High Prevalence of Celiac Disease in Apparently Healthy Blood Donors Suggests a High Prevalence of Undiagnosed Celiac Disease in the Dutch Population

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Background: In the last few years the prevalence of celiac disease (CD) seems to have increased. It is clear that subclinical and silent CD exist in a large subgroup of the celiac population.

Methods: The aim of this study was to evaluate the prevalence of CD in an apparently healthy population. Blood samples were obtained from 1000 apparently healthy blood donors at Arnhem and Nijmegen Blood Donation Centers from January 1997 through April 1998. Sera from 660 blood donors were assayed for total IgA. By means of immunofluorescence, antibodies, including those to endomysium (EMA), were determined. Serum immunoglobulin levels (IgA) were assayed by means of nephelometry. All donors who had positive serology for EMA underwent small-intestinal biopsy.

Results: Of the 1000 healthy blood donors 3 had positive EMA. Small-intestinal biopsy of two of these showed subtotal villous atrophy (Marsh IIIb), and the third had intraepithelial lymphocytosis and crypt hyperplasia (Marsh II). The prevalence of gluten sensitivity was 1 of 330. Low IgA (0.60–0.23 g/l) in our study group was found in 9 of 660 (1%), but no one showed an IgA < 0.02 g/l.

Conclusion: Our study shows that the prevalence of gluten-sensitivity in apparently healthy blood donors is 3 of 1000, which suggests a high prevalence of CD in the Dutch population, in contrast to the results of the last published Dutch epidemiologic studies. The recorded prevalence will increase further with greater recognition of subclinical and asymptomatic forms detected by screening tests.

Key words: Blood donors; celiac disease; diagnosis; prevalence; serology

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The diagnosis of celiac disease (CD) is often difficult because most patients may present with only subtle, or atypical, non-gastrointestinal symptoms (1, 2). It is not easy to estimate the true prevalence of CD, and many cases probably go undiagnosed (3). One factor may be that they will be missed because of false-negative results in serology testing (4). In European countries the prevalence of CD is based on symptomatic cases and has been calculated to be between 1:300 and 1:1000 subjects (5, 6). The classic clinical presentation of CD is weight loss and diarrhea (7, 8). However, the diagnosis of CD is often missed because of atypical presentation, and its actual prevalence is probably higher (9). It is clear that the submerged part of the ‘celiac iceberg’, consisting mostly of subclinical and silent cases, is five times bigger than the emerged part, consisting of the detected cases (5, 10). Monosymptomatic presentations of the disease is common (11, 13). CD can be excluded in subjects who have a normal small-intestinal mucosal morphology when they are on a normal gluten-containing diet. However, gluten sensitivity is probably no longer restricted to total villous atrophy. Small-intestinal mucosal damage develops gradually from normal mucosal morphology to overt atrophy with hyperplasia of crypts (14). The final diagnosis of CD can be made or excluded only by upper gastrointestinal endoscopy and biopsy. CD probably causes chronic ill health even in patients with mild disease activity and may lead to development of malignant disease in patients left untreated and in those with latent CD (15–17). The prevalence of the disease affects the outcome of the test results, and screening tests are needed to identify celiacs with atypical presentations (18). However, unfortunately, it has been reported that it is utopian to obtain optimal sensitivity and specificity for...
serology testing in population studies (18–21). This fact makes us aware of the limited values of the serologic results for detection of celiacs.

The aims of this study was to update the epidemiologic behavior of CD in an apparently healthy blood donor population and to investigate the prevalence of undiagnosed celiacs.

Materials and Methods

Serology

The values of endomysial antibodies (EMA) and immunoglobulins (total IgA) were assayed in sera from 1000 apparently healthy blood donors at Arnhem and Nijmegen Blood Donation Centers from December 1997 through April 1998. All these subjects were informed about the usefulness of serologic assays—if necessary, followed by a small-intestinal biopsy for detecting atypical CD. Informed consent was obtained from all donors. Antibodies to EMA were detected by using immunofluorescence (20). All donors who had positive EMA results underwent a small-intestinal biopsy. Serum immunoglobulin levels (IgA) were assayed by nephelometry. Our study was in accordance with the ethical standards of human experimentation approved by the Ethical Review Committee of Rijnstate Hospital.

Dexa scan

A Dexa scan was done in all three seropositive cases.

Histopathology

The three blood donors with positive EMA results were examined clinically and endoscopically and their laboratory results evaluated by a gastroenterologist. CD was defined in accordance with the revised criteria of the European Society of Pediatric Gastroenterology and Nutrition (22). Four duodenal biopsy specimens were obtained. The forceps were large and equipped with a needle (MTW). Furthermore, jejunal biopsy specimens were taken with an endoscopically guided capsule (Fujinon) (23). All biopsy specimens were reviewed and evaluated by two investigators in accordance with the modified Marsh criteria (14, 20).

The diagnosis of CD was based on the demonstration of villous atrophy, increased numbers of intraepithelial lymphocytes, and crypt hyperplasia in biopsy material by histologic evaluation of the small intestine and its response to a gluten-free diet (GFD).

HLA-DQ typing method

After the endoscopy had been performed, DNA was isolated from the blood samples of three subjects with positive serology and intestinal abnormalities. They have been screened for HLA-DQ alleles that have been associated with susceptibility to CD (24). Genomic DNA was extracted from ethylenediaminetetraacetic acid (EDTA)-anticoagulated peripheral blood in accordance with standard proteinase Q digestion and phenol/chloroform extraction technique. Generic primers were used for the amplification of the second exons of the HLA-DQA1* and -DQB1* genes on a Perkin-Elmer 9600 tamale cycler (Perkin-Elmer, Norwalk, Conn., USA). HLA-DQA1* and -DQB1* typing was performed by single-trended conformation polymorphism (CCKP)/heteroduplex methods with semi-automated electrophoresis and gel staining on the Phast system (Amersham, Pharmacia Biotech, Sweden).

Results

Of the 1000 cases 3 men had an abnormal screening result (positive EMA) and were suspected of having CD. Small-intestinal biopsy of the three EMA-positive cases showed subtotal villous atrophy (Marsh IIIb) in two and intraepithelial lymphocytosis and crypt hyperplasia (Marsh II) in one. The two patients with Marsh IIIb had recurrent abdominal complaints but otherwise normal laboratory results. One of them had mild steatorrhea (11.1 g/24 h) (Table I). The third patient had no complaints at all but had osteopenia on Dexa scan. According to the ESPGAN criteria he does not have CD but may be a latent celiac who may develop a more severe mucosal abnormality in the future. A complete clinical remission has been obtained in both cases after 3 months on a gluten-free diet (GFD). The second biopsy will be taken 6 months after starting the GFD.

The prevalence of CD was 1 in 333 to 1 in 500 (Table II), which is even higher than the range found in other European countries and significantly higher than the mean crude incidence rate of 0.54 per 1000 live births found in The Netherlands in 1990–97 (25). After the small-bowel biopsy had been performed in seropositive cases, HLA subtypes were determined. All three EMA-positive patients with intestinal

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Sex</th>
<th>EMA</th>
<th>Fecal fats/clinic</th>
<th>Dexa scan</th>
<th>HLA type</th>
<th>Pathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>34</td>
<td>M</td>
<td>Positive</td>
<td>Normal</td>
<td>Osteopenia</td>
<td>DQA<em>0501/DQB</em>0201</td>
<td>Marsh II</td>
</tr>
<tr>
<td>52</td>
<td>M</td>
<td>Positive</td>
<td>&gt;11 g/24 h/Abd. comp.</td>
<td>Normal</td>
<td>DQA<em>0501/DQB</em>0201</td>
<td>Marsh IIIb</td>
</tr>
<tr>
<td>54</td>
<td>M</td>
<td>Positive</td>
<td>Normal/Abd. comp.</td>
<td>Normal</td>
<td>DQA<em>0501/DQB</em>0201</td>
<td>Marsh IIIb</td>
</tr>
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EMA = endomysial antibodies; Abd. comp. = abdominal complaints.
damage carried the known susceptibility alleles for CD (HLA-DQA1*0501 and HLA-DQB1*0201). Total IgA was measured in only 660 of the 1000 blood donors. Low IgA (0.60–0.23 g/l) in our study group was found in 9 of the 660 (1%), but no one showed an IgA ≤ 0.02 g/l.

### Discussion

The interest in screening for undiagnosed CD has increased as a result of studies reporting the enormously varied spectrum of clinical manifestations encompassed by the disease in different countries (26). It is likely that the increased number of new cases is the outcome of the improved diagnostic methods and greater awareness of the disease and its magnitude of presentation. Screening tests are needed to detect celiacs with atypical presentation, especially individuals who are at increased risk of CD: first-degree relatives and those with deficiency diseases (monosymptomatic celiacs with anemia) of unknown causes. Members of the general population or even apparently healthy blood donors should also be considered for screening (3). There is no doubt that patients with untreated CD are at increased risk of developing malignancy, particularly lymphoma (16, 27, 28). The risk of associated ill health may also be increased in patients with clinically silent and even latent forms of CD (29–31). Early diagnosis and dietary therapy of CD may reduce the risk of the above complications—for example, bone mineral density can increase with a GFD (16, 32–34). Our study shows that the prevalence of CD in a population of apparently healthy blood donors is 2–3/1000, indicating a higher prevalence than found in the latest epidemiologic studies from The Netherlands (35, 36). The recorded prevalence will increase further with better recognition of subclinical and asymptomatic forms detected by screening tests.

Although the prevalence of CD is twofold higher in women than in men, all seropositive cases detected in apparently healthy blood donors by us, and seven of the eight cases detected by Not et al. (37) were in men. This is in contrast to the prevalence of CD in the general population, for which we do not have an explanation. However, it may be that women with mild CD develop anemia more easily due to menstruation, and they have probably been and will continue to be excluded from the blood donor population, in accordance with the blood donor criteria.

Until now, serologic analyses have been believed to be optimally sensitive methods and efficient for screening. However, screening that uses only antibody tests will underestimate the prevalence of CD due to false-negative results, as has been shown previously (18–21, 38). This is caused by the low sensitivity of serologic tests in a subgroup of patients. It is suggested that use of undetectable IgA as a selection criterion for small-bowel biopsy in addition to a positive EMA would improve sensitivity in cases of suspected CD (39). Patients with selective IgA deficiency have at least a tenfold risk of CD compared with the population in general (40). In this study we have not tried to establish a relationship between CD and the etiology of the IgA deficiency, even though some relationship may in the future be found to exist. On this occasion we have presented the results of only one screening test. However, in contrast to Not et al. (37), for reliable screening we advise performing a combination of tests and not a so-called two-step analysis, in which a second test is performed only when the first is positive (41). Measurement of only one antibody would exclude CD and miss up to 50% of patients with CD (42). Moreover, it should be also considered that screening only by means of antibodies will underestimate the prevalence of CD due to false-negative results caused by the low sensitivity of the tests (43). These approaches indicate that, by using a combination of tests, we could find more than three new celiacs among apparently healthy blood donors, suggesting a higher prevalence (>3/1000), even though in the general population it is more than this. If the prevalence of CD in a selected healthy population without symptoms of nutritional deficiency diseases is almost 1:300, the true prevalence of CD is undoubtedly higher in a non-selected population, taking into account the presence of asymptomatic multideficiency diseases in the general population. We conclude that the prevalence of antibodies is lower than the prevalence of CD, which may suggest that the true prevalence of CD might be higher than the 3:1000 in the Dutch population.

### Acknowledgements

The authors would like to thank the personnel of the Arnhem/Nijmegen Blood Bank for their help, especially Dr. H. Hopman. They also thank the laboratory technicians of the Eemland Hospital Amersfoort for their work and contribution in this study. This study was supported by Princess Irene Children’s Hospital Foundation.

### References


