Anti-\textit{Saccharomyces cerevisiae} antibody titres correlate well with disease activity in children with Crohn’s disease

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ABSTRACT

\textbf{Aim}: The role of noninvasive biologic markers for disease activity is very important in children with Crohn’s disease. The aim of this study was to assess an association between disease activity and quantitative serum \textit{anti-Saccharomyces cerevisiae} antibody (ASCA) titres.

\textbf{Methods}: \textit{Anti-Saccharomyces cerevisiae} antibody immunoglobulin (Ig) A and immunoglobulin G titres, paediatric Crohn’s disease activity index (PCDAI), serum albumin and C-reactive protein (CRP) were repeatedly measured simultaneously in children with Crohn’s disease. A possible association between ASCA IgA and IgG titres and changes in PCDAI was examined.

\textbf{Results}: Serial 136 measurements of ASCA IgA and IgG titres were documented in 57 children with Crohn’s disease over a mean duration of 3.1 ± 2.1 years. In a univariate linear regression model, there were significant correlations between ASCA IgA titres and PCDAI ($p < 0.001$), CRP ($p < 0.01$) and low serum albumin ($p < 0.001$), respectively. Similarly, ASCA IgG titres significantly correlated with PCDAI, CRP and low serum albumin.

\textbf{Conclusion}: Both ASCA IgA and IgG titres seemed to correlate well with clinical Crohn’s disease activity in children. Measuring these antibodies may be considered during routine clinical care for those patients.

INTRODUCTION

Inflammatory bowel disease (IBD) encompasses two disorders of unknown aetiology, Crohn’s disease and ulcerative colitis, and approximately 25% of patients present as children or young adults (1,2). A study from Manitoba, Canada, reported a steady increase in the incidence and prevalence of paediatric IBD (3).

Establishing the diagnosis of IBD depends on clinical, laboratory, endoscopic and radiological findings (2). However, making this diagnosis can be difficult because other common clinical conditions, such as irritable bowel syndrome, may have similar symptoms (4,5). The role of noninvasive biological markers for IBD is expanding. Serological markers, such as \textit{anti-Saccharomyces cerevisiae} antibodies (ASCA), have been helpful in establishing the diagnosis of IBD (6,7). The sensitivity and specificity of these antibodies in diagnosing Crohn’s disease range from 40% to 70% and 82% to 89%, respectively (7–9). These antibodies are more common in younger age groups, ileal location and more aggressive and complex disease behaviour (9,10). Few studies have examined whether ASCA titres changed with disease activity and showed that antibody titres were either stable or decreased with successful treatment (11,12). One paediatric study, however, showed that ASCA titres dropped after surgery related to Crohn’s disease (13).

The aim of this study was to longitudinally assess whether ASCA immunoglobulin A (IgA) and immunoglobulin G

**Key Notes**

- The role of noninvasive biologic markers for disease activity is very important in patients with Crohn’s disease.
- This study confirmed a significant correlation between \textit{anti-Saccharomyces cerevisiae} antibody immunoglobulin A and immunoglobulin G titres and clinical disease activity in children with Crohn’s disease.
- Measuring these antibodies may be considered as part of the routine clinical care of children with Crohn’s disease.

**Abbreviations**

ASCA, \textit{anti-Saccharomyces cerevisiae} antibodies; CRP, C reactive protein; ESR, erythrocyte sedimentation rate; IBD, inflammatory bowel disease; IgA, immunoglobulin A; IgG, immunoglobulin G; PCDAI, paediatric Crohn’s disease activity index.
(IgG) antibody levels changed with any changes in clinical disease activity in children with Crohn’s disease.

MATERIALS AND METHODS
A comprehensive medical chart review was performed for all children with Crohn’s disease who were under 17 years old at the Children’s Hospital, Winnipeg, Manitoba, Canada, and had quantitative ASCA IgA and IgG titres at diagnosis and/or follow-up. The diagnosis of Crohn’s disease was confirmed based on clinical, laboratory, radiological and endoscopic evidence (2).

The Paris classification was used to determine the phenotype of the disease (14). The paediatric Crohn’s disease activity index (PCDAI), serum albumin and C-reactive protein (CRP) values were determined at the same time point as the ASCA antibodies were measured. The PCDAI score ranges from zero to 100, with a score >10 indicating active disease (15).

All blood samples were drawn, and activity indices scores were calculated at the same hospital visit. The PCDAI was routinely measured for all patients during their routine clinic visits when all blood investigations were normally requested. Erythrocyte sedimentation rate (ESR), serum albumin and haematocrit values were available before each clinic visit when all blood investigations were normally requested.

ASCA IgA and IgG measurements
Anti-Saccharomyces cerevisiae antibody IgA and IgG were measured in serum on a Best 2000 (DSX) automated enzyme-linked immunosorbent assay (ELISA) system from DYNE (Magellan Biosciences, Tampa, FL, USA) using indirect noncompetitive ELISA (EUROIMMUN Medizinische Labordiagnostika AG, Lubeck, Germany) for the detection and semi-quantification of ASCA IgA and IgG antibodies. Diluted samples were incubated and coated with mannan from Saccharomyces cerevisiae for 30 min.

Results were expressed in relative units per millimetre of serum (RU/mL). ASCA antibodies were considered positive if titres measured over 20 RU/mL. The coefficients of variation for ASCA IgA and IgG were 5% and 6%, respectively.

Statistics
Calculations and data analysis were performed using STATA 9.1™ (Data Analysis and Statistical Software, Austin, TX, USA) and NCSS (Number Cruncher Statistical Systems, Kaysville, UT, USA). Univariate summaries including means, ranges and standard deviation (SD) were calculated for continuous variables. Frequencies were calculated for categorical variables, along with 95% confidence intervals (CIs) for the means and proportions. Variables were examined for normal distribution. Regression analysis was used to determine any possible relationship between ASCA IgA and IgG antibody titres as dependent variables and PCDAI, CRP and serum albumin as independent variables. Other variables included the presence or absence of immunosuppression.

RESULTS
Serial 136 measurements of ASCA IgA and IgG titres were documented in 57 children with Crohn’s disease. The mean age at diagnosis was 11.9 ± 2.2, their age ranged from 6.5 to 17.9 years, and 36 boys were included. The mean duration of follow-up was 3.1 ± 2.1 years. All patients included had normal serum IgA and IgG levels. The patients’ characteristics are summarised in Table 1. Disease phenotype was determined following the Paris classification (16).

The mean level of ASCA IgA antibody titres was 91.1 ± 84.6 RU/mL. The mean level of ASCA IgG titres was 63.9 ± 69.4 RU/mL (p = 0.06).

In a univariate linear regression model, there were significant correlations between ASCA IgA titres and PCDAI (p < 0.001, 95% CI 0.36–0.61) (Fig. 1), CRP (p < 0.01, 95% CI 0.10–0.41) and low serum albumin (p < 0.001, 95% CI −0.27 to 0.55), respectively. Similarly, ASCA IgG titres correlated with PCDAI (p < 0.001, 95% CI 0.21–0.50) (Fig. 2), CRP (p < 0.05, 95% CI 0.04–0.36) and low serum albumin (p < 0.001, 95% CI −0.19 to 0.49), respectively. These associations were independent of immunosuppressive medications. A subgroup analysis was performed that included the 25 patients who had three or more measurements of ASCA antibody titres. Figure 3 shows the longitudinal changes in the mean ASCA IgA antibody titres.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Number (%)</th>
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<tbody>
<tr>
<td>Boys</td>
<td>36 (63)</td>
</tr>
<tr>
<td>Mean age (SD)</td>
<td>11.9 ± 2.2</td>
</tr>
<tr>
<td>Paris classification</td>
<td></td>
</tr>
<tr>
<td>A1a (&lt;10 years)</td>
<td>13 (23)</td>
</tr>
<tr>
<td>A1b (10–17 years)</td>
<td>44 (77)</td>
</tr>
<tr>
<td>G0 (growth delay)</td>
<td>33 (58)</td>
</tr>
<tr>
<td>G1 (no growth delay)</td>
<td>24 (42)</td>
</tr>
<tr>
<td>L1 (ileocolic)</td>
<td>5 (8)</td>
</tr>
<tr>
<td>L2 (colonic)</td>
<td>10 (17)</td>
</tr>
<tr>
<td>L3 (ileocolonic)</td>
<td>41 (72)</td>
</tr>
<tr>
<td>L4 (isolated small bowel)</td>
<td>2 (4)</td>
</tr>
<tr>
<td>L4 (concomitant with L3 or L2)</td>
<td>13 (23)</td>
</tr>
<tr>
<td>B1 (inflammatory)</td>
<td>47 (82)</td>
</tr>
<tr>
<td>B2 (stricturing)</td>
<td>8 (14)</td>
</tr>
<tr>
<td>B3 (penetrating)</td>
<td>3* (5)</td>
</tr>
<tr>
<td>P (concomitant perianal)</td>
<td>17 (29)</td>
</tr>
<tr>
<td>Crohn’s related surgery</td>
<td>6 (10)</td>
</tr>
<tr>
<td>Immunosuppression/biologic</td>
<td>37 (65)</td>
</tr>
<tr>
<td>Exclusive nutritional therapy</td>
<td>23</td>
</tr>
<tr>
<td>Systemic steroids</td>
<td>22</td>
</tr>
<tr>
<td>5-ASA medications</td>
<td>7</td>
</tr>
</tbody>
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*One patient had B2+B3 disease.
in those children with mean PCDAI scores at the same time points over the follow-up period.

Differences between repeated measurements of ASCA IgA and IgG antibodies over the follow-up period were calculated and regressed against differences in concomitant PCDAI scores. There was a significant association between changes in antibody levels for both ASCA IgA and IgG and changes in PCDAI scores ($p = 0.03$, 95% CI 0.87–1.52 and $p = 0.01$, 95% CI 0.25–1.76, respectively). This association again seemed to be independent of immunosuppression.

**DISCUSSION**

Since the first report linking ASCA antibodies to Crohn’s disease, several other studies have confirmed this link. ASCA antibodies have been proven to be helpful in diagnosing and predicting the disease course in patients with Crohn’s disease (10,11,13). Nonetheless, the difference in diagnostic value between ASCA IgA and ASCA IgG has not been well characterised. Moreover, it is not clear whether serum ASCA antibody titre levels longitudinally change with changes in Crohn’s disease clinical activity or not. A proposed hypothesis for this association is an increased intestinal permeability by severe disease that may lead to significant exposure to intestinal antigens. Our study showed that both ASCA IgA and IgG antibodies could be useful in assessing disease activity in children with Crohn’s disease. The variations of serum ASCA IgA and IgG antibody titres seemed to be significantly affected by variations in PCDAI.

Data on changes in antibody levels over time in patients with IBD are limited. Ruemmele et al. (13) demonstrated that ASCA antibody levels dropped significantly following surgery for Crohn’s disease. These findings were confirmed in a large paediatric study by Canani et al., (16) who reported a significant reduction in ASCA titres after intestinal resection for Crohn’s disease. Neither disease location nor medical treatment, including immunosuppression, had any impact on ASCA levels. However, similar to our study, they were able to demonstrate a significant correlation between both IgA and IgG ASCA antibody titres and PCDAI scores (16). Another study showed that ASCA IgA levels went down following mesalamine treatment for Crohn’s disease (12).

On the other hand, Teml et al. looked at ASCA levels in adult patients with Crohn’s disease before and after treatment with either steroids or mesalamine. The antibody levels did not change over a period of 2–9 months (11). Other investigators showed that other antibodies were stable after 4 months of infliximab therapy (17). The majority of these studies used different markers for disease activity.

The results of our study are important and add to the value of ASCA antibodies as a relatively noninvasive tool for monitoring disease activity in children with Crohn’s disease. On the other hand, our findings are limited by the small sample size and retrospective nature of the study.

**Figure 1** Correlation between paediatric Crohn’s disease activity index (PCDAI) and ASCA IgA antibodies. ASCA, anti-Saccharomyces cerevisiae antibody.

**Figure 2** Correlation between paediatric Crohn’s disease activity index (PCDAI) and ASCA IgG antibodies. ASCA, anti-Saccharomyces cerevisiae antibody.

**Figure 3** Mean ASCA IgA titres with mean paediatric Crohn’s disease activity index (PCDAI) scores in 23 patients who had three or more measurements over the study period. ASCA, anti-Saccharomyces cerevisiae antibody.
CONCLUSION
Both ASCA IgA and IgG antibodies may be good markers for monitoring Crohn’s disease activity in children, and routine measurements of these antibodies should be considered. Large, prospective well-designed studies are needed to confirm our findings. A possible correlation between ASCA and other disease markers, such as faecal calprotectin, can also be examined.

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References