Serological screening suggests that adult coeliac disease is underdiagnosed in the UK and increases the incidence by up to 12%

D J Unsworth, D L Brown

Abstract
Because coeliac disease often presents atypically it is underdiagnosed. It is suggested that the detection rate may be increased by 12% if serology is used to identify cases of occult enteropathy. All adults noted incidentally to be R1 anti-reticulin antibody (ARA) positive in the course of routine autoantibody testing of 6532 sera over one year were followed. None of the eight patients with seropositive serum was suspected of having coeliac disease. All eight had high titres of IgA anti-gliadin and IgA anti-endomysial antibodies, neither of which is detected in a routine autoantibody test, in addition to IgA R1-ARA. On clinical review coeliac disease was considered probable in only one patient, but because of the strong serological evidence of gluten sensitivity, jejunal biopsy was advised in all eight. Seven agreed and all had villous atrophy and crypt hyperplasia in keeping with coeliac disease. Six of the seven presented initially with vague symptoms such as tiredness or arthralgia. These symptoms disappeared after several weeks of gluten withdrawal. Forty two sera showing reticulin staining patterns other than R1 were used as controls. Low titre IgA anti-gliadin was noted in two of 42 but none had IgA anti-endomysial antibody. These 42 cases were not recommended for biopsy. During our study 58 other new adult cases of coeliac disease were diagnosed, primarily on clinical rather than serological grounds, at the four hospitals that request autoantibody studies. Occult coeliac disease detected serologically thus increased the overall incidence of coeliac disease by 12% from 58 to 65 cases. R1-ARA, even in the absence of the expected symptoms and signs of coeliac disease, is an indication for jejunal biopsy and is a reliable indicator of occult coeliac disease.

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IgA anti-endomysial antibody is useful when selecting suspected cases of coeliac disease for confirmatory biopsy.1,2 Anti-reticulin antibody of the R1 type (R1-ARA), however, which also is known to be strongly associated with untreated gluten sensitive enteropathy,3 has been regarded as less useful because its sensitivity for coeliac disease is only 50% and its specificity for this disorder is questioned.1,4 Sensitivity and specificity of 97% and 98% respectively are claimed when the conventional test is adapted to detect IgA R1-ARA.5 Nonetheless, doubts over the reliability of the R1-ARA test have meant that it is now hardly ever requested in its own right. R1-ARA is occasionally discovered in the course of routine autoantibody testing using cryostat sections of rodent tissue as substrate. It is important to note that anti-endomysial and anti-gliadin antibody do not show up in this conventional autoantibody test. Patients in whom R1-ARA is found fortuitously may have none of the expected symptoms or signs of gluten sensitive enteropathy. In some cases a firm alternative diagnosis has already been made, and these patients may be presumed to represent ‘false positives’. Untreated coeliac disease carries an increased risk of malignancy6 and there are therefore sound medical and ethical reasons why cases of unsuspected disease should be diagnosed and treated. In this study we show that IgA R1-ARA is reliable and valuable in assisting the early recognition and treatment of occult coeliac disease.

Patients and methods
Altogether 6532 adult sera were tested in our routine autoantibody test in the 12 months from July 1991 to June 1992. We receive very few requests for autoantibody studies on children, and these were excluded from our study. Ninety two per cent of the sera came from four hospitals in the East Anglia region – namely Hinchinbrooke (Huntingdon); The West Suffolk (Bury St Edmunds); Addenbrooke's, Cambridge; and the Norfolk and Norwich. These hospitals together serve a population of approximately 1·16 million. Only 16% of the requests came from people suspected of gastroenterostestinal disease, particularly autoimmune liver disease. Patients known or suspected at the time of autoantibody testing to have coeliac disease were excluded from our study.

Diagnosis of coeliac disease was based on jejunal biopsy specimen showing the appropriate histopathological features, and clinical improvement on a gluten free diet, in keeping with the recently revised diagnostic criteria.7 Five of the seven patients with R1-ARA seropositivity agreed to undergo confirmatory biopsy between three and six months after starting a gluten free diet, and all five showed considerable histological improvement. Full blood counts, liver enzyme activities, and albumen and calcium were measured in all cases before starting a gluten free diet. Serum iron or ferritin, or both, and serum and red cell folate were measured in five patients before gluten free diet. Because no patient had diarrhoea, measurement of faecal fat loss was not indicated. The total number of newly presenting adults with coeliac disease at
the four hospitals during this one year study was
determined by consultation with the gastro-
enterologists and histopathologists at those
hospitals. Local population numbers are from
mid-1990 census data to the nearest 10,000.

METHODS
Indirect immunofluorescence for ARA was per-
formed as previously described, with cryostat
sections (6 μm) of a composite block of rat tissues
as substrate and patient’s sera diluted 1/10 in
phosphate buffered saline (PBS). ARA positive
sera were subdivided into five groups – R1,
partial R1, R2, Rs (sinusoidal), and heterophile,
according to the pattern of immunofluorescence
staining seen when using a polyclonal anti-
immunoglobulin reagent. All ARA positive
sera were retested using IgA and IgG specific
reagents. The five R1, five partial R1, and 15 R2
positive sera represented the total number that
showed these reactivities over the year. The
remaining 25 sera studied showed Rs or hetero-
phile patterns and were collected over a two
month period. Forty two sera were ultimately
classed as non-R1 after isotype specific testing
and these served as the control group. Four of
these 42 sera had been sent to us by other
laboratories for an opinion on whether they were
R1-ARA positive.

The R1 and R2 patterns were as defined by
Rizzetto and Doniach.18 R1 positive sera were
required to stain fibrillar connective tissue exten-
sively in rat liver, kidney, and stomach. Sera
failing to stain any one of these three tissues but
giving R1 staining in the remaining tissues were
classified as showing a partial R1 pattern. Stain-
ing of discrete thread like reticulin fibrils in rat
liver parenchyma was the most distinctive and
reliable feature of the R1 antibody. Rs positive
sera stained liver sinusoids, and in most cases
kidney tubule brush border and stomach sub-
mucosal reticulin fibres also, but failed to give R1
staining in the kidney and between the gastric
parietal cells. The Rs group includes the anti-
sinusoid and anti-Kupffer cell reactivities reported
by Rizzetto and Doniach.18 Heterophile antibodies
stained gastric parietal cells and kidney tubule
brush border, and gave R1-ARA like staining
restricted to kidney medulla.

Anti-endomyosal antibody was detected in sera
diluted 1/10 in PBS on slides of monkey oeso-
phagus (The Binding Site, Birmingham, UK).
Anti-gliadin antibody was detected by ELISA as
described earlier, except that commercial crude
gliadin (Sigma Chemicals) was used as antigen
source. Gliadin was dissolved in 70% ethanol/
30% distilled water, and the undissolved fats and
glutenins were discarded. Gliadin at a concentra-
tion of 10 μg/ml in 70% ethanol was used to coat
the plates at room temperature overnight.
Patient’s sera were tested at a dilution of 1/100.

Results
Clinical details of the eight R1-ARA seropositive
adults are shown in Table I. The presenting
complaints were vague, and in all but case 2 did
not suggest gastrointestinal disease. Case 2 pre-
pared with weight loss and diarrhoea associated
with hypoalbuminaemia (31 g/dl) and mildly
raised liver transaminase activities. Because of
the patient’s age, malignancy was suspected.
Among the other cases, arthralgias were a com-
mon symptom. One patient was asymptomatic
but was found to be leukopenic on a routine
preoperative full blood test, justifying the initial
autoantibody test. Although only three of the
eight patients were anaemic, deficiency of iron
and or folate at presentation was found in each of
case five patients tested. In all cases serum calcium
was normal. Apart from case 2, normal serum
albumen values were found. Abnormal liver
function tests (typically, mild abnormalities of
transaminase activities but a normal γ glutamyl
transferase value) were noted in five of eight
patients. Each of these five had a raised alanine
aminotransferase activity, with the highest value
of 142 IU (normal range, <35 IU) seen in patient
six. The average alanine aminotransferase value
before gluten free diet for these five patients was
85 IU. All seven cases, including the asympto-
omatic patient, felt better within eight weeks of
beginning a gluten free diet. Aminotransferase
haematology and liver function returned to
normal in all seven cases, taking up to six months
to do this. R1-ARA, and anti-endomyosal anti-
body disappeared and anti-gliadin antibody
values fell to lie inside the normal range within
six months of beginning a gluten free diet in the
five patients for whom follow up samples were
provided.

Table II shows that R1-ARA and partial R1
positive patients tended to be of IgA isotype,
while the other ARA types tended to be of IgG
isotype. Three of the five partial R1 positive sera
became recognisable as R1-ARA when retested in
the IgA specific test. These three sera were also
IgA anti-endomyosal antibody positive. This
was not noted for any of the other non-R1 sera

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### TABLE I Clinical, haematological, and histopathological details on the eight R1-anti-reticulin antibody positive patients

<table>
<thead>
<tr>
<th>Case no</th>
<th>Sex/age (y)</th>
<th>Presenting complaint</th>
<th>Reason for auto-antibody test</th>
<th>Haemoglobin (g/L)</th>
<th>Haematocrit (%)</th>
<th>Small intestinal biopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M/45</td>
<td>Swollen wrist</td>
<td>Arthralgias</td>
<td>15.1</td>
<td>7.8</td>
<td>ND</td>
</tr>
<tr>
<td>2</td>
<td>F/79</td>
<td>Swollen ankles**</td>
<td>Abnormal LFT</td>
<td>7.8</td>
<td>ND</td>
<td>Iron and folate</td>
</tr>
<tr>
<td>3</td>
<td>M/46</td>
<td>Tiredness</td>
<td>Abnormal LFT</td>
<td>13.9</td>
<td>9.3</td>
<td>ND</td>
</tr>
<tr>
<td>4</td>
<td>F/61</td>
<td>Tiredness</td>
<td>Abnormal LFT</td>
<td>10.6</td>
<td>6.1</td>
<td>Iron and folate</td>
</tr>
<tr>
<td>5</td>
<td>F/73</td>
<td>Manic/depressive</td>
<td>Abnormal LFT</td>
<td>13.1</td>
<td>8.1</td>
<td>Folate</td>
</tr>
<tr>
<td>6</td>
<td>F/48</td>
<td>Tiredness</td>
<td>Abnormal LFT</td>
<td>13.7</td>
<td>8.1</td>
<td>Folate</td>
</tr>
<tr>
<td>7</td>
<td>F/17</td>
<td>Painful/swollen</td>
<td>Arthralgias</td>
<td>12.2</td>
<td>ND</td>
<td>Iron deficient</td>
</tr>
<tr>
<td>8</td>
<td>F/45</td>
<td>Nil*</td>
<td>Leukocytopenia*</td>
<td>ND</td>
<td>ND</td>
<td>Iron deficient</td>
</tr>
</tbody>
</table>

*Routine preoperative full blood count showed abnormalities; **albumen 31 g/l (normal 32-44).
PVA = partial villous atrophy; STVA = subtotal villous atrophy; LFT = liver function test; ND = not done.

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### TABLE II Seropositivity for IgA anti-gliadin antibody and IgA anti-endomyosal antibody in relation to anti-reticulin antibody type

<table>
<thead>
<tr>
<th>Anti-reticulin type</th>
<th>No tested</th>
<th>Immunofluorescence</th>
<th>IgA anti-gliadin antibodies</th>
<th>IgA anti-endomyosal antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>5</td>
<td>5/5</td>
<td>5/5</td>
<td>5/5</td>
</tr>
<tr>
<td>Partial R1</td>
<td>5</td>
<td>3/5</td>
<td>3/5</td>
<td>3/5</td>
</tr>
<tr>
<td>R2</td>
<td>15</td>
<td>1/15</td>
<td>1/15</td>
<td>0/15</td>
</tr>
<tr>
<td>Rs</td>
<td>2/15</td>
<td>14/15</td>
<td>14/15</td>
<td>14/15</td>
</tr>
<tr>
<td>Heterophile</td>
<td>10</td>
<td>0/10</td>
<td>10/10</td>
<td>0/10</td>
</tr>
</tbody>
</table>
Underdiagnosis of coeliac disease in UK

![IgA anti-gliadin antibody ELISA titre in relation to anti-reticulin antibody type. AEA = anti-endomysial antibody.](image)

arising from the initial screen using a polyvalent anti-immunoglobulin reagent. All eight R1-ARA positive sera were also IgA anti-endomysial antibody and high titre IgA anti-gliadin antibody positive. None of the other 42 control sera were IgA anti-endomysial antibody positive, and only two of 42 were IgA anti-gliadin antibody positive. As shown in the Figure, high titre IgA anti-gliadin antibody was restricted to those sera which were IgA anti-endomysial antibody and R1-ARA positive. Table III shows the total number of newly diagnosed adult cases of coeliac disease diagnosed over the one year study period.

Discussion

We have screened a very large number (6532) of sera blind and have followed up patients who were R1-ARA positive, irrespective of their presenting symptoms. Our data show that the R1-ARA is an extremely reliable marker of coeliac disease. The diagnosis of gluten sensitive enteropathy in two of our seven patients may be disputed because these patients did not have follow up biopsies to show mucosal improvement as a result of the gluten free diet. All patients, however, admit to feeling much better after excluding gluten from their diet, and without exception, abnormalities of liver function and haematology have returned to normal in all cases where abnormalities had been noted. Coeliac disease can present with vague symptoms of malaise. Arthralgias were common in our patients, and an association between coeliac disease and arthritis has been reported before. In one case of juvenile rheumatoid arthritis, R1-ARA was present in joint aspirate. None of our patients presented with the stigmata of recognised arthritic diseases, there was no clear pattern of joint involvement (Table I), and symptoms resolved speedily on the gluten free diet. The trend towards testing rheumatology clinic sera for anti-nuclear antibody on cell monolayers (on which R1-ARA are not detected) rather than on rat liver tissue sections may lead to these atypically presenting coeliac disease cases being missed.

Had it not been for the finding of R1-ARA in our cases, the diagnosis of coeliac disease would have been at best delayed, or worse, missed altogether. This might have exposed the patients to an increased risk of gastrointestinal malignancy. There are two previous studies similar to ours. Both quote impressive results using R1-ARA fortuitously noted in the course of routine autoantibody testing to diagnose atypically presenting coeliac disease in adults. Collin et al found 51 R1-ARA positive sera when testing 17 000 adult sera over a three year period. Of 30 patients who were successfully biopsied, only 18 proved to have coeliac disease. The remaining 12 had normal jejunal biopsy specimens and proved to have IgG and not IgA R1-ARA. Sixteen of the 18 patients with coeliac disease had IgA R1-ARA. Restricting biopsy to patients who were IgA positive would thus have yielded similar results to ours. Watson et al identified 291 suspicious sera while performing autoantibody testing but did not provide details of the reticulin antibody class. They tested their sera for anti-gliadin antibody and focused attention on the anti-gliadin antibody positive patients. Eighteen underwent jejunal biopsy and each of the 13 IgA anti-gliadin antibody positive patients proved to have coeliac disease while the five with IgG but not IgA anti-gliadin antibody had normal biopsy specimens. The difference between our study and the preceding two seems to be that by using IgA anti-endomysial antibody to confirm gluten sensitivity in our R1-ARA seropositive patients, we have improved the diagnostic accuracy to 100%. By contrast, use of IgA anti-gliadin antibody to confirm gluten sensitivity is unreliable. This point is clear from study of patients with IgA nephropathy which shows that IgA anti-gliadin antibody is not infrequently found in patients who do not have coeliac disease when sent for jejunal biopsy. Anti-endomysial antibody was associated only with R1-ARA and not with the other ARA types. To our knowledge, no-one else has checked whether the various ARA types interfere in the endomysial antibody test. The copresence of both anti-gliadin antibody and anti-endomysial antibody (of IgA class) will clearly help those who are inexperienced in recognising the R1 pattern.

During the study period 58 new adult cases of coeliac disease (excluding our seven biopsy proved cases) were diagnosed in the four East Anglian hospitals that request autoantibody studies from us. These four hospitals serve a population of 1·16 million (note that this figure includes children and adults). Serological identification of cases of occult coeliac disease allowed us to increase the incidence in our local population by 12%. As R1-ARA screening using poly-

### TABLE III Incidence of adult coeliac disease in this region

<table>
<thead>
<tr>
<th>Hospital</th>
<th>Local population</th>
<th>Identified clinically</th>
<th>Identified serologically</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hinchingbrooke</td>
<td>140 000</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>The West Suffolk</td>
<td>240 000</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>Addenbrooke’s</td>
<td>290 000</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>Norfolk and Norwich</td>
<td>490 000</td>
<td>25</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>1 160 000</td>
<td>58</td>
<td>7</td>
</tr>
</tbody>
</table>

Note: The above table provides the incidence of adult coeliac disease in the local population.
valent rather than IgA specific reagents has a sensitivity in our hands of only 50% in the context of diagnosing coeliac disease, and as we screened only 6532 sera, coeliac disease is likely to be appreciably underdiagnosed. The association between coeliac disease and selective IgA deficiency will result in negative IgA R1-ARA and may further exaggerate the underdiagnosis. Population screening for gluten sensitivity has been advocated but it is uncertain that it will be cost efficient in adults. The approach we have used is inexpensive and amounts to audit of samples being processed anyway. Our high diagnostic yield may in part reflect the fact that the sera we test for autoantibodies come from patients who are ill. Screening healthy people may prove less productive.

R1-ARA in this study was highly specific for gluten sensitive enteropathy. So what of the previous reports\(^3\) that R1-ARA is not specific? Rizzetto and Doniach\(^3\) described five types of ARA, and recognition of the R1-ARA can be difficult for non-experts. Hence R1-ARA is often misreported or worse ignored, particularly if the autoantibody test is requested for reasons unconnected with gluten sensitivity. Also, initial studies did not break down seropositive sera according to isotype. Another factor is that when atypically presenting or clinically silent gluten sensitive enteropathy coexists with or is mistaken for some other diagnosis, apparent ‘false positives’ may be reported. Because coeliac disease can present with atypical symptoms subclinical gluten sensitive enteropathy cannot be excluded unless patients with R1-ARA undergo small intestinal biopsy. The fact that R1-ARA has been reported in relatives of known coeliac disease patients with a normal jejunal biopsy specimen who subsequently go on to develop gluten sensitive enteropathy\(^5\) cautions us that even in the face of a normal jejunal biopsy specimen the possibility of ‘latent’ coeliac disease means that long term follow up of patients with serum positive for R1-ARA and normal jejunal biopsy tissues is appropriate. We believe that these factors have compounded the false belief that the R1-ARA is diagnostically unreliable. Our data suggest that whatever clinical circumstances, the presence of serum IgA R1-ARA, particularly when coexisting with IgA anti-endomysial antibody, is an absolute indication for jejunal biopsy to confirm gluten sensitive enteropathy.

Serological screening suggests that adult coeliac disease is underdiagnosed in the UK and increases the incidence by up to 12%.

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