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Non-Invasive Zinc Protoporphyrin Screening Offers Opportunities for Secondary Prevention of Iron Deficiency in Blood Donors

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Keywords
Iron deficiency · Erythropoiesis · Anemia · Zinc protoporphyrin · Blood donation

Abstract

Background: Frequent blood donors are at high risk of developing iron deficiency. Currently, there is no potent screening during blood donation to detect iron deficient erythropoiesis (IDE) before anemia develops and deferral from donation is inevitable. ***Study Design and Methods:*** In addition to capillary and venous hemoglobin, the iron status of 99 frequent blood donors was assessed by various venous blood parameters and zinc protoporphyrin IX (ZnPP). ZnPP was determined by high-performance liquid chromatography (HPLC) and a new prototype fiber-optic device was employed for non-invasive measurements of ZnPP through the blood collection tubing (NI-tubing) and on lip tissue (NI-lip). We aimed to evaluate the feasibility and diagnostic value of the NI-tubing measurement for early detection of severe iron deficiency in blood donors. ***Results:*** NI-tubing and HPLC reference measurements of ZnPP showed narrow limits of agreement of 12.2 µmol ZnPP/mol heme and very high correlation (Spearman's Rho = 0.938). Using a cutoff of 65 µmol ZnPP/mol heme, NI-tubing measurements ($n = 93$) identified 100% of donors with iron deficiency anemia (IDA) and an additional 38% of donors with IDE. Accordingly, NI-tubing measurements would allow detection and selective protection of particularly vulnerable donors. ***Conclusion:*** NI-tubing measurements are an accurate and simple method to implement ZnPP determination into the routine blood donation process. ZnPP was able to identify the majority of subjects with IDE and IDA and might therefore be a valuable tool to provide qualified information to donors about dietary measures and adjustments of the donation interval and thereby help to prevent IDA and hemoglobin deferral in the future.

Introduction

Blood donation is mostly organized by nonprofit health or social welfare organizations, such as the Red Cross. Worldwide, over one hundred million units of blood are donated each year [1]. Among all blood donors, the vast majority donates blood regularly. For example, the Bavarian Red Cross (BRK) recruits 88% of blood donations from repeat donors. In Germany, male donors are allowed to donate up to 6 times a year, female donors up to 4 times [2]. Each donation draws 500 mL of whole blood, including 200–250 mg of iron. Taking into account that iron stores in men are 500–1,000 mg and 200–400 mg in premenopausal women, each blood donation depletes the iron stores considerably [3], also in comparison to the usual daily loss of about 1 mg [4]. As only a maximum of 7 mg of iron – if ferritin levels are already

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very low – can be absorbed per day from the diet, frequent donors are at a considerable risk of gradually depleting their iron stores [5]. When laboratory tests were made in research studies among regular blood donors, iron deficiency was found in 59% of cases in a US study (log [soluble transferrin receptor/ferritin] >2.07) [6] and in up to 27.4% (men with hepcidin <0.5 nmol/L) in a Danish study [7]. Advanced iron deficiency can lead to symptoms such as headaches, difficulty concentrating, depressive symptoms, and shortness of breath. Persistent iron deficiency eventually leads to iron deficiency anemia (IDA). Only in this case, the hemoglobin (Hb) value drops below predefined eligibility thresholds and donors are deferred from donation on the basis of a pre-donation Hb measurement. The corresponding deferral rates are in the order of a few percent [8]. Rejection from blood donation has been shown to reduce the likelihood of donating again [9, 10]. Thus, early detection and mitigation of iron deficiency is not only relevant for the personal health of the blood donor but could also help prevent on-site donor deferral and the subsequent loss of valuable blood donors. Unfortunately, there is so far no practical, quick and low-cost test, which could indicate the gradual development of iron deficiency at an early stage. All attempts made so far are restricted to study scenarios, and none of the investigated parameters and related devices have found their way into routine use. Blood tests, which indicate iron depletion or – probably more relevant – iron deficient erythropoiesis (IDE) comprise numerous parameters, such as ferritin, transferrin saturation (TSAT), soluble transferrin receptor (sTfR), and erythrocyte indices, which are usually measured and interpreted jointly as an appropriate criterion to conclude about the availability of iron for erythropoiesis. Ferritin is the most frequently studied parameter [11–15] and well accepted as an indicator of iron stores among otherwise healthy people, such as blood donors. However, there is still a lack of consensus with respect to the relevant threshold values used to indicate iron deficiency and to guide iron supplementation recommendations [12, 14]. In addition, possible symptoms induced by low iron stores alone, such as fatigue, restless legs syndrome, or impaired cognitive function, have to be balanced with possible side effects of regular iron supplementation: stomach cramps, constipation, or nausea may occur and may lead to a discontinuation of the iron treatment or – more severe – prolonged iron supplementation may even increase the risk for colon cancer [14, 16]. Hence, iron depletion as defined by low ferritin alone may not be the most relevant condition to look for when aiming to improve blood donor health and availability. It seems reasonable that only individuals with prolonged iron depletion that already impairs heme production during erythropoiesis – as is the case in IDE – will develop significant morbidity and be at risk for future anemia development. These donors should be the target for proactive mitigation measures such as iron supplementation or extension of the deferral period, even when anemia is not yet apparent.

Probably the best-accepted single parameter to indicate IDE is sTfR. The German Society for Hematology and Medical Oncology (DGHO) recommends the use of sTfR for the differential diagnosis of IDE as part of an algorithm for iron deficiency assessment [3]. The diagnostic potential of zinc protoporphyrin IX (ZnPP) for IDE diagnostics is also recognized by this society, with the lack of a leading standard for ZnPP determination cited as an obstacle to routine use [3]. In addition, the most commonly used method for determining ZnPP with a hematofluorometer only yields reliable results when using washed erythrocytes and needs skilled personnel to operate the device reproducibly [17, 18]. It is therefore difficult to integrate ZnPP determination into the blood donation workflow. These reasons may explain why ZnPP is currently not used in blood donor management, despite promising results demonstrating the capability of ZnPP to identify iron-deficient donors in earlier studies [8, 19–23]. The herein proposed approach to measure ZnPP overcomes the limitations of standard hematofluorometer measurements and may be easily integrated into the routine blood donation process.

In earlier work, we have demonstrated that with the use of a high dynamic range spectrometer and two-wavelength excitation, ZnPP fluorescence can be reliably extracted from very large background signals [24, 25]. In this way, ZnPP can be detected directly from whole blood without any prior blood processing. Moreover, the developed innovative device allows for completely non-invasive (NI) measurements of ZnPP through the red vermillion of the lower lip (NI-lip measurement) with a fiber-based applicator, delivers an immediate result and can be used at the point of care [25, 26]. Therefore, it might provide a standard of measurement in the future.

Given the versatility of the device, it appears well suited for integration into the blood donation logistics. To this end, a specific applicator was developed to be clipped onto the transparent hose of the blood collection tubing for ZnPP measurement (NI-tubing measurement). This applicator fixes the optical fiber perpendicularly onto the transparent hose so that light emitted from the fiber can probe the blood flowing through the tubing. The personnel just has to fix the applicator on the tubing (see Fig. 1) and start the measurement at the beginning of blood drawing. The result is available within a few seconds.

Based on the abovementioned medical needs the aims of the study were to:

- validate the NI-tubing measurement against high-performance liquid chromatography (HPLC) reference measurements of ZnPP.

- confirm the accuracy of NI-lip measurements of ZnPP as found in previous studies.
- evaluate the diagnostic value of ZnPP compared to the routine capillary Hb measurement and whether it might offer valuable benefit for blood donors and blood donation services.

Materials and Methods

Study Design

This single-armed, multicenter study was conducted at a series of mobile blood donation events run by the blood donation service of the BRK. Between August and December 2017, 100 frequent blood donors (age ≥18 years) with more than three (women) and four (men) donations in the past 12 months were included at eleven mobile blood donation events organized by the BRK district associations of Munich and Augsburg. Exclusion criteria were blood transfusions in the preceding year (<12 months prior to examination), recent iron replacement therapy (<3 months prior to examination), diagnosis of hemochromatosis and refusal from blood donation due to reasons other than low Hb levels. Written informed consent was obtained from each participant. The study is registered at the German clinical trials register as DRKS00013102 and was approved by the Ethics Committee at the Ludwig Maximilian University of Munich, approval number 17-021.

Blood Sample Collection and Laboratory Analysis

At the mobile blood donation events, each blood donor was routinely screened for low capillary Hb levels. This was done by lancet-puncture of the earlobe (n = 90) or fingertip (n = 10) at the point of care using a Hb reader (CompoLab TM, Fresenius Kabi AG, Bad Homburg, Germany). In addition, venous blood was drawn to assess the iron status. If admitted to blood donation, venous blood samples were collected from the predonation sampling bag of the blood collection system in addition to routine blood sampling by the blood donation service. In case of rejection from blood donation, venous puncture and blood drawing were performed explicitly for the purpose of the study. The venous parameters Hb, serum ferritin, TSAT, sTfR, mean corpuscular Hb, and mean corpuscular volume were determined by the Institute of Laboratory Medicine at the Ludwig Maximilian University of Munich using clinical chemistry analyzers for venous Hb, mean corpuscular Hb and mean corpuscular volume (XN 9000, Sysmex Corporation, Kobe, Japan) and for ferritin and TSAT (AU 5800, Beckman Coulter Corporation, Brea, USA). The sTfR values were determined by BN ProSpec with Dade Behring Assay (Siemens Healthineers AG, Erlangen, Germany). An aliquot of EDTA blood allowed measuring the ZnPP/heme ratio by HPLC as a reference to the executed NI ZnPP measurements. The HPLC measurements were performed by an independent laboratory (MVZ Labor PD Dr. Volkmann und Kollegen GbR, Karlsruhe, Germany) as described in Hennig et al. [25].

NI Measurement of the ZnPP/Heme Ratio

The NI ZnPP measurements were conducted by a team of five authorized examiners with two prototype devices (FID*screen*, FerroSens GmbH, Munich, Germany). The detailed working principle of the prototypes is described elsewhere [25].

In the present study, this measurement method was adapted to application through the tubing of the blood collection system during blood donation. During the study, blood collection systems from Haemonetics (Leukotrap WB, CPD/SAG-M, product code WBT438DCG, Boston, USA) with 70 mL CPD stabilizer for 500 mL blood collection were utilized. The measurement was carried out as follows. After venipuncture, the blood flow through the blood collection tubing enabled the filling of the blood collection bag. To attach the tubing applicator of the measuring device (Fig. 1), the section of the tubing between the puncture site and the blood collection bag was used. To exclude potential interference from environmental contaminants, the measuring site of the tubing was cleaned with alcohol-based disinfectant before insertion into the applicator. The applicator was designed to fix the tubing in a defined depression, so that the blood collection tubing made direct and reliable contact with the glass fiber inside. In order to avoid incorrect measurements due to ambient light, the applicator was magnetically sealed by an opaque plastic lid. The measurement was started after a continuous blood flow into the blood donation bag was ensured and repeated at two further tubing sections for each study participant. The final result was calculated from the mean value of the three measurements. Additionally, a scaling factor was calculated from the comparison of tubing measurements and HPLC results, allowing an offset to correct for disturbances from spectral signatures of the blood collection tubing.

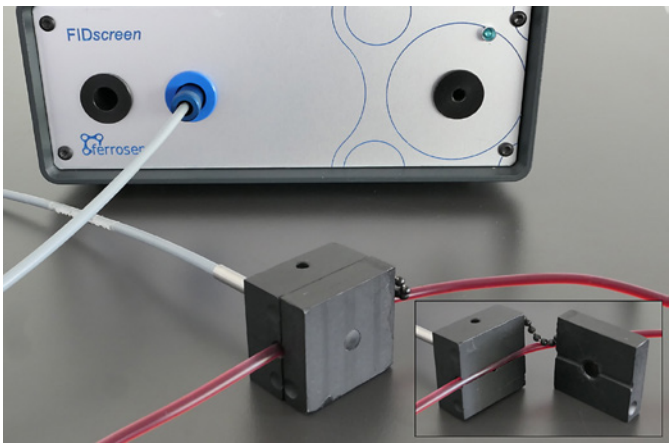

Fig. 1. Applicator for measurement of ZnPP fluorescence through the blood collection tubing. The inserted picture shows how the tubing is clicked into a groove in one part of the applicator, where the measurement fiber then is in contact with the tubing. The two halves of the applicator are fixed to each other by small magnets and shield the tubing from ambient light during measurement.

Study Procedure

If the inclusion and exclusion criteria were satisfied, the implementation of the study started with the NI-lip measurement. This was followed by capillary Hb determination. In case of confirmed eligibility for blood donation based on capillary Hb values of ≥13.5 g/dL (males) and ≥12.5 g/dL (females), the NI-tubing measurement was performed. In addition, venous blood samples were taken from the pre-donation sampling bag of the blood collection system. If the capillary Hb value was too low and the study participant was deferred from blood donation, a venipuncture was performed to obtain venous blood samples. The active study participation ended after the blood donation or after the collection of the venous blood sample.

Clinical Definitions

To enable a statement about the diagnostic performance of the NI measurements, the study population was categorized according

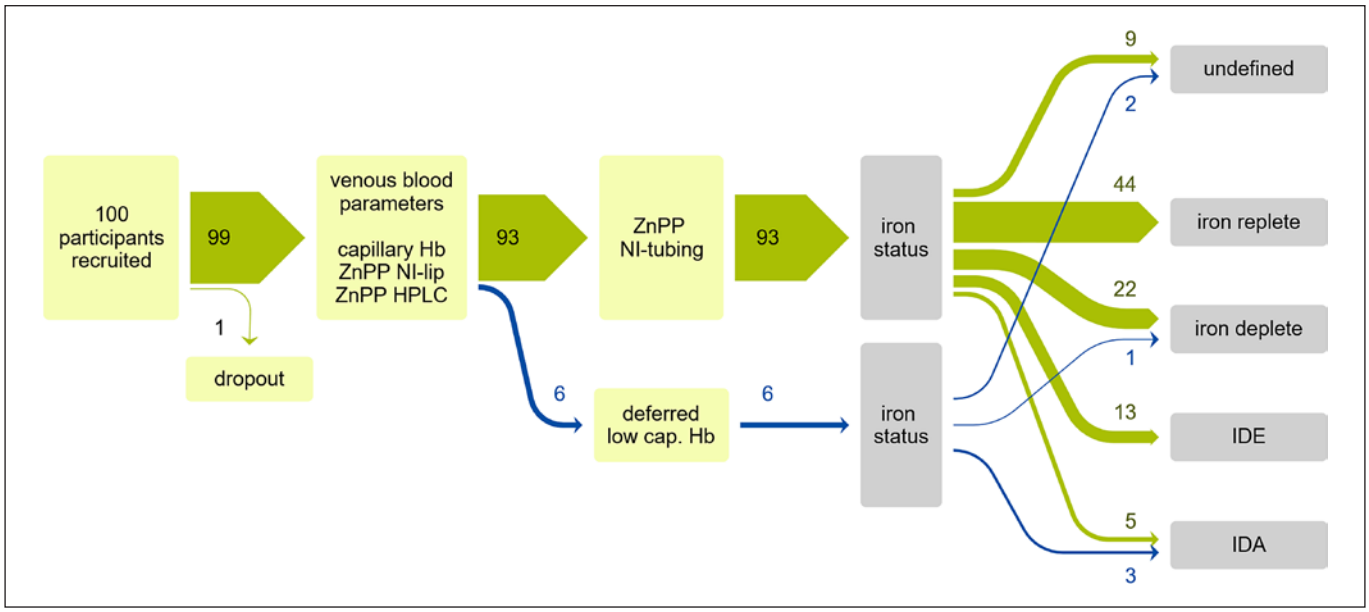

Fig. 2. Flowchart of study. From 100 recruited subjects, 1 dropped out prior to venous blood collection voluntarily. Venous blood parameters as well as capillary Hb and ZnPP measurements on the lip and by HPLC were determined from the remaining 99 subjects. 6 of the remaining 99 subjects were deferred 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.

to iron deficiency stages. The applied classification is commonly used in literature on iron deficiency diagnosis [27, 28] and is recommended by the DGHO [3]. For the purpose of this study, the iron deficiency stages are defined as follows:

- 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).

As an alternative to ferritin and sTfR, the measurement of ZnPP also provides a means to detect IDE. According to the reference values of the MVZ Laboratories Volkmann, impairment of erythropoiesis can be ruled out below 40 µmol ZnPP/mol heme. In a previous study, we have identified a "grey zone" between 40 and 65 µmol ZnPP/mol heme with ZnPP levels >65 µmol ZnPP/mol heme being indicative of IDE with a high probability [29]. In the present study, a ZnPP cutoff of 65 µmol ZnPP/mol heme was primarily applied for IDE/IDA detection, but the cutoff of 40 µmol ZnPP/mol heme was tested likewise.

Statistical Analysis

For quantitative comparison of NI-tubing, NI-lip, and HPLC measurements of ZnPP, limits of agreement (LoA) were calculated using the robust τ-estimate of the differences between the results of each two methods according to Bland-Altman analysis [30]. The 95% confidence intervals were calculated by bootstrapping (software R, version 3.2.2, functions scaleTau2, boot, boot.ci). Since none of the data sets was normally distributed, Spearman's Rho correlation coefficient was calculated to assess the quality of the correlation (software IBM SPSS Statistics, version 24).

Results

Study Population

Initially, 100 study participants, who met the inclusion and exclusion criteria, were included in the study. One subject discontinued participation before blood sampling voluntarily. Of the remaining 99 participants, 40 were females and 59 males. The mean age was 46 years for female subjects (range: 18–70 years, median: 51 years) and 51 years for male subjects (range: 20–72 years, median: 52 years).

Prevalence of Iron Deficiency in the Study Population

The enrolled 99 subjects (100%) were classified into the iron deficiency stages mentioned above according to their venous blood values. 11 subjects did not meet the predefined criteria for assignment to one of the iron deficiency stages and were therefore classified under "undefined iron status". Under these conditions, the prevalence of iron deficiency in the study population was 44% of which 23% had only depleted iron stores, 13% had IDE and 8% IDA (see Fig. 2).

Agreement between Fiber-Optic ZnPP Measurements and HPLC Reference

A Spearman Rho correlation coefficient of 0.938 ($p < 0.001$) showed significant correlation between the results of the NI-tubing and the HPLC measurement (Fig. 3a).

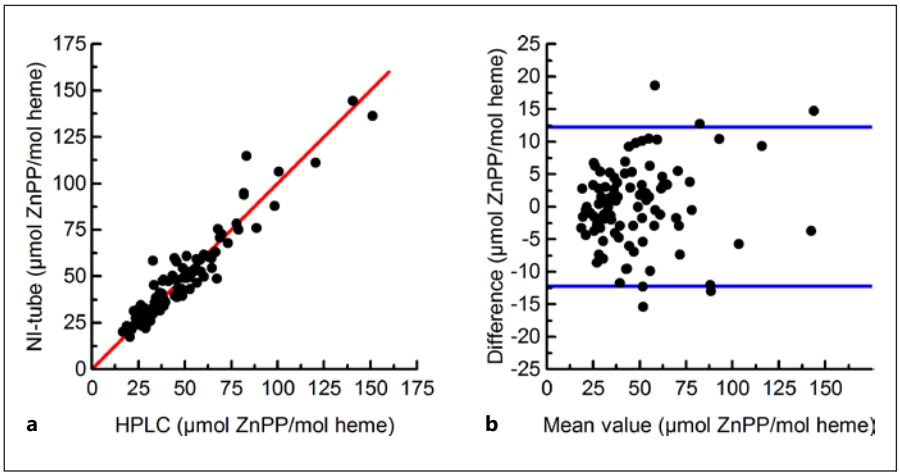

Fig. 3. Comparison of ZnPP measured in venous blood samples by high-performance liquid chromatography (HPLC measurement) and non-invasively through the blood collection tubing during blood donation (NI-tubing measurement). **a** Correlation between the two measurements. The red diagonal line represents the balance line. **b** Bland-Altman plot. The blue horizontal lines indicate the robust limits of agreement of 12.2 µmol ZnPP/mol heme.

Table 1. Comparison between ZnPP measurement methods using Bland-Altman analysis

Compared ZnPP measurement methods	*n*	LoA	CI
HPLC – NI-tubing	93	12.2	9.6–14.5
HPLC – NI-lip	99	20.9	17.6–24.1
NI-tubing – NI-lip	93	17.8	14.4–20.8

The LoA were 12.2 µmol ZnPP/mol heme, with a 95% confidence interval of 9.6–14.5 µmol ZnPP/mol heme (Fig. 3b). Thus, the LoA clearly lie below the ZnPP-difference of 25 µmol ZnPP/mol heme between manifest (>65 µmol ZnPP/mol heme) and excluded IDE (≤40 µmol ZnPP/mol heme). Table 1 additionally shows the results of the Bland-Altman Analysis performed for the comparison between the NI-lip and HPLC measurement as well as the fiber-optic measurements among each other. The best agreement was found between NI-tubing and HPLC measurement.

Diagnostic Value of NI ZnPP Measurements for the Detection of Iron Deficiency Compared to Capillary Hb Measurements

By capillary Hb determination, 3 of 8 subjects with IDA, none of 13 subjects with IDE, 1 of 23 subjects with depleted iron stores, none of 44 iron-replete subjects, and 2 of 11 subjects with undefined iron status were rejected from donation (Fig. 4a). In the cases with low capillary Hb, the NI-tubing measurements could not be conducted, due to the deferral of these subjects.

Of the remaining subjects that were accepted for donation (n = 93), the following number of subjects exceeded a cutoff of 65 µmol ZnPP/mol heme in the respective groups: 5 of 5 donors with IDA, 5 of 13 donors with IDE, 1 of 22 donors with depleted iron stores, 2 of 44 iron-replete donors, and 2 of 9 donors with undefined iron status (Fig. 4b). Using a cutoff of >65 µmol ZnPP/mol heme as indicative of IDE and IDA, 10 of 18 donors with IDE and IDA were correctly identified by this method, while only 3 of 66 donors 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 5 of 5 donors with IDA, 12 of 13 donors with IDE, 14 of 22 donors with depleted iron stores, 14 of 44 iron-replete donors and 6 of 9 donors with undefined iron status (Fig. 4c). Using a cutoff of >40 µmol ZnPP/mol heme thus identified 17 of 18 donors with IDE and IDA, while identifying 64% of subjects with only depleted iron stores. However, 32% of iron-replete donors are then classified as iron deficient. Due to the high agreement of the NI-tubing and NI-lip measurement, NI-lip measurement showed similar detection rates when applied in addition to capillary Hb measurement, as shown in Table 2.

Discussion

The primary aim of the present study was to test the feasibility of a new NI approach to measure ZnPP from whole blood as it flows through the transparent blood collection tubing during blood donation. We have previously described a novel spectroscopic method for measuring ZnPP levels, which overcomes most of the limitations of hematofluorometers [24] and is sensitive enough to measure ZnPP non-invasively from the lower lip [25]. With a modification of the light application and sensor probe, the approach proved similar agreement with reference HPLC determination of ZnPP levels as in our previous studies and may offer unique utility in the blood donation setting. These earlier studies demonstrated a good correspondence between NI-lip and reference measurements

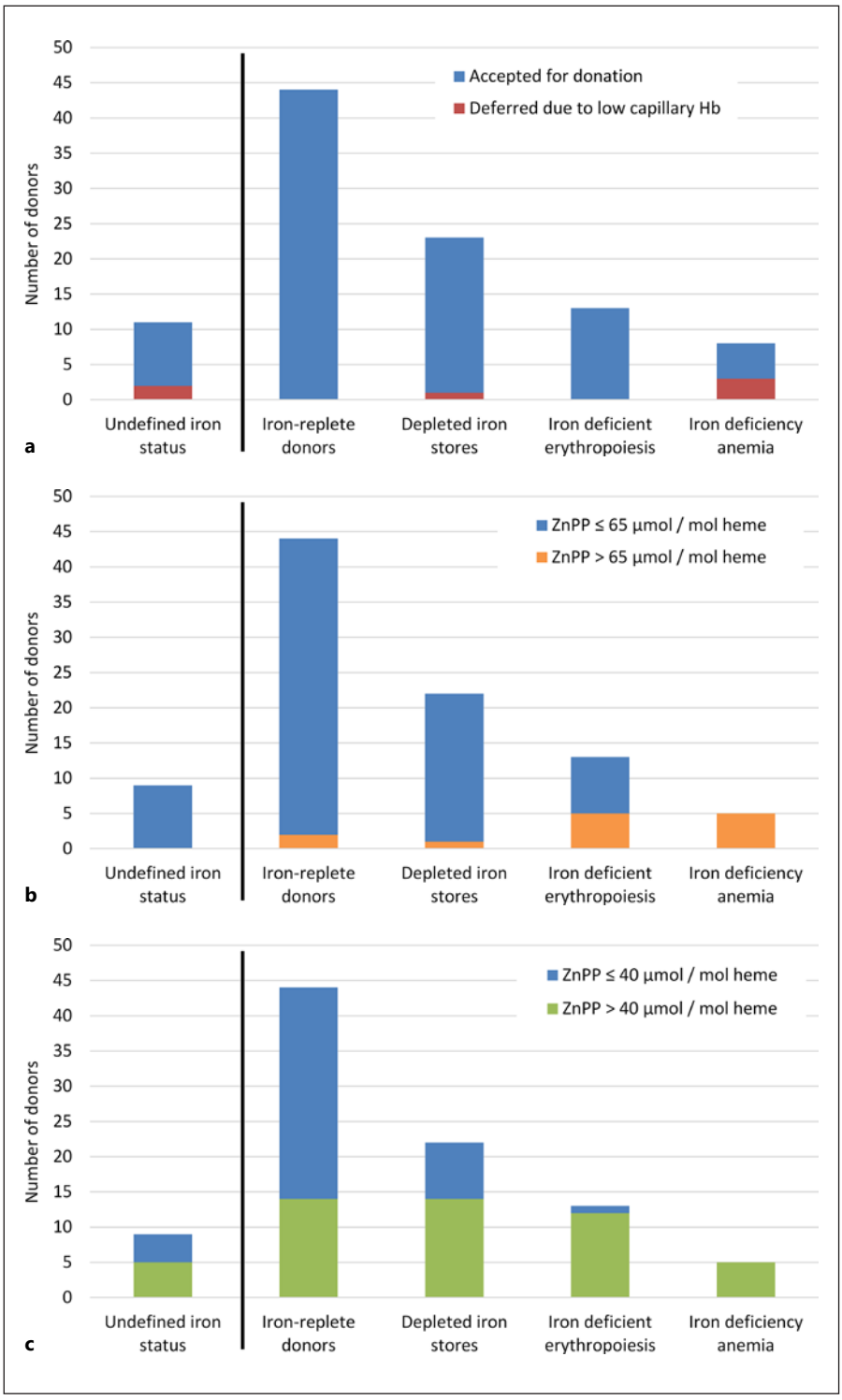

Fig. 4. Bar charts showing the assignment of potential donors to the applied iron deficiency stages. **a** Number of subjects accepted for donation (blue) and number of deferred subjects due to low capillary Hb (red) in each group. **b** Number of accepted donors (without deferred subjects) with NI-tubing measurement of ZnPP below (blue) and above (orange) the cutoff value of 65 µmol ZnPP/mol heme. **c** Number of accepted donors (without deferred subjects) with NI-tubing measurement of ZnPP below (blue) and above (green) the cutoff value of 40 µmol ZnPP/mol heme.

in blood with narrow LoAs between 14 µmol ZnPP/mol heme [31] and 19.7 µmol ZnPP/mol heme [25, 29].

In the current study, LoA between NI-lip measurement and HPLC reference were 20.9 µmol ZnPP/mol heme, comparing well to the previous results and confirming its validity as a method for NI ZnPP determination. Between NI-tubing and HPLC measurements LoA of 12.2 µmol ZnPP/mol heme were found, which demonstrates an excellent accuracy of the NI-tubing measurement. The continuous flow of blood through the tubing alleviates interfering effects of fluorescence photobleaching or sedimentation of erythrocytes, contributing to the high accuracy.

Table 2. Iron deficiency detection through NI-lip measurement

Iron deficiency stage	Detected/total subjects (cutoff at 40 µmol ZnPP/mol heme)	Detected/total subjects (cutoff at 65 µmol ZnPP/mol heme)
IDA	5/5 (100%)	3/5 (60%)
IDE	12/13 (92%)	3/13 (23%)
Depleted iron stores	13/22 (59%)	1/22 (5%)
Iron-replete	23/44 (52%)	2/44 (5%)
Undefined iron status	6/9 (66%)	2/9 (22%)

IDA, iron deficiency anemia; IDE, iron deficient erythropoiesis.

The second important aim of this study was to investigate whether NI measurement of ZnPP can contribute to secondary prevention of IDA in frequent blood donors by recognizing advanced iron deficiency at an earlier stage than routine capillary Hb. To evaluate this, all subjects were classified according to iron status based on ferritin, sTfR, and Hb obtained from venous sample laboratory measurements.

Using a cutoff of 65 µmol ZnPP/mol heme, ZnPP screening by NI-tubing measurements was able to identify all subjects with IDA and additionally 5 of 13 subjects with IDE. In contrast, capillary Hb determination did not identify any of the 13 donors with IDE. More surprisingly, capillary Hb identified only 3 of 8 donors with IDA as defined by venous blood parameters. Of note, NI-tubing measurements were not available for these 3 cases with IDA because of deferral from blood donation. However, both NI-lip and HPLC measurements yielded ZnPP values above 65 µmol ZnPP/mol heme for these subjects (online suppl. Table 2; for all online suppl. material, see www.karger.com/doi/10.1159/000528545). In summary, in the present study, NI measurements of ZnPP clearly outperformed capillary Hb determination with respect to identifying advanced iron deficiency in frequent blood donors.

Different scenarios for improved donor protection through ZnPP screening can be envisioned. A promising approach might be to combine capillary Hb and NI-tubing measurement of ZnPP to decide on future donor management strategy. Assuming donor eligibility continues to be based on capillary Hb, ZnPP measurement on the blood collection tubing could be used to identify donors at risk for future IDA and allow blood centers to propose mitigation measures such as iron supplementation or extension of donation intervals. That strategies involving iron supplementation can improve recovery of donor iron stores and lead to lower deferral rates has already been shown [13, 32].

Another option for blood centers could be to modify their screening strategy for regular donors. For example, if capillary Hb exceeded the threshold for IDA, including a safety margin, AND NI-tubing measurement of ZnPP excluded IDE, one might decide to omit capillary Hb measurements in subsequent donation visits depending on the present capillary Hb and the risk for iron deficiency reflected by the ZnPP value. Some blood establishments have already implemented similar scenarios, for example, by omitting capillary Hb determination before blood donation if the venous Hb from the previous donation exceeded 12.9 g/dL for females or 13.9 g/dL for males [33]. Used in this manner, the proposed ZnPP screening would result in fewer invasive pre-donation screening tests for regular blood donors and would automatically lead to a longitudinal record of ZnPP levels enabling even more precise and appropriate recommendations than based on single ZnPP measurements.

Other approaches to protect donors with respect to iron deficiency are based on ferritin screening. In recent years, a number of blood establishments in Europe and USA have started using routine ferritin testing as an additional measure to better identify and manage donors at risk of developing severe iron deficiency. In the present cohort, ferritin levels were below cutoff for 50% of donors (including 6 donors that were classified with "undefined iron status"). This confirms the findings of other studies [6, 7], but imposing an extended deferral period on all these donors may not be desirable and is likely also not adequate. Therefore, it might make sense to identify only donors with IDE, who are at higher risk of developing anemia than donors with just depleted iron stores. In the present study, by using a ZnPP cutoff of 65 µmol ZnPP/mol, only 19% of the donors would have been considered as needing mitigation. In addition, ferritin testing is not yet available as a point-of-care test and thus requires laboratory assessment. This does not allow for immediate feedback to the donors but instead necessitates post-donation follow-up, which may be logistically complex and not always effective. From a cost perspective, ZnPP measurements also likely offer an advantage over ferritin measurements as the presented approach is purely optical without the need for disposables. Thus, use of ZnPP rather than ferritin to guide targeted interventions allows for

a less restrictive approach, which may be easier to manage by blood centers.

The above considerations are valid for adult donors. For adolescents, the situation is presumably different. Due to their higher demand for iron, iron stores run short more quickly and whole blood donations require longer intervals. In order to determine the appropriate recommended length of intervals between donations, a large study among 30,806 teenagers in the USA has measured ferritin and found that recovery of iron stores took much longer than in adults [34]. In order to protect teenagers from severe iron depletion, ferritin may therefore be the appropriate measure.

The true benefits of a ZnPP-based donor management approach in terms of impact on the donors' iron balance, eligibility, and willingness to donate remain to be fully established in future longitudinal studies. Previous studies found added value of ZnPP measurement in the prediction of iron deficiency in blood donors and suggested that ZnPP might be a helpful parameter in improving donor management but did not yet suggest specific strategies [8, 19–22].

Results from a recent placebo-controlled interventional study [35] involving iron-deficient donors showed that iron repletion following donation dramatically reduced the risk for Hb deferral at the next donation visit, from 58% to 16% in women and from 16% to 0% in men. A recent large-scale study (n = 55,644) in the Netherlands on the consequences of a new policy involving a 6–12 month temporary deferral in case of low ferritin also found that donors managed this way were less likely to unsubscribe permanently from donation than those deferred on site due to low Hb [10]. It seems reasonable that this might also translate to iron deficiency screening based on ZnPP.

The present study has a number of limitations. One relates to the low number of individuals included. In addition, 11 of 99 donors could not be assigned to one of the predefined iron status groups (for an assessment based on additional blood parameters see online Suppl. Table 1, online Suppl. Fig. 1, 2). Furthermore, four assignable donors were deferred due to low capillary Hb, and therefore, no NI-tubing measurement of ZnPP could be performed. Another limitation relates to the ZnPP cutoff values used. The current study focused on very frequent donors as we intended to assess donors with a high-risk profile who could particularly benefit from innovative diagnostics for reliable iron deficiency screening. However, since chronic blood loss can lead to adaptation of erythropoiesis [36], the cutoffs should also be investigated for first-time donors and irregular donors in the future. Similarly, questions may be raised about sTfR thresholds used to define IDE. Although the sTfR limits applied in the study are recommended for diagnosis of suspected IDE, the threshold values may not be adequate for regular blood donors as increased erythropoiesis leads to increased sTfR levels [3, 37]. A careful reconsideration of all threshold values and assignments into iron status groups is therefore indicated for larger studies in the future.

In conclusion, the present study clearly demonstrated the technical and practical feasibility of NI ZnPP measurements both on the lower lip as well as on the blood collection tubing. It also showed that ZnPP screening in addition to capillary Hb screening detects a high percentage of donors with iron deficiency before anemia occurs. Consequently, the NI ZnPP measurement methods may enable selective implementation of secondary preventive measures to protect donors against advanced iron deficiency and IDA. Its routine deployment thus offers an opportunity to improve donor protection and to support blood donation services in retaining their regular blood donors by avoiding future iron deficiency-related Hb deferrals.

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Statement of Ethics

The study is registered at the German clinical trials register as DRKS00013102 and was approved by the Ethics Committee at the Ludwig Maximilian University of Munich, approval number 17-021. All study participants have given their written informed consent.

Conflict of Interest Statement

A.S., A.L., L.H., M.V., and R.S. have disclosed no conflicts of interest. CH is shareholder and managing director of the start-up company FerroSens GmbH (Munich, Germany), which aims at commercializing the technology for NI detection of ZnPP. C.H., G.H., and H.S. are co-inventors of the patent family with application number PCT/EP2016/053389 "Apparatus and method for fluorescence measurements on tissue for the determination of blood fluorophores." F.W. and E.Q. are affiliated to the company "Blutspendedienst des Bayerischen Roten Kreuzes gemeinnützige GmbH" (Munich, Germany).

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Authors Contributions

C.H., G.H., A.L., and H.S. developed the fiber-optic ZnPP measuring device. A.S., C.H., G.H., A.L., R.S., H.S., F.W., and E.Q. developed the concept and designed the study. A.S., C.H., A.L., and H.S. recruited study participants and carried out the fiber-optic measurements. L.H. and M.V. were responsible for the biochemical and hematological laboratory determinations. A.S. and C.H. analyzed the data and performed all statistical analyses. All authors approved the final version of the manuscript. The corresponding author A.S. had full access to all the data in the study and shared responsibility for the decision to submit for publication with C.H. and H.S.

Data Availability Statement

Anonymized source data are available on request from the corresponding author.

HPLC, ZnPP reference measurement by high performance liquid chromatography; NI-lip, non-invasive ZnPP measurement through red vermillion of lower lip; NI-tubing, non-invasive ZnPP measurement through tubing of blood collection system during blood donation; n, number of participants included; LoA, robust limits of agreement according to description under statistical analysis; CI, confidence interval.

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