Changing Patterns of Serological Testing for Celiac Disease in Latvia

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Abstract

Background and Aims. A number of recent guidelines have discouraged the use of the old anti-gliadin tests for the detection of celiac disease; tissue transglutaminase IgA (tTGA) and anti-endomysial (EMA) tests are recommended instead. Our aim was to evaluate how the current recommendations have been applied in real practice. The secondary aim was to evaluate the positivity rates provided by different test types. Methods. We analyzed the number of celiac disease tests [anti-gliadin IgA (AGA), anti-gliadin IgG (AGG), tTGA and EMA] performed by the largest laboratory in Latvia. The analysis was performed on a yearly basis for the period between 2004 and 2009. Additionally, we analyzed the percentage of the positive test results for each of the tests. Results. The number of patients being tested for celiac disease constantly increased, with the average annual growth of 16.1%; this trend was similar both in children and in adults. The majority of patients (62.6%) were tested with anti-gliadin tests only; 27.7% were tested with either tTGA or EMA, while 9.7% were tested by a combination of the above groups. There was a substantial difference in the positivity rates of the different tests from 0.94% for EMA to 21.8% for AGG. Substantial differences were also present between various manufacturers’ products. Conclusion. The current guidelines and the published evidence on the proper use of serological tests for celiac disease have been slow to be applied in clinical practice; more intensive education campaigns and change in reimbursement systems could improve the situation. Nevertheless, more clinicians in Latvia are checking patients for celiac disease; this suggests an overall increased awareness.

Key words


Introduction

Celiac disease is considered to be a common condition in Europe affecting 1 out of 150-300 of the population [1-3]. Limited evidence on the prevalence of the disease is available from East-Europe.

Several guidelines and recommendations have been published over the last years either on international or national levels. The number of related research publications has increased, e.g. on PubMed database there are 576 celiac disease related papers dated 2004, and about 760 dated 2008 or 2009. One would expect that the guidelines and evidence from these papers have been put in use by the clinical practice.

Over the period of the last 10 years substantial improvement of the serological testing has occurred. Nowadays the use of the old anti-gliadin antibody testing is discouraged due to the low sensitivity and specificity, and tests targeting endomysial antibodies or tissue transglutaminase have been recommended instead. Such recommendations have been included in the global practice guidelines by the World Gastroenterology Organization (WGO) [4] as well as various national guidelines in Europe, including the recent NICE guidelines in the United Kingdom [5], the CREST Guidelines in Ireland [6], and in the United States [7-8].

Education on adequate testing strategies has been conducted either internationally or in Latvia starting from early 2000’s. The country is small (2.3 million population), and therefore separate national guidelines were not developed, but proper use of serological tests (i.e. tissue transglutaminase instead of gliadin tests) was promoted during national meetings. In particular, this information was brought to the attention of clinicians in primary practice. After the WGO guidelines were made available in 2005 [4], the diagnostic approach included in these guidelines was promoted.

Little has been published on how these recommendations translate in actual clinical practice.
The availability of reimbursement is influencing test ordering habits in various specialties and for various tests [9-11], and therefore it is crucial to establish the right reimbursement decisions in the national health policy [12]. One would expect that reimbursement is influencing the testing strategy for celiac disease as well. At the same time, the discrepancies between the guidelines and reimbursement strategies do exist in different areas of medicine [13]. Awareness in the guidelines does not always guarantee adherence to the requirements or necessarily lead to changes in clinical practice [14].

Our intention was to analyze how the available guidelines and increasing evidence has been reflected in everyday clinical practice. We analyzed the celiac disease related test performance by the leading laboratory in Latvia covering all the major target segments.

**Methods**

We analyzed data on patients tested for celiac disease, and the number of celiac disease tests performed by the major laboratory in Latvia over the period from 2004 to 2009, in particular: anti-gliadin type IgA antibodies (AGA), anti-gliadin type IgG antibodies (AGG), anti-tissue transglutaminase type IgA antibodies (tTGA) and anti-endomysial type IgA antibodies (EMA). The total number of tests was analyzed and also the division between tests in adults and children was made (patients below the age of 18 were considered children). A separate analysis addressed combinations of tests performed in the same individuals.

Positive and negative test results as reported by the laboratory based on the routinely used cut-off values were used for the analysis: quantitative results were not considered.

The following methods and the corresponding cut-off values were used by the laboratory: two solid phase immunosorbent assays (ELISA) were used for measuring AGA during the study period. Until mid-2008 the Autostat II™ Anti-Gliadin IgA method (Hycor Biomedical Ltd., U.K.) with the positive cut-off value recommended by the manufacturer above 6 U/ml on an automated EIA instrument Hycor HY●TEC™ was applied. Afterwards, the detection was changed to Anti-Gliadin IgA (Orgentec Diagnostika GmbH, Germany) with the positive cut-off value recommended by the manufacturer above 12 U/ml on an automated EIA instrument ALEGRIA™. Similarly the method for AGG detection until mid-2008 was the Autostat II™ Anti-Gliadin IgG (Hycor Biomedical Ltd., U.K.) ELISA with the positive cut-off value above 9 U/ml on an automated EIA instrument Hycor HY●TEC™. Afterwards, the Anti-Gliadin IgG assay (Orgentec Diagnostika GmbH, Germany) with the positive cut-off value 12 U/ml on an automated EIA instrument ALEGRIA™ was introduced.

During the study period two different ELISA test-types, both for recombinant tTGA assays were used: a test-system expressed in *E.coli* (Hycor Biomedical Ltd., U.K.) with the positive cut-off value above 7 U/ml on an automated EIA instrument Hycor HY●TEC™ was used until the beginning of 2007. Afterwards the Anti-tTG IgA on the Immulite 2000 system (Siemens Healthcare Diagnostics Inc., U.K.) with the positive cut-off 6.4 U/ml was introduced. EMA was assessed by an indirect immunofluorescent test for the qualitative and semi-quantitative detection of endomysial autoantibodies in human serum by using the Nova Lite™ Endomysial testsystem (Inova Diagnostics, Inc., U.S.A.) based on primate distal esophagus tissue substrate and IgA conjugate. A sample was considered negative if the specific staining of the intracellular areas, the connective tissue component of smooth muscle bundles, was equal to or less than the negative control; while a sample was considered positive if the specific staining of the intracellular areas or endomysium of smooth muscle bundles of the esophagus was observed. The tests were performed by a reference laboratory in Belgium.

**Statistical analysis**

Methods of descriptive statistics were used to characterize the groups of patients tested. An annual growth in the number of tests was estimated as a percentage over the test number performed during the preceding year. Although the individual patients were unidentifiable, it was possible to distinguish the number of unique tested individuals (including these in whom more than one test was performed); this was used to describe the investigated group as well as the multiple-test approach.

The original data were retrieved from the Laboratory information system and analyzed by MS Excel for Windows software.

**Ethical considerations**

The study protocol was approved by the Committee of Ethics at the Institute of Experimental and Clinical Medicine of University of Latvia. This was a retrospective and anonymous study; therefore no consent form was applicable.

**Results**

The number of individuals tested for celiac disease by the laboratory (for any of the celiac disease tests) is given in Fig. 1. Out of the total number, 46% were children, and 54% were adults. This number constantly rose among children and adults (the average annual growth rate was 16.1% for the entire group, 21.5% for children, and 12.5% for adults) although during the latter years the rate of growth became slower (e.g. the growth for the entire group was 26% in 2005, but only 3.1% in 2009). The growth rates per each of the years are given in Table I. Similarly, the number of tests performed increased over the period (Fig. 2). The number of AGG and AGA throughout the period was significantly higher than that of tTGA and EMA tests. Out of the total number of tests performed, both the anti-gliadin tests put together reached 78.6%, while tTGA and EMA together, only 21.4%. In general, the increase in the number of antigliadin...
Celiac disease testing

123

tests was more rapid than that of tTGA or EMA, and only within the last year (2009) there was a decline observed in either of the gliadin tests in parallel with a more rapid increase in the tTGA tests (Fig. 2).

The majority of the patients were tested with anti-gliadin tests (AGG and/or AGA) only, while a smaller proportion of patients was tested by the newer and currently recommended (tTG, EMA) tests or by the combination of the above two groups. Over the period, only antigliadin tests were performed on average in 62.6% of the patients, and in 27.7% of the patients tTGA and/or EMA tests were performed, but 9.7% of the patients were tested by a combination of antigliadin and tTGA or EMA tests.

The test positivity rate is given in Table II. Considering that two different test types have been used for each of AGA, AGG as well tTGA tests, the table gives the positivity rate for each of these tests separately, as well as the consolidated rate for the particular test-type. The consolidated positivity rates were: 21.8% for the AGG tests (2,856 out of a total number of 13,111 tests performed), 5.6% for the AGA test (709 from 12,664), 2.6% for the tTGA tests (132 from 4,994), and only 0.9% for EMA tests (21 from 2,241).

Discussion

Previously considered a childhood problem, increasingly celiac disease is being diagnosed in adults. Although pediatricians have an important role in diagnosing the disease [15], the importance of general practitioners is increasing; e.g. in Northern Ireland half of the celiac disease patients are identified in primary practice [16]. We analyzed data from a laboratory performing the majority of celiac disease tests in Latvia. During this period the laboratory covered either hospital or out-patient segments and servicing medical establishments involved in adult and children care. Territorially it covered most of the country, including urbanized areas where the reference centers are localized as well as rural areas with dominating primary medical care institutions. Therefore, the results can be considered to be characteristic for the entire country. Our study design did not allow to analyze the proportion of test orders originating from specialists or general practice.

The number of individuals tested for celiac disease increased constantly during the period studied (Fig. 1). A more rapid growth of tests was evidenced in children than in adults, in particular at the beginning of the study. Therefore, the awareness of the disease has increased steadily and such an increase in the number of tests and patients is similar to other countries in Europe. For example, significant increase in celiac disease tests either in hospital settings or in general practice was observed in Switzerland after the year 2000 [17] as well as in the UK over the last decade [18]. The stabilization trend during the last year in Latvia could be potentially linked to the economic situation and decrease in the healthcare budgets.

Although several new tests have been suggested in the testing for celiac disease, tTGA has remained the test of choice for initial testing [19-22]. The international guidelines, including those by WGO [4] have discouraged the use of anti-gliadin tests (AGG, AGA), and such an approach has been actively promoted by the local experts in Latvia throughout the study period. Moreover, in the country the use of tTGA tests has been inadequately low if compared with the anti-gliadin test use; the latter have dominated throughout the period, achieving the average of
78.6% from all tests for the entire population, 84.0% from tests in children, and 73.6% in adults.

Frequently several celiac disease tests are performed in one patient. In particular, if some of the other test results were positive, the number of patients that have been tested by combinations of tests were analyzed, i.e. by the old anti-gliadin tests only, by the currently recommended tests (tTG and/or EMA) only, or a combination of the above. This analysis revealed that most of the patients have been tested only by anti-gliadin tests (62.6%), therefore in a large group of patients the serology test result may have been misleading due to the low sensitivity and specificity, and in a proportion of these patients false positive diagnostics of celiac disease may have occurred (the current study does not include any analysis of duodenal biopsies).

Reimbursement systems are important tools in order to regulate the test choice and performance in addition to the education of medical professionals. Optimally, the reimbursement system based on the diagnosis-treatment combination with well founded cost-efficacy estimates and the allocation of laboratory budgets should be made available to those requesting laboratory services [23]. In our situation, both the anti-gliadin tests were reimbursed throughout the study period; the tTGA test was reimbursed starting from 2007, but the EMA test was never reimbursed. The lack of reimbursement for EMA tests is well reflected in low prescription numbers for these tests. There was some increase in tTGA testing after 2007 as a probable result of both available reimbursement and education but still paradoxically the number of ordered AGG and AGA tests increased more rapidly during the period the tTGA test was awarded reimbursement. Therefore, the performed educational activities have not been able to break the previous test ordering habits in our situation. Of course, the full power of reimbursement has not been utilized, because there has been no reason to continue the reimbursement for out-of-date anti-gliadin tests. Proper coordination between the local adaptation of international guidelines and incorporating the relevant conditions with the national reimbursement system is the best method to achieve a well-balanced medical and economical benefit. There is the potential that laboratories could also become involved in proper diagnostic strategy facilitation and communication with the primary care physicians [24].

There was a striking difference observed in the positivity rates between the different tests performed (Table II). A large proportion of both the anti-gliadin tests (in particular AGG tests) were reported positive, which was completely in conflict with the tTGA, and even more – with the EMA test results.

By considering the differences in the positive predictive values between different test-types, EMA or EMA in combination with tTGA is the best choice to judge the prevalence of the disease in the study population in the absence of biopsy data [25]. Positive tTGA result is highly predictive for EMA positivity [26]. In our group, EMA was positive in 0.9%, while tTGA – in 2.6%. It is hardly credible that the patients’ higher pre-test probability of celiac disease were tested with anti-gliadin tests, and those with lower probability – with tTGA or EMA tests; therefore the only explanation of the differences in test positivity-rates is the high proportion of false positive AGG and AGA tests.

In addition, different test-systems were giving different test-positivity rates for the same test-types, e.g. the Hycor AGA tests were positive in 11.5%, while Orgentec – only in 4.5% of the cases; similarly there were differences between two recombinant tTGA test-types – Hycor test was positive

### Table II. Proportion of positive test results

<table>
<thead>
<tr>
<th>Test Method</th>
<th>Manufacturer (Instrument)</th>
<th>Total No of tests</th>
<th>No of positive results</th>
<th>Proportion of positive test results</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGA ELISA</td>
<td>HYCOR Biomedical (Hycor HY●TEC)</td>
<td>2,018</td>
<td>233</td>
<td>11.5%</td>
</tr>
<tr>
<td>AGA ELISA</td>
<td>ORGENTEC Diagnostika (ALEGRIA)</td>
<td>10,646</td>
<td>476</td>
<td>4.5%</td>
</tr>
<tr>
<td>AGA ELISA</td>
<td>Consolidated</td>
<td>12,664</td>
<td>709</td>
<td>5.6%</td>
</tr>
<tr>
<td>AGG ELISA</td>
<td>HYCOR Biomedical (Hycor HY●TEC)</td>
<td>2,079</td>
<td>506</td>
<td>24.3%</td>
</tr>
<tr>
<td>AGG ELISA</td>
<td>ORGENTEC Diagnostika (ALEGRIA)</td>
<td>11,032</td>
<td>2350</td>
<td>21.3%</td>
</tr>
<tr>
<td>AGG ELISA</td>
<td>Consolidated</td>
<td>13,111</td>
<td>2856</td>
<td>21.8%</td>
</tr>
<tr>
<td>tTGA ELISA</td>
<td>HYCOR Biomedical (Hycor HY●TEC)</td>
<td>1,875</td>
<td>83</td>
<td>4.4%</td>
</tr>
<tr>
<td>tTGA ELISA</td>
<td>SIEMENS Healthcare Diagnostics (IMMULITE)</td>
<td>3,119</td>
<td>49</td>
<td>1.6%</td>
</tr>
<tr>
<td>tTGA ELISA</td>
<td>Consolidated</td>
<td>4,994</td>
<td>132</td>
<td>2.6%</td>
</tr>
<tr>
<td>EMA Immunofluorescence</td>
<td>Inova Diagnostics</td>
<td>2,241</td>
<td>21</td>
<td>0.9%</td>
</tr>
</tbody>
</table>

AGA - anti-gliadin type IgA antibodies; AGG - anti-gliadin type IgG antibodies; tTGA - anti tissue transglutaminase type IgA antibodies; EMA - anti-endomysial type IgA antibodies.
in 4.4% of the cases, but Siemens Immulite test – only in 1.6%. The low positive result prevalence for the traditional serology EMA test (0.94%) leads us to believe that the tTGA performed on Siemens Immulite has been probably the only test of those used giving the most exact results from all the ELISA tests used.

Wide variations in sensitivity and specificity of different celiac disease tests has been reported beforehand [5, 27-28]. Therefore, the results obtained by one manufacturer test-systems cannot be translated to other manufacturers’ products unless a comparative analysis is performed.

The subjects of the study do not reflect the general population of the country, and the study group was heterogeneous. Therefore, further studies in the general population are required to judge the population prevalence of celiac disease in Latvia.

There are some other limitations in this study. The histology results were not analyzed, and therefore the proportion of patients with proven celiac disease as well as the celiac disease patients with a negative serology cannot be evaluated. It was also not possible to analyze the test prescription habits for specialists and general practice physicians separately.

Conclusion

The current guidelines and the published evidence on the proper use of serological tests for the detection of celiac disease has not been put into practice fully by the medical staff; more intensive education campaigns and changes in reimbursement systems could possibly improve the situation. The decision on the appropriate testing strategy should involve the authorities responsible for the reimbursement of the tests. Nevertheless, more clinicians in Latvia are checking patients for celiac disease – this suggests an overall increased awareness

Acknowledgements

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Conflicts of interest

Nothing to disclose.

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21. Dorn SD, Matchar DB. Cost-effectiveness analysis of strategies for