Is there any relationship between pernicious anemia and iron deficiency?

Stéphanie LAGARDE (1), Nicolas JOVENIN (2), Marie-Danièle DIEBOLD (3), Roland JAUSSAUD (4), Virginie CAHN (3), Eric BERTIN (5), Damien JOLLY (2), Gérard THIEFIN (1), Guillaume CADIOIT (1)


SUMMARY

Introduction — Previous studies have suggested that iron deficiency could be due to atrophic gastritis of the body/fundus. The aim of this study was to determine the prevalence of iron deficiency among patients with pernicious anemia and associated factors.

Patients and methods — All patients with pernicious anemia diagnosed at our institution between January 1990 and February 2005 were included. Inclusion criteria were: 1- histological diagnosis of atrophic fundic gastritis and 2- criteria of gastric autoimmune involvement. Histology of gastric biopsies was performed in a blinded manner. Iron deficiency was defined as serum ferritin level < 15 μg/L in women and < 40 μg/L in men.

Results — Ninety-five patients (69 women), mean age 60 years (range: 23-90) were included. Twenty patients (21.1 %) had normal blood cell counts; 12 patients (12.6 %) had microcytosis with or without anemia and 53 patients (55.8 %) macrocytosis with or without anemia. Serum ferritin levels were measured in 58 patients, 1.6 (27.6 %) of whom, all women, had iron deficiency. They were significantly younger (39.2 years) than patients without iron deficiency (61.6 years, P < 0.0001). Serum gastrin levels did not differ between the groups with and without iron deficiency. A significantly more severe inflammatory infiltrate of the fundus and endocrine cell hyperplasia was observed in iron deficiency patients. Multivariate analysis showed that iron deficiency was linked to female gender and age < 50 years.

Conclusion — Iron deficiency and microcytic anemia are not rare in patients with pernicious anemia and should not rule out the diagnosis. Iron deficiency does not appear to be related to the degree of atrophic fundic gastritis but is linked to female gender and young age, suggesting menstrual blood loss could play a role. Whether decreased iron absorption due to reduced acid secretion favors the expression of gynecological iron loss cannot be ascertained.

RÉSUMÉ

Existe-t-il une relation entre la maladie de Biermer et la carence martiale ?

Stéphanie LAGARDE (1), Nicolas JOVENIN (2), Marie-Danièle DIEBOLD (3), Roland JAUSSAUD (4), Virginie CAHN (3), Eric BERTIN (5), Damien JOLLY (2), Gérard THIEFIN (1), Guillaume CADIOIT (1)

Introduction — Des études antérieures ont suggéré que la gastrite atrophique fundique pouvait être à l’origine d’une carence martiale. Le but de ce travail était d’évaluer la prévalence de la carence martiale dans la maladie de Biermer ainsi que les facteurs associés à cette anémie.

Matériels et méthodes — Tous les malades atteints de maladie de Biermer diagnostiquée dans notre CHU entre janvier 1990 et février 2005 ont été inclus. Les critères d’inclusion étaient : 1- le diagnostic anatomo-pathologique de gastrite atrophique fundique et 2- un critère d’auto-immunité gastrique. Une relecture à l’aveugle des biopsies a été effectuée. La carence martiale était définie par une ferritinémie < 15 ng/L chez les femmes et < 40 ng/L chez les hommes.

Résultats — Quarante-vingt-cinq malades (69 femmes), d’âge moyen 60 ans (extrêmes : 23-90), ont été inclus. L’hémogramme était normal chez 20 malades (21,1 %); une microcytose avec ou sans anémie était notée chez 12 malades (12,6 %) et une macrocytose avec ou sans anémie chez 53 (55,8 %). La ferritinémie a été dosée chez 58 malades, 16 (27,6 %) d’entre eux avaient une carence martiale, tous des femmes, significativement plus jeunes (39,2 ans) que les sujets sans carence martiale (61,6 ans, P < 0,0001). Les taux moyens de gastrinémie n’étaient pas différents entre les 2 groupes avec et sans carence martiale. La sévérité de l’infiltrat inflammatoire fundique et la sévérité de l’hyperplasie des cellules endocrines fundiques étaient significativement plus importantes chez les malades avec carence martiale. En analyse multivariée, seuls le sexe féminin et l’âge < 50 ans étaient associés à la carence martiale.

Conclusion — La carence martiale et l’anémie microcytaire ne sont pas rares chez les malades ayant une maladie de Biermer et ne doivent pas faire méconnaître le diagnostic. La carence martiale ne semble pas liée au degré d’atrophiue fundique mais au sexe féminin et au jeune âge, suggérant le rôle des pertes menstruelles. Le rôle éventuel de l’hypochlorhydrie en tant que facteur révélateur du déficit ferrique d’origine gynécologique n’a pas été démontré.

Introduction

Pernicious anemia, also called Biermer’s disease, is an autoimmune gastritis limited to the fundus of the stomach result-
In the general population, the prevalence of pernicious anemia is estimated at 0.1%. It is higher, 1.9%, in subjects aged over 60 years [4].

Pernicious anemia is often associated with other autoimmune diseases such as insulin-dependent diabetes mellitus, autoimmune thyroiditis, or vitiligo [2, 5-7]. The estimated prevalence of pernicious anemia among subjects with insulin-dependent diabetes is 10% [6] and reaches 35% among subjects with autoimmune thyroiditis [7].

In industrialized countries, 80% of iron intake is in the ferric form, which, to be absorbed, must be transformed into the ferrous form. This chemical reduction requires the presence of ascorbic acid in the stomach and an acidic intragastric pH, a condition which is compromised in pernicious anemia. This can lead to iron malabsorption and subsequent iron deficiency [8, 9]. Two prospective studies conducted in Italy [10] and Ireland [11] demonstrated that 19.5% and 20% of patients respectively with unexplained iron deficiency presented atrophic fundic gastritis. The prevalence of iron deficiency in pernicious anemia was evaluated in two retrospective cohorts from Turkey [12] and North America [13]. These studies reported that at the time of the diagnosis of pernicious anemia the prevalence of iron deficiency was to the order of 20%. There were methodological biases in both of these studies, particularly since the diagnostic criteria for pernicious anemia were not those currently accepted [1, 2]. To date, there has been no study evaluating the prevalence of iron deficiency at de novo diagnosis of pernicious anemia established on the basis of the new diagnostic criteria (fundic atrophic gastritis with an associated autoimmune criterion).

The main objectives of this study were to determine the prevalence of iron deficiency in pernicious anemia patients and to search for relations between iron deficiency and pernicious anemia.

### Patients and methods

This was a retrospective descriptive analysis.

### Inclusion criteria

We reviewed all incident cases of pernicious anemia with histological proof of diagnosis established in the Pathology unit of the Reims University Hospital between January 1990 and February 2005.

The population was selected from the Pathology unit’s database using the key words Biermer’s gastritis and atrophic fundic gastritis. Medical files were then consulted to verify the diagnosis of pernicious anemia and select patients having the following inclusion criteria:

- atrophic fundic gastritis defined as thinning of the fundic mucosa with lengthened crypts and rarefied small-sized glands, aggressive periglandular lymphocyte inflammatory infiltrate of variable abundance and intestinal metaplasia;
- presence of anti-parietal cell antibodies and/or anti-intrinsic factor antibodies.

### Data collected

Variables of interest were recorded from the clinical files and pathology reports using a standardized data chart. The following data were recorded:

- clinical data: gender, age at diagnosis, event leading to diagnosis (autoimmune work-up, neurological disorders, hematological disorder such as anemia or isolated macrocytosis), known autoimmune disease (insulin-dependent diabetes mellitus, autoimmune thyroiditis with diagnostic ultrasound and presence of antithyroid antibodies), and presence of neurological signs at the physical examination attributable to pernicious anemia (signs of spinal cord sclerosis: ataxia, tendon areflexia, paresthesia, disorders of deep sensitivity predominating in the lower limbs);
- laboratory data: serum hemoglobin, mean corpuscular volume, serum ferritin, serum vitamin B12, serum gastrin, presence of anti-parietal and anti-intrinsic factor antibodies, Helicobacter pylori serology;
- endoscopic data: presence of gastric polyps;
- pathological data: hyperplasia of the fundic endocrine cells, hyperplastic polyps, adenocarcinoma, fundic endocrine tumors, pathological aspect of the antral and duodenal mucosa.

One of the authors (MDD) who was blinded to the clinical results of iron states reread all pathology slides of biopsy specimens obtained using a scoring system assessing: the degree of fundic atrophy (scored 1 to 3), the degree of inflammatory infiltration (scored 0 to 3), and the severity of the endocrine cell hyperplasia in the fundus (scored 0 to 3). Iron deficiency was defined as serum ferritin < 15 µg/L in women and < 40 µg/L in men [14]. Anemia was defined as serum hemoglobin < 12 g/dl in women and < 13 g/dl in men. Microcytosis was defined as mean corpuscular volume < 80 fl and macrocytosis as mean corpuscular volume > 98 fl. Vitamin B12 deficiency was defined as serum level < 160 pg/ml [15].

Data were processed with a Microsoft Excel® dataset. Coherence was checked with search for adherent and missing data.

### Statistical analysis

A descriptive analysis of events leading to diagnosis and of clinical, laboratory, endoscopic and pathological variables was conducted first to determine the number (N) and percentage (%) for qualitative variables and mean (m) and standard deviation (SD) and range for quantitative variables.

Univariate analysis used Pearson’s chi-square test or Fisher’s exact test and the Mann-Whitney test as appropriate. For multivariate analysis, variables associated with iron deficiency at univariate analysis with P < 0.20 were retained for the exact logistic regression model. The statistical analysis was performed with SAS for Windows version 8.2 (SAS Institute, Cary NC) except for exact logistic regression which was performed with LogXact version 1.3 (Cytel Software Corporation, Cambridge, MA). P < 0.05 was considered statistically significant.

### Results

The study cohort included 95 subjects with pernicious anemia. Mean age was 60 years (SD 16.7, range: 23-90 years); 69 were women (72.6%) with a M-F sex-ratio of 0.4.

### Characteristic features

According to the inclusion criteria, all subjects presented atrophic fundic gastritis and were positive for anti-parietal cell antibodies and/or anti-intrinsic factor antibodies. Search for antithyroid cell antibodies was performed in 84 subjects and 69 (82.1%) were positive. Search for anti-intrinsic factor antibodies was performed in 76 subjects and was positive in 35 (46.1%). Nine subjects were positive for both tests.

Thirty-six subjects (37.9%) presented at least one autoimmune disease, mainly insulin-dependent diabetes mellitus (52.8%) and/or autoimmune thyroiditis (58.3%).

The events leading to diagnosis were: hematological disorders (49.5%), work-up for autoimmune disease (24.2%), neurological signs related or not to pernicious anemia (15.8%), other anomalies (10.5%).

Low serum gastrin was noted in 32 of 37 subjects (86.5%) tested. Mean serum level was 621 pg/ml (SD 648; range: 40-3300). Vitamin B12 deficiency was confirmed in 59 of 87 subjects (67.8%) tested. Blood cell counts were normal at diagnosis of pernicious anemia in 20 subjects (21.1%); macrocytosis, alone or associated with anemia was detected in 53 (55.8%), and microcytosis with or with anemia in 12 (12.6%).

The initial gastroscopy revealed hyperplastic polyps in eight subjects (11.6%), adenocarcinoma in three (3.2%) and fundic endocrine tumors in three (3.2%). An antral biopsy was taken in 62 patients and 33 (53.2%) showed a non-specific inflammatory infiltration. Helicobacter pylori infection was diagnosed in six patients (6.3%), based on positive serology in three of the eleven subjects tested and a positive histological slide in three.
patients. Duodenal biopsies were normal in all 49 subjects concerned.

Women were significantly younger than men (57.4 versus 67.1 years, \( P = 0.01 \)) and had autoimmune disease more often (46.4 % versus 15.4 %, \( P = 0.005 \)). Mean corpuscular volume was different between men and women (\( P = 0.02 \)); microcytosis was found only in women (17.4 %) who had macrocytosis less often than men (49.3 % versus 73.1 %, \( P = 0.37 \)). Prevalence of anemia was similar for both genders (\( P = 0.45 \)).

Characteristics features with and without serum ferritin assay

Serum ferritin was available for 58 subjects (61.1 %). Subjects with a ferritin assay were on average 12 years younger than those who had not (55 versus 67 years, \( P = 0.0013 \)), they presented fewer neurological signs related to pernicious anemia (8.6 % versus 27 %), and had anemia more frequently (72.4 % versus 48.6 %, \( P = 0.02 \)). All patients with microcytosis had serum ferritin measured. Other laboratory and pathological findings were not significantly different in subjects with and without ferritin assay.

Characteristics features with and without iron deficiency

Iron deficiency was noted in 16 subjects (27.6 %). All 16 of these subjects were women (table I). They were younger than subjects free of iron deficiency (39.2 versus 61.8 years, \( P < 0.0001 \)). Among subjects with vitamin B12 deficiency, the prevalence of iron deficiency was 23.1 %.

There was no significant different in the prevalence of anemia (\( P = 0.8 \)) or mean serum hemoglobin level (\( P = 0.9 \)) between subjects with and without iron deficiency. Among the 16 women with iron deficiency, nine (56.3 %) had duodenal biopsies (all normal) and three (18 %) total colonoscopy (normal in all), and eight (50 %) a gynecological examination (normal). Only three of these 16 women (12.5 %) mentioned significant menstrual blood loss. Serum ferritin was available for all 16 and returned to a normal level after iron supplementation, 12 (75 %) with oral supplements and four (25 %) with parenteral infusion.

Women with iron deficiency were younger than the other women (39.3 versus 56.7 years, \( P = 0.0008 \)). Iron deficiency was more prevalent in women aged 50 years or less than in women aged 50 and older (61.2 % versus 15 %, \( P = 0.002 \)).

Serum gastrin was assayed in nine women with iron deficiency and was high in all nine (100 %). It was also assayed in 15 subjects without iron deficiency and was high in 13 of them (86.6 %). Serum gastrin level was not significantly different between subjects with and without iron deficiency (\( P = 0.2 \)).

The degree of inflammation and the severity of hyperplasia of the fundic endocrine cells were significantly more pronounced in subjects with iron deficiency, with no difference for degree of fundic atrophy (table II).

The prevalence of *Helicobacter pylori* infection was not different in subjects with or without iron deficiency: 12.5 % (16/132) versus 5.1 % (1/20) (\( P = 0.72 \)). The infection was diagnosed by serology and by histology, once each in each group. *H. pylori* serology was available for five subjects in each group.

Four variables were retained for the multivariate analysis searching for factors associated with iron deficiency: gender, age ≤ 50 years, hyperplasia of endocrine cells, inflammatory infiltration of the fundus (scores 0-1 vs 2-3). The only variables which were significantly associated with iron deficiency at multivariate analysis were female gender (\( P = 0.05 \)) and age ≤ 50 years (\( P = 0.002 \)).

### Table I

- **Characteristics features of subjects with and without iron deficiency.**

<table>
<thead>
<tr>
<th>Characteristic features</th>
<th>Patients with normal ferritin, N = 42</th>
<th>Patients with low ferritin, N = 16</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender M/F (%)</td>
<td>17/25 (40.5/59.5)</td>
<td>0/16 (0/100)</td>
<td>0.0025</td>
</tr>
<tr>
<td>Age, mean (SD)</td>
<td>61.6 (15.3)</td>
<td>39.2 (10.8)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Event leading to diagnosis, N (%)</td>
<td></td>
<td></td>
<td>NS†</td>
</tr>
<tr>
<td>- autoimmune disease</td>
<td>8 (19.1)</td>
<td>5 (31.2)</td>
<td></td>
</tr>
<tr>
<td>- neurological sign</td>
<td>5 (11.9)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>- hematological disorder</td>
<td>27 (64.3)</td>
<td>10 (62.5)</td>
<td></td>
</tr>
<tr>
<td>Associated autoimmune disease, N (%)</td>
<td></td>
<td></td>
<td>0.04a</td>
</tr>
<tr>
<td>- Type 1 diabetes mellitus</td>
<td>6 (14.3)</td>
<td>4 (25)</td>
<td>0.32</td>
</tr>
<tr>
<td>- Autoimmune thyroiditis</td>
<td>4 (9.5)</td>
<td>7 (43.7)</td>
<td>0.012</td>
</tr>
<tr>
<td>- mean corpuscular volume (fL), mean (SD)</td>
<td>106.5 (17.6)</td>
<td>83.6 (18.4)</td>
<td>0.0001</td>
</tr>
<tr>
<td>- serum vitamin B12 (pg/mL), mean (SD)</td>
<td>199 (265)</td>
<td>256 (167)</td>
<td>0.01</td>
</tr>
<tr>
<td>- serum gastrin (pg/mL, mean (SD)</td>
<td>549.3 (536)</td>
<td>784 (480)</td>
<td>NS</td>
</tr>
<tr>
<td>- microcytosis, N (%)</td>
<td>3 (7.1)</td>
<td>9 (56.2)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>- macrocytosis, N (%)</td>
<td>28 (66.7)</td>
<td>3 (18.7)</td>
<td>0.001</td>
</tr>
<tr>
<td>- vitamin B12 deficiency (≤ 160 pg/mL), N (%)</td>
<td>28/40 (70)</td>
<td>8/15 (53.3)</td>
<td>NS</td>
</tr>
<tr>
<td>- anti-parietal cell antibodies, N (%)</td>
<td>30/36 (83.3)</td>
<td>14/16 (87.5)</td>
<td>NS</td>
</tr>
<tr>
<td>- anti-intrinsic factor antibodies, N (%)</td>
<td>16/33 (48.5)</td>
<td>5/12 (41.7)</td>
<td>NS</td>
</tr>
</tbody>
</table>

*a = global test; missing data : b = 2, c = 1, d = 27, e = 7 ; NS = not significant; SD = standard deviation*
Discussion

Prevalence of iron deficiency was an estimated 27.6% in subjects who had a serum ferritin assay. This is probably an overestimation since this group is not representative of the 95 subjects in the study cohort. Serum ferritin was assayed more often in subjects with anemia and microcytosis than the others. Extrapolating from the number of subjects presenting documented iron deficiency to the entire cohort of 95 subjects, the prevalence of iron deficiency was 16.8%. It can thus be estimated that the real prevalence of iron deficiency in pernicious anemia is between 16.8% and 27.6%. This level is in agreement with reports in the literature; the two studies available were also retrospective analyses that found that the prevalence of iron deficiency at the initial diagnosis of pernicious anemia was 21% [12] and 20.7% [13]. Retaining only our population of subjects with pernicious anemia associated with vitamin B12 deficiency, the prevalence of iron deficiency was 23.1%.

In our series, all the subjects presenting iron deficiency were women. This female predominance is also found by others. Marignani et al [10] reported that 33 of 36 subjects with iron deficiency anemia explained by atrophic fundic gastritis were women, with a mean age of 44 years. Annibale et al. [16] had only 3 men among 19 subjects with atrophic fundic gastritis associated with iron deficiency. In one study examining the prevalence of iron deficiency at the initial diagnosis of pernicious anemia, 21 of 25 subjects with iron deficiency were women [13]. The question is thus whether menstrual blood loss is the only cause of iron deficiency or whether fundic atrophy has a favoring influence. Although there are pathogenic arguments which would favor this later hypothesis, our results suggest that fundic atrophy has little effect. The degree of atrophy was not significantly different between subjects with and without iron deficiency. The indirect arguments in favor of more severe fundic atrophic gastritis in subjects with iron deficiency involved variables (higher grade inflammatory infiltration and higher grade endocrine cell hyperplasia) which are no longer significant at multivariate analysis. Female gender and age below 50 years were the only variables which were independently associated with iron deficiency in the multivariate model. This observation suggests that menstrual blood loss is the main cause of iron deficiency in this population. Another argument against a role for reduced acid secretion is the absence of deficiency in patients treated with proton pump inhibitors [17-20]. The prevalence of iron deficiency in women aged 50 years or less was high in our cohort (61.2%), very much above the prevalence estimated in the literature for post-menopause women (5 to 30%) [21, 22]. This would be an argument in favor of a causal influence of gastric atrophy. There is however a probable bias since upper endoscopic procedures are more often ordered for subjects with anemia. Atrophy might also be a factor revealing iron deficiency related to menstrual blood loss, but our data cannot confirm this hypothesis.

In the general population the prevalence of iron deficiency can reach up to 8% in industrialized countries [23, 24]. The estimate is from 5 to 30% in pre-menopause women [21, 22]. In post-menopause women and men, digestive bleeding is dramatically increased in 62 to 64% of persons presenting iron deficiency [24]. Non-hemorrhagic causes of iron deficiency also include iron malabsorption. Besides celiac disease which is one of the most frequent causes of iron deficiency in this context, Helicobacter pylori gastritis appears to be the cause of authentic deficiency in certain patients [25-27]. In our study, H. pylori status was very incorrectly assessed, so no valid conclusions can be drawn. The duodenal biopsies performed in 49 subjects were all negative.

Macrocytosis with or without anemia is common in pernicious anemia patients. The prevalence reached 55.8% in our series while microcytosis, with or without anemia, was observed in only 12.6%. Consequently, the presence of macrocytosis cannot rule out the diagnosis of atrophic fundic gastritis. These results demonstrate the importance of obtaining fundic and antral biopsies in addition to duodenal biopsies in subjects with iron deficiency anemia.

In conclusion, our findings confirm the high prevalence of iron deficiency in pernicious anemia, particularly among young women. This deficiency would appeared to be related to menstrual blood loss and not to reduced acid secretion per se. A role for reduced acid secretion as a revealing sign of iron deficiency resulting from gynecological loss cannot be deduced from our data.

REFERENCES


