Do Intrinsic Factor Antibodies Assays Provide Univocal Answers in Biermer's Disease?

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The objective of the study is to evaluate the concordance of four assays for antibodies against intrinsic factor (IF-Ab). Sixty-two sera were tested with one competitive automated and three manual noncompetitive assays. Thirty-five percent patients had discordant results with at least one of the

four assays. However, any method uncovered patients with proven Biermer's disease missed by the others assays. The observed discordance partly explains the poor sensitivity of IF-Ab in studies using a single assay. J. Clin. Lab. Anal. 22:337–339, 2008. © 2008 Wiley-Liss, Inc.

Key words: intrinsic factor antibodies assays; pernicious anemia; diagnosis

INTRODUCTION

Biermer's disease (BD) *a.k.a.* pernicious anemia, is a gastric autoimmune disease resulting in fundus atrophy and vitamin B12 deficiency with neurological and haematological consequences (1–3). Biologically, it is characterized by the presence in the serum of antibodies directed against gastric antigens. Among these antibodies those against parietal cells are sensitive but not specific markers of BD (1). Antibodies against intrinsic factor have also been described: type I (IF-AbI) recognizing and blocking the vitamin B12 binding site of IF, and type II binding-only antibodies (IF-AbII) (1,3). IF-Ab are considered specific but not very sensitive markers of BD as only 2/3 patients with BD test positive for IF-Ab (1,3). The reference method to assay IF-AbI is a competitive assay using radioactive vitamin B12 as ligand (4). Its drawbacks are the regulatory constraints of isotope use and the interference of therapeutic vitamin B12 in the serum if treatment precedes the sampling. Manual enzyme-linked immunosorbent assay (ELISA) noncompetitive techniques assaying IF-AbI and IF-AbII have been proposed using intrinsic factor as ligand with no interference of vitamin B12 (5). More recently, a nonradioactive competitive IF-AbI assay has been developed on an automated analyser, UniCel DxI 800 (DxI, Beckman Coulter, Villepointe, France) in which IF-AbI compete with a monoclonal mouse antibody for intrinsic factor binding (6).

The aim of this study was to evaluate the diagnostic concordance of four different IF-Ab assays.

PATIENTS AND METHODS

We searched in our database serum from patients that were tested for the detection of IF-Ab in the past year with our routine assay at this time, an ELISA assay (D-tek, DiaSorin, France). From the leftover routine samples, we selected 29 sera that tested positive and matched them with 33 sera that tested negative. The patients were tested either because of fundus atrophy, macrocytic anemia or neurologic signs of vitamin B12 deficiency. These sera were then assayed for IF-Ab with two other sandwich ELISA immunoassays (Bioadvance, France; Aeskulisa, Aesku Lab, Germany) and with the DxI assay according to the manufacturers instructions. Positivities of IF-Ab were established above the threshold provided by each manufacturer. Vitamin B12 was assayed with the DxI.

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RESULTS

IF-Ab were detected (above the significant threshold) in 29 patients using the Diasorin method, in 12 patients using the Bioadvance method, in 12 patients using Aeskulisa ELISA kit and in 16 patients using DxI (plus two ambiguous) (Table 1). Individually, 40 out of 62 sera had identical results with all four assays (29 negative and 11 positive). One serum tested positive with three techniques, one with two techniques and two sera tested positive with one technique and ambiguous with another. Fourteen sera tested positive with Diasorin only, three with DxI only and one with Aeskulisa only. Overall, one patient out of three had at least one discordant result out of four assays.

We investigated the strength of concordance between the different assays using κ testing (MedCalc software, Belgium). The concordance between Bioadvance & Aeskulisa was very good ($k = 0.90$). The concordances between DxI & Bioadvance and between DxI & Aeskulisa were good ($k = 0.74$ and $k = 0.66$, respectively). The concordances between Diasorin & Bioadvance, between Diasorin & DxI and between Diasorin & Aeskulisa were poor ($k = 0.43$, $k = 0.39$ and $k = 0.36$, respectively).

Although this study was a retrospective one, we searched in the patients' files whether there was any

indication of BD. Among the 16 patients positive with Diasorin and negative with the three other techniques (including two ambiguous with DxI) eight had either a fundus atrophy with positive biopsy or vitamin B12 deficiency (#16,19,20,21,22,23,28,29); five other patients had vitamin B12 deficiency but no fundus biopsy was performed (14,15,24,26). For the three remaining patients, only presumption can be presented: one had neurological and biological signs of vitamin B12 deficiency (#18), one had positive antitransglutaminase autoantibodies (#25), one had autoimmune vasculitis (#27). Thus, for most of these patients (positive with Diasorin and negative with the three other techniques) the diagnosis was probably BD but remained unproven.

The patients positive with three & two assays (#12,13) had proven BD. The patients positive with one assay only (#30,31,32,33) had also proven and treated BD.

DISCUSSION

This study was initiated to answer some questions elicited by the importance of IF-Ab in some diagnosis algorithms (1,3). IF-Ab assays although specific are not very sensitive (1,3,7). Variability of the incidence can obviously be owing to the population screened but also to the method used to detect the antibodies (7). Indeed,

TABLE 1. Individual Results of Sera Tested With the Four Techniques

Patient #	IF-Ab DiaSorin	IF-Ab DxI®	IF-Ab Bio-advance	IF-Ab Aeskulisa	Vit. B12 (pg/mL) [180 à 914]	Diagnosis
1–11	POS	POS	POS	POS	0 to >1500	*
12	POS	POS	POS	NEG	375	Proven BD
13	POS	POS	NEG	NEG	> 1500	Proven BD
14	POS	NEG	NEG	NEG	65	Probable BD
15	POS	NEG	NEG	NEG	86	Probable BD
16	POS	NEG	NEG	NEG	188	Proven BD
17	POS	NEG	NEG	NEG	192	Probable BD
18	POS	NEG	NEG	NEG	200	BD?
19	POS	NEG	NEG	NEG	253	Proven BD
20	POS	NEG	NEG	NEG	288	Proven BD
21	POS	NEG	NEG	NEG	307	Proven BD
22	POS	NEG	NEG	NEG	326	Proven BD
23	POS	NEG	NEG	NEG	355	Proven BD
24	POS	NEG	NEG	NEG	424	Probable BD
25	POS	NEG	NEG	NEG	425	BD?
26	POS	NEG	NEG	NEG	454	Probable BD
27	POS	NEG	NEG	NEG	921	BD?
28	POS	Ambiguous	NEG	NEG	378	Proven BD
29	POS	Ambiguous	NEG	NEG	215	Proven BD
30	NEG	POS	NEG	NEG	> 1500	Proven BD
31	NEG	POS	NEG	NEG	> 1500	Proven BD
32	NEG	POS	NEG	NEG	> 1500	Proven BD
33	NEG	NEG	NEG	POS	313	Proven BD
34 to 62	NEG	NEG	NEG	NEG	113 to >1500	

Sera 1–11 and 34–62 had similar responses with the four techniques. For details about the methods and diagnosis see main text. BD, Biermer's disease; NEG, negative; POS, positive; ambiguous: $1.2 < (\text{sample optical density}) / (\text{cutoff sample optical density}) < 1.53$ (see Table 2).

TABLE 2. Technical Features of the Four Assays Tested

	Principle	Sample volume (μL)	Incubation time (min)	Intrinsic factor origin	Positivity threshold
DiaSorin	Sandwich 2 steps	10	70	Porcine	Sample OD/cutoff sample OD > 1.1
Aeskulisa	Sandwich 2 steps	10	65	Human recombinant	Sample OD > cutoff sample OD
Bioadvance	Sandwich 2 steps	10	75	Porcine	Sample OD > cutoff sample OD
Unicel DxI® 800	Competition 2 steps	55	30	Porcine	Sample OD/cutoff sample OD > 1.53

OD, optical density.

the commercial kits are technically different. For instance, the kits used to detect IF-Ab can either detect IF-AbI alone or IF-AbI and IF-AbII at the same time (competitive or ELISA assays), the target antigen—IF—used may differ (porcine or human recombinant), etc... (Table 2). Obviously, this has implications on the antibodies detected. Furthermore, some techniques such as competitive ones (here DxI) are prone to false positive results when vitamin B12 concentrations are elevated hence the importance of testing serum with such methods before vitamin B12 therapy (4).

In this retrospective study, the Diasorin assay uncovered more BD than the other assays but conversely the latter uncovered some BD missed by the Diasorin assay. Indeed, it is noteworthy that even when a serum tested positive with a single method (out of four) it could reveal a true BD. This could be part of the explanation for the apparent lack of sensitivity of a given assay. What can and should be done? Technically, an independent quality control exists (NEQUAS, UK) that provides positive and negative samples to laboratories participating on a voluntary basis. Similarly to other assays, a reference standard could be established, possibly recombinant human intrinsic factor. This will probably solve some but not all discrepancies as the majority of serum OD from discordant patients are not close to the cutoff OD suggesting other factors than lack of sensitivity of the assays.

Clinically, different assays could be performed in a prospective study: IF-Ab assays would have to be associated to a mandatory fundus biopsy in various groups of patient with diverse risk factors (fundus

atrophy, B12 deficiency, macrocytic anemia, etc). ROC curves for various assays could then be constructed and the place of IF-Ab detection in the diagnosis strategy of the various clinical presentations of BD could be established.

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REFERENCES

1. Toh BH, Van Driel I, Glesson P. Pernicious anemia. *N Engl J Med* 1997;337:1441–1448.
2. Annibale B, Lahner E, Negrini R et al. Lack of specific association between gastric autoimmunity hallmarks and clinical presentations of atrophic body gastritis. *World J Gastroenterol* 2005; 11:5351–5357.
3. Toh BH, Alderuccio F. Pernicious anaemia. *Autoimmunity* 2004; 37:357–361.
4. Muckerheide M, Wolfman JA, Rohde DA, McManamy GE. Studies on a radioassay for intrinsic factor antibody: comparison of methods and false positive results due to elevated serum B12 levels. *Am J Clin Pathol* 1984;82:300–304.
5. Waters HM, Smith C, Howarth JE, Dawson DW, Delamore IW. New enzyme immunoassay for detecting total, type I, and type II intrinsic factor antibodies. *J Clin Pathol* 1989;42:307–312.
6. Gomez EA, Ehresmann DD, Ledebuhr LK et al. Development and validation of an automated chemiluminometric immunoassay for human intrinsic factor antibodies in serum. *Clin Chem* 2005;51: 232–235.
7. Nimo RE, Carmel R. Increased sensitivity of detection of the blocking (type I) anti-intrinsic factor antibody. *Am J Clin Pathol* 1987;88:729–733.