Thyroid and gastric autoimmune diseases
Maladies auto-immunes thyroïdiennes et gastriques

S. Morel, A. Georges, L. Bordenave, J.-B. Corcuff*
Department of Nuclear Medicine, University of Bordeaux, University Hospital of Bordeaux, France
Available online 15 January 2009

Résumé

Objectifs. – Les maladies thyroïdiennes autoimmunes (MTAI) s’accompagnent fréquemment d’autres pathologies auto-immunes spécifiques d’organe. Nous avons étudié la fréquence de l’association MTAI-maladie de Biermer en recherchant la présence d’anticorps anti-facteurs intrinsèques (Ac-FI) chez des sujets atteints de MTAI. Patients et Méthodes. – Les sérum de 113 patients souffrant de MTAI (hypo- ou hyperthyroïdie) ont été testés pour la présence d’AcFI de type I IF-Ab avec un immunodosage par compétition automatisé. Ces sérum ont été appariés avec 113 sérum de sujets souffrant de dysthyroïdie sans anticorps antithyroïdiens à un titre élevé. Résultats. – Les sérum de quatre patients avec une MTAI présentaient des AcFI. Une maladie de Biermer était connue chez deux et fortement suspectée chez les deux autres. Aucun sujet contrôle ne présentait d’AcFI dans le sérum. Les concentrations de vitamine B12 étaient fréquemment retrouvées basses quelles que soient les sujets. Conclusion. – La prévalence des AcFI (3,5 %) est plus élevée chez des patients souffrant de MTAI que chez ceux souffrant de dysthyroïdie non auto-immune. Des études prospectives devraient chercher à établir : (i) si la correction hormonale des dysthyroïdies améliore les concentrations de vitamine B12 chez tous les sujets quelle que soit l’étiologie de la dysthyroïdie et (ii) si la recherche en routine d’AcFI doit faire partie des bilans biologiques des MTAI.

© 2008 Elsevier Masson SAS. Tous droits réservés.

Abstract

Objectives. – Autoimmune thyroid disease (AITD) is frequently accompanied by other organ-specific diseases. We investigated the frequency of the association AITD-Biermer’s disease (BD) in patients with AITD by investigating the prevalence of intrinsic factor antibodies (IF-Ab). Design and Methods. – Sera from 113 patients with AITD (hypo- or hyperthyroidism) were screened for the presence of type I IF-Ab with a competitive automated immunoassay based. Matched sera from 113 patients with dysthyroidism (not AITD) were tested. Results. – Four IF-Ab positive patients suffered from AITD. BD was known for two of them and strongly suspected in the two others. All patients with no AITD tested IF-Ab negative. B12 levels were often low whatever the etiology. Conclusion. – The prevalence of IF-AbI is higher (3.5%) in patients with AITD. Prospective studies should investigate whether correcting thyroid dysfunction improves vitamin B12 levels, and establish whether routine screening for gastric autoimmunity is clinically useful or purely academic.

© 2008 Elsevier Masson SAS. All rights reserved.

Mots clés : Maladie de Biermer ; Anémie pernicieuse ; Anticorps antifacteur intrinsèque ; Hypothyroïdie ; Hyperthyroïdie ; Vitamine B12 ; Auto-immunité

Keywords: Biermer’s disease; Pernicious anemia; Intrinsic factor antibodies; Hypothyroidism; Hyperthyroidism; Vitamin B12; Autoimmunity

1. Introduction

Autoimmune thyroid diseases (AITD) are common as the prevalence reaches 5 to 10% in a non selected population [1]. The most common way to diagnose AITD is to look for the presence of antibodies against thyroid peroxidase (Hashimoto’s thyroiditis) or against the TSH receptor (Graves’ disease) in the serum of patients. The association of AITD and other organ-specific diseases is frequent (about 14%): lupus erythematosus, adrenal insufficiency, Gougerot-Sjögren syndrome, vitiligo, Biermer’s disease (pernicious anemia), etc., for which auto-antibodies can confirm the diagnosis [1–4].

Biermer’s disease is a gastric autoimmune disease targeting H+\textsuperscript{+},K\textsuperscript{+}-ATPase and resulting in fundus atrophy and vitamin B12 deficiency [5,6]. Biologically, it is characterized by the
presence in the serum of auto-antibodies directed against different antigens. Antibodies against parietal cells are a sensitive but not specific marker of Biermer’s disease [5]. Very recently, an Elisa assay of these antibodies has been developed [7]. Antibodies against the intrinsic factor have also been described: type I antibodies (IF-AbI) recognizing and blocking the vitamin B12 binding site of IF, and type II antibodies binding but not blocking the intrinsic factor (IF-AbII) [5,8]. These IF-Ab are considered specific but not very sensitive markers of autoimmune gastritis pernicious anemia as about two-thirds of patients with Biermer’s disease are positive for IF-Ab: 70% IF-AbI and 30-45% IF-AbII [5,9–11].

The reference method to determine IF-AbI is a competitive assay using radioactive vitamin B12 as a ligand [12,13]. Its main drawbacks are its complexity and the possible interference of therapeutic vitamin B12 in the serum [14]. Manual non-competitive techniques (Elisa) assaying both types of IF-Ab have been proposed using intrinsic factor as ligand with no or reduced interference of vitamin B12 [15]. More recently, a non-radioactive competitive IF-AbI assay has been developed on an automated analyser, UniCel Dxi 800 (Beckman Coulter) in which IF-AbI auto-antibodies compete with a monoclonal mouse antibody for intrinsic factor binding [16].

We aimed to investigate the presence of IF-Ab in matched patients with thyroid dysfunction presenting or not biological signs of thyroid dysimmunity. Secondly, we wanted to evaluate the possibility and the usefulness of an automated analyser to test for the presence of IF-AbI in these patients. Should a systematic screening for atrophic gastritis appear desirable, the use of an automated analyser (that could assay vitamin B12 and IF-Ab) would be useful for population testing.

2. Patients and methods

2.1. Patients

The database of sera from the left-over of routine samples of our department was screened. We selected sera from patients with biological thyroid dysfunction (hypothyroidism, N=55 or hyperthyroidism, N=58) associated with the presence of autoimmunity stigma (thyroid peroxidase >500 U/ml or TSHR autoantibodies >5 UI/l: thresholds chosen were 30- and 3-fold the positivity threshold of the kits, respectively). These sera were matched with sera from patients with hypothyroidism (N=66) or hyperthyroidism (N=47) but no detectable peroxidase (<60 U/ml) or TSHR autoantibodies (<1.5 UI/l). The population with hypothyroidism had mild forms of disease.

2.2. Assays

The initial assays used to diagnose the thyroid diseases were those routinely used: TSH IRMA, FT4 RIA, FT3 RIA (Beckman Coulter) and thyroid peroxidase auto-antibodies, TSH receptor autoantibodies (DYNOtest anti-TPOon and DYNOtest TRAK, respectively, Brahms). Inter-series coefficients of variation were 16%, 15% and 13% for FT4, FT3 and TSH, respectively. IF-AbI and B12 were assayed using the automated analyser, UniCel Dxi 800 (Beckman Coulter) according to the manufacturer’s instructions. Positivity of IF-Ab was established above a threshold provided by the manufacturer: 1.53 AU/mL. This positive cutoff is the point where the maximum sensitivity and specificity (about 87% for each) are obtained [16].

2.3. Statistics

Intergroup concentration comparisons were performed either by the Kruskall-Wallis or the Mann-Whitney tests (Statview 5.0, SAS Institute). Comparison of proportions was performed using a $\chi^2$ test. $p<0.05$ was considered significant.

3. Results

The biological thyroid status of the different groups of patients is presented in Table 1. IF-AbIs were detected (above the significant threshold) in four patients with AITD: two with hyperthyroidism and two with hypothyroidism. For two of these patients, serum samples obtained at other dates were available and were similarly positive for IF-AbI detection (Table 2). No serum positive for IF-AbI was found in the groups of subjects with thyroid dysfunction without auto-immunity. Thus, there was a significantly different proportion of gastric auto-immunity in patients with AITD compared to matched controls (3.5 vs 0%, $p<0.05$).

Table 1 Mediane [extrêmes] des données biologiques thyroïdiennes et de vitamine B12 dans les quatre groupes de patients.

<table>
<thead>
<tr>
<th>N</th>
<th>Age (yr)</th>
<th>TSH (mUI/l)</th>
<th>FT4 (pmol/l)</th>
<th>FT3 pmol/l</th>
<th>Vitamin B12 (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal range</td>
<td></td>
<td>(0.17–4.05)</td>
<td>[11.5–23.0]</td>
<td>[2.5–5.8]</td>
<td>[180–914]</td>
</tr>
<tr>
<td>Hypothyroidism</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyperthyroidism</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Autoimmune</td>
<td>58</td>
<td>45 [16–88]</td>
<td>&lt;0.06</td>
<td>33.0 [10.4–43.9]</td>
<td>11.9 [4.0–31.5]</td>
</tr>
<tr>
<td>Non autoimmune</td>
<td>47</td>
<td>64 [27–94]</td>
<td>&lt;0.06</td>
<td>21.4 [12.7–36.8]</td>
<td>5.0 [2.4–29.2]</td>
</tr>
</tbody>
</table>

* $p<0.05$; ** $p<0.005$ autoimmune hypothyroidism vs non autoimmune hypothyroidism; *** $p<0.0001$ autoimmune hyperthyroidism vs non autoimmune hyperthyroidism.

* $p<0.05$; ** $p<0.005$ hypothyroidie auto-immune vs hypothyroidie non auto-immune ; *** $p<0.0001$ hyperthyroidie auto-immune vs hyperthyroidie non auto-immune auto-immune.
As gastric autoimmunity elicits the gastric atrophy of Biermer’s disease that in turn leads to vitamin B12 deficiency, this vitamin was assayed in all sera. There was no difference between the vitamin B12 concentrations between the four groups of patients ($p>0.05$). However, one must note that the proportion of patients with decreased vitamin B12 concentrations ($< normal$ range) was higher in patients suffering from hyperthyroidism than in patients suffering from hypothyroidism, irrespective of the immunity status ($p<0.05$). We specifically searched for clinical or biological signs of vitamin B12 deficiency in the four patients who tested positive for IF-AbI (Table 2). Two patients (no.2 and no.4) indeed had a diagnosis of Biermer’s disease years earlier and were treated, hence the normal vitamin B12 serum levels. For the two other patients (no.1 and no.3) the diagnosis was plausible. Patient no.1 had Hashimoto’s thyroiditis in a familial context of thyroid dysfunction and his serum was tested positive for antiparietal cell antibodies. An improbable false positive detection by the competitive assay due to normal vitamin B12 serum levels was ruled out as this serum also tested positive using a non competitive Elisa assay (Aeskulisa, Germany). Patient no.3 had low serum vitamin B12 concentration in a personal and familial context of autoimmune diseases.

### 4. Discussion

We investigated the association of biological signs of autoimmune gastritis and thyroid dysfunction by assaying IF-AbI in patients withAITD compared to matched control patients. This study was initiated to answer some biological as well as clinical points.

Does the use of an automated analyser for the detection of IF-Ab disclose patients with autoimmune gastritis within the expected range of frequency in patients withAITD? The answer is yes, as our results demonstrate that the patients suffering fromAITD are more likely to test positively for IF-AbI. This is consistent with prior studies where pernicious anaemia was diagnosed from among 6.3% of patients with type I diabetes andAITD to 16% of patients withAITD alone, although with various diagnostic tools [2,17,18]. Variability of the prevalence can obviously be due to the population screened but also to the method used to diagnose the disease. Indeed, various IF-Ab detection kits are likely to provide different answers [19]. As far as the detection of IF-Ab is concerned, the kits used to assay IF-Ab can either detect both types of antibodies or only one type. Furthermore, the target antigen used in the kits can be porcine or recombinant human intrinsic factor. Unfortunately, the method used is infrequently mentioned in the literature. In this paper, the detection of IF-AbI – expected in about 50% patients with Biermer’s disease – was positive in 3.5% patients withAITD. One should note that detection of IF-AbI alone would miss patients with Biermer’s disease exhibiting only IF-AbII thus slightly underestimating the prevalence of the disease inAITD.

Are the consequences of positive detection of IF-Ab in a context of normal concentrations of vitamin B12 clinically relevant? In this study, the two patients with yet undetected autoimmune gastritis had normal levels of vitamin B12. Indeed elevated B12 levels ($>1000\text{ pg/ml}$ [14] or $>444\text{ pg/ml}$ [20]) could falsify the detection of IF-AbI in competitive assays (RIA and chemiluminescent). Here, the presence of IF-Ab was confirmed by another technique – Elisa – independent of B12 concentrations. What are then the consequences of a positive detection of IF-Ab in a context of normal concentrations of vitamin B12? The detection of IF-AbI is considered specific of autoimmune gastritis although the antibodies are by themselves not immediately threatening the patient’s health (no vitamin B12 deficiency). A gastric endoscopy could be performed to confirm the diagnosis, although there could be a delay between positive antibody detection and the apparition of gastric atrophy.

---

**Table 2**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age</th>
<th>Diagnosis</th>
<th>Personal auto-immune context</th>
<th>Familial autoimmune context</th>
<th>Vitamin B12 (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1 Male</td>
<td>58</td>
<td>Graves’ disease</td>
<td>Type I IF-Ab</td>
<td>Autoimmune thyroid diseases</td>
<td>529</td>
<td></td>
</tr>
<tr>
<td>#2 Female</td>
<td>73</td>
<td>Known pernicious anaemia</td>
<td>Type I IF-Ab</td>
<td>Thyroglobulin Ab</td>
<td>872 (treated)</td>
<td></td>
</tr>
<tr>
<td>#3 Female</td>
<td>23</td>
<td>Hashimoto's thyroiditis</td>
<td>Type I IF-Ab</td>
<td>Thyroid peroxysdase Ab</td>
<td>153</td>
<td></td>
</tr>
<tr>
<td>#4 Female</td>
<td>51</td>
<td>Hashimoto's thyroiditis</td>
<td>Type I IF-Ab</td>
<td>Thyroid peroxysdase Ab</td>
<td>477 (treated)</td>
<td></td>
</tr>
</tbody>
</table>

For assays see material and methods.
Do patients with AITD have to be systematically tested for pernicious anemia by looking for the presence of IF-Ab? Indeed, IF-Ab are considered as specific of this disease. The answer is still ambiguous as half of the patients tested positive had been diagnosed previously and systematic testing would have resulted in the discovery of the remaining half. A positive result (independently of the vitamin B12 level) could justify iterative gastric endoscopies to detect the occurrence of gastric cancer associated with fundus atrophy. Cost-efficiency of such a systematic screening has to be established before launching the ample task of testing all subjects with AITD. Could systematic screening of IF-Ab be replaced by another systematic assay in patients with dysthyroidism? Assaying vitamin B12 does not seem to be the answer as many patients (12%) with hyperthyroidism or hypothyroidism had decreased levels of vitamin B12 independently of the presence of IF-Ab. By itself this is a result that has to be investigated. Whether this situation reflects the levels of the general population [21,22] or is related to thyroid activity (and thus is reversible with the cure of thyroid disease) remains to be investigated. Other studies have used a gastrin assay to diagnose with success fundus atrophy in patients with AITD [18]. In patients with type I diabetes, Alonso et al. investigated pepsinogen I secretion [23]. Ness-Abramof and colleagues proposed a 2-step analysis: B12 and gastrin assays before referring patients for gastroscopy [17]. Indeed, elevated gastrin levels reflect the gastric atrophy but do not provide an etiological diagnosis. One should also note the need for proton pump therapy wash out before assaying serum gastrin. Only a cost-efficiency study could truly differentiate these approaches in various populations.

5. Conclusion

In this study, we showed that the prevalence of pernicious anemia is modestly increased in patients with AITD compared with matched patients with non-autoimmune thyroid disease. The prevalence of true pernicious anemia and atrophic gastritis could possibly be higher as we detected specific but not very sensitive IF-Ab. We also found a high prevalence of low vitamin B12 serum concentration confirming Ness-Abramof and colleagues’ study [17]. Whether patients with autoimmune thyroid diseases have B12 malabsorption or whether our patients displayed deficiency of the normal population of our region is yet unknown. We propose to analyze the existence of autoimmune gastritis and related B12 deficiency after initiating thyroid dysfunction treatment. The best screening strategy in this population remains to be defined.

Acknowledgements

We wish to thank Immunotech Beckman Coulter France for providing the UniCel Dxi 800 analyser and reagents for this study.

References