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SHORT REPORT

Iron deficiency and thrombocytosis

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According to many textbooks, iron deficiency (ID) is associated with reactive thrombocytosis. In this study, we aimed to investigate the correlation between serum ferritin levels and platelet counts in a large cohort of healthy blood donors. We included all whole blood and apheresis donors aged 18 years or older with at least one ferritin measurement and one platelet count performed at the same visit between 1996 and 2014. A total of 130 345 blood counts and ferritin measurements obtained from 22 046 healthy donors were analysed. Overall, no correlation between serum ferritin and platelet count was observed ($r = -0.03$, $\rho = 0.04$ for males, and $r = 0.01$, $\rho = -0.02$ for females, respectively). Associations remained clinically negligible after adjusting for age, time since previous blood donation, number of donations and restricting the analysis to ferritin deciles. In this large, retrospective single-centre study, correlations between low ferritin and platelet count in a large and homogeneous cohort of healthy donors were negligible. Further studies in patients with more severe anaemia and patients with inflammation are warranted.

Key words: iron, platelet count, blood donors.

Introduction

Trainees in internal medicine and haematology learn that iron deficiency (ID) with or without anaemia (iron deficiency anaemia, IDA) is often accompanied by thrombocytosis. Accordingly, most of the textbooks report on this association. For example, in *Harrison's Principle of Internal Medicine* and *Wintrobe's Clinical Hematology*, the authors describe thrombocytosis as a common finding in ID and state that the platelet count returns within normal values after iron substitution [1, 2]. The same is true for other standard haematology textbooks, that is *Williams Hematology* and *Postgraduate Haematology,* and for different guidelines [3, 4]. The British Committee for Standards in Haematology lists ID as a common cause of reactive thrombocytosis and includes the evaluation of iron status in the diagnostic work-up of patients presenting with thrombocytosis [5]. Furthermore, it has been suggested that reactive thrombocytosis may represent a risk factor for thromboembolic complications and optimal management is still a matter of debate [6].

Most of the currently available data are obtained from patient collectives and small studies, including children [7]. Recent results show that thrombocytosis seems to actually occur only in a minority of patients with IDA [8]. Other authors investigated the occurrence of thrombocytosis in patients with inflammatory bowel disease and IDA [9, 10]. To the best of our knowledge, large studies analysing the association between thrombocytosis and ID without anaemia in otherwise healthy individuals have not been published so far [7].

The pathophysiologic link between ID and thrombocytosis also remains unclear. Cell line and animal studies suggested that ID leads to megakaryocytic expansion and stimulates megakaryocytic differentiation, independently from thrombopoietin, interleukin-6 and interleukin-11 [11, 12].

For the detection of ID and IDA, the most reliable parameter remains serum ferritin, with low values being sufficient for the diagnosis [13, 14].

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In this study, we aimed to investigate the correlation between serum ferritin levels and platelet counts in a large cohort of healthy blood donors.

Methods

In this single-centre, retrospective study, all whole-blood and apheresis donors aged 18 years or older, both first-time and repeat donors, with at least one ferritin measurement and one platelet count performed at the same visit between 1996 and 2014 were included in the analysis.

As of 2004, serum ferritin measurements were routinely implemented in our centre in order to evaluate iron stores of repeat donors and improve donor care [15]. Before 2004, serum ferritin was measured only in single cases. Further iron parameters were not assessed.

All donors gave written informed consent. Donor eligibility criteria in accordance with Swiss regulations and the Council of Europe blood directives had to be fulfilled.

At each visit, a complete blood count taken from finger prick samples was run on a haematology analyser (Sysmex K-4500, Sysmex Digitana AG, Horgen, Switzerland). Serum ferritin was routinely determined on a venous sample either obtained from the predonation diversion pouch in eligible donors, or by venous puncture in donors deferred because of low haemoglobin in the capillary measurement. Serum ferritin measurements were conducted with a chemiluminescence assay (CMIA) on an integrated serum analyser for photometric, electrochemical and immunological assays (Architect ci8200, Abbott Diagnostics Division, Baar, Switzerland).

We assessed the association between serum ferritin and platelet count using Pearson's product–moment correlation coefficients (r) and Spearman's rank correlation coefficients (ρ). The latter are more robust when extreme values are present or when the association between variables is not linear but monotonic. Correlation coefficients below ±0·3 were considered negligible [16].

While potential heteroscedasticity and autocorrelation in our data do not result in biased parameter estimates, that is correlations, standard errors and hence inference are likely to be biased. Therefore, we applied a linear mixed model to further investigate the association between ferritin and platelet counts with platelet counts (G/l) as outcome and serum ferritin (μg/l) as predictor. Age at blood donation (years), a linear and quadratic term of the time since the previous donation (days), and the number of donations were added as covariates to the model. Random intercept and random slope for number of donations were used to capture the heterogeneity between donors, that is the different initial platelet counts and platelet trajectories between individual donors. The outcome was analysed separately for men and women. Furthermore, we assessed the association between ferritin and platelet counts overall and within ferritin deciles. We compared mean platelet counts and the association between ferritin levels and platelet counts in 10 similarly sized groups of donors with progressively lower ferritin values in order to assess the sensitivity of our findings. We reported unstandardized regression coefficients (b) with corresponding 95% confidence intervals and probability values (p). We employed robust standard errors in all models.

The linear mixed model for platelet counts of blood donors can be expressed as following:

$$Plt_{ij} = \beta_1 + \beta_2 \mathrm{Fe}_{ij} + \beta_5 \mathrm{TSPD}_{ij} + \beta_6 \mathrm{TSPD}_{ij}^2 + \beta_7 \mathrm{NOD}_{ij} + \beta_8 \mathrm{Age}_{ij} + \zeta_{1j} + \zeta_{2j} \mathrm{NOD}_{ij} + \varepsilon_{ij} \quad (1)$$

Eqn 1: overall association of ferritin with platelet counts adjusting for covariates

$$Plt_{ij} = \beta_1 + \beta_2 \mathrm{Fe}_{ij} + \beta_3 \mathrm{FeD}_{ij} + \beta_4 (\mathrm{Fe}_{ij} \times \mathrm{FeD}_{ij}) + \beta_5 \mathrm{TSPD}_{ij} + \beta_6 \mathrm{TSPD}_{ij}^2 + \beta_7 \mathrm{NOD}_{ij} + \beta_8 \mathrm{Age}_{ij} + \zeta_{1j} + \zeta_{2j} \mathrm{NOD}_{ij} + \varepsilon_{ij} \quad (2)$$

Eqn 2: association of ferritin with platelet counts within ferritin deciles adjusting for covariates.

Plt: platelet count (G/l); Fe: ferritin level (μg/l); FeD: ferritin decile; TPSD: time since previous blood donation; NOD: number of blood donations; age (years).

All statistical analyses were carried out in Stata 14 (StataCorp, College Station, TX). Statistical significance was established at $p \leq 0{\cdot}05$.

Results

A total of 130,345 blood counts and ferritin measurements obtained from 22,046 healthy volunteers were analysed. Median donor age at first donation was 30 years (interquartile range: 22–41 years); 52% were female donors.

Overall, correlations between serum ferritin and platelet count were negligible ($r = -0{\cdot}03$, $\rho = 0{\cdot}04$ for males, and $r = 0{\cdot}01$, $\rho = -0{\cdot}02$ for females, respectively; Fig. 1a,b). For donors with ID, defined as a serum ferritin level below 30 μg/l without anaemia (57,429 donations), correlations yielded $r = -0{\cdot}12$ and $\rho = -0{\cdot}10$. In cases with IDA, defined as haemoglobin <120 g/l in females and <140 g/l in males and a ferritin value <30 μg/l, the correlations for male donors (3,623 donations) were $r = -0{\cdot}03$, $\rho = -0{\cdot}03$, and for female donors (1,763 donations) were $r = -0{\cdot}13$, $\rho = -0{\cdot}16$ (Fig. 2a,b). Furthermore, if restricted to donors with a platelet count of >450 G/l (204 donations) or donors with a ferritin value <15 μg/l (22 863 donations), correlations remained negligible (for donors with a platelet count >450 G/l $r = 0{\cdot}00$, $\rho = -0{\cdot}09$; for

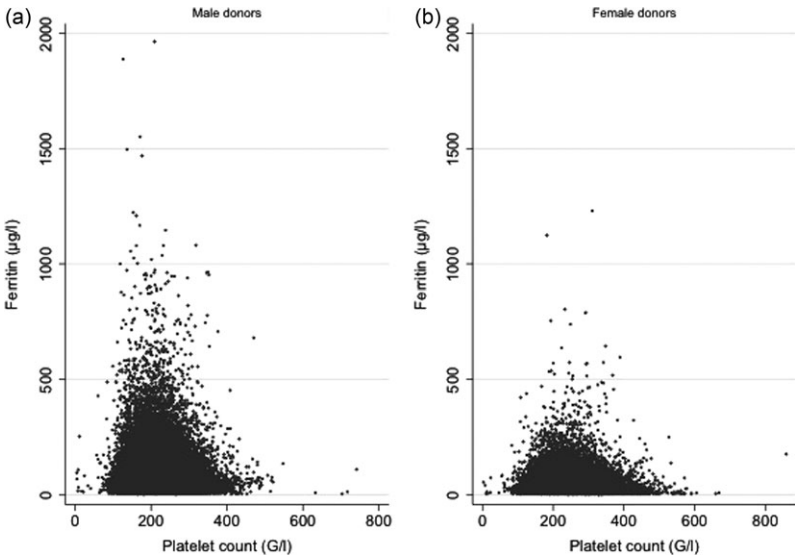

Fig. 1 Correlation between ferritin value (µg/l) and platelet count (G/l) for male (a) and female (b) donors.

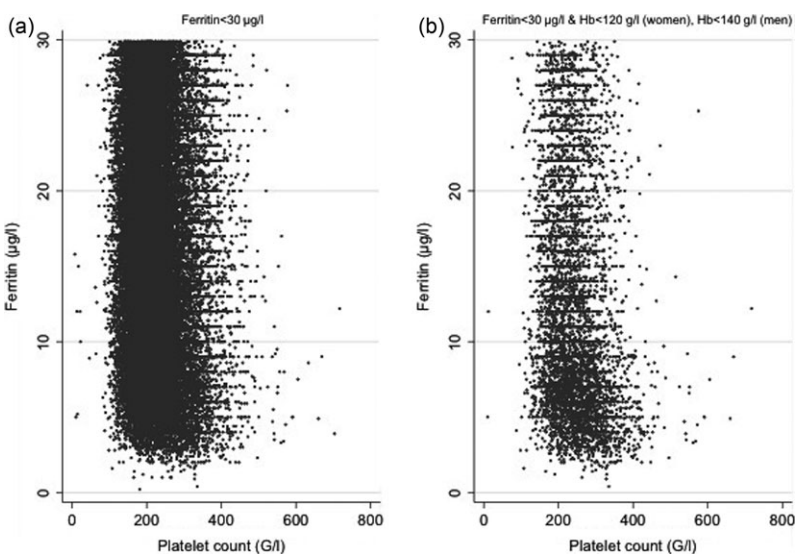

Fig. 2 Correlation between donors with a ferritin value of <30 µg/l and platelet count (a) and donors with IDA and platelet count (b).

donors with serum ferritin levels <15 µg/l $r = -0{\cdot}15$, $\rho = -0{\cdot}13$).

Consistent with the findings described above, no substantial overall association was found between ferritin levels and platelet counts adjusting for all covariates in our linear mixed model ($b = 0{\cdot}01$, $p = 0{\cdot}000$ for men; $b = 0{\cdot}00$, $p = 0{\cdot}749$ for women). The large sample size is responsible for the highly significant p-value in male donors. However, the overall effect is negligible (i.e. increase in platelet counts of 1 G/l for an increase of 100 µg/l in ferritin) and hence clinically insignificant. Mean platelet counts varied only marginally between ferritin deciles (Fig. 3). For women, mean platelet counts were between 10 and 15 G/l lower in the 2nd to 10th deciles as compared to the 1st ferritin decile. For men, this difference was smaller, with mean platelet counts between −5 G/l and +2 G/l from the 1st ferritin decile. Figure 3 clearly illustrates that in men, mean platelet counts between ferritin deciles are not statistically different from each other (overlapping 95% CI). On the other hand, for women we observe a statistically significant difference in the mean platelet count only between the 1st decile and the remaining deciles. Furthermore, the latter are not statistically different from each other. In order to better understand the correlation, we analysed the association between ferritin and platelet counts within the build ferritin deciles. Within each ferritin decile, associations between ferritin levels and platelet counts were also not substantial (Fig. 4) and the association between ferritin and platelet counts was statistically significant in only 4

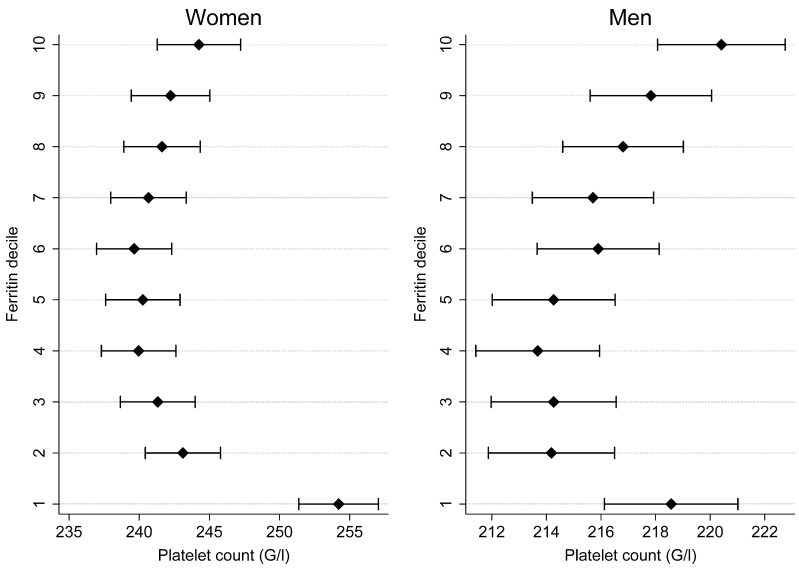

Fig. 3 Estimated mean platelet counts with 95% confidence interval by ferritin decile and gender. Linear mixed model adjusted for all covariates (age, number of days since last donation and number of donations).

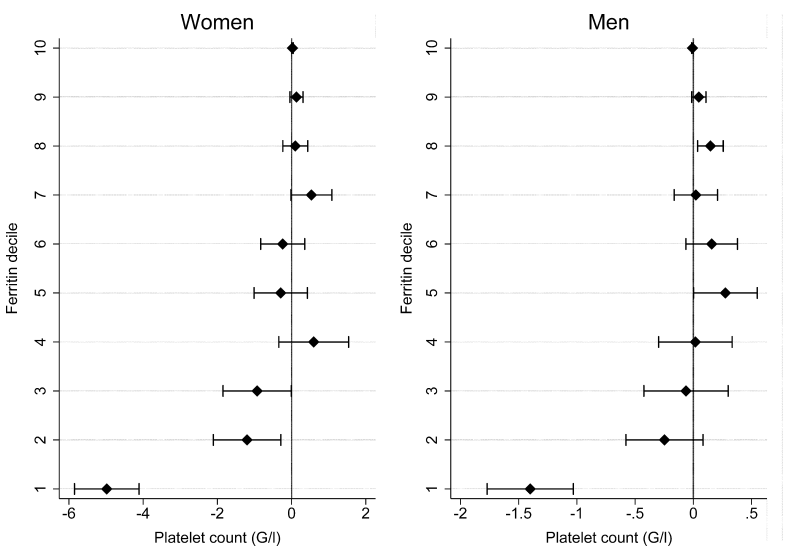

Fig. 4 Estimated effects of a 1 μg/l increase in ferritin on platelet counts with 95% confidence interval by ferritin decile and gender. Linear mixed model adjusted for all covariates (age, number of days since last donation and number of donations).

out of 10 deciles for both gender (men: 1st, 5th, 8th and 10th deciles; women: 1st, 2nd, 3rd and 10th deciles). The association between ferritin level and platelet counts was negative and more pronounced in the first decile (men: $b = -1{\cdot}4$, $p = 0{\cdot}000$; women: $b = -5{\cdot}0$, $p = 0{\cdot}000$). Given the range of observed ferritin values in the first decile (men: 0·2–13·6 μg/l; women: 0·4–8·0 μg/l), the expected reduction in platelet counts due to ferritin level ranged from −2·0 to −39·9 G/l for women and from −0·3 to −19·1 G/l for men, respectively. Corresponding expected reductions in platelet counts at the mean ferritin level in the first decile was −29·5 G/l for women and −13·8 G/l for men. However, reductions in platelet counts due to increasing ferritin levels were relatively small as compared to estimated initial platelet counts. Consequently, mean platelet counts differed only marginally between ferritin deciles and were in the range of 239–254 G/l for women and 213–220 G/l for men (Fig. 3).

With respect to the covariates, platelet counts tended to slightly increase with the number of donations on average for men ($b = 0{\cdot}28$; $p = 0{\cdot}000$) and women ($b = 0{\cdot}64$; $p = 0{\cdot}000$). However, the individual donors' platelet counts trajectory over several donations varied considerably for men ($-1{\cdot}11 \leq b \leq 1{\cdot}62$) and women ($-1{\cdot}67 \leq b \leq 2{\cdot}31$), indicating different courses of platelet counts over time (increasing, decreasing or stable). Moreover, intraclass correlations were 0·64 for women and 0·66 for men, that is platelet counts of donations from individual blood donors were moderately correlated adjusting for ferritin and all covariates. In other words, differences in platelet counts between donors were more

pronounced than differences of platelet counts within individual donors' blood donations.

The time since the last donation showed a weak negative association with platelet counts resulting in a reduction of less than 3 G/l for all donors returning within 548 days after their last donation. Similarly, platelet counts tended to be lower with increasing age in both genders. Compared with the values at the age of 18, platelet counts at age 70 years were 6·6 G/l lower for women and 4·8 G/l lower for men, respectively.

Discussion

The association between ID with or without anaemia and reactive thrombocytosis is described in many textbooks and distinguished guidelines [2–5]. However, data suggesting this correlation derive from works performed in the seventies and on small patient populations, including children [7]. More recent data have been obtained from patient groups with additional underlying diseases, mainly inflammatory bowel disease [10]. In this large, retrospective single-centre study, we aimed to investigate whether there is an association between serum ferritin values and platelet counts in healthy individuals. Correlations between low ferritin and platelet count were negligible, even if the analysis was restricted to donors with ID without anaemia, IDA, and donors with high platelet counts. In a linear mixed model, accounting for age, number and interval of donations and ferritin decile, the lack of substantial association was confirmed. Based on our results, one may speculate that there is a potentially nonlinear association and only when ferritin levels are very low, we would observe a statistically significant effect. Latter should however be confirmed in a larger cohort of subjects with very low ferritin values and perhaps IDA.

An interesting finding of our analysis is that platelet counts show variable trends in the course of repeated blood donations. Irrespective of ferritin levels, and thus also in subjects with ID, platelet count can increase, remain stable or even decrease. This observation suggests that factors other than iron levels influence changes in platelet count and that probably the degree of ID or even IDA as encountered in blood donors is not sufficiently strong to significantly stimulate platelet production in the majority of the subjects. In a previous study in iron-deficient female donors, who switched from whole-blood donation to platelet apheresis, platelet counts after correction of ID were also not significantly lower compared with baseline [17].

Limitations of our analysis include the retrospective setting and the inclusion of only healthy blood donors. Moreover, ID or IDA in our population is mostly of mild degree. Despite strict donor selection, it is a fact that conditions associated with subclinical inflammation (e.g. metabolic syndrome) are frequent among blood donors and may therefore represent an unavoidable bias. Nevertheless, our cohort represents a homogeneous healthy population, where ferritin can be used as an overall index of ID. Additionally, the large number of ferritin and simultaneous platelet measurements and observations strengthens our conclusions.

However, our results may not be extended to patients, patients with severe IDA and anaemia of inflammation, where the platelet response might be different [18]. Thus, our findings should be confirmed in future prospective studies. In addition, further studies in clinical settings including elderly patients and different forms of anaemia and ID as well as prospective observations on the effect of iron substitution on platelet counts should be encouraged.

We conclude that in healthy individuals, in the absence of evident inflammatory conditions, ID with or without anaemia does not correlate with higher platelet counts. This finding needs confirmation in specific patient subgroups.

Author contributions

AH, LI and AB designed the study and drafted the manuscript. AH, LI, JPS, TV and AB collected the data. TV performed statistical analysis. All authors contributed to data analysis, data interpretation and writing of the manuscript.

Conflict of interests

The authors declare no conflict of interests.

References

1 Kasper DL, Harrison TR: *Harrison's Principles of Internal Medicine*, 19th edn. New York, McGraw Hill Education Medical, 2015

2 Wintrobe MM, Greer JP: *Wintrobe's Clinical Hematology*, 11th edn. Philadelphia, Lippincott Williams & Wilkins, 2013

3 Kaushansky K, Williams WJ: *Williams hematology, 8th ed.* New York, McGraw-Hill, 2010

4 Hoffbrand AV: *Postgraduate Haematology*, 7th edition, Wiley Blackwell, 2016

5 Harrison CN, Bareford D, Butt N, *et al.*: Guideline for investigation and management of adults and children presenting with a thrombocytosis. *Br J Haematol* 2010; **149**:352–375

6 Alberio L: Do we need antiplatelet therapy in thrombocytosis? Pro. Diagnostic and pathophysiologic considerations for a treatment choice. *Hamostaseologie* 2015; **35**: (Epub ahead of print)

7 Hicsonmez G, Suzer K, Suloglu G, *et al.*: Platelet counts in children with iron deficiency anemia. *Acta Haematol* 1978; **60**:85–89
8 Kuku I, Kaya E, Yologlu S, *et al.*: Platelet counts in adults with iron deficiency anemia. *Platelets* 2009; **20**:401–405
9 Voudoukis E, Karmiris K, Oustamanolakis P, *et al.*: Association between thrombocytosis and iron deficiency anemia in inflammatory bowel disease. *Eur J Gastro Hepatol* 2013; **25**:1212–1216
10 Kulnigg-Dabsch S, Schmid W, Howaldt S, *et al.*: Iron deficiency generates secondary thrombocytosis and platelet activation in IBD: the randomized, controlled thromboVIT trial. *Inflamm Bowel Dis* 2013; **19**: 1609–1616
11 Jimenez K, Khare V, Evstatiev R, *et al.*: Increased expression of HIF2alpha during iron deficiency-associated megakaryocytic differentiation. *J Thromb Haemost* 2015; **13**:1113–1127
12 Evstatiev R, Bukaty A, Jimenez K, *et al.*: Iron deficiency alters megakaryopoiesis and platelet phenotype independent of thrombopoietin. *Am J Hematol* 2014; **89**:524–529
13 Knovich MA, Storey JA, Coffman LG, *et al.*: Ferritin for the clinician. *Blood Rev* 2009; **23**:95–104
14 Camaschella C: Iron-deficiency anemia. *N Engl J Med* 2015; **373**:485–486
15 O'Meara A, Infanti L, Stebler C, *et al.*: The value of routine ferritin measurement in blood donors. *Transfusion* 2011; **51**:2183–2188
16 Jacob C: *Statistical Power Analysis for the Behavioral Sciences*, 2nd edn, Hillsdale, NJ, L. Erlbaum Associates, 1988.
17 O'Meara A, Infanti L, Sigle J, *et al.*: Switching iron-deficient whole blood donors to plateletpheresis. *Transfusion* 2012; **52**:2183–2188
18 Park MJ, Park PW, Seo YH, *et al.*: The relationship between iron parameters and platelet parameters in women with iron deficiency anemia and thrombocytosis. *Platelets* 2013; **24**:348–351