Follow-up of adult celiac patients: which noninvasive test reflects mucosal status most reliably?1

Introduction

Celiac disease is an autoimmune disorder requiring a gluten-free diet (GFD) for life if acute and chronic complications are to be avoided [1]. Achieving patient adherence to a GFD is difficult in some cases, because drastic changes in eating habits are required. Serological tests have become important screening tools for celiac disease in the past 20 years. However, diagnosis is still based on histological criteria [1]. The use of anti-gliadin antibodies (AGA) is problematic due to the extremely low specificity of AGA-IgG and low sensitivity of AGA-IgA. For this reason AGA are no longer important in the diagnosis of celiac disease [2], but they could be useful in patients already established as having celiac disease, to monitor their adherence to a GFD [3]. In these situations, anti-endomysial antibodies (EMA) are less sensitive [4]. Regarding the use of IgA anti-tissue-transglutaminase antibodies (anti-tTG), various studies have given contradictory results. Hansson et al. found that anti-tTG are reliable indicators of even brief dietary transgressions [5], whilst Vahedi et al. challenged their usefulness in monitoring GFD adherence [6].

Among other noninvasive tests, the intestinal permeability test (IPT) using orally administered inert sugars such as lactulose and mannitol turned out to be an unreliable screening tool in children at diagnosis of celiac disease [7] because

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of its low specificity for discriminating between healthy subjects and those with celiac disease. However, for follow-up in adults, IPT recently was proven superior to AGA-IgA in detecting persistent mucosal alterations during a GFD [8]. Furthermore, intestinal permeability normalizes in the majority of individuals with celiac disease who are on a strict GFD. Gluten ingestion as measured by a 3-day food record correlates with changes in the IPT [9]. Nevertheless, biopsy remains the gold standard for monitoring the effect of a GFD. The value of noninvasive tests for follow-up of celiac disease after gluten withdrawal can only be assessed by comparison with simultaneously conducted biopsies [10]. Therefore, the aim of this study was to investigate which noninvasive test best reflects the mucosal status by comparing the results of serology and IPT with histology on follow-up. In addition, we addressed the question: Does the performance of various noninvasive tests vary with the length of the interval between diagnosis and follow-up?

Patients and methods

Subjects

Data regarding all patients diagnosed with celiac disease at the Department of Gastroenterology, Medical University of Vienna, between December 1989 and July 2006, and followed by dietary history, clinical examination, and blood tests including celiac serology and IPT were retrieved from a computerized database. Serology and permeability testing was performed in all patients regularly on a yearly basis, whereas re-biopsies were not part of their routine follow-up. Only in the case of persistent EMA positivity (> 1 year), suspected noncompliance, dietary resistance, or silent celiac disease at diagnosis, or else at their own request, did patients undergo repeat endoscopy at irregular intervals [11]. For final analysis, only patients with biopsy-proven celiac disease followed by biopsy and simultaneous noninvasive tests were included. Patients were divided into two groups (A, B) according to follow-up time. Patients allocated to group A were followed for up to 2 years after diagnosis, patients from group B for longer. Subgroups were defined in respect of normal villous architecture (groups A1 and B1) and persistent mucosal damage (groups A2 and B2) on follow-up histology.

Dietary assessment

Adherence to a GFD was assessed by a physician at follow-up. Patients were classified as either keeping a strict GFD or admitting dietary transgressions at least once a month.

Biopsy

Upper gastrointestinal endoscopy was performed using an Olympus gastroscope with an Endoflex KF225B (Endoflex, Voerde, Germany) and Olympus FB24K–1 Forceps (Olympus, Heidelberg, Austria), with an open-cup diameter of 7 mm. Four biopsies were taken from the second part of the duodenum and two from the bulb, the latter having shown equal or superior diagnostic value to more distal biopsies [12]. The intestinal biopsy specimens were fixed in 4% phosphate-buffered formalin (pH 7.4) for histological analysis. Celiac disease was diagnosed according to modified ESPGHAN (European Society of Pediatric Gastroenterology, Hepatology, and Nutrition) criteria [13]. Morphological characteristics of the crypts and villi and the number of intraepithelial lymphocytes (IEL) were included in the report. The stages of histological changes were classified using a modified Marsh classification [14].

Serology

EMA-IgA levels were determined by an immunofluorescence assay using monkey esophagus sections (Biosystems, Barcelona, Spain) according to the manufacturer’s instructions. Sera were tested starting with a screening dilution of 1:10. Results were rated as positive if an apple-green luminescent fluorescence pattern of the intermyofibrillar substance of the smooth muscle was observed. All slides were assessed by the same experienced observer.

AGA-IgA levels were measured by enzyme-linked immunosorbent assay (ELISA) (1989–2003: Gluten IgA EIA, Pharmacia Diagnostics, Freiburg, Germany; 2003–2005: Pharmacia CAP System, Uppsala, Sweden; since 2005: QUANTA Lite Gliadin IgA, Inova Diagnostics, Inc., San Diego, USA). Cut-offs were 25 U/ml, 2.5 U/ml, and 30 U/ml, respectively for the three different assays.

AGA-IgG levels were assessed by ELISA (materials sourced as for AGA-AgA). Cut-offs were 25 U/ml, 25 U/ml, and 30 U/ml, respectively.

Intestinal permeability test

A sugar test solution containing 20 g saccharose, 10 g lactulose, and 5 g mannitol dissolved in 100 ml water was administered to the patients. Lactulose and mannitol concentrations in a 5-hour urine collection were measured by high-performance liquid chromatography as previously described [15]. An L/M ratio above 0.03 was considered a positive test result indicating increased intestinal permeability. For gastroduodenal permeability, the urinary concentration of saccharose was determined by UV spectrometry (Sucrose/D-Glucose/D-Fructose UV Test Assay, Boehringer Mannheim, Mannheim, Germany) [16]. Values above 43 mg were indicative of increased gastroduodenal permeability.

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences for Windows 15.0. The x2 test was used for comparison of frequencies (positivity of various tests, gender) between groups A and B. The results of noninvasive tests were evaluated by calculating specificity, sensitivity, PPV, and NPV for the detection of villous atrophy with binomial 95% exact confidence intervals (CI). For comparison of permeability parameters, the Mann–Whitney U test was performed. A P-value less than 0.05 was considered statistically significant.

Results

Study population

Between December 1989 and July 2006, 305 patients were diagnosed with celiac disease (80 men and 225 women, mean age at diagnosis 38.9 ± 16.6 years, median age 36 years, age range 16–84 years). Of these 305 patients, 250 attended for at least one follow-up in our clinic. Out of the 250, 47 (16 men and 31 women, mean age 44.1 ± 14.7 years, median age 45 years, age range 16–74 years) with follow-up biopsies and simultaneously performed IPT were eligible for final analysis. None of these had IgA deficiency. On histological examination, 57.5% showed complete villous recovery.
Patients were further subdivided into two groups according to the interval between diagnosis and follow-up. Three patients had two or more follow-up biopsies and were allocated to both groups. The results of the first follow-up within each follow-up period were evaluated.

Group A (interval from diagnosis to follow-up /C1772 years, median 15 months, range 1 /C17724 months) consisted of 23 patients (7 men and 16 women, mean age 38.5 /C12718.0 years, median age 40 years, age range 16 /C17774 years). Group B (interval from diagnosis to follow-up > 2 years, median 40 months, age range 26 /C17791 months) consisted of 27 patients (12 men and 15 women, mean age 44.52 /C12716.1 years, median age 47 years, age range 17 /C17774 years).

Fourteen group A patients (60.9%) had normal or mildly altered mucosa (group A1); the remaining 9 exhibited persistent villous atrophy (group A2). Group B1 consisted of 13 patients (48.6 %) with Marsh stage 0 /C1772 disease while group B2 comprised 14 individuals. No difference in persistence of villous atrophy was found between group A and group B (<i>P</i> = 0.407). A strict GFD was not adhered to by 17.4% of group A and 34.6% of group B (<i>P</i> = 0.173). Similarly, no differences of adherence to GFD were found in subgroup analysis (group A1 vs. A2 and B1 vs. B2; <i>P</i> = 0.624 and 0.075, respectively) (<i>l</i> Table 1 and 2).

Results of noninvasive tests at diagnosis and follow-up
At diagnosis, L/M ratio, saccharose, and EMA proved to be most sensitive (91.3%, 88% and 84%, respectively) for detecting mucosal atrophy (Marsh /C1793).

On follow-up, L/M ratio and saccharose values were lower than the pre-GFD values (median 0.053 vs. 0.177, <i>P</i> = < 0.001; and median 43 vs. 88 mg, <i>P</i> = 0.038, respectively). In addition, patients with normal villous architecture had a lower L/M ratio than those with persistent villous atrophy, whereas saccharose showed no difference (<i>l</i> Tables 1 and 2). However, there was no difference between these patients in the rate of positivity for both IPT parameters. Regarding late follow-up, only EMA reliably discriminated between villous atrophy and normal villous architecture (group B1 vs. B2, <i>P</i> = 0.009) (<i>l</i> Table 2).

Test characteristics of noninvasive tests on follow-up
The sensitivity of the L/M ratio decreased from 88.9 % at early to 85.7 % at late follow-up, whereas the specificity of EMA increased from 71.4 % to 91.7 % (<i>l</i> Table 4).
Only one-sixth of the patients diagnosed in our clinic were celiac disease. We observed that IPT in particular the L/M ratio (follow-up within 2 years), and group B (follow-up after 2 years).

Table 3  Comparison of results of intestinal permeability test with respect to persistence of villous atrophy in all patients with follow-up biopsies, in group A (follow-up within 2 years), and group B (follow-up after 2 years).

<table>
<thead>
<tr>
<th>Marsh stage ≤ 2</th>
<th>Marsh stage ≥ 3</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>All patients with follow-up biopsies</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L/M</td>
<td>Median IR</td>
<td>Median IR</td>
</tr>
<tr>
<td>Saccharose, mg</td>
<td>0.032 0.043</td>
<td>0.115 0.145</td>
</tr>
<tr>
<td>Group A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L/M</td>
<td>0.033 0.048</td>
<td>0.110 0.090</td>
</tr>
<tr>
<td>Saccharose, mg</td>
<td>37 90</td>
<td>43 49</td>
</tr>
<tr>
<td>Group B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L/M</td>
<td>0.066 0.188</td>
<td>0.146 0.145</td>
</tr>
<tr>
<td>Saccharose, mg</td>
<td>68 78</td>
<td>44.5 41</td>
</tr>
</tbody>
</table>

Table 4  Sensitivity, specificity, positive predictive value, and negative predictive value with 95% confidence intervals of noninvasive follow-up procedures in all patients with follow-up biopsies, in group A (follow-up within 2 years) and group B (follow-up after 2 years).

<table>
<thead>
<tr>
<th>Sensitivity</th>
<th>Specificity %</th>
<th>NPV %</th>
<th>PPV %</th>
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<tbody>
<tr>
<td><strong>Total study population</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EMA</td>
<td>50</td>
<td>27.2–72.8</td>
<td>77.8</td>
</tr>
<tr>
<td>AGA-IgG</td>
<td>20</td>
<td>5.7–43.7</td>
<td>100</td>
</tr>
<tr>
<td>AGA-IgA</td>
<td>15</td>
<td>3.2–37.9</td>
<td>100</td>
</tr>
<tr>
<td>L/M</td>
<td>85</td>
<td>62.1–96.8</td>
<td>46.2</td>
</tr>
<tr>
<td>Saccharose</td>
<td>60</td>
<td>32.3–83.7</td>
<td>52.6</td>
</tr>
<tr>
<td><strong>Group A</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EMA</td>
<td>66.6</td>
<td>29.2–92.5</td>
<td>71.4</td>
</tr>
<tr>
<td>AGA-IgG</td>
<td>33.3</td>
<td>7.5–70.1</td>
<td>100</td>
</tr>
<tr>
<td>AGA-IgA</td>
<td>22.2</td>
<td>2.8–60</td>
<td>100</td>
</tr>
<tr>
<td>L/M ratio</td>
<td>88.9</td>
<td>51.8–99.7</td>
<td>42.9</td>
</tr>
<tr>
<td>Saccharose</td>
<td>66.7</td>
<td>9.4–99.2</td>
<td>57.1</td>
</tr>
<tr>
<td><strong>Group B</strong></td>
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<td></td>
</tr>
<tr>
<td>EMA</td>
<td>57.1</td>
<td>28.9–82.3</td>
<td>91.7</td>
</tr>
<tr>
<td>AGA-IgG</td>
<td>23.1</td>
<td>5.0–53.8</td>
<td>100</td>
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<tr>
<td>AGA-IgA</td>
<td>23.1</td>
<td>5.0–53.8</td>
<td>100</td>
</tr>
<tr>
<td>L/M ratio</td>
<td>85.7</td>
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<td>23.1</td>
</tr>
<tr>
<td>Saccharose</td>
<td>58.3</td>
<td>27.7–84.8</td>
<td>36.4</td>
</tr>
</tbody>
</table>

NPV, negative predictive value; PPV, positive predictive value; EMA, endomysial antibodies; AGA, antigliadin antibodies; L/M, lactulose/mannitol.

Influence of age on follow-up histology and permeability tests

No difference was found between older and younger patients (> 45 years, n = 26 vs. < 45 years, n = 21) with respect either to follow-up histology or to L/M ratio (P = 0.137 and 0.204, respectively).

Discussion

In this study we investigated which noninvasive test is best suited to reflect mucosal status and therefore might be used as a substitute for small-bowel biopsy for follow-up of patients with celiac disease. We observed that IPT – in particular the L/M ratio – performed best in terms of sensitivity, especially if follow-up was early, but had low specificity. A normal L/M ratio excluded villous atrophy in 80% of cases. EMA achieved the highest specificity, exceeding even 90% in patients with late follow-up.

Only one-sixth of the patients diagnosed in our clinic were completely lost to follow-up, which is lower than numbers given in the literature [17]. Only one-fifth of the patients underwent small-bowel biopsy for assessment of the recovery of the duodenal mucosa, especially those with suspected noncompliance, dietary resistance, or silent celiac disease at diagnosis. Thus, the results from our study might be subject to a selection bias because they particularly reflect the status of a difficult patient group. However, this selection bias, however, should not have any influence on the correlation of invasive with noninvasive test results. In addition, this patient group is particularly in need of easy follow-up procedures such as establishing reliable noninvasive methods as an alternative to intestinal biopsy.

Slightly more than half of these patients showed no mucosal atrophy on follow-up biopsy. No difference in the proportion of patients with normalized mucosal architecture was found between those followed up early as compared to those with late follow-up. Persistent villous atrophy in celiac disease, even in the absence of symptoms, carries a risk of subsequent severe complications [18]. For this reason a few centers recommend at least one follow-up biopsy to study the response to a GFD [18]. Wahab et al. reported histological remission in 65.0% of patients...
within 2 years, in 85.3% within 5 years, and in 89.9% after 5 years of follow-up [10]. Our results did not show this improvement over the years, but were still superior to those found by Lee et al., who observed no villous atrophy in only 21% of patients with celiac disease patients who adhered to a GFD for an average of 8.5 years [19]. The tendency to deterioration on long-term follow-up in our study population might be due to the selection of patients with whose compliance was doubtful, as previously mentioned. Furthermore, the longer the interval between diagnosis and follow-up visits, the higher the expected proportion of patients not who do not adhere to a strict GFD.

One-third of our patients in the long-term follow-up group admitted not keeping a strict GFD – a proportion also reported in other follow-up studies [20,21].

Regarding the IPT as a follow-up tool, only the L/M ratio achieved acceptable sensitivity, which slightly weakened over the years. There is a clear relation between the extent of mucosal alteration and the results of the IPT at the diagnosis of celiac disease [22,23]. Changes in the IPT after the introduction of a GFD have been reported, e.g., by comparison with the duration and extent of a GFD [9] or with serological tests [8]. Duerksen et al. demonstrated that the IPT normalizes after adherence to a GFD for more than 1 year and correlated well with ingestion of trace amounts of gluten [9]. Comparing the IPT with serological tests during a GFD, Vilela et al. found a lower L/M ratio in AGA-negative patients but results remained above cutoff [8]. Among follow-up studies using intestinal biopsy as the gold standard, Uil et al. demonstrated a higher L/M ratio in villous atrophy than in normalized mucosa [24]. This finding is in accordance with our results (Table 3). However, only in the early follow-up period (<2 years after diagnosis) a higher L/M ratio was observed in patients with villous atrophy. Regarding the second parameter of the IPT, Vogelsang et al. showed a significant correlation of the urinary recovery of saccharose and the presence of lymphocytic gastritis in untreated celiac disease [16]. Furthermore, it was demonstrated that saccharose permeation occurs mainly through the gastric mucosa in celiac disease [16]. As can be expected from these data, the saccharose test was not suitable for assessment of the status of the intestinal mucosa in our patients and showed low sensitivity and specificity, possibly due to rapid restoration of gastric mucosa.

Our findings confirmed the lack of reliability of EMA for use in monitoring either compliance or histological response to treatment, as reported elsewhere [4,25]. Dickey et al. demonstrated absence of EMA in 87% of patients after 12 months on a GFD [26]. Only 40% of these seronegative patients had complete villous recovery after 12 months, and in only 33% with villous atrophy did EMA remain positive. No patient who had recovered normalized villous structure showed persistent EMA positivity. In our study, a smaller percentage (63.2%) of compliant patients who had been followed up early became EMA-negative. Normalized villous architecture was found in 76.9% of our EMA-negative patients, and two-thirds of our patients with persistent Marsh stage 3 lesions had positive results for EMA. Four out of 14 patients with Marsh lesions of stage 2 or less still were positive for EMA, indicating faster mucosal recovery than EMA seroconversion. Indeed, all four developed EMA negativity on further follow-up within the next 3 months. Paradoxically, Dickey et al. [26], who took a titer of ≥1:5 as showing positivity, had a higher rate of false negative EMA results in their patients after 12 months on a GFD than we had with the higher cutoff of 1:10. This might be due to variations between the performance of different EMA test kits in detecting low autoantibody titers, and might also be related to very early histological restaging in some patients. However, the performance of EMA testing changed in our study in relation to the follow-up interval. Beyond 2 years after diagnosis, significantly more positive results were found in patients with persistent villous atrophy, possibly due to persistent dietary transgressions, and the specificity of EMA for detecting villous atrophy exceeded 90%. However, it can take more than 2 years for EMA to disappear following commencement of a GFD, especially if titers were very high to begin with [27]. This might explain the single false positive EMA result in our group of patients who had their follow-up later than 2 years after diagnosis.

Wauters et al. showed excellent sensitivity of AGA-IgA in 17 children after a gluten challenge, based on jejunal biopsies [3]. In contrast to these findings, AGA showed the lowest sensitivity of all noninvasive tests used in our adult patients. Its high specificity may be explained by the extremely low rate of positive titers. However, the changing the AGA testing kit twice during the study period limits the validity of these findings.

Regarding newer serological tests in the assessment of mucosal recovery, Vahedi et al. demonstrated that anti-tTG are poor predictors of dietary transgressions in adult celiac disease patients on a GFD [6]. Unfortunately, we were not able to correlate these antibodies with histological changes since anti-tTG results were available for only a few patients.

In conclusion, our results indicate that serology and IPT is far less sensitive and specific for follow-up of patients with celiac disease than for screening purposes. The L/M ratio had an acceptable sensitivity in patients followed up within 2 years after diagnosis, whereas EMA showed good specificity in those investigated later than that. However, there is no replacement for intestinal biopsy for the accurate detection of persistent mucosal atrophy in celiac patients on a GFD.

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References


Valentini RA, Andreani ML, Corazza GR, Gasbarrini G. IgA endomysium antibody: a valuable tool in the screening of coeliac disease but not its follow-up. Ital J Gastroenterol 1994; 26: 279 – 282
