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Full title: Iron deficiency is common in idiopathic pulmonary arterial hypertension

Short title: Iron deficiency in pulmonary arterial hypertension

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Abstract

Introduction

The aims of this study were to assess the prevalence of iron deficiency in idiopathic pulmonary arterial hypertension (IPAH), and to investigate whether oral iron suppletion has effects in iron deficient patients.

Methods

From all IPAH patients attending our center between May 2009 and February 2010, iron parameters were measured. Iron data were related to clinical parameters including six minute walking distance (6MWD) and haemodynamic parameters measured during right heart catheterisation. In a subset of iron deficient patients, the uptake of iron from the bowel was studied after administering oral iron for four weeks.

Results

Iron deficiency was found in 30 of 70 patients (43%). 6MWD was reduced in iron deficient patients compared to non-iron deficient patients (390 ± 138 m vs 460 ± 143 m, $p<0.05$) irrespective of the existence of anaemia. In a subset of 18 patients that received oral iron, ferritin levels were significantly increased although 8 patients only slightly increased their iron storage.

Conclusion

This study shows that iron deficiency is frequently present in idiopathic pulmonary arterial hypertension and is associated with a lower exercise capacity. The small response to oral iron in 44% of the treated patients suggests impaired iron absorption in these patients.

Introduction

Iron is an important cofactor in oxygen transport; it is needed to produce haemoglobin in erythrocytes, and myoglobin in the heart and skeletal muscles which facilitates oxygen diffusion and oxygen storage. Moreover, iron functions in several mitochondrial oxidative enzymes and the respiratory chain. Consequently, when iron deficiency occurs, oxygen supply to the heart and skeletal muscles is reduced, resulting in decreased exercise performance. (1-5)

In chronic left heart failure, iron deficiency is frequently encountered.(6-9) The cause of iron depletion is thought to be inflammation mediated and/or due to renal failure. Both conditions cause an increase in hepcidin concentration (increased production in the liver or reduced breakdown by the kidney), which causes a downregulation of iron release from storage sites and decreased uptake of iron from the gut mucosa. (10-13) It was also shown that restoring iron levels, improved quality of life and New York Health Association (NYHA) functional class in iron deficient patients with chronic left heart failure. (6-9) In contrast to iron deficiency in left ventricular failure, no clinical data exists for right ventricular failure, although Krasuski et al. reported that anaemia in pulmonary hypertension decreased survival. (14) Pulmonary hypertension is the most common cause of right ventricular failure. Since iron is important in increasing oxidative metabolism of the hypertrophied right ventricle to cope with the increased afterload and for skeletal muscles to perform physical exercise, iron deficiency might thus have detrimental effects in pulmonary hypertension. However, data are lacking about the prevalence and effect of iron deficiency in right heart failure.

Therefore, we performed a study aimed to find the prevalence of iron deficiency in idiopathic pulmonary arterial hypertension patients (IPAH). In addition, we compared iron deficient with non-iron deficient IPAH patients to investigate whether iron deficiency is related to disease severity and exercise capacity. Finally, we studied whether digestive uptake of oral iron is impaired in iron deficient IPAH patients.

Methods

In this observational descriptive study, from all consecutive IPAH patients attending the VU University Medical Center outpatient clinic for their annual or four month check up visit and all hospitalised IPAH patients between May 2009 and February 2010 iron status was determined. Haemoglobin (Hb), haematocrit (Ht), mean corpuscular volume (MCV), kidney function, and NT-proBNP are measured routinely during every visit in our clinic. Assessment of the iron status including serum iron, transferrin saturation, total iron binding capacity (TIBC) and serum ferritin levels was performed from the same material of the routinely drawn blood samples. Hb and MCV were measured with spectrophotometry (Cell-Dyn Sapphire, Abbott) with Ht calculation from the product of MCV and erythrocyte number. Using sandwich immunoassays with electrochemical luminescence technology, serum ferritin and NT-proBNP were determined (Modular E170 system, Roche). Serum creatinin, iron and total iron binding capacity were measured using photometry and transferrin saturation was calculated from serum iron divided by TIBC (Modular P800 system, Roche).

Iron deficiency was defined as serum iron below 10 μmol/l and decreased transferrin saturation (<15% in females and <20% in males) irrespective of the coexistence of anaemia (Hb <12.0 g/dl in females and Hb <13.0 g/dl in males). (8,9,15) Serum ferritin was not used to define iron deficiency because of possible false-high values due to increased inflammatory markers.

In case of iron deficiency, patients were questioned about pathological blood loss (gastrointestinal or menstrual blood loss), known haematological comorbidities or previous anaemia to obtain information about possible causes for the iron deficiency. Standard oral iron treatment was started in the newly diagnosed iron deficient IPAH patients as part of good clinical practice. These patients (n=21, all females, with or without anaemia) received oral iron (ferrous fumarate 200 mg 3 times a day, 4 weeks (Teva Pharmaceuticals Industries, The Netherlands)). Patients were encouraged to complete these four weeks through telephone calls from specialized pulmonary hypertension nurses. After four weeks of treatment, iron parameters were measured again.

Iron data were related to simultaneously measured clinical parameters (age, gender, NYHA functional class, body mass index, current treatment, NT-proBNP). Haemodynamic parameters from right heart catheterisation (mean pulmonary artery pressure, mean right atrial pressure, cardiac output, pulmonary vascular resistance, arterial and mixed venous saturation) and 6MWD were obtained less than one year before iron measurement.

The study protocol was approved by the Institutional Review Board on Research Involving Human Subjects (Amsterdam, The Netherlands) and it was stated that this protocol did not require informed consent since all measurements were performed within the routine measurements and oral iron was given after clinical indication.

Statistical analysis

To compare parameters between iron deficient and non-iron deficient patients normal distribution of the data was tested with D'Agostino-Pearson testing and unpaired t-tests were performed. Paired t-tests were used to determine the effects of oral iron and non-parametric Mann-Whitney tests were performed to compare iron deficient anaemic with iron deficient non-anaemic patients. To compare categorical data, Fisher's exact tests or Chi-square tests were performed. All data are presented as mean ± SD, unless stated otherwise with a p-value <0.05 as statistically significant. Analyses were performed with SPSS 15.0 (SPSS Inc., Chicago, IL, USA).

Results

Of 70 IPAH patients (15 male, 55 female) measured iron parameters and baseline characteristics are shown in table 1. The haemodynamic characteristics of the patients showed that the patients had pulmonary hypertension with a mean pulmonary artery pressure of 50.2 ± 14.1 mmHg, and a pulmonary vascular resistance of 786 ± 500 dyn.s/cm^5. All patients were treated with pulmonary hypertension medication and 61 patients used oral anticoagulants. Iron deficiency was found in 30 patients (43%). None of these patients reported current or past pathological gastrointestinal or abnormal gynaecological blood loss. The female patients were divided into pre-menopausal (n=28) and post-menopausal (n=27), mean age 39.1 and 62.9 years, respectively. There were no significant differences in iron deficiency incidence between these groups: of the premenopausal patients 50% were iron deficient vs 44% in the post-menopausal group (p=0.8).

Right heart catheterisation information and six minute walking distance (6MWD) tests were available in 67 (39 non-iron deficient and 28 iron deficient patients) and 68 patients (40 non-iron deficient and 28 iron deficient patients) respectively. In two patients no 6MWD data were available because one patient declined to perform the 6MWD in the last years and data were missing from the second patient. As presented in table 1, Hb and Ht were significantly lower in the iron deficient patients, although anaemia was only present in 12 of 30 iron deficient patients. There were no significant differences in haemodynamic data between the iron deficient and the non-iron deficient group. However, 6MWD was significantly lower in iron deficient patients compared to non-iron deficient patients (390 ± 138 m vs 460 ± 143 m, $p<0.05$) but similar in iron deficient anaemic (n=12) and non-anaemic (n=18) patients (397 ± 132 m vs 385 ± 147 m, p=0.82). (figure 1) There was no difference between the clinical and haemodynamic parameters between iron deficient anaemic and non-anaemic patients.

Oral iron uptake in iron deficient IPAH patients

Of the 21 patients who received oral iron treatment, 18 patients completed the 4 weeks treatment. The other three patients did not complete the course due to side effects secondary to oral iron intake consisting of gastrointestinal constipation and nausea. Baseline characteristics of the 18 patients showed that they were representative for the iron deficient group.

In figure 2 iron parameters are shown before and after 4 weeks of oral iron. Only 2 patients increased their serum iron levels significantly and 14 patients did not reach normal transferrin

saturation values (9 ± 3 % vs 12 ± 4 %). Serum ferritin levels were significantly increased after 4 weeks (12 ± 7 μg/l vs 32 ± 20 μg/l, $p<0.05$). Despite this overall increase in serum ferritin, a subset of the patients (n=8; 44%) was not able to significantly increase serum ferritin and did not reach normal serum ferritin values (11 ± 5 μg/l vs 19 ± 14 μg/l, $p=0.09$), while the other 56% did, despite similar haemodynamic, clinical and haematological parameters.

Discussion

To the best of our knowledge this is the first study that describes iron deficiency in idiopathic pulmonary arterial hypertension. We demonstrated that iron deficiency is a common feature (43%) in IPAH patients and is associated with a decreased 6MWD regardless of the presence of anaemia. After administration of oral iron, 8 of 18 patients did not restore ferritin levels to normal values.

Patients

This study was performed in idiopathic PAH patients since those patients have pulmonary hypertension without comorbidities which might explain iron deficiency. The population was predominantly female (15:55, M:F), however no significant differences were found between gender in the iron deficient and non-iron deficient group.

The prevalence of iron deficiency of 43% in this study corresponds to data from chronic left heart failure patients where iron deficiency prevalence varies between 15-61% in anaemic and 20-43% in non-anaemic patients depending on the definition of iron deficiency. (16-20)

Possible mechanisms of iron deficiency in IPAH

IPAH patients can become iron deficient by reduced intake, increased loss, or impaired uptake. Although BMI values were normal, we cannot exclude reduced iron intake as a cause of iron deficiency. Also, 61 patients were known to use anticoagulants, indicating that increased blood (iron) loss might be considered a possible cause, but no significant differences in iron deficiency incidence were found between pre- and post-menopausal women. (21) Concerning iron uptake, we found that 8 of 18 patients who received oral iron did not seem to increase serum ferritin levels as much as the other 10 patients. (11) Although doses were similar and clinical and haemodynamic parameters were similar as well, iron absorption may be different between the patients, resulting in the reduced response to oral iron. This impaired iron absorption could be due to both gastrointestinal edema, although right atrial pressures were similar, and decreased iron release from storage sites or enterocytes due to inflammation. In IPAH interleukin-6 (IL-6) levels are known to be increased and IL-6 induces hepcidin release from the liver which reduces iron release from iron storage cells and duodenal cells. (22-26) Whether this contributes to iron deficiency in IPAH has yet to be determined.

Iron deficiency and exercise capacity

The significantly reduced 6MWD in iron deficient IPAH patients indicate that iron plays an important role in maintaining exercise performance. The decreased 6MWD can not be explained by differences in disease severity, since haemodynamic characteristics of both groups were similar. However, it cannot be decided whether iron deficiency has an effect on RV function, on skeletal muscle function or both. Additionally, Smith et al. showed that iron suppletion in healthy humans with iron deficiency and hypoxia-induced pulmonary hypertension at high altitude, decreased mean pulmonary artery pressure. (27;28) Whether iron treatment also has effects on the pulmonary vasculature in patients with IPAH is unknown. However, data from iron deficiency in left heart failure showed that administration of intravenous iron improved both quality of life and NYHA functional class. (6-9) Anker et al. also showed that 6MWD significantly increased after intravenous iron. (9) The present data cannot answer the question if iron deficiency is a cause of more advanced symptoms or if it results from a more severe disease state. However, it appears that iron deficiency is the cause for the reduced 6MWD since all other parameters are similar between the iron deficient and non-iron deficient group. Our findings warrant further research aimed to study the effects of restoration of iron levels for instance by means of intravenous iron suppletion in iron deficient IPAH patients on exercise capacity.

Limitations

This study is an observational prospective study which therefore has several limitations. There is no information about inflammatory status of the patients since CRP is not measured routinely in our IPAH patients Also haemodynamic measurements were not measured at the same time as iron status determination. Since right heart catheterisation was not repeated, implying no change in clinical condition, we assumed that the right heart catheterisation data provided are representative for the clinical state. Additionally, NT-proBNP values obtained at the same time of iron measurement and at time of right heart catheterisation were used as a marker of disease progression. Because of the design of the study no clinical measurements were repeated after the oral iron administration.

Conclusion

In this study, iron deficiency is described for the first time in idiopathic pulmonary arterial hypertension with a prevalence of 43%. The small response to oral iron in 44% of the

patients, suggests problems with iron absorption or release. The exact mechanisms of the iron deficiency in IPAH and whether intravenous iron administration is useful has yet to be determined.

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Figure 1. A. The six minute walking distances (6MWD) of IPAH patients with (n=30) and without (n=40) iron deficiency. B. Iron deficient patients are subdivided into anaemic (Hb<12.0 g/dl in females and <13.0 g/dl in males) (n=12) and non anaemic (n=18). Data is presented as mean ± SEM. (ID: iron deficiency)

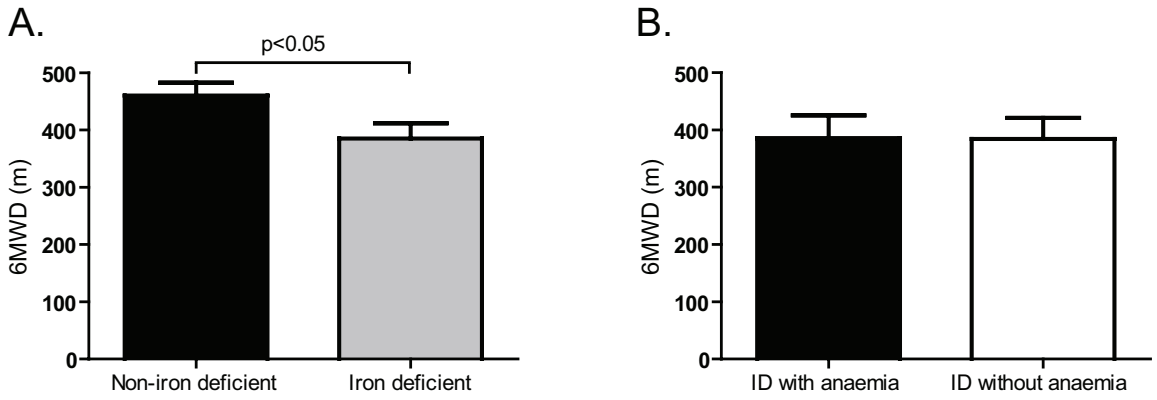

Figure 2. Effects of 4 weeks oral iron on A) haemoglobin concentration, B) haematocrit, C) serum iron concentration, D) total iron binding capacity, E) transferrin saturation, F) serum ferritin. Every dot represents one patient with a connecting line between the values for one patient before (○) and after (●) four weeks (n=18).

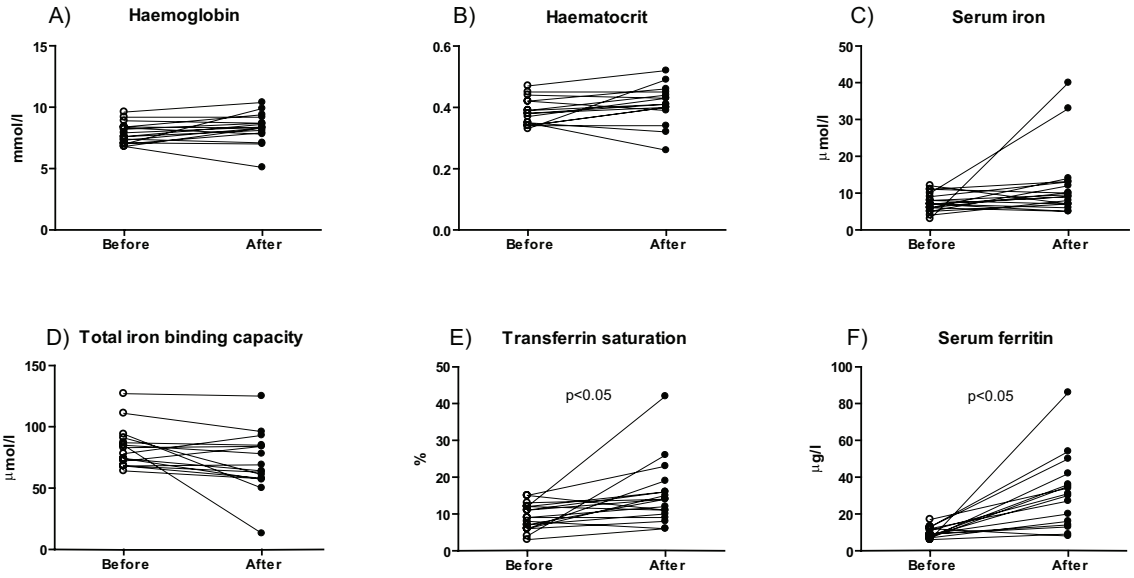
A)
Haemoglobin
mmol/l
15
10
5
0
Before
After
B)
Haematocrit
0.6
0.4
0.2
0.0
Before
After
C)
Serum iron
µmol/l
50
40
30
20
10
0
Before
After
D)
Total iron binding capacity
µmol/l
150
100
50
0
Before
After
E)
Transferrin saturation
%
p<0.05
50
40
30
20
10
0
Before
After
F)
Serum ferritin
µg/l
p<0.05
100
80
60
40
20
0
Before
After

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Table 1. Patient characteristics

	All patients n=70	Non-iron deficient n=40	Iron deficient n=30	
Clinical parameters	Mean ± SD	Mean ± SD	Mean ± SD	**p-value***
Age	52.2 ± 15.6	51.8 ± 16.6	52.8 ± 14.5	NS
Gender male:female	15:55	11:29	4:26	NS
NYHA classification II / III / IV	34 / 29 / 7	22 / 16 / 2	12 / 13 / 5	NS
Body mass index (kg/m^2)	26.1 ± 5.3	26.4 ± 5.1	25.7 ± 5.8	NS
Oral anticoagulants use (VKA / PAI / unknown / none)$^{\$}$	58 / 3 / 8 / 1	32 / 2 / 6 / 0	26 / 1 / 2 / 1	NS
Single / combination treatment†	25 / 45	18 / 22	7 / 23	NS
Duration of treatment (months)	155.8 ± 129.8	127.6 ± 86.7	192.5 ± 164.9	NS$^{\#}$
Haemodynamic parameters				
Mean pulmonary arterial pressure (mmHg)	50.2 ± 14.1	51.0 ± 13.6	49.1 ± 14.9	NS
Mean right atrial pressure (mmHg)	6.9 ± 5.6	6.6 ± 6.5	7.3± 4.2	NS$^{\#}$
Pulmonary vascular resistance (dyn.s/cm^5)	786 ± 500	808 ± 516	751 ± 484	NS$^{\#}$
Cardiac output (Fick method) (l/min)	5.3 ± 1.7	5.2 ± 1.4	5.6 ±2.1	NS
Cardiac Index (l/min/m^2)	2.7 ± 0.9	2.6 ± 0.8	2.9 ± 0.9	NS
Arterial saturation (%)	93 ± 4	93 ± 3	93 ± 5	NS
Mixed venous saturation (%)	65 ± 8	66 ± 8	63 ± 9	NS
6 minute walking distance (m)	431 ± 144	460 ±143	390 ± 138	p<0.05
NT-proBNP (ng/l)	1022 ± 1592	941 ± 1717	1128 ± 1435	NS$^{\#}$
Haematological parameters				
Hemoglobin (g/dl)	13.9 ± 1.9	14.9 ± 1.4	12.6 ± 1.6	p<0.05
Hematocrit	0.42 ± 0.05	0.45 ± 0.04	0.39 ± 0.05	p<0.05
Mean corposcular volume (fl)	86 ± 12	91 ± 5	80 ± 16	p<0.05
Serum iron (µmol/l)	12 ± 6	16 ± 5	7 ± 3	p<0.05
Total iron binding capacity (µmol/l)	72 ± 13	68 ± 9	77 ± 16	p<0.05
Transferrin saturation (%)	17 ± 9	23 ± 7	10 ± 5	p<0.05
Ferritin (µg/l)	43 ± 38	66 ± 37	12 ± 6	p<0.05
Creatinin (µmol/l) (male/female)	98 ± 22 / 75 ± 18	103 ± 24 / 79 ± 21	87 ± 8 / 71 ± 14	NS

* independent sample t-testing to compare non-iron deficient with iron deficient idiopathic pulmonary arterial hypertension patients and Fisher's exact or Chi-square testing for categorical data

$^{\#}$ independent sample t-testing after log transformation to obtain normal distribution

$^{\$}$VKA: vitamin K antagonist, PAI: platelet aggregation inhibitor

† type of IPAH treatment (number of non-iron deficient / iron deficient patients): endothelin receptor antagonists (24 / 19), phospodiesterase 5 inhibitor (20 / 26), prostanoids (14 / 16)