Clinical Nutrition 43 (2024) 1025–1032

Contents lists available at ScienceDirect

Clinical Nutrition

journal homepage: http://www.elsevier.com/locate/clnu

Original article

Iron deficiency in pernicious anemia: Specific features of iron deficient patients and preliminary data on response to iron supplementation

Juliette Rogez [a], Geoffrey Urbanski [a, b, c], Emeline Vinatier [d], Christian Lavigne [a], Léa Emmanuel [a], Iris Dupin [a], Camille Ravaiau [a], Valentin Lacombe [a, e, *]

[a] Department of Internal Medicine and Clinical Immunology, Angers University Hospital, Angers, France
[b] Department of Immunology and Allergology, Geneva University Hospital, Geneve, Switzerland
[c] Department of Orofacial Sciences, School of Dentistry, University of California, San Francisco, CA, USA
[d] Laboratory of Immunology, Angers University Hospital, Angers, France
[e] Univ Angers, MitoLab, Unité MITOVASC, UMR CNRS 6015, INSERM U1083, SFR ICAT, Angers, France

ARTICLE INFO

Article history:
Received 4 February 2024
Accepted 16 March 2024

Keywords:
Iron
Iron deficiency
Pernicious anemia
Vitamin B12
Atrophic gastritis

SUMMARY

Background & aims: While vitamin B12 (B12) deficiency is considered as the hallmark of pernicious anemia (PA), iron deficiency (ID) is also prevalent. Indeed, this auto immune gastritis is responsible for parietal cell atrophy and increase in gastric pH, leading to impaired iron absorption. We compared PA patients' features according to their iron status at PA diagnosis, and we assessed the iron status recovery after oral or intravenous iron supplementation.

Methods: We prospectively included patients presenting with a newly diagnosed PA in a tertiary referral hospital between November 2018 and October 2020. Iron status was assessed at PA diagnosis then regularly during a standardized follow-up. In case of ID, the decision of treatment with oral and/or intravenous iron supplementation was left to the clinician convenience.

Results: We included 28 patients with newly diagnosed PA. ID was observed in 21/28 (75.0%) patients: from the PA diagnosis in 13 patients, or during the follow-up in 8 patients. Iron deficient PA patients had higher plasma B12 ($p = 0.04$) and lower homocysteine levels ($p = 0.04$). Also, ID was independently associated with the 'APCA (anti-parietal cell antibodies) alone' immunological status (absence of anti-intrinsic factor antibodies) after adjustment for age, gender and B12 level (aOR 12.1 [1.1–141.8], $p = 0.04$). High level of APCA was associated with lower ferritin level. After 3 months of supplementation, 3/11 PA patients normalized the iron status with oral iron supplementation, versus 7/8 with intravenous iron supplementation ($p = 0.02$).

Conclusion: The high frequency of iron deficiency in PA highlights the interest of regular assessment of iron status in this condition. ID was associated with a profile including APCA alone and less pronounced B12 deficiency. Intravenous iron supplementation seemed to be more efficient than an oral supplementation in these preliminary data.

© 2024 Elsevier Ltd and European Society for Clinical Nutrition and Metabolism. All rights reserved.

1. Introduction

Iron is a critical component of heme groups for binding oxygen to hemoglobin, and of enzymes involved in crucial pathways such as mitochondrial respiration and DNA synthesis [1,2]. This is therefore a key nutrient in human diet. Iron deficiency (ID) is a widespread nutritional deficiency responsible for microcytic hypochromic anemia and non-specific clinical features such as fatigue, poor concentration, headache, alopecia, koilonychia, and restless legs [3].

Two major mechanisms may lead to ID: an increase in iron needs (during pregnancy and childhood, or after a blood loss) and a decrease in iron bioavailability, which could be related to a lack of intake, an impaired absorption, or systemic inflammation [3]. Diet provides iron in two chemical forms: a ferrous haeminic form from meat, and a ferric non haeminic which is present in a variety of animal and vegetable foods. Iron is mainly absorbed in its ferrous form in the duodeum and proximal jejunum, which therefore

* Corresponding author. Service de Médecine interne et immunologie clinique, Centre Hospitalier Universitaire d'Angers, 4 rue Larrey, 49000 Angers, France.
E-mail address: valentin.lacombe@chu-angers.fr (V. Lacombe).

https://doi.org/10.1016/j.clnu.2024.03.011
0261-5614/© 2024 Elsevier Ltd and European Society for Clinical Nutrition and Metabolism. All rights reserved.

require the change from ferric to ferrous form [4]. This change is achieved by a ferrireductase (duodenal cytochrome *b*, Dcytb) in acid pH condition [4,5]. Gastric acid therefore promotes reduction and solubilization of dietary ferric iron and thus improve its absorption [6]. Consequently, increase in gastric pH contributes to ID, as observed in patients with chronic proton pump inhibitors (PPIs) use which inhibits the H^+/K^+ATPase of gastric parietal cells [7].

PA is an autoimmune atrophic gastritis whose diagnosis is based on the association of gastric histological features and immunological markers: anti-intrinsic factor antibodies (IFA) and anti-parietal cell antibodies (APCA) [8,9]. In PA, both the parietal cell damages and the clearance or inactivation of the intrinsic factor lead to a lack of intrinsic factor, the enteral cobalamin (vitamin B12, B12) carrier. As intrinsic factor is needed for the active enteral B12 absorption, PA therefore leads to a B12 deficiency. However, even if B12 deficiency is a hallmark of PA, ID is also observed in up to 52% of PA [10–12]. Indeed, the autoimmune gastritis leads to an increase in gastric pH [13] and therefore impairs the iron absorption. The disruption in iron absorption also raises questions regarding the effectiveness of oral iron supplementation in PA.

This study aimed at analyzing the frequency of iron deficiency in PA patients, then comparing phenotypes of PA patients, notably their clinical, biological and immunological features, according to their iron status at the time of PA diagnosis. Also, we assessed the iron status recovery after oral or intravenous iron supplementation.

2. Methods

2.1. Study population and PA diagnosis

We included inpatients and outpatients over 18 years presenting at the Department of Internal medicine and clinical immunology in a tertiary referral hospital (Angers University Hospital, Angers, France), between November 2018 and October 2020. We conducted prospective screening for PA in cases of B12 deficiency and subsequently included patients with a confirmed diagnosis of PA.

Among B12 deficient patients, the diagnosis of PA was defined in case of.

i) B12 deficiency associated with IFA, due to the very high specificity of IFA for PA diagnosis [14],
ii) B12 deficiency associated with either IFA and/or APCA in case of typical gastric histology with fundic atrophy and antral sparing [15,16],
iii) B12 deficiency associated with either IFA and/or APCA in case of compatible gastric histology (pangastric atrophy, or fundic gastritis with intense inflammatory infiltrates but no atrophy [15,16]) or in the absence of gastric biopsy (in case were gastroscopy was not suitable), with a low oral B12 absorption defined as a high daily oral B12 supplementation of cyanocobalamin 1000 μg requirement for maintaining a normal plasma B12 with at least 1 year of follow-up [17]. Indeed, we previously demonstrated that the evolution of plasma vitamin B12 during oral supplementation help to differentiate true and false positives APCA for PA diagnosis [17].

Diagnosis of PA was therefore ruled out in patients presenting neither IFA nor APCA, and in patients with isolated APCA without typical fundic atrophy and having low or transient oral B12 needs.

2.2. Definitions of biological abnormalities

B12 deficiency was defined by a total plasma B12 $\leq$ 200 ng/l, or the combination of total plasma B12 between 201 and 350 ng/l and elevated homocysteine ($\geq$13 μmol/l for women, $\geq$15 μmol/l for men, $\geq$20 μmol/l in patients older than 65 years), plasma methylmalonic acid (pMMA, $\geq$0.35 μmol/l) [18], or a urinary methylmalonic acid/creatinine ratio (uMMA/C, $\geq$1.45 μmol/mmol) [19], according to expert recommendations [13,20,21]. In severe renal failure with MDRD (Modification of Diet in Renal Disease) clearance <30 ml/min/1.73 m^2, only total plasma B12 and MMAu/C were used to define B12 deficiency [19].

Iron deficiency was defined as: i) ferritin $\leq$30 μg/l, or ii) ferritin $\leq$100 μg/l and transferrin saturation <20% in patients with C-reactive protein (CRP) $\geq$10 mg/l [3,22].

Anemia was defined as hemoglobin <120 g/l in women and <130 g/l in men. Microcytosis was defined as MCV (mean corpuscular volume) < 80 fl, and macrocytosis as MCV >100 fl.

2.3. Follow-up, biological assessments, and iron supplementation

Patients were prospectively followed during 1 year with standardized clinical and biological assessment at the PA diagnosis (visit M0, baseline), then over the months following the introduction of oral B12 supplementation at visit M1, M3, M6, M9 and M12 (1, 3, 6, 9, 12 months).

Biological assessment included parameters of B12 status (total plasma B12, homocysteine, pMMA, uMMA/C), iron status (ferritin, transferrin saturation coefficient), blood count, and parameters needed for interpreting other results (CRP, creatinine and MDRD clearance). All patients were tested for *Helicobacter pylori* gastritis at baseline with urea breath test.

When iron deficiency was detected, the clinician had the discretion to determine the chosen route of administration, the molecule and the dosage to be employed.

2.4. Immunological bioassays

IFA were tested using immunodot (Biermer atrophic gastritis dot, Alphadia, Mont-Saint-Guibert, Belgium). ACPA were tested using immunodot (Biermer atrophic gastritis dot, Alphadia, Mont-Saint-Guibert, Belgium) and by indirect immunofluorescence (IIF) on rat stomach tissue (Biosystems, Barcelona, Spain).

2.5. Statistical analysis

The quantitative variables were presented as medians and quartiles, and compared by the Student t-test or the Mann–Whitney U test according to the normality of the distribution, assessed by the Shapiro–Wilk normality test. Categorical variables were presented as absolute values and percentages, and compared by the Fisher's exact test or Chi-square test. The alpha risk was set at 5%.

To assess the independent association between a variable and the iron status, multivariate analysis was performed by means of logistic regression. Multivariate models were adjusted for age and sex. We only included in multivariate logistic regression the variables associated with iron deficiency in univariate analysis. For logistic regression, continuous variables were considered as binary variables by using the median values as thresholds. The adjusted odds ratios (aOR) were presented with a 95% confidence interval (CI).

In order to represent ferritin level according to the APCA titer, different linear, logarithmic, and semi-logarithmic mathematical models were compared and selected according to the information criteria of Akaike [23].

Time-to-event curves for occurrence of iron deficiency during follow-up were presented as Kaplan–Meier curves and were compared with a log-rank test. Loss of follow-up was censored.

The analyses were performed using Graphpad Prism v6.01 (Graphpad Software, La Jolla, CA, USA) and Jamovi software v2.3.9.

2.6. Ethics

This study was approved by the bioethics committee of the Angers University Hospital (n°2018/63). It was conducted in compliance with the Declaration of Helsinki and according to the STROBE (Strenghtening The Reporting of OBservational studies in Epidemiology) recommendations for cohort studies. Written consent was systematically obtained from patients.

3. Results

3.1. Study population

We included 28 newly diagnosed PA patients (Fig. 1), whose characteristics are detailed in Table 1. The study cohort had a median age of 62 [45–76] years and comprised 19/28 (67.9%) women. Among the 24/28 participants who underwent gastric endoscopy, 19/24 (79.2%) exhibited typical fundic atrophy with antral sparing. In the 4 patient who did not underwent gastric endoscopy, 2 patients had IFA (± APCA), and 2 patients had isolated APCA with demonstrated high oral vitamin B12 needs in the absence of other cause of B12 deficiency. The gastric endoscopy and biopsies were either contra-indicated or refused by these 4 patients. About immunological markers of PA, 2/28 (7.1%) patients had IFA alone, 19/28 (67.9%) had APCA alone, and 7/28 (25.0%) exhibited both IFA and APCA concurrently. At PA diagnosis, 14/28 (50.0%) patients presented with anemia, and 8 of them experienced related symptoms such as asthenia, pallor, and dyspnea.

3.2. Iron deficiency in PA

ID was observed in 13/28 (46.4%) patients at PA diagnosis, and 8 other patients developed ID during the one-year follow-up (Fig. 2).

A concurrent cause of iron deficiency was identified in 6 patients: chronic PPI use in 3 patients, menorrhagia or metrorrhagia in 2 patients, and a vegetarian diet in 1 patient. No patient presented *H. pylori*-related gastritis, coeliac disease, Crohn's disease, ulcerative colitis, colorectal cancer, peptic ulcer, severe undernutrition, or gastrointestinal bleeding.

3.3. Features associated with iron deficiency in pernicious anemia

The comparison between patients having PA with ID at diagnosis and PA with normal iron status is detailed in Table 2. ID tended to be associated with a younger age, female sex, and the presence of a second cause of ID, however without statistical significance ($p = 0.10$, $p = 0.11$, and $p = 0.07$, respectively).

The iron deficient PA patients also differed from PA patients with normal iron status according to the degree of B12 deficiency (Table 2): iron deficient patients had higher plasma B12 ($p = 0.04$) and lower homocysteine levels ($p = 0.04$). Higher hemoglobin levels ($p = 0.05$) and lower proportion of anemic patients ($p = 0.02$) were also observed in PA patients with ID and so less profound B12 deficiency. Clinical consequences of B12 deficiency however did not differ between the two groups.

In univariate analysis, ID was associated with the 'isolated APCA' (APCA without IFA) serological status ($p = 0.03$, Table 2 and Fig. 3A). In multivariate analysis, the immunological status 'APCA alone' was independently associated with iron deficiency at PA diagnosis (aOR 12.1 [95%CI: 1.1–141.8], $p = 0.04$) after adjustment for age (<62 versus ≥62 years, 62 years being the median value in whole population), gender, and vitamin B12 level at PA diagnosis (<205 versus ≥205 ng/l, 205 being the median value in whole population). Also, high level of APCA in IIF assay was associated with lower ferritin level (Fig. 3B). The semi-logarithmic model was the most appropriate to represent the ferritin level according to the APCA titer (Fig. 3C).

Histological gastric features did not differ according to the iron status in PA, a majority of patients in both groups having fundus atrophy with antral sparing and fundus metaplasia (Table 2). Also, gastrin level did not statistically differ according to the iron status: 908 [602–1336] ng/l in case of ID versus 719 [365–921] ng/l in case of normal iron status ($p = 0.20$).

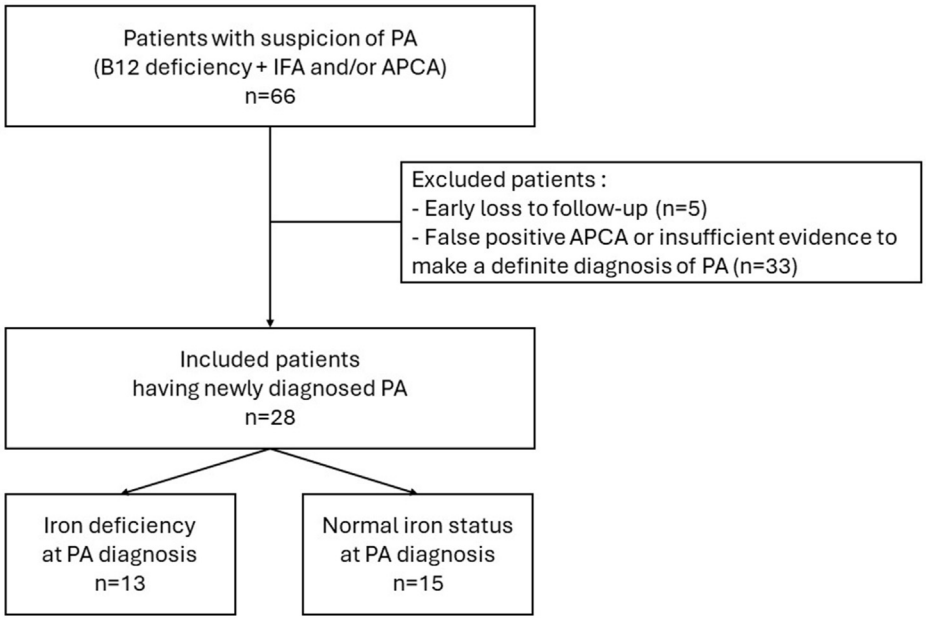

Fig. 1. Flowchart. Notes: PA: pernicious anemia; IFA: anti-intrinsic factor antibodies; APCA: anti-parietal cell antibodies.

Table 1
Characteristics of the study population.

	Pernicious anemia (whole population, n = 28)
Demographic characteristics	
Age (years)	62.0 [45.5–76.0]
Gender (women)	19 (67.9%)
Duration of follow-up after PA diagnosis (months)	21 [12–27]
Other causes of iron deficiency	
Gynecologic bleeding (menorragy, metrorragy)	2 (7.1%)
Gastrointestinal bleeding (rectorragy, melena)	0 (0%)
Chronic use of Proton Pump Inhibitor	3 (10.7%)
Helicobacter pylori gastritis	0 (0%)
Vegetarian or vegan diet	1 (3.6%)
Factors favoring a potential bleeding	
Anticoagulant treatment	4 (14.3%)
Antiaggregant treatment	4 (14.3%)
Baseline features of pernicious anemia	
Gastric histology	
Mild inflammation in fundus without atrophy[a]	3/24 (12.5%)
Fundic atrophy with antral sparing[a]	19/24 (79.2%)
Pangastric atrophy[a]	2/24 (8.3%)
Fundic metaplasia[a]	20/24 (83.3%)
Fundic dysplasia[a]	1/24 (4.2%)
Immunological markers of pernicious anemia	
IFA with immunodot assay	9 (32.1%)
APCA with immunodot assay	26 (92.9%)
APCA with indirect immunofluorescence assay	17 (60.7%)
Assessment of iron and B12 deficiencies	
Plasma total vitamin B12 (ng/l)	205.5 [159.0–295.5]
Homocysteine (μmol/l)	22.0 [13.7–4.67]
Plasma methylmalonic acid (μmol/l)	0.56 [0.32–1.08]
Urinary methylmalonic acid/creatinine ratio (μmol/mmol)	0.39 [0.30–2.35]
Vitamin B12 deficiency	28 (100%)
Ferritin (μg/l)	43.5 [17.3–159.3]
Transferrin saturation (%)	20 [10–28]
Iron deficiency	13 (46.4%)
Biological and clinical consequences of iron and B12 deficiencies	
Hemoglobin (g/l)	122 [108–131]
Mean corpuscular volume (MCV, fl)	94.2 [90.5–100.4]
Anemia *(hemoglobin < 120 g/l in women, < 130 g/l in men)*	14 (50.0%)
Microcytosis *(MCV < 80 fl)*	1 (3.6%)
Macrocytosis *(MCV > 100 fl)*	7 (25.0%)
Clinical signs of anemia (asthenia, pallor, dyspnea)	8 (28.6%)
Glossitis	5 (17.8%)
Polyneuropathy	9 (32.1%)
Pyramidal involvement	7 (25.0%)
Proprioceptive involvement	2 (7.1%)

Notes: IFA: Anti-Intrinsic Factor Antibodies; APCA: Anti-Parietal Cell Antibodies.
[a] Among patients in which gastric endoscopy was performed.

3.4. Changes in iron status after oral or intravenous iron supplementation

Nineteen patients with iron deficiency were supplemented with iron during follow-up: 11 patients received oral iron supplementation and 8 patients received intravenous supplementation as first line therapy. Oral iron supplementation included ferrous sulfate 80 mg/day in 10 patients, and ferrous fumarate 198 mg/day in 1 patient. All the 8 patients treated intravenously received ferric carboxymaltose 1000 mg.

Normalization of iron status was observed three months after initiating supplementation in 3/11 patients with oral iron medication and 7/8 patients who received iron infusion (p = 0.02, Fig. 4). Among the 8 patients orally supplemented who did not achieve a normal iron status after 3 months of supplementation, 2 achieved a normal iron status after 3 extra months, while 6/11 (54.5%) patients treated with oral iron supplementation never corrected their ID during follow-up, and were thereafter treated intravenously.

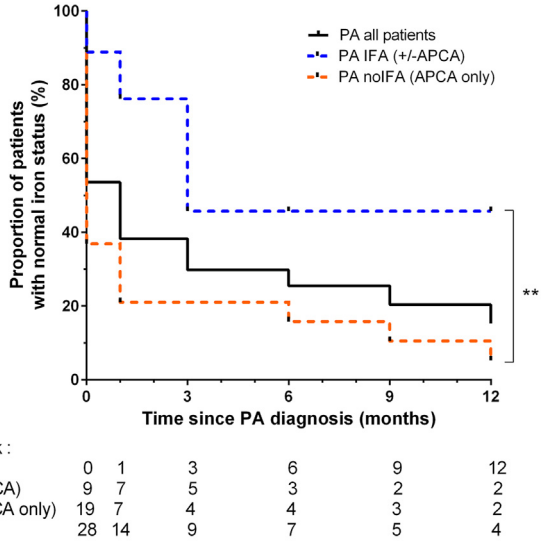

Fig. 2. Occurrence of iron deficiency at diagnosis and during the first year of follow-up.

4. Discussion

B12 deficiency is considered as the hallmark of PA, but PA is also responsible for iron deficiency. Only few studies assessed ID in PA, mainly with descriptive objectives [11,12,24–26] and several studies included heterogeneous causes of gastritis [5,10,27]. We hypothesized that the subgroup of PA patients having ID could have specific biological, immunological or histological features, which could be either the cause or the consequence of ID. In our study, we demonstrated that i) ID is frequent at PA diagnosis and during the 1 first year of follow-up, ii) ID is mainly associated in PA patients having APCA alone and a high titer of APCA in IIF assay, iii) ID occurs in PA patients having less pronounced B12 deficiency, and iv) oral iron supplementation is only marginally effective to correct ID in PA.

ID was very frequent in our cohort, affecting 13/28 (46.4%) of patients at PA diagnosis, and 8/28 (28.6%) other patients during the first year of follow-up. In previous cohorts, the frequency of ID in PA was estimated between 24% and 52%, with various diagnosis criteria of PA and various duration of follow-up [10,11,24–26]. These data strongly argue for careful assessment of iron status at PA diagnosis and during follow-up. Some authors even hypothesized that ID could be unmasked during the first months of B12 supplementation with renewed erythropoiesis [28–30]. We therefore recommend dosing the ferritin in PA at time of diagnosis and regularly during follow-up, at the same times as plasma B12 measurements.

Some studies noted a predominance of women among PA patients having ID [11,12,26], mainly young non-menopausal women [11,12]. In our work, ID tended to be more frequent in women and in younger patients, however without statistical significance. The absence of statistical difference could be explained by the median age of our population (62.0 [45.5–76.0] years) which mainly included post-menopausal women. Although it was not surprising to find more ID in non-menopausal women, our results highlighted that it is not the only explanation for ID in PA, thus suggesting a specific role of PA in ID.

Table 2
Comparison of features between iron deficient and others among PA patients.

	Pernicious anemia with iron deficiency n = 13	Pernicious anemia with normal iron status n = 15	p-value
Demographic characteristics			
Age (years)	56.0 [42.0–66.0]	68.0 [49.5–82.5]	0.10
Gender (women)	11 (84.6%)	8 (53.3%)	0.11
Women <50 years	4 (30.8%)	2 (13.3%)	0.37
Concurrent causes			
Presence of other cause (non-PA) of iron deficiency	5 (38.5%)	1 (6.7%)	0.07
Gastric histology			
Mild inflammation in fundus without atrophy[a]	2/12 (16.7%)	1/12 (8.3%)	0.25
Fundus atrophy with antral sparing[a]	8/12 (66.6%)	11/12 (91.7%)	
Pangastric atrophy[a]	2/12 (16.7%)	0/12 (0%)	
Immunological markers of pernicious anemia			
Isolated IFA	0 (0%)	2 (13.3%)	**0.03**
Association of IFA and APCA	1 (7.7%)	6 (40.0%)	
Isolated APCA	12 (92.3%)	7 (46.7%)	
Other biological markers			
Vitamin B12 (ng/l)	276.0 [199.0–308.0]	173.0 [132.0–228.0]	**0.04**
Homocysteine (μmol/l)	14.3 [11.3–17.7]	36.0 [17.3–54.8]	**0.04**
pMMA (μmol/l)	0.57 [0.24–1.03]	0.55 [0.32–1.71]	0.44
uMMA/C (μmol/mmol)	0.38 [0.34–0.87]	0.44 [0.25–3.22]	0.86
Gastrine (ng/l)	908.4 [602.3–1336.4]	718.6 [364.8–920.7]	0.20
Chromogranine A (ng/ml)	162.8 [121.0–258.9]	98.1 [78.4–208.3]	0.15
Biological and clinical consequences of iron and B12 deficiencies			
Hemoglobin (g/l)	12.4 [12.1–13.1]	10.9 [9.8–12.9]	0.053
MCV (fl)	91.4 [87.6–94.3]	95.5 [93.8–103.3]	0.052
Anemia *(Hb < 120 g/l in women, < 130 g/l in men)*	3 (23.1%)	11 (73.3%)	**0.02**
Microcytosis *(MCV < 80 fl)*	1 (7.7%)	0 (0%)	0.46
Macrocytosis *(MCV > 100 fl)*	2 (15.4%)	5 (33.3%)	0.40
Clinical signs of anemia	3 (23.1%)	5 (33.3%)	0.69
Neurological features of B12 deficiency	6 (46.2%)	7 (46.7%)	0.99
Mucosal features of B12 deficiency	1 (7.7%)	6 (40.0%)	0.12

Notes: PA: Pernicious anemia; PPI: Proton pump inhibitor; IFA: Anti-Intrinsic Factor Antibodies; APCA: Anti-Parietal Cell Antibodies; pMMA: plasma methylmalonic acid; uMMA/C: urinary methylmalonic acid/creatinine ratio.

[a] Among the patients who had gastric endoscopy and biopsies (12/13 PA patients with iron deficiency, and 12/15 PA patients with normal iron status). Clinical signs of anemia included asthenia, pallor, and dyspnea. Neurological features of B12 deficiency included polyneuropathy, pyramidal and/or proprioceptive involvement. Mucosal features of B12 deficiency included glossitis, buccal ulcerations and/or tongue dysesthesia.

We demonstrated that PA patients having ID had a less severe B12 deficiency with a lower plasma homocysteine, this probably explain why patients were less prompt to have anemia in ID group. The association between ID and a milder B12 deficiency and milder hematological consequences of B12 deficiency interrogated about the time of the development of ID in the PA history. Indeed, B12 deficiency and its hematological consequences progressively worsen as the disease progresses until the diagnosis and the beginning of the B12 supplementation. Our data were therefore in line with those of Hershko et al. who postulated that iron deficiency would develop earlier than B12 deficiency in the disease history of atrophic gastritis [10]. Also, we demonstrated that the “isolated APCA” serological status was associated with ID, and high titer of APCA was associated with lower ferritin level. Our results therefore led to two major hypotheses. First, one could hypothesize two distinct PA subgroups: PA with APCA alone responsible for ID and milder B12 deficiency in one hand, and PA with FI associated with less ID and an important B12 deficiency. However, this would imply more severe gastritis and atrophy in PA-APCA subgroups, which was not obvious in our cohort. Second, we could hypothesize that ID could be an early sign of in the PA disease history, whose the immunological markers vary during the disease evolution. Indeed, titer of APCA is known to decrease in the disease history of PA, until the APCA negativation, due to the progressive target autoantigen disappearance when gastric atrophy gradually increases [31].

Whereas IFA results in clearance or inactivation of intrinsic factor [32], APCA targets the H^+/K^+ATPase of gastric parietal cells. It is not clear if APCA contributes to the inactivation of this membrane enzyme, to the parietal cell destruction through antibody-dependent cell-mediated cytotoxicity, or both [33]. Even if PA involves a T CD8+ immune reaction responsible for fundic atrophy common to the different serological profiles [33,34], the different targets of the two antibodies could explain mild phenotypic differences among a same disease. Notably, the 2 PA patients having isolated IFA in our study were exempt of ID, whereas most of ‘isolated APCA’ patients had ID. As the increase in gastric pH is considered to be the key element of ID in PA [4,6,10,27,35], the inverse relationship between APCA titer and ferritin is supported by physiological hypotheses related to the target of these autoantibodies.

Gastric achlorhydria related to parietal cell atrophy is indeed pointed as the main explanation of ID in PA by impairing of the transformation from ferric to ferrous iron forms. Among patients with atrophic gastritis from different causes, gastrin levels were higher in patients having ID [10,27]. In our study restricted to PA, gastrin levels were also higher in patients with ID, however without statistical significance. Also, the low efficiency of oral ferrous iron supplementation argued for the existence of other mechanisms, because the absorption of ferrous iron form does not require acid pH conditions. Other hypotheses have been formulated to explain the ID in PA, which could be associated: the co-deficiency of vitamin C (as vitamin C plays an important role in the iron absorption) [5,36], a decrease in the conversion of pepsinogen to pepsin (as pepsin acts in iron absorption) [37], and the increased iron requirements to assume the restart of erythropoiesis after the initiation of B12 supplementation [38].

We finally described the efficiency of oral and intravenous iron supplementation in PA. In our study, a 3-month course of

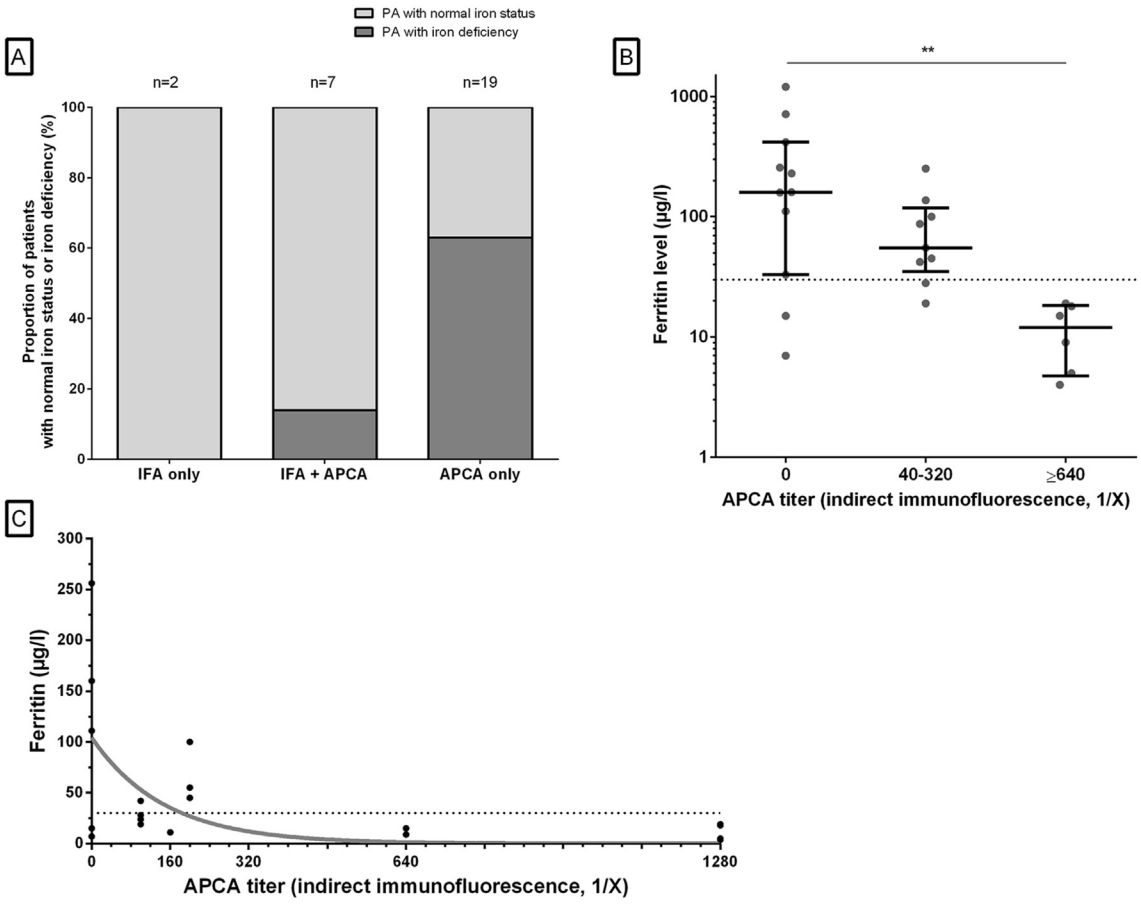

Fig. 3. Association between the serological markers of PA and the iron status or ferritin level. Notes: PA: pernicious anemia; IFA: anti-intrinsic factor antibodies; APCA: anti-parietal cell antibodies.

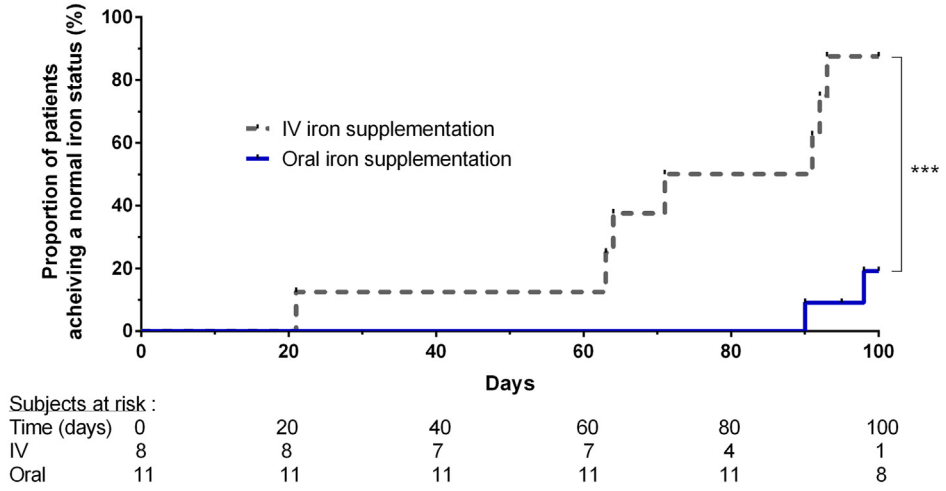

Fig. 4. Correction of the iron deficiency under iron supplementation during the first 100 days following the beginning of oral iron supplementation of after the infusion of IV iron supplementation.

oral supplementation was ineffective to correct ID in most PA patients. We did not compare the efficiency of oral iron supplementation in PA and in other conditions, but the proportion of our PA patients achieving a normal status after a 3-months oral treatment (3/11, 27.3%) was lower than previously described in other conditions. Indeed, in non-menopausal women having ID, the response rate for ID correction was between 76.9% and 97.3% after an oral supplementation with ferrous sulfate 30–80 mg during 10–16 weeks [39–41]. This supported the interest of intravenous iron supplementation in PA, all the more so as the intravenous iron supplementation is better tolerated than the oral route [42,43].

Our study had some limitations. Despite being one of the largest PA cohorts, we were limited by our population size, which could have prevented us to identify other specific features associated to ID in PA. However, we were voluntarily strict in the inclusion criteria to only include patients with a diagnosis of PA, to strictly focus on this disease, contrary to previous works which often included patients with different causes of atrophic gastritis or probable false positive APCA [5,10,27]. Second, we only included PA patients with B12 deficiency. This avoided to include patients with false positive APCA, but this prevented us to explore the possible ID appearing before the B12 deficiency. Indeed, our results supported that ID appeared early in the disease history of most patients, so it would be of interest in further studies to explore the consequences of PA on iron status before the B12 deficiency occurred. This would be of interest to assess if ID is an early sign of PA whatever the initial immunological profile or only when APCA are present (associated or not with IFA). Finally, both the route of iron supplementation and the monitoring of iron status after the first year of follow-up were left to the clinician convenience. The comparison of efficiency between the oral and the IV route should therefore be interpreted with caution.

5. Conclusion

Iron deficiency was very frequent in our cohort of PA patients, affecting 46.4% of patients at PA diagnosis, and other 28.6% patients during the first year of follow-up. ID was associated with an immunological profile including APCA alone, high titer of APCA, and less pronounced B12 deficiency, which could argue for the occurrence of ID early in the disease history. The gastric achlorhydria could explain the iron deficiency and the low rate of response to oral iron supplementation, but other mechanisms unrelated to gastric pH could be involved. Our results argued for searching an ID at the PA diagnosis then monitoring the iron status during follow-up, and to rather propose an iron supplementation through IV route due to the low efficiency rate of oral iron supplementation.

Funding

None.

Conflict of interest

None.

Author contribution

Juliette Rogez: Investigation, Formal analysis, Writing - original draft. Geoffrey Urbanski: Validation, Writing - review & editing. Emeline Vinatier: Investigation, Writing - review & editing. Christian Lavigne: Writing - review & editing. Léa Emmanuel: Investigation. Iris Dupin: Investigation. Camille Ravaiau: Writing - review & editing. Valentin Lacombe: Conceptualization, Methodology, Supervision, Project administration, Formal analysis, Writing - original draft.

Acknowledgments

We gratefully thank the physicians who helped us to screen and include patients in the present study: Estelle Delattre, Sami Hammi, Jonathan Pehlivan, Robin Echerbault, Carole Lacout, Benoit Prouveur, Guillaume Roquin, Cédric Annweiler, Jean Barré. We also thank the medical biologists who enabled the regular biological follow-up: Pascal Reynier, Floris Chabrun and Chadi Homedan.

References

[1] Steinbicker AU, Muckenthaler MU. Out of balance–systemic iron homeostasis in iron-related disorders. Nutrients 2013;5:3034–61. https://doi.org/10.3390/nu5083034.
[2] Muckenthaler MU, Rivella S, Hentze MW, Galy B. A red carpet for iron metabolism. Cell 2017;168:344–61. https://doi.org/10.1016/j.cell.2016.12.034.
[3] Pasricha S-R, Tye-Din J, Muckenthaler MU, Swinkels DW. Iron deficiency. Lancet 2021;397:233–48. https://doi.org/10.1016/S0140-6736(20)32594-0.
[4] Kumar A, Sharma E, Marley A, Samaan MA, Brookes MJ. Iron deficiency anaemia: pathophysiology, assessment, practical management. BMJ Open Gastroenterol 2022;9:e000759. https://doi.org/10.1136/bmjgast-2021-000759.
[5] Annibale B, Capurso G, Delle Fave G. The stomach and iron deficiency anaemia: a forgotten link. Dig Liver Dis 2003;35:288–95. https://doi.org/10.1016/s1590-8658(03)00067-7.
[6] Gulec S, Anderson GJ, Collins JF. Mechanistic and regulatory aspects of intestinal iron absorption. Am J Physiol Gastrointest Liver Physiol 2014;307:G397–409. https://doi.org/10.1152/ajpgi.00348.2013.
[7] Lam JR, Schneider JL, Quesenberry CP, Corley DA. Proton pump inhibitor and histamine-2 receptor antagonist use and iron deficiency. Gastroenterology 2017;152:821–829.e1. https://doi.org/10.1053/j.gastro.2016.11.023.
[8] Bizzaro N, Antico A. Diagnosis and classification of pernicious anemia. Autoimmun Rev 2014;13:565–8. https://doi.org/10.1016/j.autrev.2014.01.042.
[9] Mohamed M, Thio J, Thomas RS, Phillips J. Pernicious anaemia. BMJ 2020;369:m1319. https://doi.org/10.1136/bmj.m1319.
[10] Hershko C, Ronson A, Souroujon M, Maschler I, Heyd J, Patz J. Variable hematologic presentation of autoimmune gastritis: age-related progression from iron deficiency to cobalamin depletion. Blood 2006;107:1673–9. https://doi.org/10.1182/blood-2005-09-3534.
[11] Zulfiqar A-A, Dramé M, Pennaforte J-L, Novella J-L, Vogel T, Andres E. [Iron deficiency and pernicious anemia: a rare association?]. Ann Biol Clin (Paris) 2015;73:420–6. https://doi.org/10.1684/abc.2015.1056.
[12] Lagarde S, Jovenin N, Diebold M-D, Jaussaud R, Cahn V, Bertin E, et al. Is there any relationship between pernicious anemia and iron deficiency? Gastroenterol Clin Biol 2006;30:1245–9. https://doi.org/10.1016/s0399-8320(06)73532-1.
[13] Stabler SP. Vitamin B12 deficiency. N Engl J Med 2013;368:2041–2. https://doi.org/10.1056/NEJMc1304350.
[14] Lahner E, Norman GL, Severi C, Encabo S, Shums Z, Vannella L, et al. Reassessment of intrinsic factor and parietal cell autoantibodies in atrophic gastritis with respect to cobalamin deficiency. Am J Gastroenterol 2009;104:2071–9. https://doi.org/10.1038/ajg.2009.231.
[15] Neumann WL, Coss E, Rugge M, Genta RM. Autoimmune atrophic gastritis–pathogenesis, pathology and management. Nat Rev Gastroenterol Hepatol 2013;10:529–41. https://doi.org/10.1038/nrgastro.2013.101.
[16] Coati I, Fassan M, Farinati F, Graham DY, Genta RM, Rugge M. Autoimmune gastritis: pathologist's viewpoint. World J Gastroenterol 2015;21:12179–89. https://doi.org/10.3748/wjg.v21.i42.12179.
[17] Lacombe V, Roquin G, Vinatier E, Lavigne C, Urbanski G. Parietal cell antibodies: evolution of plasma vitamin B12 during oral supplementation to differentiate true and false positives for pernicious anemia. Pol Arch Intern Med 2020;130:813–5. https://doi.org/10.20452/pamw.15478.
[18] Miller JW, Garrod MG, Rockwood AL, Kushnir MM, Allen LH, Haan MN, et al. Measurement of total vitamin B12 and holotranscobalamin, singly and in combination, in screening for metabolic vitamin B12 deficiency. Clin Chem 2006;52:278–85. https://doi.org/10.1373/clinchem.2005.061382.
[19] Supakul S, Chabrun F, Genebrier S, N'Guyen M, Valarche G, Derieppe A, et al. Diagnostic performances of urinary methylmalonic acid/creatinine ratio in vitamin B12 deficiency. J Clin Med 2020;9:2335. https://doi.org/10.3390/jcm9082335.
[20] Olson SR, Deloughery TG, Taylor JA. Time to abandon the serum cobalamin level for diagnosing vitamin B12 deficiency. Blood 2016;128. https://doi.org/10.1182/blood.V128.22.2447.2447. 2447–2447.
[21] Hannibal L, Lysne V, Bjørke-Monsen A-L, Behringer S, Grünert SC, Spiekerkoetter U, et al. Biomarkers and algorithms for the diagnosis of vitamin B12 deficiency. Front Mol Biosci 2016;3:27. https://doi.org/10.3389/fmolb.2016.00027.
[22] Dignass AU, Gasche C, Bettenworth D, Birgegård G, Danese S, Gisbert JP, et al. European consensus on the diagnosis and management of iron deficiency and anaemia in inflammatory bowel diseases. J Crohns Colitis 2015;9:211–22. https://doi.org/10.1093/ecco-jcc/jju009.
[23] Bozdogan H. Model selection and Akaike's Information Criterion (AIC): the general theory and its analytical extensions. Psychometrika 1987;52:345–70. https://doi.org/10.1007/BF02294361.
[24] Carmel R, Weiner JM, Johnson CS. Iron deficiency occurs frequently in patients with pernicious anemia. JAMA 1987;257:1081–3.
[25] Demiroğlu H, Dündar S. Pernicious anaemia patients should be screened for iron deficiency during follow up. N Z Med J 1997;110:147–8.
[26] Atrah HI, Davidson RJ. Iron deficiency in pernicious anaemia: a neglected diagnosis. Postgrad Med J 1988;64:110–1. https://doi.org/10.1136/pgmj.64.748.110.
[27] Marignani M, Delle Fave G, Mecarocci S, Bordi C, Angeletti S, D'Ambra G, et al. High prevalence of atrophic body gastritis in patients with unexplained microcytic and macrocytic anemia: a prospective screening study. Am J Gastroenterol 1999;94:766–72. https://doi.org/10.1111/j.1572-0241.1999.00949.x.

[28] Solmaz S, Özdoğu H, Boğa C. Cobalamin deficiency can mask depleted body iron reserves. Indian J Hematol Blood Transfus 2015;31:255–8. https://doi.org/10.1007/s12288-014-0417-x.
[29] Hillman RS, Adamson J, Burka E. Characteristics of vitamin B12 correction of the abnormal erythropoiesis of pernicious anemia. Blood 1968;31:419–32.
[30] Carmel R, MacPhee RD. Erythropoietin levels in cobalamin deficiency: comparison of anemic and non-anemic, subtly deficient patients. Eur J Haematol 1992;48:159–62. https://doi.org/10.1111/j.1600-0609.1992.tb00589.x.
[31] Nishizawa T, Watanabe H, Yoshida S, Toyoshima A, Kataoka Y, Kanazawa T, et al. Decreased anti-parietal cell antibody titer in the advanced phase of autoimmune gastritis. Scand J Gastroenterol 2022;57:143–8. https://doi.org/10.1080/00365521.2021.1994642.
[32] Schade SG, Abels J, Schilling RF. Studies on antibody to intrinsic factor. J Clin Invest 1967;46:615–20. https://doi.org/10.1172/JCI105563.
[33] Lenti MV, Rugge M, Lahner E, Miceli E, Toh B-H, Genta RM, et al. Autoimmune gastritis. Nat Rev Dis Prim 2020;6:56. https://doi.org/10.1038/s41572-020-0187-8.
[34] D'Elios MM, Bergman MP, Azzurri A, Amedei A, Benagiano M, De Pont JJ, et al. H(+),K(+)-atpase (proton pump) is the target autoantigen of Th1-type cytotoxic T cells in autoimmune gastritis. Gastroenterology 2001;120:377–86. https://doi.org/10.1053/gast.2001.21187.
[35] Hershko C, Hoffbrand AV, Keret D, Souroujon M, Maschler I, Monselise Y, et al. Role of autoimmune gastritis, Helicobacter pylori and celiac disease in refractory or unexplained iron deficiency anemia. Haematologica 2005;90:585–95.
[36] Cavalcoli F, Zilli A, Conte D, Massironi S. Micronutrient deficiencies in patients with chronic atrophic autoimmune gastritis: a review. World J Gastroenterol 2017;23:563–72. https://doi.org/10.3748/wjg.v23.i4.563.
[37] Horimoto Y, Lim L-T. Effects of different proteases on iron absorption property of egg white hydrolysates. Food Res Int 2017;95:108–16. https://doi.org/10.1016/j.foodres.2017.02.024.
[38] Fernández-Fernández FJ, Ameneiros-Lago E, Sesma P. Pernicious anemia: normal ferritin levels and possible hidden iron deficiency. Rev Clin Esp (Barc) 2015;215:478. https://doi.org/10.1016/j.rce.2015.07.005.
[39] Leonard AJ, Chalmers KA, Collins CE, Patterson AJ. Comparison of two doses of elemental iron in the treatment of latent iron deficiency: efficacy, side effects and blinding capabilities. Nutrients 2014;6:1394–405. https://doi.org/10.3390/nu6041394.
[40] Viteri FE, Ali F, Tujague J. Long-term weekly iron supplementation improves and sustains nonpregnant women's iron status as well or better than currently recommended short-term daily supplementation. J Nutr 1999;129:2013–20. https://doi.org/10.1093/jn/129.11.2013.
[41] McClung JP, Karl JP, Cable SJ, Williams KW, Nindl BC, Young AJ, et al. Randomized, double-blind, placebo-controlled trial of iron supplementation in female soldiers during military training: effects on iron status, physical performance, and mood. Am J Clin Nutr 2009;90:124–31. https://doi.org/10.3945/ajcn.2009.27774.
[42] Tolkien Z, Stecher L, Mander AP, Pereira DIA, Powell JJ. Ferrous sulfate supplementation causes significant gastrointestinal side-effects in adults: a systematic review and meta-analysis. PLoS One 2015;10:e0117383. https://doi.org/10.1371/journal.pone.0117383.
[43] Onorati M, Nicola M, Renda A, Lancia M, Di Nuovo F. Iron overload in gastric mucosa: underdiagnosed condition rarely documented in clinical and pathology reports. Cureus 2020;12:e8234. https://doi.org/10.7759/cureus.8234.