Supplementary material

Manuscript: **Non-invasive zinc protoporphyrin screening offers opportunities for secondary prevention of iron deficiency in blood donors**

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In the main manuscript, subjects were categorized for their iron deficiency status strictly according to the following classification (see "Clinical definitions" in the Materials & Methods sections of the main manuscript):

- Iron depletion: reduction of ferritin levels (males <30 µg/L, females <15 µg/L)
- IDE: reduction of ferritin levels and increase of sTfR (>1.760 mg/L)
- IDA: reduction of ferritin levels, increase of sTfR and drop of venous Hb (males <13 g/dL, females <12 g/dL)

Subjects that did not fall into these categories were either classified as "iron replete" if all of the above parameters were in normal range or as "undefined iron status" if the parameters were conflicting (e.g. increased sTfR but normal Ferritin). As further blood parameters like transferrin saturation, MCH and MCV that are connected to iron status were also available, a classification of the iron status of some of the subjects with "undefined iron status" seemed justified based on these additional parameters. Supplementary Table 1 summarizes the blood parameters of these subjects and the classification chosen. Supplementary Figure 1 shows the flow chart of the study including subjects assigned to iron deficiency stages according to Supplementary Table 1.

Taking the newly assigned subjects into account for the evaluation, the section "Diagnostic value of non-invasive ZnPP measurements for the detection of iron deficiency compared to capillary Hb measurements" in the Results section of the manuscript reads as follows. By capillary Hb determination, 3 of 11 donors with IDA, none of 14 donors with IDE, 1 of 23 with depleted iron stores, none of 46 iron-replete donors, and 2 of 5 donors with undefined iron status were rejected from donation (Suppl. Fig. 2a). In the cases of low capillary Hb, the NI-tubing measurements could not be conducted, due to the deferral of these donors. Given the high correlation between the HPLC and NI-tubing measurements (Fig. 2a of the main manuscript), we replaced the six missing ZnPP values of the NI-tubing measurements with the ZnPP values of the HPLC measurements in the further evaluation. Using NI-lip measurements instead yielded identical results, further justifying this approach.

The following number of subjects exceeded a cutoff of 65 µmol ZnPP/mol heme in the respective groups: 9 of 11 donors with IDA, 6 of 14 donors with IDE, 2 of 23 donors with depleted iron stores, 2 of 46 iron-replete donors, and none of 5 donors with undefined iron status (Suppl. Fig. 2b). Using a cutoff of >65 µmol ZnPP/mol heme as indicative of IDE and IDA, 15 of 25 donors with IDE and IDA were correctly identified by this method, while only 4 of 69 subjects with either only depleted or replete iron stores also exceeded this threshold.

A cutoff of 40 µmol ZnPP/mol heme was exceeded for the following number of subjects in the respective groups: All 11 donors with IDA, 13 of 14 subjects with IDE, 15 of 23 with depleted iron stores, 14 of 46 iron-replete donors and 4 of 5 subjects with undefined iron status (Suppl. Fig. 2c). As expected, lowering the cutoff level leads to a higher sensitivity and the identification of 24 of 25 subjects with IDE and IDA with the additional benefit of identifying 65% of subjects with only depleted iron stores. However, 30% of iron-replete donors are then classified as iron deficient.

The inclusion of the previously discarded subjects does not significantly change the overall result, but further confirms that ZnPP is more potent in identifying donors with severe iron deficiency (IDE or IDA) than capillary Hb.

Supplementary Table 1: Iron deficiency classification of study participants with inconclusive blood parameters according to the "Clinical definitions" in the Materials & Methods sections of the main manuscript.

Gender	Parameter							
	Venous Hb [mg/dL]	Ferritin [µg/L]	TSAT [%]	sTfR [mg/L]	MCH [pg]	MCV [fL]	ID - Grade	Explanation for ID grading
m	14.4	37	Not determined	↑1.99	↓24.4	↓74.5	IDE	Advanced iron deficiency with impending venous Hb drop indicated by microcytic, hypochromic erythrocytes and high sTfR
m	↓12.7	39	Not determined	↑1.8	↓25.8	↓79.7	IDA	Microcytic, hypochromic anemia and high sTfR
f	↓11	↓9	↓8	1.75	↓25.6	80.9	IDA	Hypochromic anemia with borderline MCV, low ferritin and borderline sTfR
m	↓11.2	↓10	↓13	1.44	29.8	91.8	unclear	values remain inconclusive
m	↓12.7	47	27	1.37	31.4	92.3	No ID	No indications of iron deficiency, anemia of other cause
f	↓11.7	↓9	↓7	1.63	27.3	82.5	IDA	Anemia with low ferritin, borderline erythrocyte indices and high normal sTfR
f	↓11.7	↓11	24	1.26	28.1	86.1	unclear	values remain inconclusive
m	14.6	38	19	↑1.84	31.1	88.9	No ID	No indications of iron deficiency other than slightly increased sTfR
f	↓11.4	↓12	↓15	1.39	27.8	85.6	unclear	values remain inconclusive
m	13.8	293	25	↑2.63	↓25.3	80.9	unclear	values remain inconclusive
f	↓11.9	↓11	17	1.6	27.3	84.6	unclear	values remain inconclusive

TSAT = transferrin saturation, sTfR = soluble transferrin receptor, MCH = mean corpuscular hemoglobin,

MCV = mean corpuscular volume, ID = iron deficiency, IDE = iron deficient erythropoiesis, IDA = iron deficiency anemia, m = male, f = female.

Normal ranges for the studied parameters were as follows: Venous Hb ≥13 g/dL (men)/≥12 g/dL (women) (according to iron deficiency classification recommended by the German Society for Hematology and Medical Oncology (DGHO) [3]). Ferritin 30-400 µg/L (men)/15-150 µg/L (women), TSAT 16-45%, sTfR 0,760-1,760 mg/L, MCH 27,6-32,8 pg (men)/26.1-32.6 (women), MCV 80-95,5 fL (reference values of the Institute of Laboratory Medicine at the Ludwig Maximilian University of Munich). Arrows denote if the respective value is below or above normal range.

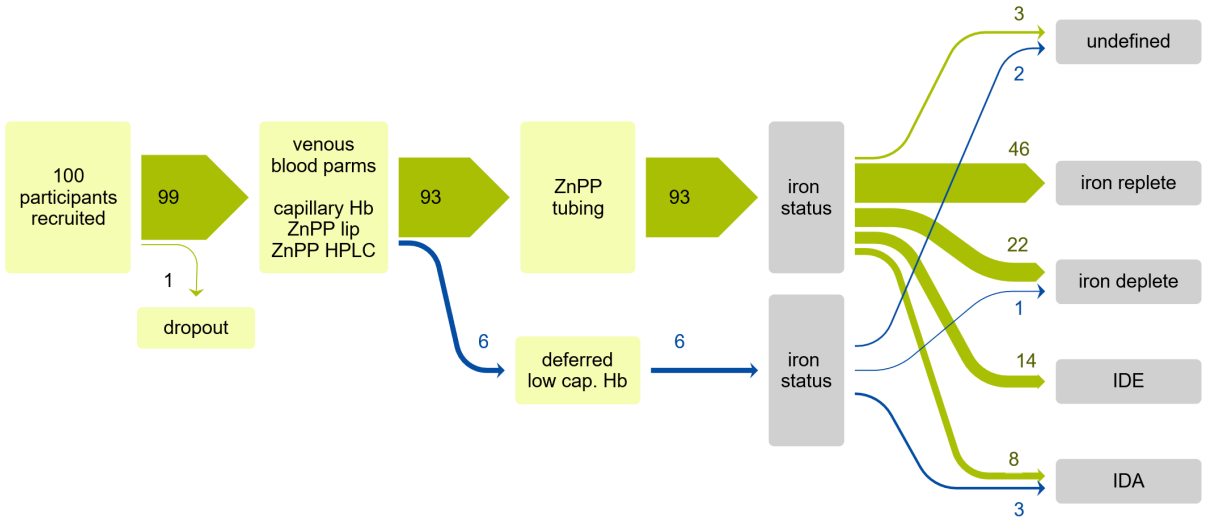
Due to the possibility of incorrectly high values of the acute phase protein ferritin during inflammation, this parameter was considered secondary to more specific iron parameters.

Not determined values originated from incorrect order submission to the laboratory.

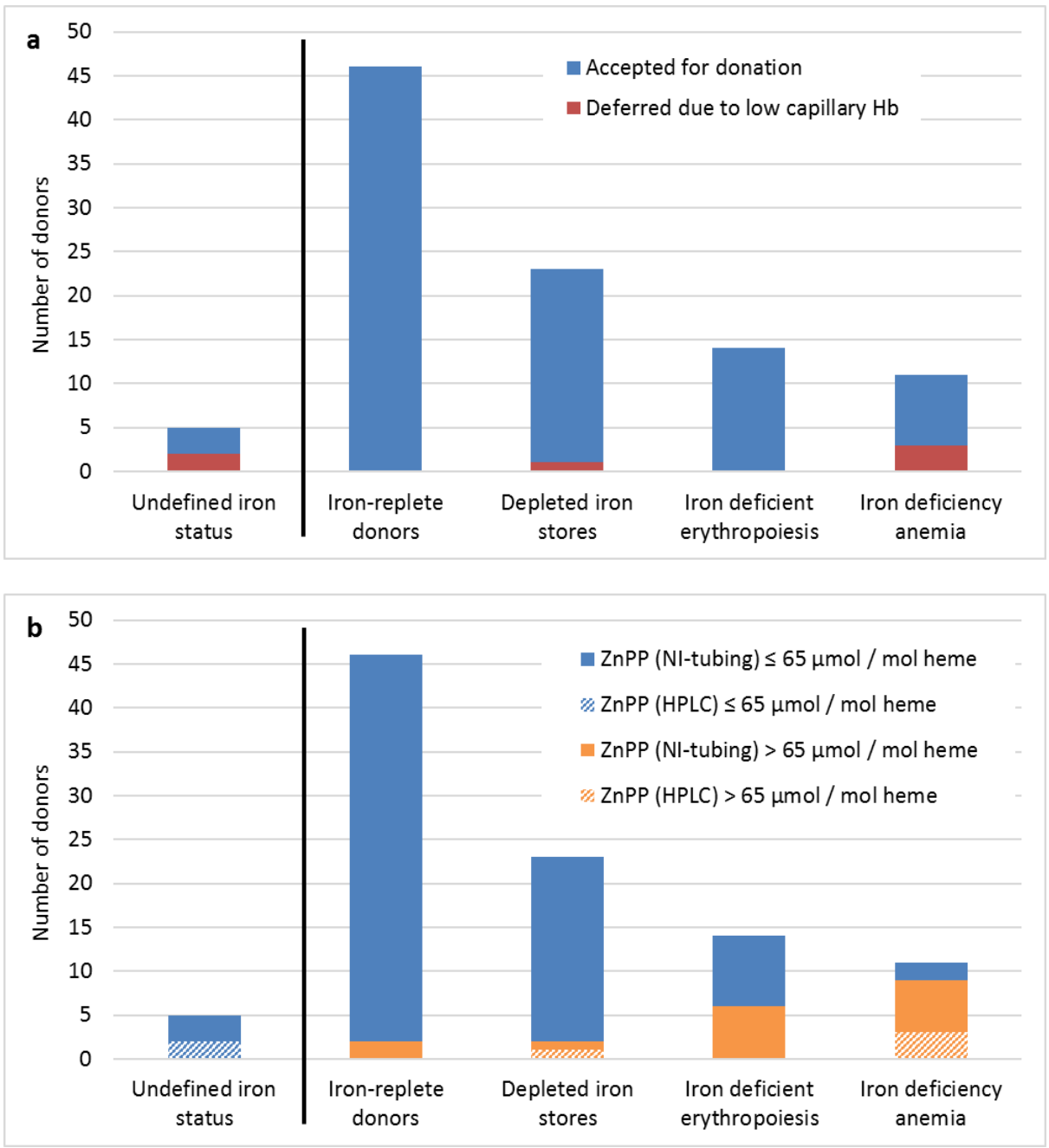
Supplementary Table 2: ZnPP values of the deferred subjects

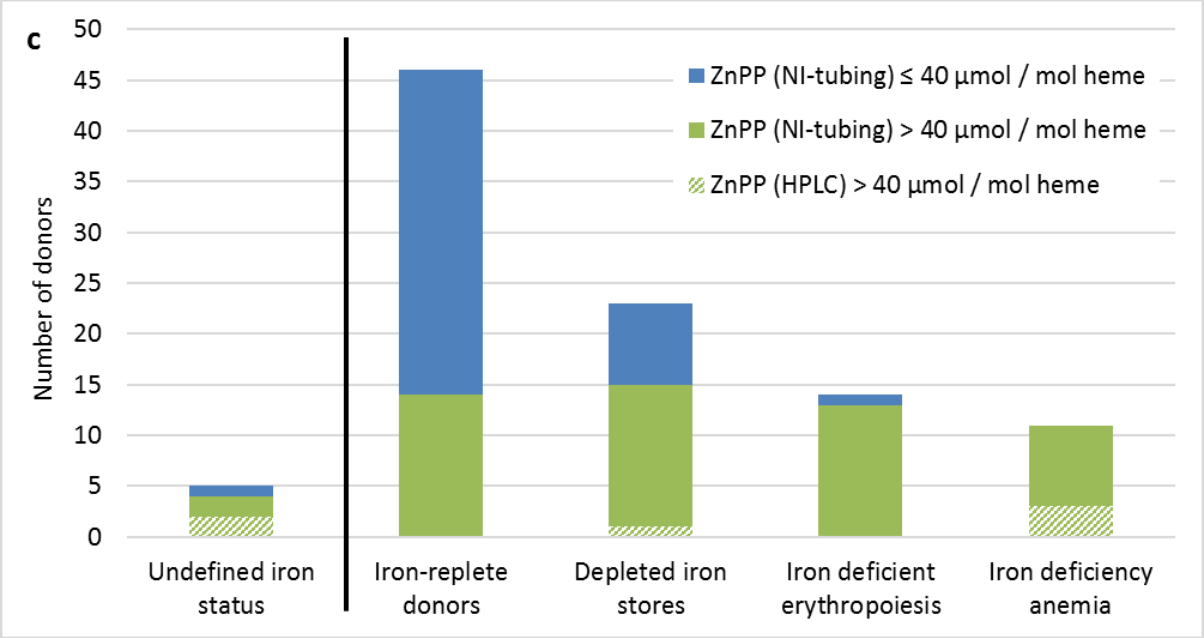
	Parameter		
ƒ	ZnPP HPLC [µmol/mol heme]	ZnPP NI-lip [µmol/mol heme]	ID-Grade
11.2	43	52.2	undefined iron status
11.4	169.8	177.4	IDA
11.9	175.5	161.4	IDA
10.9	150.6	128.1	IDA
11.9	53.1	47.2	undefined iron status
12.4	75.1	90.5	depleted iron stores

Supplementary Figure 1: Flow chart of study including subjects assigned to iron deficiency stages according to Supplementary Table 1. From 100 recruited subjects, 1 dropped out prior to venous blood collection voluntarily. Venous blood parameters as well as capillary Hb and ZnPP measurements on the lip and by HPLC were determined from the remaining 99 subjects. 6 of the remaining 99 subjects were deferred from blood donation due to low capillary Hb and no NI-tubing measurement of ZnPP could be performed. The right side of the chart shows the assignment of the subjects to the applied iron deficiency stages.



Supplementary Figure 2. Bar charts showing the classification of potential donors according to iron status. Fig. 2a number of subjects accepted for donation (blue) and number of deferred subjects due to low capillary Hb (red) in each group. **Fig. 2b** Number of subjects with ZnPP below (blue) and above (orange) the cutoff of 65 µmol ZnPP / mol heme. For the six subjects which were deferred from donation and hence no NI-tubing measurement was available, highly correlating ZnPP HPLC values were used instead (n=6, shown with stripes). **Fig. 2c** number of subjects with ZnPP below (blue) and above (green) the cutoff of 40 µmol ZnPP / mol heme. For the six subjects which were deferred from donation and hence no NI-tubing measurement was available, highly correlating ZnPP HPLC values were used instead (n=6, shown with stripes).




c
50
45
40
35
30
25
20
15
10
5
0
Number of donors
ZnPP (NI-tubing) ≤ 40 µmol / mol heme
ZnPP (NI-tubing) > 40 µmol / mol heme
ZnPP (HPLC) > 40 µmol / mol heme
Undefined iron status
Iron-replete donors
Depleted iron stores
Iron deficient erythropoiesis
Iron deficiency anemia