Parietal cell antibody identified by ELISA is superior to immunofluorescence, rises with age and is associated with intrinsic factor antibody

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Abstract
Parietal cell antibody is a marker for autoimmune gastritis. With identification of gastric H/K ATPase as its molecular target, ELISAs have been introduced. We compared performance of ELISA with immunofluorescence in a retrospective and prospective sera set and correlated the results with intrinsic factor antibody. In 138 retrospective sera selected for positivity or negativity for intrinsic factor antibody, 87 reacted with gastric H/K ATPase by Euroimm ELISA but only 62 reacted by immunofluorescence. Similar results were obtained with Inova ELISA with 78 positives that were also positive by Euroimm ELISA. In 161 prospective sera, 29 sera tested positive by ELISA compared to 24 by immunofluorescence. ELISA positive but immunofluorescence negative sera are bona fide positives because a representative set of 16 sera reacted with both 95kD α and 60–90kD β subunits of gastric H/K ATPase. ELISA values rose with age regardless of whether immunofluorescence tests were positive or negative. Of 53 sera containing antibody to intrinsic factor, 46/53 (87%) reacted to gastric H/K ATPase by ELISA. Taken together, the data indicates an enhanced detection rate by ELISA over immunofluorescence and validates it as a robust diagnostic assay for parietal cell antibody. As parietal cell antibody marks asymptomatic autoimmune gastritis that may progress to end stage gastric atrophy and haematological complications, and as autoimmune gastritis is associated with autoimmune thyroiditis and type 1 diabetes mellitus, early detection of parietal cell antibody by a sensitive ELISA will enable early follow-up of at risk subjects.

Keywords: Parietal cell H/K ATPase antibody, autoimmune gastritis, intrinsic factor antibody

Introduction
Autoimmune gastritis is an asymptomatic chronic inflammatory disease restricted to the fundus of the stomach sparing the antrum [1–3]. It is characterized by a chronic inflammatory infiltrate in the gastric mucosa that progresses over 10–20 years to gastric atrophy recognized as chronic atrophic gastritis. Chronic atrophic gastritis is evident macroscopically as a flat and thinned mucosa with loss of gastric folds. Its histopathology of intestinal metaplasia is characterized by total loss of gastric parietal cells, zymogenic cells and endocrine cells and their replacement by mucus-secreting cells. Intestinal metaplasia predisposes to gastric cancer. The development of gastric atrophy heralds the symptomatic phase of the disease, with haematologic manifestations of iron deficient anemia [4,5] that may precede B12-deficient pernicious anemia [1].

With discontinuation of the Schilling test to identify vitamin B12 malabsorption and scarcity of gastric biopsies undertaken to investigate autoimmune gastritis, reliance is today placed primarily on serological markers of parietal and intrinsic factor antibody for diagnosis of pernicious anemia.
Parietal cell antibody is the serological hallmark of symptomatic autoimmune gastritis in humans [1–3] and in mouse models of gastric autoimmunity [6,7]. The α and β subunits of gastric H/K ATPase (proton pump) have been identified as molecular targets of parietal cell antibody [8,9]. The α subunit is a 95 kDa catalytic subunit responsible for secretion of H⁺ in exchange for K⁺ and the consequent acidification of gastric juice. The β subunit is a 60–90 kDa heavily glycosylated 35 kDa core protein which acts as a chaperone to assemble the αβ gastric H/K ATPase complex in gastric secretory membranes [8,10,11].

In assessing reactivity of parietal cell antibody identified by immunofluorescence with gastric H/K ATPase, we previously reported that parietal cell antibody concordantly targets both the α and β subunits of the gastric H/K ATPase [10,12]. We also showed that autoantibody reactivity with the β subunit is critically dependent on a full complement of N-linked glycans because partially deglycosylated protein, and recombinant β subunit expressed in COS cells, bearing high mannose N-glycans, failed to bind to the autoantibody [10].

Using gastric H/K ATPase purified by tomato lectin affinity chromatography [13,14], we previously described an ELISA for detection of antibody to the gastric H/K ATPase [15]. Since then commercial ELISAs have been developed to identify antibody to gastric H/K ATPase. However the performance of the ELISA using purified gastric H/K ATPase compared to immunofluorescence for demonstration of parietal cell antibody has not previously been ascertained. In this study, we compared ELISA to that of immunofluorescence for detection of antibody to the gastric H/K ATPase. As intrinsic factor antibody is the other serological marker for autoimmune gastritis and pernicious anemia and, we also compared the presence of intrinsic factor antibody in the presence or absence of antibody to gastric H/K ATPase by ELISA.

Methods

Sera Selection

Two sets of sera at Healthscope Pathology were selected for study. The first set comprised 138 sera selected on the basis of positivity (n = 48) or negativity (n = 90) for intrinsic factor antibody identified by ELISA (Genesis Diagnostics Ltd, UK). The second set of sera (Figure 1b) comprised 161 sera sent for testing for autoantibodies to antigens in rodent tissue sections of stomach, liver and kidney (IMMCO Diagnostics, USA).

Parietal cell antibody detection by immunofluorescence

Parietal cell antibody was detected by indirect immunofluorescence on fixed tissue sections of mouse kidney/stomach/liver (IMMCO Diagnostics, USA), slides. Sera were tested at a 1:40 serum dilution and bound antibody detected by an IgG conjugate. Slides were read by a junior scientist and positive and negative test results confirmed by a senior scientist and the immunopathologist. Immunofluorescence tests were blinded and not biased by the ELISA results.

Gastric H/K ATPase antibody and intrinsic factor antibody detection by ELISA

IgG antibody to gastric H/K ATPase and to intrinsic factor was detected using ELISA kits for gastric H/K ATPase (Euroimmun AG, Lübeck, Germany; INOVA Diagnostics, Inc., USA) and intrinsic factor (Genesis Diagnostics Ltd, UK). Gastric H/K ATPase was purified by affinity chromatography from porcine stomachs. Bound antibody was detected by an IgG conjugate. Gastric H/K ATPase was purified by chromatography from porcine stomachs. Tests were carried out on an Euroimmun ELISA workstation, using manufacturer's recommended cut-off of 20 AU for the Euroimmun and the Inova kit as positive test results.

Immunoblotting sera positive for gastric H/K ATPase antibody by ELISA

Parietal cell antigen, purified from porcine gastric mucosa, 0.1 μg protein per test strip) was separated by discontinuous polyacrylamide gel electrophoresis according to molecular mass and transferred onto a nitrocellulose membrane. Human antibodies against parietal cell antibody were detected according to standard conditions using anti-human IgG from goat.
Table I. Gastric H/K ATPase antibody identified by Euroimmun ELISA compared with Parietal Cell Antibody identified by immunofluorescence in a retrospective set of 138 sera assessed for intrinsic factor antibody.

<table>
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<th>Positive</th>
<th>Negative</th>
<th>Total</th>
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<tbody>
<tr>
<td>Parietal Cell Antibody (Immunofluorescence)</td>
<td>59</td>
<td>3</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>48</td>
<td>76</td>
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<tr>
<td>Total</td>
<td>87</td>
<td>52</td>
<td>138</td>
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In 138 retrospective sera, Euroimmune ELISA identifies 87 positives compared to 62 positives by immunofluorescence.

We tested 138 retrospective sera for reactivity with parietal cells by immunofluorescence and for antibody to gastric H/K ATPase by ELISA. The 138 sera were selected on the basis of positivity (n = 48) or negativity (n = 90) for intrinsic factor antibody. A total of 59 sera tested positive by ELISA and by immunofluorescence, 28 sera tested positive by ELISA and negative by immunofluorescence, yet 3 sera tested positive by immunofluorescence and negative by ELISA (Table I). All 3 sera that tested positive by immunofluorescence and negative by ELISA were weak positives. Thus there were 87 positive test results by ELISA compared to 62 positive results by immunofluorescence giving a 40% increase in detection of antibody to gastric H/K ATPase.

In the same 138 retrospective sera, Inova ELISA identifies 78 positives compared to 62 positives by immunofluorescence.

To ascertain that this outcome is kit independent, we re-tested the same set of 138 retrospective sera for antibody to gastric H/K ATPase using an Inova ELISA kit. Using this kit, 57 sera tested positive by ELISA and by immunofluorescence, 21 tested positive by ELISA and negative by immunofluorescence while 4 tested positive by immunofluorescence and negative by ELISA (Table II). There was excellent concordance with the Euroimmune kit, with all 57 concordant positives, 21 concordant negatives and 3 out of 4 sera being concordant negative with a positive parietal cell antibody. Thus using the Inova kit, there were 78 positive test results by ELISA compared to the 62 positives by immunofluorescence giving a 25% increase in detection of antibody to gastric H/K ATPase.

In 161 prospective sera, ELISA identifies 29 positives compared to 24 positives by immunofluorescence.

We then assessed the performance of the Euroimmune ELISA compared to immunofluorescence for parietal cell antibody in 161 prospective samples sent to Healthscope pathology for testing for tissue antibodies. In this prospective set of sera, 21 sera tested positive by ELISA and by immunofluorescence, 8 sera tested positive by ELISA and negative by immunofluorescence while 4 sera tested positive by immunofluorescence and negative by ELISA (Table III). Thus 29 sera tested positive by ELISA compared to 24 by immunofluorescence giving a 20% increase in detection of antibody to gastric H/K ATPase.

Table II. Gastric H/K ATPase antibody identified by Inova ELISA compared with Parietal Cell Antibody identified by immunofluorescence in a retrospective set of 138 sera assessed for intrinsic factor antibody.

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<th>Total</th>
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<tbody>
<tr>
<td>Parietal Cell Antibody (Immunofluorescence)</td>
<td>58</td>
<td>4</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>56</td>
<td>76</td>
</tr>
<tr>
<td>Total</td>
<td>78</td>
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<td>138</td>
</tr>
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Results

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Representative sera reactive to gastric H/K ATPase by ELISA and negative by immunofluorescence immunoblots both the 95kD α and 60–90 kD β subunits of gastric H/K ATPase.

To determine that sera that tested positive by ELISA and negative by immunofluorescence are not false positives, we randomly selected 16 of these sera from the retrospective set of 138 sera for immunoblot reactivity with gastric H/K ATPase. All 16 ELISA positive sera concordantly reacted with both a 95 kD and 60–90 kD molecular species consistent respectively with the α and β subunits of gastric H/K ATPase (Figure 1). A random set of 4 sera from the same retrospective set of 138 sera that were negative both by immunofluorescence for parietal cell antibody and by ELISA to the gastric H/K ATPase did not react by immunoblot to either the 95kD nor the 60–90kD molecular species.

Absorbance values of gastric H/K ATPase antibody identified by ELISA increases with age regardless of the presence or absence of parietal cell antibody identified by immunofluorescence.

Subjects that were positive by Euroimmune ELISA and negative by immunofluorescence for gastric H/K ATPase comprised of 28 individuals (8 males and 20 females ranging in age from 20–80 years. ELISA
values were elevated above the manufacturer’s cut-off of 20 arbitrary units (E)/ml with 55 E/ml recorded as the lowest value and 200 as the highest value.

Segregating subjects into age groups revealed that ELISA values for gastric H/K ATPase rose with age, peaking at the 40–50-yr age group and then leveling off (Figure 2). However, the rise with age is not statistically significant.

Subjects that were positive both by ELISA and by immunofluorescence for gastric H/K ATPase comprised of 59 individuals (13 males and 46 females) ranging in age from 18–95 years (Figure 2). ELISA values were also elevated above the manufacturer’s cut-off of 20 E/ml (arbitrary units) with 22 E/ml recorded as the lowest value and 200 as the highest value. Segregating subjects into age groups also showed that ELISA values rose with age peaking at the 40–50-year age group and then leveling off (Figure 2).

Sera reactive by ELISA to gastric H/K ATPase segregates with intrinsic factor antibody identified by ELISA

Correlating sera reactive with the gastric H/K ATPase with intrinsic factor antibody revealed that of 87 sera reactive to gastric H/K ATPase by Euroimm ELISA, 46 also contained antibody reactive with intrinsic factor identified by ELISA. In contrast of 52 sera that did not react with the gastric H/K ATPase by Euroimm ELISA, only 6 contained reactivity to intrinsic factor. Thus, of 52 sera containing antibody to intrinsic factor, 46/52 (88%) also had reactivity to the gastric H/K ATPase.

Correlation of antibody to gastric H/K ATPase with, macrocytosis, vitamin B12 deficiency and intrinsic factor antibody

In the ELISA positive and IIF negative group, full blood analysis revealed a normocytic blood picture in 27 out of 28 subjects while low B12 levels of 153–176 (normal range >180) was recorded in 10 of the 28 subjects. Two subjects in the group with low B12 levels had intrinsic factor antibody.

In the group positive both by ELISA and IIF, and where data was available, macrocytosis was found in 18/39 (46%) and low B12 levels in 29/39 (74%) of subjects. Within this group, 12 subjects had intrinsic factor antibody.

Discussion

Our study demonstrates that ELISA has a higher detection rate than immunofluorescence for identifying antibody to gastric H/K ATPase. Immunoblot studies with representative sera that tested positive by ELISA but negative by immunofluorescence showed concordant and specific reactivity to both a 95 kD and a 60–90 kD molecular species in a gastric H/K ATPase preparation that is consistent respectively with the molecular weights of the α and β subunits of gastric H/K ATPase. The immunoblot data indicate that these ELISA positive and immunofluorescence negative sera are not false positives but bona fide positives for antibody to gastric H/K ATPase. These findings are consistent with our previous report that parietal cell antibody concordantly targets both subunits of the gastric H/K ATPase [15] and those of others [16–18]. Our findings of enhanced sensitivity of the ELISA over immunofluorescence are also consistent with an earlier study that compared an ELISA to a vesicular gastric membrane rich in H/K ATPase to immunofluorescence [18].

Our conclusion that the ELISA positive but immunofluorescence negative sera are bona fide positives is further supported by our observation that the age-related elevation of ELISA absorbance values of ELISA positive but immunofluorescence negative sera is similar to the age-related rise in ELISA values of sera that were positive both by immunofluorescence and by ELISA to the gastric H/K ATPase. The findings an age-related rise in antibody levels is consistent with a previous report of an age-related rise in parietal cell antibody identified by immunofluorescence [19].

<table>
<thead>
<tr>
<th>Gastric H/K ATPase antibody (Euroimm ELISA)</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
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</thead>
<tbody>
<tr>
<td>Parietal Cell Antibody (Immunofluorescence) Positive</td>
<td>21</td>
<td>3</td>
<td>24</td>
</tr>
<tr>
<td>Negative</td>
<td>8</td>
<td>129</td>
<td>137</td>
</tr>
<tr>
<td>Total</td>
<td>29</td>
<td>132</td>
<td>161</td>
</tr>
</tbody>
</table>

Table III. Gastric H/K ATPase antibody identified by Euroimm ELISA compared with Parietal Cell Antibody identified by immunofluorescence in 161 prospective sera sent for testing for tissue-reactive antibodies.

Figure 2. Comparison between ELISA +/Immunofluorescence + (white bars) and ELISA +/immunofluorescence- (black bars) correlated with age. Data shows ELISA absorbance values rising with age of subjects, regardless of whether corresponding immunofluorescence tests are positive or negative.
The vast majority (27/28) of subjects who were positive by ELISA but negative by immunofluorescence for gastric H/K antibody were normocytic. In contrast, where data was available, macrocytosis was found in 18/39 (46%) of subjects positive both by immunofluorescence for parietal cell antibody and by ELISA to the gastric H/K ATPase. Low vitamin B12 levels and intrinsic factor antibody was also less frequent in subjects who were ELISA positive but immunofluorescence negative. The data suggest that the ELISA may identify subjects at an earlier stage in the progression of autoimmune gastritis to its subsequent development of gastric atrophy. However, the lack of clinical details, the lack of gastric biopsy evidence of autoimmune gastritis and the lack of evidence of vitamin B12 malabsorption are major limitations in drawing a conclusive clinical interpretation of the results presented herein.

The value of intrinsic factor antibody for serological diagnosis of pernicious anemia has also recently been revisited. Parietal cell antibody and intrinsic factory antibody were tested by ELISA in a study of patients with biopsy-proven chronic atrophic gastritis [17]. The authors concluded that combining the two tests increased their diagnostic performance for pernicious anemias. The findings contrast with a retrospective study that found only a limited value for intrinsic factor antibody in parietal cell antibody-negative patients [17,20]. In our study, we found that 96.5% of intrinsic factor antibody positive subjects also had antibody to the gastric H/K ATPase. Our findings suggest that it remains useful to continue to screen for both parietal as well as intrinsic factor antibody in the setting of autoimmune gastritis.

Antibody to gastric H/K ATPase is an established serological marker for asymptomatic autoimmune gastritis [1–3]. In a study of the gastric mucosa and parietal cell antibody, an excellent correlation was found between parietal cell antibody and histological evidence of autoimmune gastritis [21]. Given that pernicious anemia develops as a consequence of mucosal atrophy arising from autoimmune gastritis, parietal cell antibody is also segregates with patients with pernicious anemia. Indeed, elevated gastric H/K ATPase antibody titers were found in 93% of pernicious anemia probands [22]. Antibody levels did not change over 1–4 years, but gradually decreased in titers over decades consistent with progressive mucosal destruction and loss of antigenic drive.

Autoimmune gastritis is also associated with other organ-specific autoimmune diseases and in particular with autoimmune thyroiditis and type 1 diabetes mellitus. In a prospective 5 year study of patients with autoimmune thyroiditis, parietal H.K ATPase antibody predicted the development of chronic atrophic gastritis [23]. Autoantibody levels rose progressively over time, peaked and then fell, following progressive gastric mucosal destruction and disappearance of the target autoantigen.

Similarly, in a retrospective study of autoimmune thyroiditis, antibody to gastric H/K ATPase also progressively increased in patients from 13% in the first-second decade to 42% in the ninth decade [24]. During follow up, 21% of antibody-positive patients converted to antibody-negative status. A positive parietal cell antibody by ELISA also identified type 1 diabetic patients with a higher risk to decreased vitamin B12 levels during follow-up over 5 years [25]. In a long-term follow-up over 20 years of patients with type 1 diabetes mellitus, the associated presence of parietal cell antibody and thyroid antibodies was associated with dysfunction of the corresponding organ [26].

Taken together, our findings indicate that ELISA to detect antibody to gastric H/K ATPase is a robust immunoassay that identifies more positive sera than immunofluorescence. The ELISA can therefore be applied instead of immunofluorescence for routine use in diagnostic immunology laboratories for detecting antibody to gastric H/K ATPase. We suggest that asymptomatic subjects with autoimmune gastritis marked by antibody to the gastric H/K ATPase should be monitored, at least yearly, for the development of iron deficiency or B12-deficient pernicious anemia and for the presence of associated autoimmune thyroiditis and type 1 diabetes mellitus. The ability to more readily detect autoimmune gastritis by an ELISA for antibody to the gastric H/K ATPase allows earlier detection of these at-risk subjects.

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References


