or  $<$ 15  $\mu$ g/L are deferred for 6 or 12 months, respectively.

### 129 ***Study population***

130 Whole blood donors who have successfully donated before, donate at a participating blood  
131 bank location, are fluent in Dutch, and whose ferritin levels are measured during their next  
132 donation are invited by email to participate in the study. Donors are excluded from  
133 participation when regularly taking iron supplementation within the three months before  
134 enrolment. During their next donation, baseline measurements are taken, and a questionnaire  
135 is sent to donors who have indicated to be willing to participate. Inclusion into the trial is based  
136 on the baseline ferritin levels. Donors with baseline ferritin levels of  $\leq$ 30  $\mu$ g/L are eligible for  
137 the trial; for the other donors, the study ends after their next donation (i.e., baseline visit). All  
138 exclusion criteria are presented in figure 1.

139  
140 *Figure 1 Flowchart of the donor inclusion for the baseline questionnaire and the randomized controlled trial.*

### 141 **Study design**

142 This is a 6-armed placebo-controlled double-blind, randomized controlled trial (RCT). Of the  
143 donors included at baseline, only those with ferritin levels  $\leq 30$   $\mu\text{g/L}$  are eligible for participation  
144 in the trial and are randomly allocated to one of the six trial arms. The arms vary in (1) intake  
145 frequency; high frequency, or daily supplementation (HF), versus low frequency, or alternate  
146 day supplementation (LF), and (2) dosage; high dose (60mg elemental iron) iron supplements  
147 (HD), versus low dose (30mg elemental iron) iron supplements (LD), versus placebo (P). The  
148 inclusion of donors is continued until each trial arm consists of two hundred donors. We aim  
149 for an equal distribution of male and female participants (*Figure 2*).

150 *Figure 2 Schematic diagram of the participant randomization. LF, low frequency supplementation (alternate day);*  
151 *HF, high frequency supplementation (daily); LD, low dose supplements (30mg elemental iron); HD, high dose*  
152 *supplements (60mg elemental iron); P, placebo supplements.*

### 152 **Study procedures**

153 Shortly before the baseline and follow-up visits, donors are asked to complete an online  
154 questionnaire through Castor EDC<sup>37</sup> (Castor Electronic Data Capture, the Netherlands) and are  
155 provided with a hyperlink to the Wageningen University & Research page for an iron-specific  
156 food frequency questionnaire (FFQ).

157 At baseline, donors will visit one of the selected donation centers for a regular whole blood  
158 donation. When donors meet all eligibility criteria to donate, blood samples are taken from  
159 the blood donation sampling pouch. The collected blood samples are sent to the Sanquin  
160 National Screening Laboratory in Amsterdam for analysis. At least twice per week, the donor  
161 database containing donor ferritin levels is examined, and participating donors with ferritin  
162 levels  $\leq 30$   $\mu\text{g/L}$  are selected. These donors are randomized and will receive iron or placebo  
163 supplements and additional study information through postal mail. Donors with ferritin levels  
164  $> 30$   $\mu\text{g/L}$  are informed that their ferritin levels are sufficient and, therefore, they are not eligible

1  
2  
3 165 to participate in the RCT. During the follow-up visits, blood samples are collected through  
4  
5 166 venipuncture.  
6  
7

### 8 167 **Intervention and timeline**

9  
10 168 For this study, ferrous bisglycinate iron supplements are used with capsules containing 0, 30,  
11  
12 169 or 60 mg of elemental iron. Participants are asked to adhere to the study product  
13  
14 170 supplementation protocol as strictly as possible for 56 days after the baseline visit. The  
15  
16 171 supplementation protocol instructs the participants to take the capsules at least three hours  
17  
18 172 before and after eating products that interfere with iron uptake (e.g., dairy, coffee, tea, and  
19  
20 173 soy). Therefore, participants are advised to take the capsules shortly before going to bed and  
21  
22 174 asked to refrain from taking any additional iron supplements.  
23  
24

25  
26 175 Follow-up visits will occur at 56, 122, and 182 days corresponding to the minimum donation  
27  
28 176 interval for men, the minimum donation interval for women, and the minimum ferritin-guided  
29  
30 177 deferral period in the Netherlands, respectively (*Figure 3*). The participants are asked to return  
31  
32 178 any unused capsules at the first follow-up visit to determine their treatment adherence.  
33  
34

35  
36 179 *Figure 3 Schematic diagram of the study timeline with t being the time since the baseline visit.*  
37  
38

### 39 180 **Sample size**

40  
41 181 Based on previous research performed by Kiss *et al.* and Waldvogel *et al.*, who used a similar  
42  
43 182 randomized trial design in smaller groups of whole blood donors, sample size calculations were  
44  
45 183 made for the primary outcome parameters, being ferritin and Hb, and the secondary outcome  
46  
47 184 parameters, being adverse events, mental health, and physical health (Table 2)<sup>18,38,39</sup>. For the  
48  
49 185 sample size calculation, we used the following formula:  
50  
51

$$52$$

$$53$$

$$54$$

$$55 \quad n_1 = \frac{(z_{\frac{1-\alpha}{2}} + z_{1-\beta})^2 * \sigma^2 * (r + 1)}{v^2 * r}$$

$$56$$

$$57$$

$$58$$

$$59$$

$$60$$

Here,  $n_1$  is the sample size of the intervention groups,  $r$  is the ratio between the control groups and the intervention groups,  $\sigma^2$  is the variance of the continuous variable,  $v$  describes the expected difference between the continuous variables between the intervention and control groups (Table 2),  $\frac{z_{1-\alpha}}{2}$  is the two-tailed alternative hypothesis with significance level  $\alpha = 0.05$  (1.96),  $z_{(1-\beta)}$  is the probability of rejecting the null hypothesis when it is one minus probability of type II error ( $\beta$ ) with a power of 0.90 (1.28).

It is expected that the chosen sample size is sufficient to reach statistical power for nearly all main outcome parameters (Table 2)<sup>39</sup>. For mental health, a lack of power is expected. However, indications of trends towards an effect might be observed, and data may be used in future meta-analyses to reach statistical significance. Furthermore, we expect to observe small differences in outcomes between low- and high-dose iron supplementation groups. To observe an expected difference ( $v$ ) of 4  $\mu\text{g/l}$  in ferritin and 2.5  $\text{g/l}$  in hemoglobin between the iron supplementation groups, at least 126 and 151 donors should be included in each intervention group, respectively. Therefore, to reduce the risk of underpowering the study and considering possible dropout, we will include 200 participants per intervention group. Moreover, this will allow us to perform subgroup analysis based on sex, treatment adherence, and dietary intake.

	Iron supplementation	Placebo	Required sample size	Sufficiency
<b>Hb</b>	13.4 (1.1) g/dL	12.0 (1.2) g/dL	19	Sufficient
<b>Ferritin</b>	28.0 (9.8) $\mu\text{g/L}$	12.9 (8.3) $\mu\text{g/L}$	14	Sufficient
<b>Adverse events*</b>	39.2%	15.5%	144	Sufficient
<b>Mental health**</b>	40.1 (4.8)	40.7 (4.8)	2016	Not sufficient
<b>Physical condition**</b>	54.8 (3.3)	52.4 (5.2)	60	Sufficient

\* Adverse events included gastrointestinal symptoms, dizziness, headache, acne, palpitations, and renal lithiasis.

\*\* The outcome parameters mental health and physical condition were based on construct scores from the SF-12 questionnaire.

## Recruitment

Donors will first be recruited for the baseline questionnaire and blood sample measurements.

The invitation is sent by email at least three weeks before the end of the donor's standard

1  
2  
3 207 donation interval or deferral period. Donors are provided with study information and an  
4  
5 208 example of the informed consent form (appendix A). The information folder contains  
6  
7 209 information about the study procedures and the rights of the participant. The invited donors  
8  
9  
10 210 are asked to respond to the invitation through email to indicate if they would like to participate  
11  
12 211 in the study before signing the informed consent form. During the blood donation visit (i.e.,  
13  
14 212 baseline), donors who have agreed to participate are asked to sign the written informed  
15  
16 213 consent form in a blood bank employee's presence. By signing the informed consent form,  
17  
18 214 donors officially agree to participate and confirm that their personal information and material  
19  
20 215 can be used for research purposes. Finally, the consent form is signed by the blood bank  
21  
22 216 employee to affirm being the study representative, after which the donor can continue with  
23  
24 217 the regular whole blood donation. Participation in this study is voluntary, and donors will not  
25  
26 218 receive any compensation besides travel expenses as part of regular Sanquin Blood Bank  
27  
28 219 policies.

31  
32  
33 220 We expect that fifty percent of the donors recruited for the baseline measurements have  
34  
35 221 ferritin levels  $\leq 30$   $\mu\text{g/L}$  and are eligible to participate in the trial<sup>3</sup>. Recruitment will continue  
36  
37 222 until 1200 donors have been included in the trial. Data on demographics such as sex, age,  
38  
39 223 donation history, and region are collected from the blood bank information system eProgesa  
40  
41 224 (MAK systems, Paris, France). We aim to include all participants within one year and expect to  
42  
43 225 finalize all follow-up visits at the end of 2022.

44  
45  
46 226 The trial is initiated at one blood collection center. Based on donor response- and inclusion  
47  
48 227 rates, additional centers are added. The additional centers are added based on their capacity  
49  
50 228 and ability to cope with the additional study-related workload, accessibility, the number of  
51  
52 229 regularly donating donors, and the availability of direct transport to the National Screening  
53  
54 230 Laboratory of Sanquin (NSS). Based on previous studies performed at Sanquin Research, we  
55  
56 231 expect a response rate between 50% and 75%<sup>40,41</sup>. The expected response rate corresponds  
57  
58  
59  
60

1  
2  
3 232 with the inclusion of 1614 to 2421 eligible donors for the RCT per year, based on recruitment  
4  
5 233 from two large Sanquin Blood Bank locations. In case of lower response rates, donors from  
6  
7 234 other locations will also be included.  
8  
9

### 10 235 ***Blinding and randomization***

11  
12 236 Participant randomization is done on the individual level using a block randomization method.  
13  
14 237 This procedure is realized through Castor EDC, with randomized block sizes set at 12 and 18.  
15  
16 238 Furthermore, the randomization is stratified by age (18-49 years versus 50 years and older)  
17  
18 239 and sex to account for menopausal effects in women, differences in ferritin levels between  
19  
20 240 men and women, and the impact of aging on iron absorption<sup>42-44</sup>. Supplements and  
21  
22 241 information are sent to the participants based on the number corresponding with the group  
23  
24 242 they are allocated to after randomization. The responsible researcher is blinded for which  
25  
26 243 group number corresponds with which study product. The 30mg iron, 60 mg iron, and placebo  
27  
28 244 capsules are identical in terms of appearance and weight to guarantee the blinding of the  
29  
30 245 participants and involved researchers. The participants will not be blinded for the varying  
31  
32 246 intake frequency.  
33  
34  
35  
36  
37

### 38 247 ***Data collection***

39 248 Data are collected at baseline and during three follow-up visits. The baseline questionnaire  
40  
41 249 consists of a combination of previously used or validated questionnaires. Participants are  
42  
43 250 characterized by social-economic status, medical background, physical fitness, menstrual  
44  
45 251 status, and smoking, as described in previous research<sup>41</sup>. To determine the effects of iron  
46  
47 252 supplementation, we will use the *36-Item Short-Form Health Survey (SF-36)*; to determine  
48  
49 253 baseline status and changes in general health<sup>45</sup>, the *International Physical Activity*  
50  
51 254 *Questionnaire Short Form (IPAQ)*; to assess changes in levels of physical activity<sup>46</sup>, the *Fatigue*  
52  
53 255 *Assessment Scale*; to assess changes in self-reported fatigue<sup>47</sup>, donation intention-specific  
54  
55 256 *Theory of Planned Behavior* questions; to determine if donors are more or less willing to donate  
56  
57  
58 257 as a consequence of iron supplementation<sup>48</sup>, and the *Gastrointestinal Symptom Rating Scale*  
59  
60

1  
2  
3 258 and the *Bristol Stool Chart*; to assess the gastrointestinal side effects potentially caused by iron  
4  
5 259 supplementation<sup>49</sup>. To determine any effects of iron supplementation on iron deficiency-  
6  
7 260 related symptoms, we use the *Cambridge Hopkins Restless Legs Syndrome Questionnaire*; to  
8  
9  
10 261 assess the presence of and changes in restless legs, the *PICA questionnaire*; to assess a  
11  
12 262 potential appetite for non-nutritive substances, and the *Cognitive Failure Questionnaire*; to  
13  
14 263 determine changes in cognitive function<sup>50-52</sup>.

15  
16 264 Questionnaires that will also determine possible confounding and effect modification are the  
17  
18 265 Treatment Adherence Questionnaire<sup>53</sup> to determine the participants' compliance with the  
19  
20 266 intake protocols and an iron specific Food Frequency Questionnaire (FFQ) to determine their  
21  
22 267 dietary iron, macro-, and micronutrient intake<sup>41,54,55</sup>. The FFQ allows us to assess whether or  
23  
24 268 not iron supplements are more effective for donors who have low dietary (heme) iron intake  
25  
26 269 and will only be completed at baseline and before the final follow-up visit. The follow-up visit  
27  
28 270 questionnaires are similar to the baseline questionnaire, excluding the questions related to  
29  
30 271 demographic characteristics and the addition of the adherence questionnaire for the first  
31  
32 272 follow-up measurement. Furthermore, participants are asked to use the MedApp (MedApp  
33  
34 273 Nederland B.V., Eindhoven, The Netherlands, <https://medapp.nl>) to assist with compliance  
35  
36 274 with the supplementation protocol and to accurately assess treatment adherence. The  
37  
38 275 MedApp will provide participants with daily or alternate daily notifications to remind them  
39  
40 276 about their capsule intake and to indicate if the intake protocol was successfully followed.  
41  
42  
43  
44  
45

46 277 At baseline and during follow-up, whole blood and serum samples are collected using 2ml, and  
47  
48 278 6ml coated EDTA (VACUETTE®, K3EDTA, Greiner Bio-one International GmbH, Austria) and  
49  
50 279 3.5ml and 5ml serum separating (VACUETTE®, Serum gel, Greiner Bio-one International GmbH,  
51  
52 280 Austria) tubes, respectively. Ferritin measurements (Architect Ci8200, Abbott Laboratories, IL,  
53  
54 281 USA), using serum samples, are performed routinely within 24 hours after the donation. The  
55  
56 282 Architect Ci8200 is calibrated yearly for ferritin measurements by the manufacturer (Abbott  
57  
58 283 Laboratories) and traceable to the the first WHO Human Liver Ferritin International Standard  
59  
60

284 (80/602). Furthermore, quality assurance assessments are performed daily by the laboratory  
 285 staff, using low (20 µg/L), medium (150 µg/L), and high (400 µg/L) ferritin quality controls,  
 286 provided by the manufacturer. When the daily quality assurance measurements do not meet  
 287 the predefined acceptance criteria , the Architect Ci8200 will be recalibrated by the  
 288 manufacturer (Abbott Laboratories). Quality management is in accordance with ISO 15189.  
 289 Complete blood count measurements, including Hb and red blood cell parameters (Advia  
 290 2120, Siemens Medical Solutions Diagnostics, Breda, the Netherlands), are performed within  
 291 24 hours after the whole blood samples are taken. DNA is isolated using 400 µl buffy coat from  
 292 EDTA- whole bloodsamples (QIASymphony® DSP DNA Mini Kit, Qiagen GmbH, Hilden,  
 293 Germany)<sup>56</sup> and stored at -20°C, for later use. Additional processed and aliquoted plasma and  
 294 serum samples are collected from the EDTA- and serum separating tubes, respectively. The  
 295 additional samples will be stored at -80°C and used for potential post-study measurements of  
 296 parameters which might affect the iron hemeostasis (e.g., inflammatory markers).

### 297 **Statistical analysis**

298 Descriptive statistics are presented for intervention and control groups and for men and  
 299 women separately as means ± standard deviation for normally distributed data and median  
 300 and interquartile range in case of a skewed distribution. For the analysis of the primary study  
 301 parameters, the following multiple regression model is used if all assumptions for the model  
 302 are fulfilled:

$$\begin{aligned}
 \text{Effect} &= \text{Intercept} + \beta_1 * \text{Dose}_1 + \beta_2 * \text{Dose}_2 + \beta_3 * \text{Frequency} + \beta_4 * \text{Dose}_1\text{Frequency} \\
 &+ \beta_5 * \text{Dose}_2\text{Frequency} + e
 \end{aligned}$$

304 Here, *Intercept* represents the expected mean value when all explanatory variables have a  
 305 value of 0, *Dose<sub>1</sub>* the low dose iron capsules (30mg), *Dose<sub>2</sub>* the high dose iron capsules (60mg),  
 306 both dummy variables have 0mg as a reference, *frequency* the every other day versus daily  
 307 intake, and *e* the error term or difference between observed and expected values. The  
 308 assessed assumptions are the normality distributed random error term and the assumption of



1  
2  
3 309 the equality of variance. All the analyses will consist of two-sided tests, with a p-value <0.05  
4  
5 310 considered statistically significant. Statistical data analyses are performed with SPSS (IBM®  
6  
7 311 SPSS® Statistics 23.0 or newer) and R (R Core Team, Windows)<sup>57</sup>.

9  
10 312 Potential effect modification by age, BMI, donation history, dietary intake (e.g., heme- and  
11  
12 313 non-heme iron, dairy products, alcohol, tea, coffee, and total energy), and menstrual status  
13  
14 314 are examined. These potential effect modifiers have been selected due to their role in iron  
15  
16 315 hemoestasis<sup>58,59</sup>. We will test for effect modification by adding the variable of interest and an  
17  
18 316 interaction term with the iron different iron dosages and intake frequency to the model. A  
19  
20 317 variable is treated as an effect modifier when it has an interaction term with a p-value <0.10  
21  
22 318 for more than half of the associations. When effect modification is observed, stratified results  
23  
24 319 will be reported in addition to the overall results. The following potential confounders are  
25  
26 320 examined; age, sex, BMI, donation history, menstrual status, smoking, season, compliance with  
27  
28 321 the study products, social-economic status, ethnicity, recent infections (including Sars-CoV-2),  
29  
30 322 and baseline ferritin and Hb levels. A change of more than 10% in the regression coefficient is  
31  
32 323 considered confounding, and variables are added to all the models.

### 38 324 **Monitoring**

39  
40 325 Study monitoring is performed by the TAPAS Group, an independent monitoring bureau. The  
41  
42 326 FORTE project has been labeled as a negligible risk study by the involved medical ethics  
43  
44 327 committee based on a risk assessment. Study monitoring will consist of an initiation visit, two  
45  
46 328 monitoring visits, and a close-out visit.

### 50 329 **Patient and public involvement**

51  
52 330 Donors, as well as non-donors, are involved in the FORTE research project through focus group  
53  
54 331 interviews. The focus group interviews will consist of interactive group discussions involving  
55  
56 332 frequent donors; donors who have donated at least five times, new donors; donors who have  
57  
58 333 signed up for donation but have not yet donated, and blood bank staff, including donor  
59  
60

1  
2  
3 334 physicians. During the focus group interviews, the participants are asked to discuss their  
4  
5 335 perceptions, opinions, and attitude towards iron supplementation, current or potential  
6  
7 336 alternative blood bank policies regarding iron management, and any potential effects of these  
8  
9  
10 337 aspects on their willingness to donate. Based on the outcomes of the focus group interviews,  
11  
12 338 we will design a questionnaire to quantify the findings from the focus group interviews. The  
13  
14 339 questionnaire is developed based on the recurrent constructs and topics observed during the  
15  
16 340 group discussions and distributed amongst a larger group of donors and new donors. The  
17  
18 341 results from the focus group interviews and the questionnaire are used to determine the  
19  
20  
21 342 optimal approach and potential areas of concern for implementing iron supplementation as a  
22  
23 343 blood bank policy, if shown effective based on the trial's outcome.  
24  
25  
26 344  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

## 345 ETHICS AND DISSEMINATION

### 346 Ethical considerations

347 This study is performed according to the Declaration of Helsinki and Good Clinical Practice  
348 guidelines. The Medical Research Ethics Committee (METC) of the Academic Medical Centre  
349 (AMC) Amsterdam has approved the study protocol (trial ID NL8590). All participants are asked  
350 to provide their written informed consent and are informed that they can discontinue  
351 participation at any time. Data collection is compliant with the General Data Protection  
352 Regulation (GDPR), and all data are pseudonymized to prevent the identification of study  
353 participants. To ensure the donors' safety, invasive actions such as venipuncture are  
354 performed by trained blood bank employees, following the routine blood donation protocols  
355 of Sanquin Blood Bank, the Netherlands, whenever applicable.

### 356 Dissemination

357 Study results are published in peer-reviewed journals after evaluation of scientific relevance  
358 and quality by the involved researchers. Furthermore, the results are presented at  
359 (inter)national conferences, shared with study participants, and communicated with donors  
360 and different Sanquin departments. Data that can lead to the identification of the participants  
361 will not be published.

362

363

## 364 **SIGNIFICANCE AND OUTLOOK**

365 This study's outcomes will provide evidence to lead the debate if and how iron  
366 supplementation should be implemented to support iron repletion for whole blood donors  
367 with low ferritin levels. Blood donation and adequate blood availability are of great  
368 importance to guarantee patients' health in need of blood transfusions. However, frequent  
369 blood donation increases the risk of iron deficiency in repeat whole blood donors. To ensure  
370 sufficient availability of blood and blood products and safeguard donor health, the donors' iron  
371 status should be monitored and managed appropriately. However, international uniformity  
372 amongst blood services regarding policies to address the donors' iron stores is lacking.  
373 Therefore, the FORTE study aims to provide new insights regarding the efficacy of iron  
374 supplementation for whole blood donors with low ferritin levels. We do this by comparing the  
375 effectiveness of high and low-dose iron capsules versus placebo for daily and alternate-day  
376 supplementation protocols in whole blood donors, thereby investigating laboratory and  
377 health-related outcomes. The thorough characterization of participating donors will add to an  
378 enhanced understanding of the effectiveness of iron supplements under various  
379 circumstances.

380

381

1  
2  
3 382 **Author contributions**  
4  
5

6 383 J.K., K.v.d.H., M.S. designed the study protocol. J.K. wrote the manuscript with input from  
7  
8 384 K.v.d.H., F.Q., M.S., D.S., and V.N., H.Z. and H.W. were consulted for medical and ethical input.  
9

10  
11 385 **Funding statement**  
12  
13

14 386 Sanquin supported this research project: Product and Process Development Cellular Products  
15  
16 387 Grant, project id: PPOC19-02/1-239.  
17  
18

19 388 **Competing interests statement**  
20  
21

22 389 The authors declare that they have no competing interests.  
23  
24

25 390 **Corresponding author**  
26  
27

28 391 Correspondence to Jan Karregat.  
29  
30

31 392 **Word count**  
32  
33

34 393  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

## References

1. Simon TL, Garry PJ, Hooper EM. Iron stores in blood donors. *JAMA*. 1981;245(20):2038-2043.
2. Finch CA, Cook JD, Labbe RF, Culala M. Effect of blood donation on iron stores as evaluated by serum ferritin. *Blood*. 1977;50(3):441-447.
3. Vinkenog M, van den Hurk K, van Kraaij M, van Leeuwen M, Janssen MP. First results of a ferritin-based blood donor deferral policy in the Netherlands. *Transfusion*. n/a(n/a).
4. Greig AJ, Patterson AJ, Collins CE, Chalmers KA. Iron deficiency, cognition, mental health and fatigue in women of childbearing age: a systematic review. *J Nutr Sci*. 2013;2:e14-e14.
5. Houston BL, Hurrie D, Graham J, et al. Efficacy of iron supplementation on fatigue and physical capacity in non-anaemic iron-deficient adults: a systematic review of randomised controlled trials. *BMJ open*. 2018;8(4):e019240-e019240.
6. Avni T, Reich S, Lev N, Gafter-Gvili A. Iron supplementation for restless legs syndrome - A systematic review and meta-analysis. *Eur J Intern Med*. 2019;63:34-41.
7. Pasricha SR, Low M, Thompson J, Farrell A, De-Regil LM. Iron supplementation benefits physical performance in women of reproductive age: a systematic review and meta-analysis. *J Nutr*. 2014;144(6):906-914.
8. Miao D, Young SL, Golden CD. A meta-analysis of pica and micronutrient status. *Am J Hum Biol*. 2015;27(1):84-93.
9. (CD-P-TS) ECoBT. *Guide to the preparation, use and quality assurance of blood components. Recommendation no. R(95)15*. 16th ed: Council of Europe Publishing; 2020.
10. Cable RG. Hemoglobin determination in blood donors. *Transfus Med Rev*. 1995;9(2):131-144.
11. Custer B, Chinn A, Hirschler NV, Busch MP, Murphy EL. The consequences of temporary deferral on future whole blood donation. *Transfusion*. 2007;47(8):1514-1523.
12. Halperin D, Baetens J, Newman B. The effect of short-term, temporary deferral on future blood donation. *Transfusion*. 1998;38(2):181-183.
13. Cable RG, Brambilla D, Glynn SA, et al. Effect of iron supplementation on iron stores and total body iron after whole blood donation. *Transfusion*. 2016;56(8):2005-2012.
14. Bialkowski W, Kiss JE, Wright DJ, et al. Estimates of total body iron indicate 19 mg and 38 mg oral iron are equivalent for the mitigation of iron deficiency in individuals experiencing repeated phlebotomy. (1096-8652 (Electronic)).
15. Magnussen K, Bork N, Asmussen L. The effect of a standardized protocol for iron supplementation to blood donors low in hemoglobin concentration. *Transfusion*. 2008;48(4):749-754.
16. Group AAHDW. AABB donor iron deficiency risk-based decision-making assessment report supplemental material. 2018.
17. Szczepiorkowski Z. Updated Strategies to Limit or Prevent Iron Deficiency in Blood Donors. In: Members A, ed. aabb.org: American Association of Blood Banks; 2017.
18. Kiss JE, Brambilla D, Glynn SA, et al. Oral iron supplementation after blood donation: a randomized clinical trial. *Jama*. 2015;313(6):575-583.
19. Milman N, Jønsson L, Dyre P, Pedersen PL, Larsen LG. Ferrous bisglycinate 25 mg iron is as effective as ferrous sulfate 50 mg iron in the prophylaxis of iron deficiency and anemia during pregnancy in a randomized trial. *J Perinat Med*. 2014;42(2):197-206.
20. Bovell-Benjamin AC, Viteri FE, Allen LH. Iron absorption from ferrous bisglycinate and ferric trisglycinate in whole maize is regulated by iron status. *Am J Clin Nutr*. 2000;71(6):1563-1569.
21. Szarfarc SC, de Cassana LM, Fujimori E, Guerra-Shinohara EM, de Oliveira IM. Relative effectiveness of iron bis-glycinate chelate (Ferrochel) and ferrous sulfate in the control of iron deficiency in pregnant women. *Arch Latinoam Nutr*. 2001;51(1 Suppl 1):42-47.
22. Layrisse M, García-Casal MaN, Solano L, et al. Iron Bioavailability in Humans from Breakfasts Enriched with Iron Bis-Glycine Chelate, Phytates and Polyphenols. *The Journal of Nutrition*. 2000;130(9):2195-2199.
23. Ashmead HD. The absorption and metabolism of iron amino acid chelate. *Arch Latinoam Nutr*. 2001;51(1 Suppl 1):13-21.
24. Duque X, Martinez H, Vilchis-Gil J, et al. Effect of supplementation with ferrous sulfate or iron bis-glycinate chelate on ferritin concentration in Mexican schoolchildren: a randomized controlled trial. *Nutr J*. 2014;13:71.
25. Vuk T, Magnussen K, De Kort W, et al. International forum: an investigation of iron status in blood donors. *Blood transfusion = Trasfusione del sangue*. 2017;15(1):20-41.
26. Magnussen K, Bork N, Asmussen L. The effect of a standardized protocol for iron supplementation to blood donors low in hemoglobin concentration. *Transfusion*. 2008.
27. Ganz T, Nemeth E. Hepcidin and iron homeostasis. *Biochim Biophys Acta*. 2012;1823(9):1434-1443.
28. Moretti D, Goede JS, Zeder C, et al. Oral iron supplements increase hepcidin and decrease iron absorption from daily or twice-daily doses in iron-depleted young women. *Blood*. 2015;126(17):1981-1989.
29. Stoffel NU, Cercamondi CI, Brittenham G, et al. Iron absorption from oral iron supplements given on consecutive versus alternate days and as single morning doses versus twice-daily split dosing in iron-

- 1  
2  
3 459 depleted women: two open-label, randomised controlled trials. *Lancet Haematol.* 2017;4(11):e524-  
4 460 e533.
- 5 461 30. Cable RG, Birch RJ, Spencer BR, et al. The operational implications of donor behaviors following  
6 462 enrollment in STRIDE (Strategies to Reduce Iron Deficiency in blood donors). *Transfusion.*  
7 463 2017;57(10):2440-2448.
- 8 464 31. Pittori C, Buser A, Gasser UE, et al. A pilot iron substitution programme in female blood donors with  
9 465 iron deficiency without anaemia. *Vox Sang.* 2011;100(3):303-311.
- 10 466 32. Pasricha SR, Marks DC, Salvin H, et al. Postdonation iron replacement for maintaining iron stores in  
11 467 female whole blood donors in routine donor practice: results of two feasibility studies in Australia.  
12 468 *Transfusion.* 2017;57(8):1922-1929.
- 13 469 33. Mirrezaei SM, Parsi R, Askarian M. Low Dose, Short-Term Iron Supplementation in Female Blood Donors  
14 470 of Childbearing Age: a Randomized, Double-Masked, Placebo-Controlled Study. *Iranian Journal of*  
15 471 *Medical Sciences.* 2008;33.
- 16 472 34. Radtke H, Tegtmeier J, Rocker L, Salama A, Kiesewetter H. Daily doses of 20 mg of elemental iron  
17 473 compensate for iron loss in regular blood donors: a randomized, double-blind, placebo-controlled study.  
18 474 *Transfusion.* 2004;44(10):1427-1432.
- 19 475 35. Wet inzake bloedvoorziening. 1997.
- 20 476 36. de Kort W, Prinsze F, Nuboer G, Twisk J, Merz EM. Deferral rate variability in blood donor eligibility  
21 477 assessment. *Transfusion.* 2019;59(1):242-249.
- 22 478 37. Castor EDC. Castor Electronic Data Capture. 2019; <https://castoredc.com>. Accessed August 28, 2019.
- 23 479 38. Smith GA, Fisher SA, Doree C, Di Angelantonio E, Roberts DJ. Oral or parenteral iron supplementation  
24 480 to reduce deferral, iron deficiency and/or anaemia in blood donors. *Cochrane Database Syst Rev.*  
25 481 2014(7):CD009532.
- 26 482 39. Waldvogel S, Pedrazzini B, Vaucher P, et al. Clinical evaluation of iron treatment efficiency among non-  
27 483 anemic but iron-deficient female blood donors: a randomized controlled trial. *BMC Med.* 2012;10:8.
- 28 484 40. van den Hurk K, Merz E-M, Prinsze F, et al. *Low awareness of past SARS-CoV-2 infection in healthy*  
29 485 *adults.* 2020.
- 30 486 41. Timmer TC, de Groot R, Habets K, et al. Donor InSight: characteristics and representativeness of a Dutch  
31 487 cohort study on blood and plasma donors. *Vox Sang.* 2018.
- 32 488 42. Vanasse GJ, Berliner N. Anemia in Elderly Patients: An Emerging Problem for the 21st Century.  
33 489 *Hematology.* 2010;2010(1):271-275.
- 34 490 43. Busti F, Camprostrini N, Martinelli N, Girelli D. Iron deficiency in the elderly population, revisited in the  
35 491 hepcidin era. *Front Pharmacol.* 2014;5:83-83.
- 36 492 44. den Elzen WPJ, de Craen AJM, Wiegerinck ET, Westendorp RGJ, Swinkels DW, Gussekloo J. Plasma  
37 493 hepcidin levels and anemia in old age. The Leiden 85-Plus Study. *Haematologica.* 2013;98(3):448-454.
- 38 494 45. Brazier JE, Harper R Fau - Jones NM, Jones Nm Fau - O'Cathain A, et al. Validating the SF-36 health  
39 495 survey questionnaire: new outcome measure for primary care. (0959-8138 (Print)).
- 40 496 46. Lee PH, Macfarlane DJ, Lam TH, Stewart SM. Validity of the international physical activity questionnaire  
41 497 short form (IPAQ-SF): A systematic review. *International Journal of Behavioral Nutrition and Physical*  
42 498 *Activity.* 2011;8(1):115.
- 43 499 47. Michielsen HJ, De Vries J, Van Heck GL. Psychometric qualities of a brief self-rated fatigue measure:  
44 500 The Fatigue Assessment Scale. *J Psychosom Res.* 2003;54(4):345-352.
- 45 501 48. Giles M, McClenahan C, Cairns E, Mallet J. An application of the Theory of Planned Behaviour to blood  
46 502 donation: the importance of self-efficacy. *Health Education Research.* 2004;19(4):380-391.
- 47 503 49. Revicki DA, Wood M, Wiklund I, Crawley J. Reliability and validity of the Gastrointestinal Symptom  
48 504 Rating Scale in patients with gastroesophageal reflux disease. *Quality of life research : an international*  
49 505 *journal of quality of life aspects of treatment, care and rehabilitation.* 1998;7(1):75-83.
- 50 506 50. Allen RP, Burchell B Fau - MacDonald B, MacDonald B Fau - Hening WA, Hening Wa Fau - Earley CJ,  
51 507 Earley CJ. Validation of the self-completed Cambridge-Hopkins questionnaire (CH-RLSq) for  
52 508 ascertainment of restless legs syndrome (RLS) in a population survey. (1878-5506 (Electronic)).
- 53 509 51. Walters AS, Frauscher B, Allen R, et al. Review of diagnostic instruments for the restless legs  
54 510 syndrome/Willis-Ekbom Disease (RLS/WED): critique and recommendations. *Journal of clinical sleep*  
55 511 *medicine : JCSM : official publication of the American Academy of Sleep Medicine.* 2014;10(12):1343-  
56 512 1349.
- 57 513 52. Broadbent DE, Cooper PF, FitzGerald P, Parkes KR. The Cognitive Failures Questionnaire (CFQ) and its  
58 514 correlates. *The British journal of clinical psychology.* 1982;21(1):1-16.
- 59 515 53. Sidorkiewicz S, Tran VT, Cousyn C, Perrodeau E, Ravaud P. Development and validation of an instrument  
60 516 to assess treatment adherence for each individual drug taken by a patient. *BMJ Open.*  
517 2016;6(5):e010510.
- 518 54. Streppel MT, de Vries JH, Meijboom S, et al. Relative validity of the food frequency questionnaire used  
519 55 to assess dietary intake in the Leiden Longevity Study. *Nutr J.* 2013;12:75.
- 520 55. Timmer TC, de Groot R, Rijnhart JJM, et al. Dietary intake of heme iron is associated with ferritin and  
521 56 hemoglobin levels in Dutch blood donors: results from Donor InSight. *Haematologica.* 2019.
- 522 56. QIAsymphony DSP DNA Instructions for Use (Handbook)2015.
- 523 57. R Core Team (2013). R: A language and environment for statistical  
524 computing. R Foundation for Statistical Computing, Vienna, Austria.

1  
2  
3 525 URL <http://www.R-project.org/>.

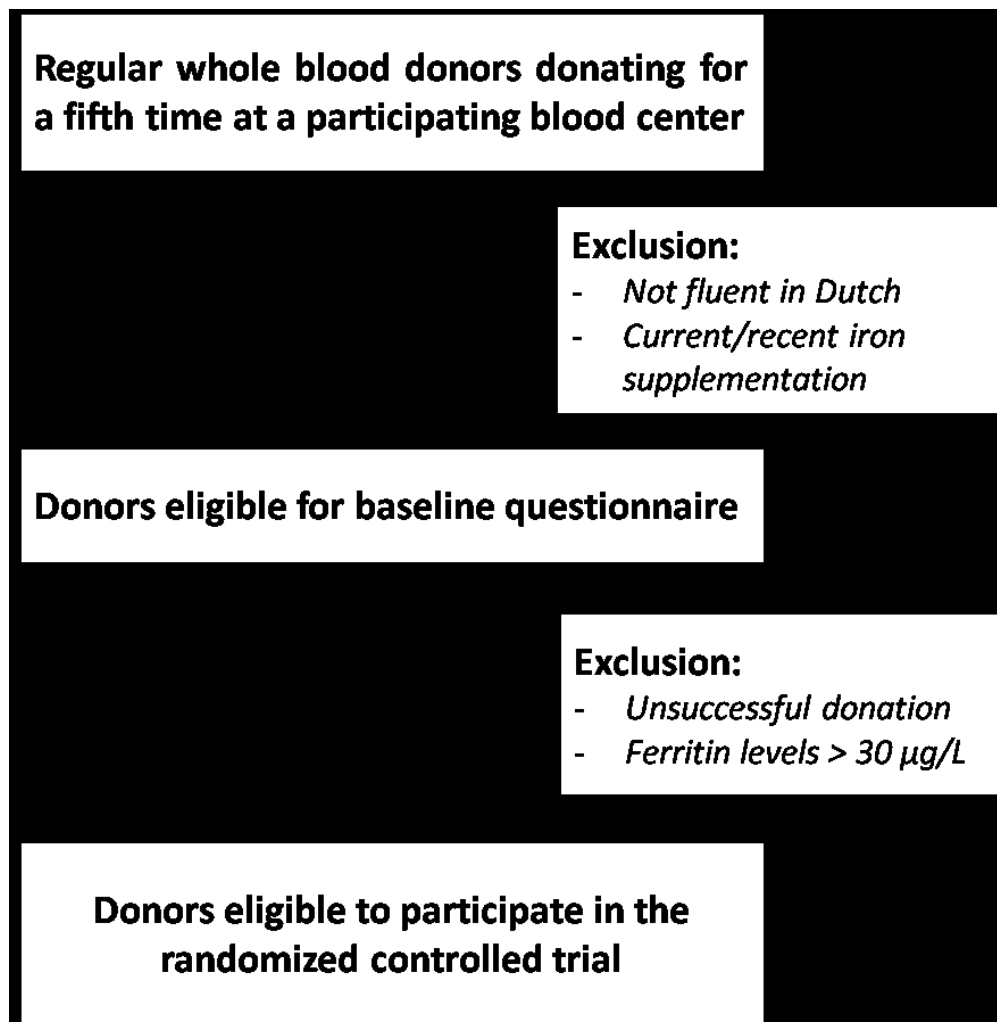
4 526 58. Yanoff LB, Menzie CM, Bi D, et al. Inflammation and iron deficiency in the hypoferremia of obesity.  
5 527 *International journal of obesity (2005)*. 2007;31:1412-1419.

6 528 59. Schotten N, Pasker-de Jong PC, Moretti D, et al. The donation interval of 56 days requires extension to  
7 529 180 days for whole blood donors to recover from changes in iron metabolism. *Blood*. 2016.

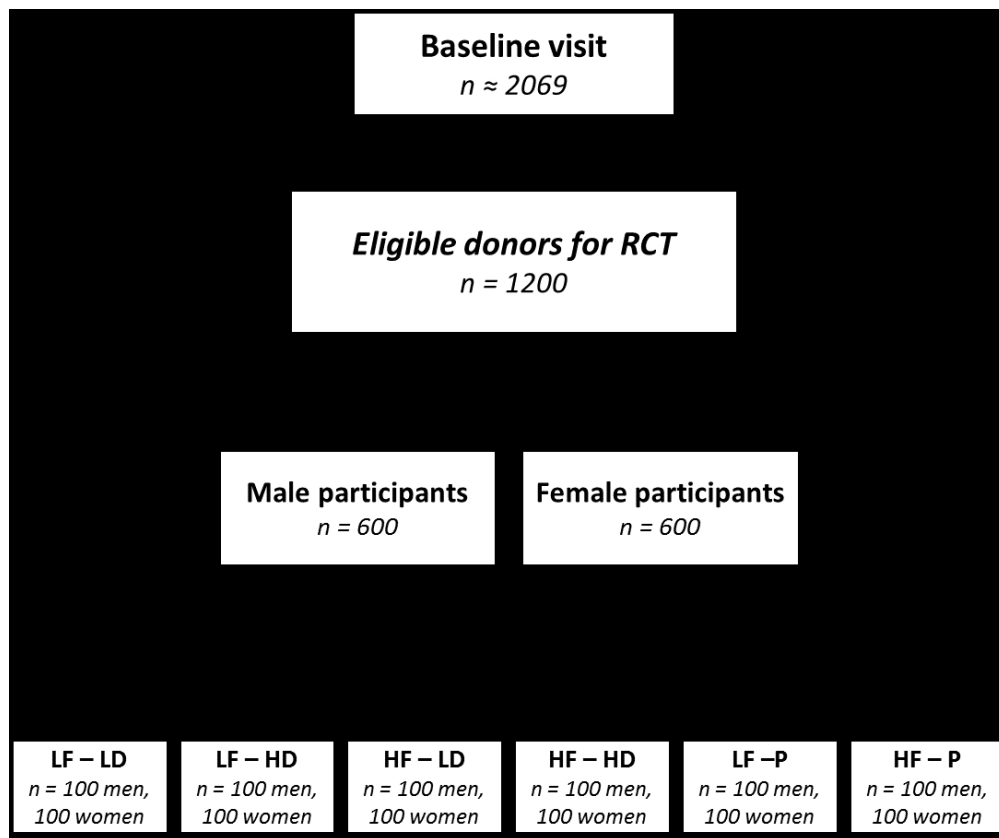
8 530  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

For peer review only





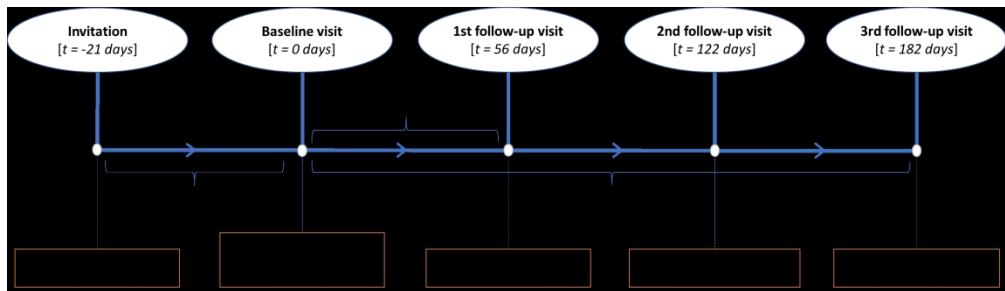
392x399mm (57 x 57 DPI)



467x389mm (59 x 59 DPI)

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60



1081x308mm (59 x 59 DPI)

## Toestemmingsformulier FORTE

### Onderzoek naar ijzersuppletie bij bloeddonors met een laag ferritine

- Ik heb de informatiebrief gelezen. Ook kon ik vragen stellen. Mijn vragen zijn voldoende beantwoord. Ik had genoeg tijd om te beslissen of ik meedoe.
- Ik weet dat meedoen vrijwillig is. Ook weet ik dat ik op ieder moment kan beslissen om toch niet mee te doen of te stoppen met het onderzoek. Daarvoor hoef ik geen reden te geven.
- Ik geef toestemming dat er informatie kan worden opgevraagd bij een medisch centrum, bij mijn huisarts, of het Centraal Bureau voor de Statistiek (CBS) over mijn gezondheid, een ziekenhuisopname, of overlijden tijdens of na afloop van het onderzoek.
- Ik geef toestemming voor het koppelen van mijn donatiegegevens aan de onderzoeksgegevens.
- Ik geef toestemming dat mijn gegevens en lichaamsmateriaal worden verzameld, gebruikt, en bewaard voor de beantwoording van de onderzoeksvraag in dit onderzoek.
- Ik weet dat voor de controle van het onderzoek sommige mensen toegang tot al mijn gegevens kunnen krijgen. Deze mensen staan vermeld in deze informatiebrief. Ik geef toestemming voor inzage door deze personen.
- Ik geef toestemming dat Sanquin mijn gegevens en lichaamsmateriaal mag bewaren tot 15 jaar na het onderzoek.
- Ik geef toestemming dat mijn gecodeerde gegevens en lichaamsmaterialen mogen worden gedeeld met andere binnen- en buitenlandse laboratoria en onderzoekers voor doelen zoals beschreven in de informatiebrief.
- ❖ Ik geef  wel  geen toestemming om DNA uit mijn bloed te halen en dit te bewaren en te gebruiken voor dit onderzoek.
- ❖ Ik geef  wel  geen toestemming om mij opnieuw te benaderen voor een mogelijk vervolgonderzoek.
- ❖ Ik geef  wel  geen toestemming voor nog 15 jaar extra opslag, na de 15 jaar waarin het lichaamsmateriaal, DNA en de onderzoeksgegevens reeds in het kader van deze studie worden bewaard, in totaal dus 30 jaar.
- ❖ Ik wil deelnemen aan het onderzoek.

1  
2  
3 Naam proefpersoon:.....

4  
5 Donornummer: .....

6  
7 Handtekening:..... Datum: \_\_ / \_\_ / \_\_

8  
9 -----  
10  
11 Ik verklaar dat ik deze proefpersoon volledig heb geïnformeerd over het genoemde onderzoek.

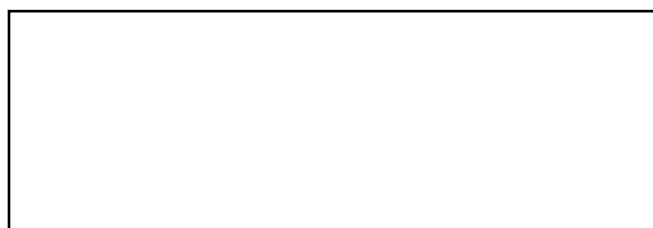
12  
13  
14  
15 Als er tijdens het onderzoek informatie bekend wordt die de toestemming van de proefpersoon zou  
16 kunnen beïnvloeden, dan breng ik hem/haar daarvan tijdig op de hoogte.

17  
18  
19  
20 Naam onderzoeker (of diens vertegenwoordiger): .....

21  
22 Handtekening:..... Datum: \_\_ / \_\_ / \_\_

23  
24 -----  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50

51 EIN-sticker:



For peer review only



STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents\*

Section/item	Item No	Description	Addressed on page number
<b>Administrative information</b>			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	___ 1 ___
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	___ 1 ___
	2b	All items from the World Health Organization Trial Registration Data Set	___ x ___
Protocol version	3	Date and version identifier	___ 1 ___
Funding	4	Sources and types of financial, material, and other support	___ 19 ___
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	___ 1 ___
	5b	Name and contact information for the trial sponsor	___ 19 ___
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	___ x ___
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	___ x ___

6/bmjopen-2021-056316 on 9 March 2022. Downloaded from <http://bmjopen.bmj.com/> on April 17, 2024 by guest. Protected by copyright.

1	<b>Introduction</b>			
2				
3	Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4
4				
5				
6		6b	Explanation for choice of comparators	5
7				
8	Objectives	7	Specific objectives or hypotheses	5
9				
10	Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	6
11				
12				
13				
14	<b>Methods: Participants, interventions, and outcomes</b>			
15				
16	Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	7
17				
18				
19	Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	7
20				
21				
22	Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	8-10
23				
24		11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	17
25				
26		11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	9, 13
27				
28		11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	7
29				
30	Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	9
31				
32				
33				
34	Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	9
35				
36				
37				
38				
39				
40				
41				
42				
43				
44				
45				
46				

1	Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	_____ 9-10 _____
2				
3				
4	Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	_____ 12 _____
5				

**Methods: Assignment of interventions (for controlled trials)**

Allocation:

10	Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	_____ 12 _____
11				
12				
13				
14				
15				
16	Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	_____ 12 _____
17				
18				
19				
20				
21	Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	_____ 12 _____
22				
23				
24	Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	_____ 12 _____
25				
26				
27		17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	_____ x _____
28				
29				
30				

**Methods: Data collection, management, and analysis**

33	Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	_____ 12-14 _____
34				
35				
36				
37				
38				
39		18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	_____ 13 _____
40				
41				
42				

http://bmjopen.bmj.com/ on April 17, 2024 by guest. Protected by copyright.



6/bmjopen-2022-01056316 on 9 March 2023. Downloaded from <http://bmjopen.bmj.com/> on April 17, 2024 by guest. Protected by copyright.

1	Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	_____ 8 _____
2				
3				
4				
5	Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	_____ 14-15 _____
6				
7				
8		20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	_____ 14-15 _____
9				
10		20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	_____ x _____
11				
12				
13				
14	<b>Methods: Monitoring</b>			
15				
16	Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	_____ 15 _____
17				
18				
19				
20				
21				
22		21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	_____ 15 _____
23				
24				
25	Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	_____ x _____
26				
27				
28	Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	_____ 15 _____
29				
30				
31				
32	<b>Ethics and dissemination</b>			
33				
34	Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	_____ 17 _____
35				
36				
37	Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	_____ 17 _____
38				
39				
40				
41				
42				
43				
44				
45				
46				

1	Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	_____ 17 _____
2				
3				
4		26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	_____ 17 _____
5				
6				
7	Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	_____ 17 _____
8				
9				
10	Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	_____ 19 _____
11				
12				
13	Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	_____ 17 _____
14				
15				
16	Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	_____ x _____
17				
18				
19				
20	Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	_____ 17 _____
21				
22				
23				
24		31b	Authorship eligibility guidelines and any intended use of professional writers	_____ 17 _____
25				
26		31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	_____ x _____
27				
28				
29	<b>Appendices</b>			
30				
31	Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	_____ A _____
32				
33				
34	Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	_____ x _____
35				
36				

\*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons “Attribution-NonCommercial-NoDerivs 3.0 Unported” license.

6/bmjopen-2021-056356 on 9 March 2022. Downloaded from <http://bmjopen.bmj.com/> on April 17, 2022 by guest. Protected by copyright.

# BMJ Open

## Ferritin-guided iron supplementation in whole blood donors: Optimal dosage, donor Response, return, and Efficacy (FORTE) – a randomized controlled trial protocol

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2021-056316.R1
Article Type:	Protocol
Date Submitted by the Author:	14-Jan-2022
Complete List of Authors:	Karregat, Jan; Sanquin Research, Department of Donor Medicine Research Sweegers, Maike G; Sanquin Research, Department of Donor Medicine Research Quee, Franke A; Sanquin Research, Department of Donor Medicine Research Weekamp, Henriëtte H; Sanquin Blood Supply Foundation, Medical Donor Affairs Swinkels, Dorine W; Radboud University Nijmegen, Department of Laboratory Medicine; Sanquin Blood Supply Foundation, Center for Iron Disorders Sanquin Novotny, Věra M J; Sanquin Blood Supply Foundation, Department of Transfusion Medicine Zaaijer, Hans L; Sanquin Research, Department of Donor Medicine Research; Amsterdam UMC Location AMC, Department of Clinical Virology van den Hurk, Katja; Sanquin Research, Department of Donor Medicine Research
<b>Primary Subject Heading</b>:	Haematology (incl blood transfusion)
Secondary Subject Heading:	Epidemiology, Public health
Keywords:	Blood bank & transfusion medicine < HAEMATOLOGY, Anaemia < HAEMATOLOGY, Clinical trials < THERAPEUTICS

SCHOLARONE™  
Manuscripts

1  
2  
3 1 **Ferritin-guided iron supplementation in whole blood donors: Optimal dosage, donor Response,**  
4 **return, and Efficacy (FORTE) – a randomized controlled trial protocol**

5 2  
6  
7 3 Jan H M Karregat<sup>1</sup>, [j.karregat@sanquin.nl](mailto:j.karregat@sanquin.nl)

8  
9 4 Maïke G Sweegers<sup>1</sup>, [m.sweegers@sanquin.nl](mailto:m.sweegers@sanquin.nl)

10 5 Franke A Quee<sup>1</sup>, [f.quee@sanquin.nl](mailto:f.quee@sanquin.nl)

11 6 Henriëtte H Weekamp<sup>2</sup>, [h.weekamp@sanquin.nl](mailto:h.weekamp@sanquin.nl)

12 7 Dorine W Swinkels<sup>3,4</sup>, [Dorine.Swinkels@Radboudumc.nl](mailto:Dorine.Swinkels@Radboudumc.nl)

13 8 Věra M J Novotny<sup>5</sup>, [v.novotny@sanquin.nl](mailto:v.novotny@sanquin.nl)

14 9 Hans L. Zaaijer<sup>1,6</sup>, [h.zaaijer@sanquin.nl](mailto:h.zaaijer@sanquin.nl)

15 10 Katja van den Hurk<sup>1</sup>, [k.vandenhurk@sanquin.nl](mailto:k.vandenhurk@sanquin.nl)

- 11 1. Department of Donor Medicine Research, Sanquin Research, Amsterdam, The  
12 Netherlands  
13 2. Medical Donor Affairs, Sanquin, Zwolle, The Netherlands  
14 3. Translational Metabolic Laboratory, Department of Laboratory Medicine, Radboud  
15 university medical center, Nijmegen, The Netherlands  
16 4. Center for Iron Disorders, Sanquin, Amsterdam, The Netherlands  
17 5. Department of Transfusion Medicine, Sanquin Blood Supply, Amsterdam, The  
18 Netherlands  
19 6. Department of Clinical Virology, Amsterdam UMC, location AMC, Amsterdam, The  
20 Netherlands

21 Protocol version 2, 13-01-2022

22 Word count: 3770

23  
24 **Corresponding author**

25 Jan Karregat, Donor Medicine Research, Sanquin Research, Plesmanlaan 125, 1066 CX  
26 Amsterdam, the Netherlands

27 E-mail: [j.karregat@sanquin.nl](mailto:j.karregat@sanquin.nl)

28 Telephone: (+31) 0650093376

1  
2  
3 **29 Abstract**  
4

5 30 *Background:* Frequent whole blood donors have an increased risk of developing iron  
6  
7  
8 31 deficiency. Iron deficiency can have detrimental health effects when left untreated. Donation  
9  
10 32 intervals are commonly too short to replenish iron stores, and extending these reduces donor  
11  
12 33 availability. Oral iron supplementation is known to shorten iron store recovery time but may  
13  
14 34 also induce gastrointestinal complaints. We aim to optimize the effectiveness of iron  
15  
16 35 supplements while minimizing the risks of side effects. Therefore, we will evaluate the impact  
17  
18 36 of different iron supplementation protocols in terms of dosage and frequency on ferritin and  
19  
20 37 hemoglobin levels, gastrointestinal side-effects, iron deficiency-related symptoms, and donor  
21  
22 38 return compared to placebo supplementation.  
23

24  
25 39 *Methods:* Twelve hundred whole blood donors with ferritin levels  $\leq 30$   $\mu\text{g/L}$  are included into a  
26  
27 40 double-blind, randomized controlled trial. Participants are randomly allocated to one of six  
28  
29 41 arms, administering capsules containing 0, 30, or 60 mg of iron, either on alternate days or  
30  
31 42 daily for 56 days. At baseline and 56, 122, and 182 days of follow-up, ferritin and hemoglobin  
32  
33 43 levels are measured, and compliance, donor return, dietary iron intake, gastrointestinal, iron  
34  
35 44 deficiency-related symptoms, and general health are assessed by questionnaire.  
36  
37

38  
39 45 *Ethics and dissemination:* This study will provide a comprehensive overview of the effects of  
40  
41 46 different frequencies and dosages of administration of iron supplements on iron status and  
42  
43 47 health effects, thereby considering individual differences in treatment adherence and lifestyle.  
44  
45 48 The outcome will provide scientific evidence to guide the debate if and how oral iron  
46  
47 49 supplements may support the recovery of whole blood donors with low ferritin levels.  
48  
49

50 50  
51  
52  
53 51  
54  
55  
56 52 *Trial registration:* The Dutch trial registry NL8590.  
57  
58  
59 53  
60

1  
2  
3 54 **Strengths and limitations of this study**  
4  
5

- 6 55 • This is a large (n = 1200), double-blind, randomized controlled trial to determine the  
7  
8 56 optimal iron supplementation protocol in terms of both intake frequency and dosage.  
9  
10  
11 57 • Outcome variables include ferritin and hemoglobin levels, complete blood counts, iron  
12  
13 58 deficiency-related symptoms, and gastrointestinal side effects.  
14  
15 59 • Participants are thoroughly characterized at baseline regarding social-economic  
16  
17 60 status, medical background, physical fitness, dietary intake, and smoking status.  
18  
19  
20 61 • A limitation is the limited number of follow-up visits; however, these time points are  
21  
22 62 the most relevant, and temporal patterns are modeled based on earlier studies.  
23  
24  
25  
26  
27 63  
28  
29 64  
30  
31 65  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

## 67 Introduction

68 Due to the hemoglobin (Hb)-bound iron loss during donation, regular whole blood donors are  
69 prone to developing iron deficiency, often in the absence of anemia<sup>1,2</sup>. Iron deficiency without  
70 anemia is characterized by reduced serum ferritin levels. It is highly prevalent amongst  
71 frequent whole blood donors, with 15.0% of the female and 9.4% of male Dutch donors having  
72 ferritin levels <15 µg/L<sup>3</sup>. Reduced ferritin levels increase the risk of developing anemia and are  
73 associated with iron deficiency-related symptoms, including fatigue, reduced exercise  
74 endurance, restless legs, PICA (appetite for non-nutritious substances), and reduced  
75 neurocognitive functioning<sup>4-8</sup>.

76 At most international blood banks, including the Netherlands, Hb levels are measured before  
77 donation to safeguard donor health and blood product quality<sup>9</sup>. However, Hb levels do not  
78 reflect the amount of stored iron, which is significantly impacted by whole blood donations<sup>10</sup>.  
79 Therefore, ferritin measurements have become more common amongst blood banks to assess  
80 the donor's iron storage. Sanquin incorporated ferritin-guided donation intervals, deferring  
81 donors for 6 or 12 months when ferritin levels are ≥15 and ≤30 µg/L or <15 µg/L, respectively.  
82 These cut-off values are based on WHO standards, as described previously<sup>3</sup>. However, donor  
83 deferral has been shown to demoralize donors and reduce donor return rates<sup>3,11,12</sup>.

84 Several studies have shown that oral iron supplementation reduces the post-donation  
85 recovery time of ferritin and Hb levels to pre-donation levels<sup>13,14</sup>. While donors in the countries  
86 like the United States, Finland, and Denmark are already advised about iron supplementation  
87 for iron storage recovery after donation, other blood services are hesitant, often due to ethical  
88 concerns<sup>15-17</sup>. Kiss *et al.* showed in a randomized controlled trial that post-donation iron  
89 supplementation led to full recovery of ferritin levels within 56 days, whereas the non-iron  
90 supplementing group did not reach full recovery after 160 days<sup>18</sup>.

1  
2  
3 91 In several clinical trials, the iron supplement ferrous bisglycinate has been shown to result in a  
4  
5 92 relatively high fractional iron uptake. This has been attributed to its iron-bound chelates that  
6  
7 93 prevent the iron from binding to other dietary compounds (e.g., tannins, catechols, and  
8  
9  
10 94 phytates) that inhibit iron absorption<sup>19-22</sup>. Furthermore, supplementation with ferrous  
11  
12 95 bisglycinate has been shown to lower the risk of gastrointestinal discomfort compared to other  
13  
14 96 iron formulations, possibly due to the increased uptake by intestinal mucosal cells, leading to  
15  
16 97 less iron entering the colon<sup>19,23,24</sup>. Therefore, ferrous bisglycinate is already used by the Danish  
17  
18 98 blood bank for donors who suffer from gastrointestinal side-effects<sup>25,26</sup>.

19  
20  
21 99 Iron absorption in the duodenum and its entry into the plasma compartment is regulated by  
22  
23  
24 100 the peptide hormone hepcidin<sup>27</sup>. It has been shown that iron supplementation leads to an  
25  
26 101 increased hepatic production of hepcidin in iron-depleted women, causing a reduced intestinal  
27  
28 102 iron uptake up to 24 hours post-supplementation<sup>28,29</sup>. While the dosages of elemental iron  
29  
30 103 used in previous studies range from 19 to 240 mg/day, lower dosed iron supplements are  
31  
32  
33 104 shown effective in recovering the iron stores post-donation<sup>28,30</sup>. These findings suggest that  
34  
35 105 alternate-day supplementation with low-dose ferrous bisglycinate capsules may lead to higher  
36  
37 106 fractional iron uptake and fewer side effects compared to high-dose iron supplements taken  
38  
39 107 daily or twice daily.

40  
41  
42 108 The effects of oral iron supplementation on iron deficiency-related symptoms in donors with  
43  
44 109 low ferritin levels have currently only been studied in one non-blinded, non-randomized pilot  
45  
46  
47 110 study<sup>31</sup>. While many studies have shown beneficial effects of iron supplementation on iron  
48  
49 111 store recovery time after whole blood donation, the optimal intake frequency and dosages are  
50  
51 112 still unknown<sup>13,32-34</sup>. Similarly, the influence of dietary status and treatment adherence on  
52  
53 113 post-donation iron store recovery using iron supplements has not been thoroughly assessed.



1  
2  
3 114 This study aims to determine the effect of iron supplementation on hemoglobin and ferritin  
4  
5 115 levels, side effects, donor return, and iron deficiency-related symptoms in whole blood donors  
6  
7 116 with low ferritin levels, thereby comparing varying intake frequencies and iron dosages.  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

For peer review only

## 117 **Methods and analysis**

### 118 ***Setting***

119 Sanquin Blood Bank is a non-profit organization with a legal duty to collect, process, and  
120 provide blood products throughout the Netherlands<sup>35</sup>. Before every blood donation, the  
121 donor's eligibility to donate is assessed using a donor health questionnaire (DHQ)<sup>36</sup>. Donors  
122 should be in good health, aged between 18 and 79, and not at risk for any blood-borne  
123 infections. In accordance with European and international legislation, male and female whole  
124 blood donors with Hb  $\leq$  13.5 g/dL and Hb  $\leq$  12.5 g/dL (measured with the HemoCue 201,  
125 Angelholm, Sweden), respectively, are not eligible to donate for three months. Furthermore,  
126 routine ferritin measurements have been introduced since November 2017 for newly  
127 registered donors and at every 5<sup>th</sup> whole blood donation. Donors with ferritin levels  $\geq$ 15 and  
128  $\leq$ 30  $\mu$ g/L or  $<$ 15  $\mu$ g/L are deferred for 6 or 12 months, respectively.

### 129 ***Study population***

130 Whole blood donors who have successfully donated before, donate at a participating blood  
131 bank location, are fluent in Dutch, and whose ferritin levels are measured during their next  
132 donation are invited by email to participate in the study. Donors are excluded from  
133 participation when regularly taking iron supplementation within the three months before  
134 enrolment. During their next donation, baseline measurements are taken, and a questionnaire  
135 is sent to donors who have indicated to be willing to participate. Inclusion into the trial is based  
136 on the baseline ferritin levels. Donors with baseline ferritin levels of  $\leq$ 30  $\mu$ g/L are eligible for  
137 the trial; for the other donors, the study ends after their next donation (i.e., baseline visit). All  
138 exclusion criteria are presented in figure 1.

139

140

1  
2  
3 141 **Study design**  
4

5 142 This is a 6-armed placebo-controlled double-blind, randomized controlled trial (RCT). Of the  
6  
7 143 donors included at baseline, only those with ferritin levels  $\leq 30$   $\mu\text{g/L}$  are eligible for participation  
8  
9  
10 144 in the trial and are randomly allocated to one of the six trial arms. The arms vary in (1) intake  
11  
12 145 frequency; high frequency, or daily supplementation (HF), versus low frequency, or alternate  
13  
14 146 day supplementation (LF), and (2) dosage; high dose (60mg elemental iron) iron supplements  
15  
16 147 (HD), versus low dose (30mg elemental iron) iron supplements (LD), versus placebo (P). The  
17  
18 148 inclusion of donors is continued until each trial arm consists of two hundred donors. We aim  
19  
20  
21 149 for an equal distribution of male and female participants (*Figure 2*).  
22

23  
24 150  
25

26  
27 151  
28

29  
30 152 **Study procedures**  
31

32  
33 153 Shortly before the baseline and follow-up visits, donors are asked to complete an online  
34  
35 154 questionnaire through Castor EDC<sup>37</sup> (Castor Electronic Data Capture, the Netherlands) and are  
36  
37 155 provided with a hyperlink to the Wageningen University & Research page for an iron-specific  
38  
39 156 food frequency questionnaire (FFQ).  
40

41  
42 157 At baseline, donors will visit one of the selected donation centers for a regular whole blood  
43  
44 158 donation. When donors meet all eligibility criteria to donate, blood samples are taken from  
45  
46 159 the blood donation sampling pouch. The collected blood samples are sent to the Sanquin  
47  
48  
49 160 National Screening Laboratory in Amsterdam for analysis. At least twice per week, the donor  
50  
51 161 database containing donor ferritin levels is examined, and participating donors with ferritin  
52  
53 162 levels  $\leq 30$   $\mu\text{g/L}$  are selected. These donors are randomized and will receive iron or placebo  
54  
55 163 supplements and additional study information through postal mail. Donors with ferritin levels  
56  
57  
58 164  $> 30$   $\mu\text{g/L}$  are informed that their ferritin levels are sufficient and, therefore, they are not eligible  
59  
60

165 to participate in the RCT. During the follow-up visits, blood samples are collected through  
 166 venipuncture.

### 167 **Intervention and timeline**

168 For this study, ferrous bisglycinate iron supplements are used with capsules containing 0, 30,  
 169 or 60 mg of elemental iron. Participants are asked to adhere to the study product  
 170 supplementation protocol as strictly as possible for 56 days after the baseline visit. The  
 171 supplementation protocol instructs the participants to take the capsules at least three hours  
 172 before and after eating products or taking medicine that interfere with iron uptake (e.g., dairy,  
 173 coffee, tea, soy, antacids, and anti-biotics). Therefore, participants are advised to take the  
 174 capsules shortly before going to bed and asked to refrain from taking any additional iron  
 175 supplements.

176 Follow-up visits will occur at 56, 122, and 182 days corresponding to the minimum donation  
 177 interval for men, the minimum donation interval for women, and the minimum ferritin-guided  
 178 deferral period in the Netherlands, respectively (*Figure 3*). The participants are asked to return  
 179 any unused capsules at the first follow-up visit to determine their treatment adherence.

### 181 **Sample size**

182 Based on previous research performed by Kiss *et al.* and Waldvogel *et al.*, who used a similar  
 183 randomized trial design in smaller groups of whole blood donors, sample size calculations were  
 184 made for the primary outcome parameters, being ferritin and Hb, and the secondary outcome  
 185 parameters, being adverse events, mental health, and physical health (Table 1)<sup>18,38,39</sup>. For the  
 186 sample size calculation, we used the following formula:

$$187 \quad n_1 = \frac{\left(z_{\frac{1-\alpha}{2}} + z_{(1-\beta)}\right)^2 * \sigma^2 * (r + 1)}{v^2 * r}$$

Here,  $n_1$  is the sample size of the intervention groups,  $r$  is the ratio between the control groups and the intervention groups,  $\sigma^2$  is the variance of the continuous variable,  $\nu$  describes the expected difference between the continuous variables between the intervention and control groups (Table 1),  $\frac{z_{1-\alpha}}{2}$  is the two-tailed alternative hypothesis with significance level  $\alpha = 0.05$  (1.96),  $z_{(1-\beta)}$  is the probability of rejecting the null hypothesis when it is one minus probability of type II error ( $\beta$ ) with a power of 0.90 (1.28).

It is expected that the chosen sample size is sufficient to reach statistical power for nearly all main outcome parameters (Table 1)<sup>39</sup>. For mental health, a lack of power is expected. However, indications of trends towards an effect might be observed, and data may be used in future meta-analyses to reach statistical significance. Furthermore, we expect to observe small differences in outcomes between low- and high-dose iron supplementation groups. To observe an expected difference ( $\nu$ ) of 4  $\mu\text{g/l}$  in ferritin and 2.5  $\text{g/l}$  in hemoglobin between the iron supplementation groups, at least 126 and 151 donors should be included in each intervention group, respectively. Therefore, to reduce the risk of underpowering the study and considering possible dropout, we will include 200 participants per intervention group. Moreover, this will allow us to perform subgroup analysis based on sex, treatment adherence, and dietary intake.

	Iron supplementation	Placebo	Required sample size	Sufficiency
<b>Hb</b>	13.4 (1.1) g/dL	12.0 (1.2) g/dL	19	Sufficient
<b>Ferritin</b>	28.0 (9.8) $\mu\text{g/L}$	12.9 (8.3) $\mu\text{g/L}$	14	Sufficient
<b>Adverse events*</b>	39.2%	15.5%	144	Sufficient
<b>Mental health**</b>	40.1 (4.8)	40.7 (4.8)	2016	Not sufficient
<b>Physical condition**</b>	54.8 (3.3)	52.4 (5.2)	60	Sufficient

\* Adverse events included gastrointestinal symptoms, dizziness, headache, acne, palpitations, and renal lithiasis.

\*\* The outcome parameters mental health and physical condition were based on construct scores from the SF-12 questionnaire.

### Recruitment

Donors will first be recruited for the baseline questionnaire and blood sample measurements.

The invitation is sent by email at least three weeks before the end of the donor's standard

1  
2  
3 208 donation interval or deferral period. Donors are provided with study information and an  
4  
5 209 example of the informed consent form (appendix A). The information folder contains  
6  
7 210 information about the study procedures and the rights of the participant. The invited donors  
8  
9  
10 211 are asked to respond to the invitation through email to indicate if they would like to participate  
11  
12 212 in the study before signing the informed consent form. During the blood donation visit (i.e.,  
13  
14 213 baseline), donors who have agreed to participate are asked to sign the written informed  
15  
16 214 consent form in a blood bank employee's presence. By signing the informed consent form,  
17  
18 215 donors officially agree to participate and confirm that their personal information and material  
19  
20 216 can be used for research purposes. Finally, the consent form is signed by the blood bank  
21  
22  
23 217 employee to affirm being the study representative, after which the donor can continue with  
24  
25 218 the regular whole blood donation. Participation in this study is voluntary, and donors will not  
26  
27  
28 219 receive any compensation besides travel expenses as part of regular Sanquin Blood Bank  
29  
30 220 policies.

31  
32  
33 221 We expect that fifty percent of the donors recruited for the baseline measurements have  
34  
35 222 ferritin levels  $\leq 30$   $\mu\text{g/L}$  and are eligible to participate in the trial<sup>3</sup>. Recruitment will continue  
36  
37 223 until 1200 donors have been included in the trial. Data on demographics such as sex, age,  
38  
39 224 donation history, and region are collected from the blood bank information system eProgesa  
40  
41  
42 225 (MAK systems, Paris, France). Recruitment will start in July 2022. We aim to include all  
43  
44 226 participants within one year and expect to finalize all follow-up visits at the end of 2022.

45  
46  
47 227 The trial is initiated at one blood collection center. Based on donor response- and inclusion  
48  
49 228 rates, additional centers are added. The additional centers are added based on their capacity  
50  
51 229 and ability to cope with the additional study-related workload, accessibility, the number of  
52  
53  
54 230 regularly donating donors, and the availability of direct transport to the National Screening  
55  
56 231 Laboratory of Sanquin (NSS). Based on previous studies performed at Sanquin Research, we  
57  
58 232 expect a response rate between 50% and 75%<sup>40,41</sup>. The expected response rate corresponds  
59  
60

1  
2  
3 233 with the inclusion of 1614 to 2421 eligible donors for the RCT per year, based on recruitment  
4  
5 234 from two large Sanquin Blood Bank locations. In case of lower response rates, donors from  
6  
7 235 other locations will also be included.  
8  
9

### 10 236 **Blinding and randomization**

11  
12 237 Participant randomization is done on the individual level using a block randomization method.  
13  
14 238 This procedure is realized through Castor EDC, with randomized block sizes set at 12 and 18.  
15  
16 239 Furthermore, the randomization is stratified by age (18-49 years versus 50 years and older)  
17  
18 240 and sex to account for menopausal effects in women, differences in ferritin levels between  
19  
20 241 men and women, and the impact of aging on iron absorption<sup>42-44</sup>. Supplements and  
21  
22 242 information are sent to the participants based on the number corresponding with the group  
23  
24 243 they are allocated to after randomization. The responsible researcher is blinded for which  
25  
26 244 group number corresponds with which study product. The 30mg iron, 60 mg iron, and placebo  
27  
28 245 capsules are identical in terms of appearance and weight to guarantee the blinding of the  
29  
30 246 participants and involved researchers. The participants will not be blinded for the varying  
31  
32 247 intake frequency.  
33  
34  
35  
36  
37

### 38 248 **Data collection**

39 249 Data are collected at baseline and during three follow-up visits. The baseline questionnaire  
40  
41 250 consists of a combination of previously used or validated questionnaires. Participants are  
42  
43 251 characterized by social-economic status, medical background, physical fitness, menstrual  
44  
45 252 status, and smoking, as described in previous research<sup>41</sup>. To determine the effects of iron  
46  
47 253 supplementation, we will use the *36-Item Short-Form Health Survey (SF-36)*; to determine  
48  
49 254 baseline status and changes in general health<sup>45</sup>, the *International Physical Activity*  
50  
51 255 *Questionnaire Short Form (IPAQ)*; to assess changes in levels of physical activity<sup>46</sup>, the *Fatigue*  
52  
53 256 *Assessment Scale*; to assess changes in self-reported fatigue<sup>47</sup>, donation intention-specific  
54  
55 257 *Theory of Planned Behavior* questions; to determine if donors are more or less willing to donate  
56  
57 258 as a consequence of iron supplementation<sup>48</sup>, and the *Gastrointestinal Symptom Rating Scale*  
58  
59  
60

1  
2  
3 259 and the *Bristol Stool Chart*; to assess the gastrointestinal side effects potentially caused by iron  
4  
5 260 supplementation<sup>49</sup>. To determine any effects of iron supplementation on iron deficiency-  
6  
7 261 related symptoms, we use the *Cambridge Hopkins Restless Legs Syndrome Questionnaire*; to  
8  
9  
10 262 assess the presence of and changes in restless legs, the *PICA questionnaire*; to assess a  
11  
12 263 potential appetite for non-nutritive substances, and the *Cognitive Failure Questionnaire*; to  
13  
14 264 determine changes in cognitive function<sup>50-52</sup>.

15  
16 265 Questionnaires that will also determine possible confounding and effect modification are the  
17  
18 266 Treatment Adherence Questionnaire<sup>53</sup> to determine the participants' compliance with the  
19  
20  
21 267 intake protocols and an iron specific Food Frequency Questionnaire (FFQ) to determine their  
22  
23 268 dietary iron, macro-, and micronutrient intake<sup>41,54,55</sup>. The FFQ allows us to assess whether or  
24  
25 269 not iron supplements are more effective for donors who have low dietary (heme) iron intake  
26  
27  
28 270 and will only be completed at baseline and before the final follow-up visit. The follow-up visit  
29  
30 271 questionnaires are similar to the baseline questionnaire, excluding the questions related to  
31  
32 272 demographic characteristics and the addition of the adherence questionnaire for the first  
33  
34 273 follow-up measurement. Furthermore, participants are asked to use the MedApp (MedApp  
35  
36 274 Nederland B.V., Eindhoven, The Netherlands, <https://medapp.nl>) to assist with compliance  
37  
38  
39 275 with the supplementation protocol and to accurately assess treatment adherence. The  
40  
41 276 MedApp will provide participants with daily or alternate daily notifications to remind them  
42  
43 277 about their capsule intake and to indicate if the intake protocol was successfully followed.

44  
45  
46 278 At baseline and during follow-up, whole blood and serum samples are collected using 2ml, and  
47  
48 279 6ml coated EDTA (VACUETTE®, K3EDTA, Greiner Bio-one International GmbH, Austria) and  
49  
50  
51 280 3.5ml and 5ml serum separating (VACUETTE®, Serum gel, Greiner Bio-one International GmbH,  
52  
53 281 Austria) tubes, respectively. Ferritin measurements (Architect Ci8200, Abbott Laboratories, IL,  
54  
55 282 USA), using serum samples, are performed routinely within 24 hours after the donation. The  
56  
57 283 Architect Ci8200 is calibrated yearly for ferritin measurements by the manufacturer (Abbott  
58  
59 284 Laboratories) and traceable to the the first WHO Human Liver Ferritin International Standard



1  
2  
3 285 (80/602). Furthermore, quality assurance assessments are performed daily by the laboratory  
4  
5 286 staff, using low (20 µg/L), medium (150 µg/L), and high (400 µg/L) ferritin quality controls,  
6  
7 287 provided by the manufacturer. When the daily quality assurance measurements do not meet  
8  
9  
10 288 the predefined acceptance criteria , the Architect Ci8200 will be recalibrated by the  
11  
12 289 manufacturer (Abbott Laboratories). Quality management is in accordance with ISO 15189.  
13  
14 290 Complete blood count measurements, including Hb and red blood cell parameters (Advia  
15  
16 291 2120, Siemens Medical Solutions Diagnostics, Breda, the Netherlands), are performed within  
17  
18  
19 292 24 hours after the whole blood samples are taken. DNA is isolated using 400 µl buffy coat from  
20  
21 293 EDTA- whole bloodsamples (QIASymphony® DSP DNA Mini Kit, Qiagen GmbH, Hilden,  
22  
23 294 Germany)<sup>56</sup> and stored at -20°C, for later use. Additional processed and aliquoted plasma and  
24  
25 295 serum samples are collected from the EDTA- and serum separating tubes, respectively. The  
26  
27  
28 296 additional samples will be stored at -80°C and used for potential post-study measurements of  
29  
30 297 parameters which might affect the iron hemeostasis (e.g., inflammatory markers).

### 31 32 33 298 **Statistical analysis**

34  
35 299 Descriptive statistics are presented for intervention and control groups and for men and  
36  
37 300 women separately as means ± standard deviation for normally distributed data and median  
38  
39 301 and interquartile range in case of a skewed distribution. For the analysis of the primary study  
40  
41 302 parameters, the following multiple regression model is used if all assumptions for the model  
42  
43 303 are fulfilled:

$$44$$

$$45$$

$$46$$

$$47 304 \quad \textit{Effect} = \textit{Intercept} + \beta_1 * \textit{Dose}_1 + \beta_2 * \textit{Dose}_2 + \beta_3 * \textit{Frequency} + \beta_4 * \textit{Dose}_1\textit{Frequency}$$

$$48 \quad + \beta_5 * \textit{Dose}_2\textit{Frequency} + e$$

$$49$$

$$50$$

51 305 Here, *Intercept* represents the expected mean value when all explanatory variables have a  
52  
53 306 value of 0, *Dose*<sub>1</sub> the low dose iron capsules (30mg), *Dose*<sub>2</sub> the high dose iron capsules (60mg),  
54  
55 307 both dummy variables have 0mg as a reference, *frequency* the every other day versus daily  
56  
57  
58 308 intake, and *e* the error term or difference between observed and expected values. The  
59  
60 309 assessed assumptions are the normality distributed random error term and the assumption of

1  
2  
3 310 the equality of variance. All the analyses will consist of two-sided tests, with a p-value <0.05  
4  
5 311 considered statistically significant. Statistical data analyses are performed with SPSS (IBM®  
6  
7 312 SPSS® Statistics 23.0 or newer) and R (R Core Team, Windows)<sup>57</sup>.  
8  
9  
10 313 Potential effect modification by age, BMI, donation history, dietary intake (e.g., heme- and  
11  
12 314 non-heme iron, dairy products, alcohol, tea, coffee, and total energy), and menstrual status  
13  
14 315 are examined. These potential effect modifiers have been selected due to their role in iron  
15  
16 316 hemoestasis<sup>58,59</sup>. We will test for effect modification by adding the variable of interest and an  
17  
18 317 interaction term with the iron different iron dosages and intake frequency to the model. A  
19  
20 318 variable is treated as an effect modifier when it has an interaction term with a p-value <0.10  
21  
22 319 for more than half of the associations. When effect modification is observed, stratified results  
23  
24 320 will be reported in addition to the overall results. The following potential confounders are  
25  
26 321 examined; age, sex, BMI, donation history, menstrual status, smoking, season, compliance with  
27  
28 322 the study products, social-economic status, ethnicity, recent infections (including Sars-CoV-2),  
29  
30 323 and baseline ferritin and Hb levels. A change of more than 10% in the regression coefficient is  
31  
32 324 considered confounding, and variables are added to all the models.  
33  
34  
35  
36  
37

### 38 325 **Monitoring**

39  
40 326 Study monitoring is performed by the TAPAS Group, an independent monitoring bureau. The  
41  
42 327 FORTE project has been labeled as a negligible risk study by the involved medical ethics  
43  
44 328 committee based on a risk assessment. Study monitoring will consist of an initiation visit, two  
45  
46 329 monitoring visits, and a close-out visit.  
47  
48  
49

### 50 330 **Patient and public involvement**

51  
52 331 Donors, as well as non-donors, are involved in the FORTE research project through focus group  
53  
54 332 interviews. The focus group interviews will consist of interactive group discussions involving  
55  
56 333 frequent donors; donors who have donated at least five times, new donors; donors who have  
57  
58 334 signed up for donation but have not yet donated, and blood bank staff, including donor  
59  
60

1  
2  
3 335 physicians. During the focus group interviews, the participants are asked to discuss their  
4  
5 336 perceptions, opinions, and attitude towards iron supplementation, current or potential  
6  
7 337 alternative blood bank policies regarding iron management, and any potential effects of these  
8  
9 338 aspects on their willingness to donate. Based on the outcomes of the focus group interviews,  
10  
11 339 we will design a questionnaire to quantify the findings from the focus group interviews. The  
12  
13 340 questionnaire is developed based on the recurrent constructs and topics observed during the  
14  
15 341 group discussions and distributed amongst a larger group of donors and new donors. The  
16  
17 342 results from the focus group interviews and the questionnaire are used to determine the  
18  
19 343 optimal approach and potential areas of concern for implementing iron supplementation as a  
20  
21 344 blood bank policy, if shown effective based on the trial's outcome.  
22  
23  
24  
25  
26 345  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

## 346 **ETHICS AND DISSEMINATION**

### 347 **Ethical considerations**

348 This study is performed according to the Declaration of Helsinki and Good Clinical Practice  
349 guidelines. The Medical Research Ethics Committee (METC) of the Academic Medical Centre  
350 (AMC) Amsterdam has approved the study protocol (trial ID NL8590). All participants are asked  
351 to provide their written informed consent and are informed that they can discontinue  
352 participation at any time. Data collection is compliant with the General Data Protection  
353 Regulation (GDPR), and all data are pseudonymized to prevent the identification of study  
354 participants. To ensure the donors' safety, invasive actions such as venipuncture are  
355 performed by trained blood bank employees, following the routine blood donation protocols  
356 of Sanquin Blood Bank, the Netherlands, whenever applicable.

### 357 **Dissemination**

358 Study results are published in peer-reviewed journals after evaluation of scientific relevance  
359 and quality by the involved researchers. Furthermore, the results are presented at  
360 (inter)national conferences, shared with study participants, and communicated with donors  
361 and different Sanquin departments. Data that can lead to the identification of the participants  
362 will not be published.

363

364

## 365 **SIGNIFICANCE AND OUTLOOK**

366 This study's outcomes will provide evidence to lead the debate if and how iron  
367 supplementation should be implemented to support iron repletion for whole blood donors  
368 with low ferritin levels. Blood donation and adequate blood availability are of great  
369 importance to guarantee patients' health in need of blood transfusions. However, frequent  
370 blood donation increases the risk of iron deficiency in repeat whole blood donors. To ensure  
371 sufficient availability of blood and blood products and safeguard donor health, the donors' iron  
372 status should be monitored and managed appropriately. However, international uniformity  
373 amongst blood services regarding policies to address the donors' iron stores is lacking.  
374 Therefore, the FORTE study aims to provide new insights regarding the efficacy of iron  
375 supplementation for whole blood donors with low ferritin levels. We do this by comparing the  
376 effectiveness of high and low-dose iron capsules versus placebo for daily and alternate-day  
377 supplementation protocols in whole blood donors, thereby investigating laboratory and  
378 health-related outcomes. The thorough characterization of participating donors will add to an  
379 enhanced understanding of the effectiveness of iron supplements under various  
380 circumstances.

381

382

1  
2  
3 383 **Author contributions**

4  
5  
6 384 J.K., K.v.d.H., M.S. designed the study protocol. J.K. wrote the manuscript with input from  
7  
8 385 K.v.d.H., F.Q., M.S., D.S., V.N., H.Z. and H.W. were consulted for medical and ethical input.  
9

10  
11 386 **Statement of Ethics Approval**

12  
13  
14 387 This study involves human participants and was approved by the Medical Research Ethics  
15  
16 388 Committee (METC) of the Academic Medical Centre (AMC) Amsterdam (reference number:  
17  
18 389 2020\_206); trial ID NL8590. All participants are asked to provide their written informed  
19  
20  
21 390 consent and are informed that they can discontinue participation at any time.  
22

23  
24 391 **Funding statement**

25  
26 392 Sanquin supported this research project: Product and Process Development Cellular Products  
27  
28  
29 393 Grant, project id: PPOC19-02/1-239.  
30

31  
32 394 **Competing interests statement**

33  
34  
35 395 The authors declare that they have no competing interests.  
36

37  
38 396 **Corresponding author**

39  
40 397 Correspondence to Jan Karregat.  
41  
42  
43 398  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

## 399 References

- 400  
401 1. Simon TL, Garry PJ, Hooper EM. Iron stores in blood donors. *JAMA*. 1981;245(20):2038-2043.  
402 2. Finch CA, Cook JD, Labbe RF, Culala M. Effect of blood donation on iron stores as evaluated by serum  
403 ferritin. *Blood*. 1977;50(3):441-447.  
404 3. Vinkenog M, van den Hurk K, van Kraaij M, van Leeuwen M, Janssen MP. First results of a ferritin-based  
405 blood donor deferral policy in the Netherlands. *Transfusion*. n/a(n/a).  
406 4. Greig AJ, Patterson AJ, Collins CE, Chalmers KA. Iron deficiency, cognition, mental health and fatigue  
407 in women of childbearing age: a systematic review. *J Nutr Sci*. 2013;2:e14-e14.  
408 5. Houston BL, Hurrie D, Graham J, et al. Efficacy of iron supplementation on fatigue and physical capacity  
409 in non-anaemic iron-deficient adults: a systematic review of randomised controlled trials. *BMJ open*.  
410 2018;8(4):e019240-e019240.  
411 6. Avni T, Reich S, Lev N, Gafter-Gvili A. Iron supplementation for restless legs syndrome - A systematic  
412 review and meta-analysis. *Eur J Intern Med*. 2019;63:34-41.  
413 7. Pasricha SR, Low M, Thompson J, Farrell A, De-Regil LM. Iron supplementation benefits physical  
414 performance in women of reproductive age: a systematic review and meta-analysis. *J Nutr*.  
415 2014;144(6):906-914.  
416 8. Miao D, Young SL, Golden CD. A meta-analysis of pica and micronutrient status. *Am J Hum Biol*.  
417 2015;27(1):84-93.  
418 9. (CD-P-TS) ECoBT. *Guide to the preparation, use and quality assurance of blood components*.  
419 *Recommendation no. R(95)15*. 16th ed: Council of Europe Publishing; 2020.  
420 10. Cable RG. Hemoglobin determination in blood donors. *Transfus Med Rev*. 1995;9(2):131-144.  
421 11. Custer B, Chinn A, Hirschler NV, Busch MP, Murphy EL. The consequences of temporary deferral on future  
422 whole blood donation. *Transfusion*. 2007;47(8):1514-1523.  
423 12. Halperin D, Baetens J, Newman B. The effect of short-term, temporary deferral on future blood  
424 donation. *Transfusion*. 1998;38(2):181-183.  
425 13. Cable RG, Brambilla D, Glynn SA, et al. Effect of iron supplementation on iron stores and total body iron  
426 after whole blood donation. *Transfusion*. 2016;56(8):2005-2012.  
427 14. Bialkowski W, Kiss JE, Wright DJ, et al. Estimates of total body iron indicate 19 mg and 38 mg oral iron  
428 are equivalent for the mitigation of iron deficiency in individuals experiencing repeated phlebotomy.  
429 (1096-8652 (Electronic)).  
430 15. Magnussen K, Bork N, Asmussen L. The effect of a standardized protocol for iron supplementation to  
431 blood donors low in hemoglobin concentration. *Transfusion*. 2008;48(4):749-754.  
432 16. Group AAHDW. AABB donor iron deficiency risk-based decision-making assessment report supplemental  
433 material. 2018.  
434 17. Szczepiorkowski Z. Updated Strategies to Limit or Prevent Iron Deficiency in Blood Donors. In: Members  
435 A, ed. aabb.org: American Association of Blood Banks; 2017.  
436 18. Kiss JE, Brambilla D, Glynn SA, et al. Oral iron supplementation after blood donation: a randomized  
437 clinical trial. *Jama*. 2015;313(6):575-583.  
438 19. Milman N, Jønsson L, Dyre P, Pedersen PL, Larsen LG. Ferrous bisglycinate 25 mg iron is as effective as  
439 ferrous sulfate 50 mg iron in the prophylaxis of iron deficiency and anemia during pregnancy in a  
440 randomized trial. *J Perinat Med*. 2014;42(2):197-206.  
441 20. Bovell-Benjamin AC, Viteri FE, Allen LH. Iron absorption from ferrous bisglycinate and ferric trisglycinate  
442 in whole maize is regulated by iron status. *Am J Clin Nutr*. 2000;71(6):1563-1569.  
443 21. Szarfarc SC, de Cassana LM, Fujimori E, Guerra-Shinohara EM, de Oliveira IM. Relative effectiveness of  
444 iron bis-glycinate chelate (Ferrochel) and ferrous sulfate in the control of iron deficiency in pregnant  
445 women. *Arch Latinoam Nutr*. 2001;51(1 Suppl 1):42-47.  
446 22. Layrisse M, García-Casal MaN, Solano L, et al. Iron Bioavailability in Humans from Breakfasts Enriched  
447 with Iron Bis-Glycine Chelate, Phytates and Polyphenols. *The Journal of Nutrition*. 2000;130(9):2195-  
448 2199.  
449 23. Ashmead HD. The absorption and metabolism of iron amino acid chelate. *Arch Latinoam Nutr*. 2001;51(1  
450 Suppl 1):13-21.  
451 24. Duque X, Martinez H, Vilchis-Gil J, et al. Effect of supplementation with ferrous sulfate or iron bis-  
452 glycinate chelate on ferritin concentration in Mexican schoolchildren: a randomized controlled trial.  
453 *Nutr J*. 2014;13:71.  
454 25. Vuk T, Magnussen K, De Kort W, et al. International forum: an investigation of iron status in blood  
455 donors. *Blood transfusion = Trasfusione del sangue*. 2017;15(1):20-41.  
456 26. Magnussen K, Bork N, Asmussen L. The effect of a standardized protocol for iron supplementation to  
457 blood donors low in hemoglobin concentration. *Transfusion*. 2008.  
458 27. Ganz T, Nemeth E. Hepcidin and iron homeostasis. *Biochim Biophys Acta*. 2012;1823(9):1434-1443.  
459 28. Moretti D, Goede JS, Zeder C, et al. Oral iron supplements increase hepcidin and decrease iron  
460 absorption from daily or twice-daily doses in iron-depleted young women. *Blood*. 2015;126(17):1981-  
461 1989.  
462 29. Stoffel NU, Cercamondi CI, Brittenham G, et al. Iron absorption from oral iron supplements given on  
463 consecutive versus alternate days and as single morning doses versus twice-daily split dosing in iron-



1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

- depleted women: two open-label, randomised controlled trials. *Lancet Haematol.* 2017;4(11):e524-e533.
30. Cable RG, Birch RJ, Spencer BR, et al. The operational implications of donor behaviors following enrollment in STRIDE (Strategies to Reduce Iron Deficiency in blood donors). *Transfusion.* 2017;57(10):2440-2448.
  31. Pittori C, Buser A, Gasser UE, et al. A pilot iron substitution programme in female blood donors with iron deficiency without anaemia. *Vox Sang.* 2011;100(3):303-311.
  32. Pasricha SR, Marks DC, Salvin H, et al. Postdonation iron replacement for maintaining iron stores in female whole blood donors in routine donor practice: results of two feasibility studies in Australia. *Transfusion.* 2017;57(8):1922-1929.
  33. Mirrezaei SM, Parsi R, Askarian M. Low Dose, Short-Term Iron Supplementation in Female Blood Donors of Childbearing Age: a Randomized, Double-Masked, Placebo-Controlled Study. *Iranian Journal of Medical Sciences.* 2008;33.
  34. Radtke H, Tegtmeier J, Rocker L, Salama A, Kiesewetter H. Daily doses of 20 mg of elemental iron compensate for iron loss in regular blood donors: a randomized, double-blind, placebo-controlled study. *Transfusion.* 2004;44(10):1427-1432.
  35. Wet inzake bloedvoorziening. 1997.
  36. de Kort W, Prinsze F, Nuboer G, Twisk J, Merz EM. Deferral rate variability in blood donor eligibility assessment. *Transfusion.* 2019;59(1):242-249.
  37. Castor EDC. Castor Electronic Data Capture. 2019; <https://castoredc.com>. Accessed August 28, 2019.
  38. Smith GA, Fisher SA, Doree C, Di Angelantonio E, Roberts DJ. Oral or parenteral iron supplementation to reduce deferral, iron deficiency and/or anaemia in blood donors. *Cochrane Database Syst Rev.* 2014(7):CD009532.
  39. Waldvogel S, Pedrazzini B, Vaucher P, et al. Clinical evaluation of iron treatment efficiency among non-anemic but iron-deficient female blood donors: a randomized controlled trial. *BMC Med.* 2012;10:8.
  40. van den Hurk K, Merz E-M, Prinsze F, et al. *Low awareness of past SARS-CoV-2 infection in healthy adults.* 2020.
  41. Timmer TC, de Groot R, Habets K, et al. Donor InSight: characteristics and representativeness of a Dutch cohort study on blood and plasma donors. *Vox Sang.* 2018.
  42. Vanasse GJ, Berliner N. Anemia in Elderly Patients: An Emerging Problem for the 21st Century. *Hematology.* 2010;2010(1):271-275.
  43. Busti F, Camprostrini N, Martinelli N, Girelli D. Iron deficiency in the elderly population, revisited in the hepcidin era. *Front Pharmacol.* 2014;5:83-83.
  44. den Elzen WPJ, de Craen AJM, Wiegerinck ET, Westendorp RGJ, Swinkels DW, Gussekloo J. Plasma hepcidin levels and anemia in old age. The Leiden 85-Plus Study. *Haematologica.* 2013;98(3):448-454.
  45. Brazier JE, Harper R Fau - Jones NM, Jones Nm Fau - O'Cathain A, et al. Validating the SF-36 health survey questionnaire: new outcome measure for primary care. (0959-8138 (Print)).
  46. Lee PH, Macfarlane DJ, Lam TH, Stewart SM. Validity of the international physical activity questionnaire short form (IPAQ-SF): A systematic review. *International Journal of Behavioral Nutrition and Physical Activity.* 2011;8(1):115.
  47. Michielsen HJ, De Vries J, Van Heck GL. Psychometric qualities of a brief self-rated fatigue measure: The Fatigue Assessment Scale. *J Psychosom Res.* 2003;54(4):345-352.
  48. Giles M, McClenahan C, Cairns E, Mallet J. An application of the Theory of Planned Behaviour to blood donation: the importance of self-efficacy. *Health Education Research.* 2004;19(4):380-391.
  49. Revicki DA, Wood M, Wiklund I, Crawley J. Reliability and validity of the Gastrointestinal Symptom Rating Scale in patients with gastroesophageal reflux disease. *Quality of life research : an international journal of quality of life aspects of treatment, care and rehabilitation.* 1998;7(1):75-83.
  50. Allen RP, Burchell Bj Fau - MacDonald B, MacDonald B Fau - Hening WA, Hening Wa Fau - Earley CJ, Earley CJ. Validation of the self-completed Cambridge-Hopkins questionnaire (CH-RLSq) for ascertainment of restless legs syndrome (RLS) in a population survey. (1878-5506 (Electronic)).
  51. Walters AS, Frauscher B, Allen R, et al. Review of diagnostic instruments for the restless legs syndrome/Willis-Ekbom Disease (RLS/WED): critique and recommendations. *Journal of clinical sleep medicine : JCSM : official publication of the American Academy of Sleep Medicine.* 2014;10(12):1343-1349.
  52. Broadbent DE, Cooper PF, FitzGerald P, Parkes KR. The Cognitive Failures Questionnaire (CFQ) and its correlates. *The British journal of clinical psychology.* 1982;21(1):1-16.
  53. Sidorkiewicz S, Tran VT, Cousyn C, Perrodeau E, Ravaud P. Development and validation of an instrument to assess treatment adherence for each individual drug taken by a patient. *BMJ Open.* 2016;6(5):e010510.
  54. Streppel MT, de Vries JH, Meijboom S, et al. Relative validity of the food frequency questionnaire used to assess dietary intake in the Leiden Longevity Study. *Nutr J.* 2013;12:75.
  55. Timmer TC, de Groot R, Rijnhart JJM, et al. Dietary intake of heme iron is associated with ferritin and hemoglobin levels in Dutch blood donors: results from Donor InSight. *Haematologica.* 2019.
  56. QIAsymphony DSP DNA Instructions for Use (Handbook)2015.
  57. R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.



1  
2  
3 530 URL <http://www.R-project.org/>.

4 531 58. Yanoff LB, Menzie CM, Bi D, et al. Inflammation and iron deficiency in the hypoferrremia of obesity.  
5 532 *International journal of obesity (2005)*. 2007;31:1412-1419.

6 533 59. Schotten N, Pasker-de Jong PC, Moretti D, et al. The donation interval of 56 days requires extension to  
7 534 180 days for whole blood donors to recover from changes in iron metabolism. *Blood*. 2016.

8 535  
9

10  
11 536 *Figure 1 Flowchart of the donor inclusion for the baseline questionnaire and the randomized controlled trial.*

12  
13 *Figure 3 Schematic diagram of the participant randomization. LF, low frequency supplementation (alternate day);*  
14 *HF, high frequency supplementation (daily); LD, low dose supplements (30mg elemental iron); HD, high dose*  
15 *supplements (60mg elemental iron); P, placebo supplements.*

16  
17 *Figure 2 Schematic diagram of the study timeline with t being the time since the baseline visit.*  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

Regular whole blood donors donating for a fifth time at a participating blood center

**Exclusion:**

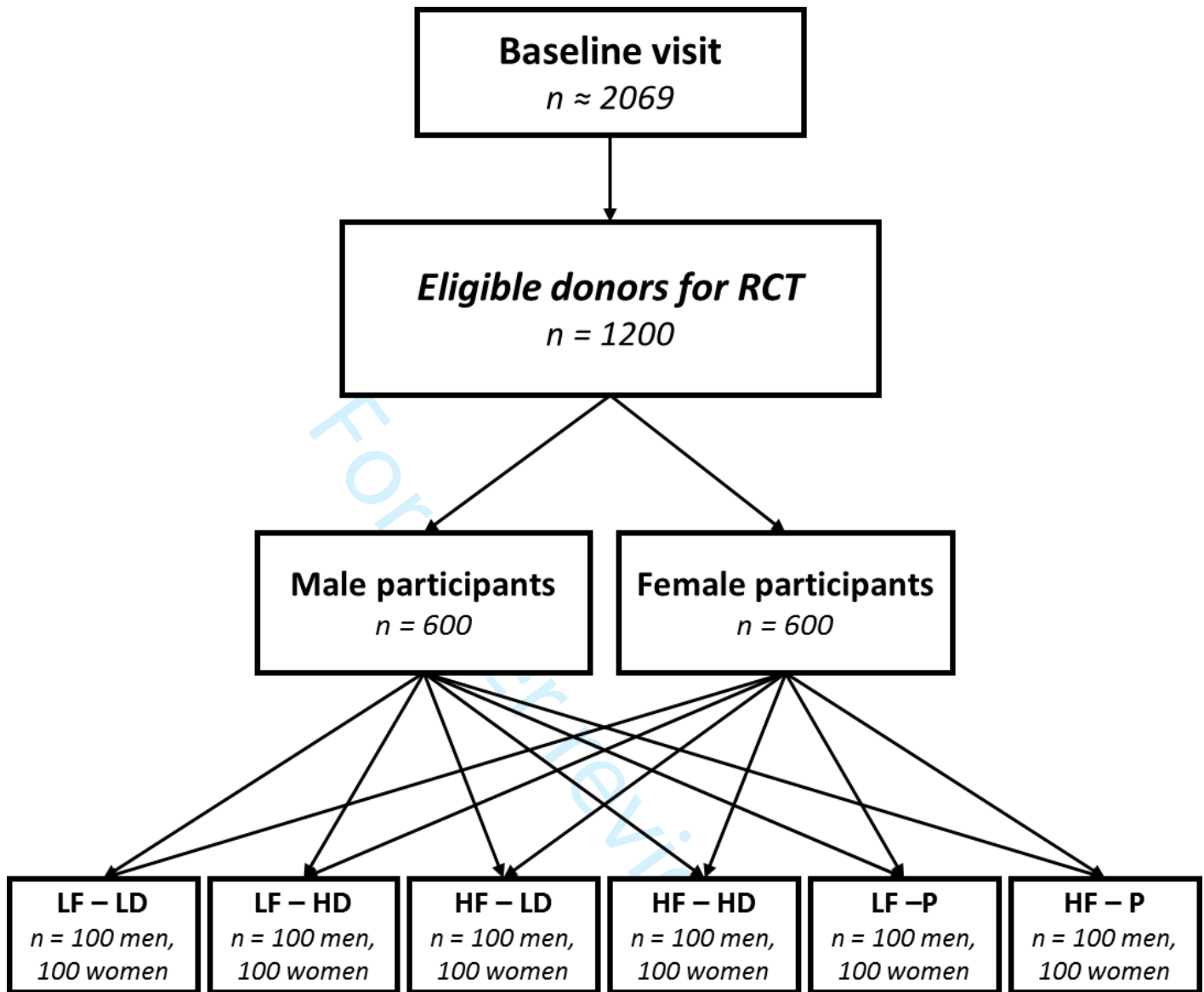
- *Not fluent in Dutch*
- *Current/recent iron supplementation*

Donors eligible for baseline questionnaire

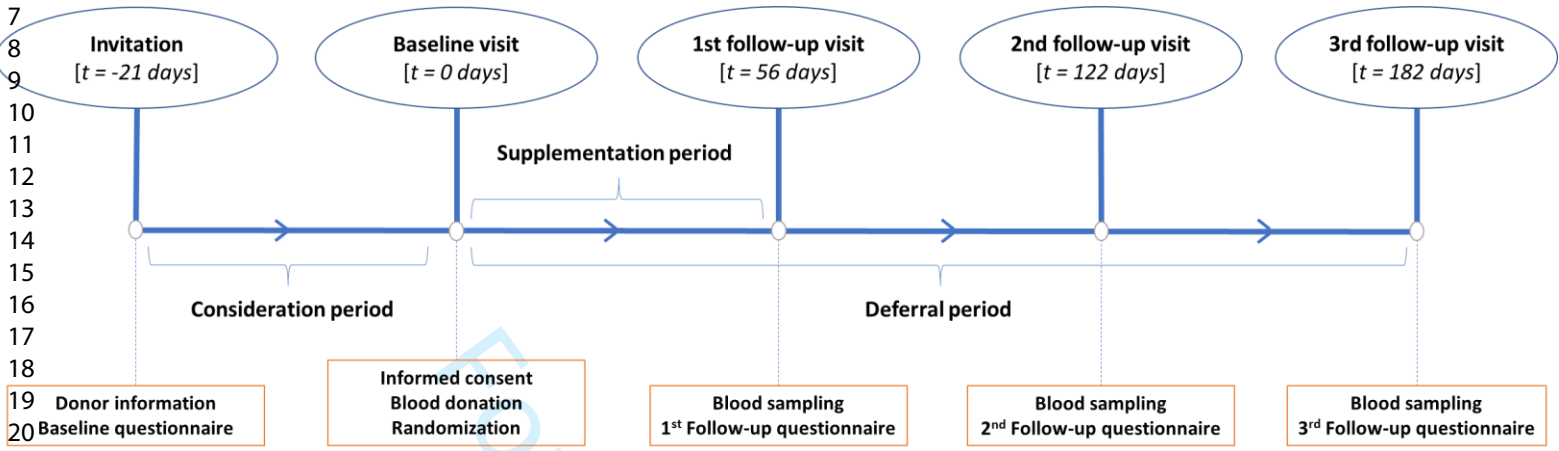
**Exclusion:**

- *Unsuccessful donation*
- *Ferritin levels > 30 µg/L*

Donors eligible to participate in the randomized controlled trial



1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60



## Informed consent form FORTE

### Research regarding iron supplementation for blood donors with low ferritin levels

- I have read the information letter. I was also able to ask questions. My questions have been answered adequately. I have had enough time to consider if I would like to participate.
- I understand that participation is voluntarily. I also know that I can decide to quit participating at any moment. For this I don't need to provide any reasons.
- I give permission to request information regarding my health, a hospitalization, or death from a medical centre, my physician, or the Central Bureau for Statistics, during or after the study.
- I give permission to link my donation information with the research information.
- I give permission to collect, use, and store my information and human tissue to answer the research questions in this study.
- I know that some persons will have access to all my information for study monitoring. These persons are mentioned in the information letter. I give these persons permission for access.
- I give permission to Sanquin to store my information and human tissue for 15 years after the study.
- I give permission to share my encrypted information and human tissue with domestic and international laboratories and researchers for purposes described in the information letter.
- ❖ I
  - do
  - do not
 give permission to collect DNA from my blood and to store and use it for this study
- ❖ I
  - do
  - do not
 give permission to approach me again for a potential follow-up study.
- ❖ I
  - do
  - do not
 give permission to store the human tissue, DNA, and research data for an additional 15 years, after the initial 15 years of storage as part of this study, so in total 30 years.
- ❖ I would like to participate in this study.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

Name subject:.....

Donor number: .....

Signature:..... Date: \_\_ / \_\_ / \_\_

-----

I declare that I have fully informed the subject about the afore mentioned study.

If information becomes available which could affect the informed consent, then the subject will be informed timely.

Name researcher (or the latter's representative ): .....

Signature:..... Date: \_\_ / \_\_ / \_\_

-----

For peer review only

EIN-sticker:





STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents\*

Section/item	Item No	Description	Addressed on page number
<b>Administrative information</b>			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	___1___
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	___1___
	2b	All items from the World Health Organization Trial Registration Data Set	___x___
Protocol version	3	Date and version identifier	___1___
Funding	4	Sources and types of financial, material, and other support	___19___
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	___1___
	5b	Name and contact information for the trial sponsor	___19___
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	___x___
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	___x___

6/bmjopen-2021-056316 on 9 March 2022. Downloaded from <http://bmjopen.bmj.com/> on April 17, 2024 by guest. Protected by copyright.

1	<b>Introduction</b>			
2				
3	Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4
4				
5				
6		6b	Explanation for choice of comparators	5
7				
8	Objectives	7	Specific objectives or hypotheses	5
9				
10	Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	6
11				
12				
13				
14	<b>Methods: Participants, interventions, and outcomes</b>			
15				
16	Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	7
17				
18				
19	Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	7
20				
21				
22	Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	8-10
23				
24		11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	17
25				
26		11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	9, 13
27				
28		11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	7
29				
30	Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	9
31				
32				
33				
34	Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	9
35				
36				
37				
38				
39				
40				
41				
42				
43				
44				
45				
46				



1	Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	_____ 9-10 _____
2				
3				
4	Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	_____ 12 _____
5				

### 6 **Methods: Assignment of interventions (for controlled trials)**

#### 7 Allocation:

8				
9				
10	Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	_____ 12 _____
11				
12				
13				
14				
15				
16	Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	_____ 12 _____
17				
18				
19				
20	Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	_____ 12 _____
21				
22				
23				
24	Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	_____ 12 _____
25				
26				
27		17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	_____ x _____
28				
29				
30				

### 31 **Methods: Data collection, management, and analysis**

32				
33	Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	_____ 12-14 _____
34				
35				
36				
37				
38				
39		18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	_____ 13 _____
40				
41				
42				

6/bmjopen-2024-01056316 on 9 March 2025. Downloaded from <http://bmjopen.bmj.com/> on April 4, 2024 by guest. Protected by copyright.

1	Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	_____8_____
2				
3				
4				
5	Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	_____14-15_____
6				
7				
8		20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	_____14-15_____
9				
10		20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	_____x_____
11				
12				
13				
14	<b>Methods: Monitoring</b>			
15				
16	Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	_____15_____
17				
18				
19				
20				
21				
22		21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	_____15_____
23				
24				
25	Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	_____x_____
26				
27				
28	Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	_____15_____
29				
30				
31				
32	<b>Ethics and dissemination</b>			
33				
34	Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	_____17_____
35				
36				
37	Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	_____17_____
38				
39				
40				
41				
42				
43				
44				
45				
46				

1	Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	_____ 17 _____
2				
3				
4		26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	_____ 17 _____
5				
6				
7	Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	_____ 17 _____
8				
9				
10	Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	_____ 19 _____
11				
12				
13	Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	_____ 17 _____
14				
15				
16	Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	_____ x _____
17				
18				
19				
20	Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	_____ 17 _____
21				
22				
23				
24		31b	Authorship eligibility guidelines and any intended use of professional writers	_____ 17 _____
25				
26		31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	_____ x _____
27				
28				
29	<b>Appendices</b>			
30				
31	Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	_____ A _____
32				
33				
34	Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	_____ x _____
35				
36				

\*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "[Attribution-NonCommercial-NoDerivs 3.0 Unported](https://creativecommons.org/licenses/by/4.0/)" license.