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Observational Study

Location-based prediction model for Crohn's disease regarding a novel serological marker, anti-chitinase 3-like 1 autoantibodies

Sipeki N *et al*. aCHI3L1 in IBD

Abstract

BACKGROUND

Defective neutrophil regulation in inflammatory bowel disease (IBD) is thought to play an important role in the onset or manifestation of IBD, as it could lead to damage of the intestinal mucosal barrier by the infiltration of neutrophils in the inflamed mucosa and the accumulation of pathogens. Like neutrophils in the context of innate immune responses, immunoglobulin A (IgA) as an acquired immune response partakes in the defense of the intestinal epithelium. Under normal conditions, IgA contributes to the elimination of microbes, but in connection with the loss of tolerance to chitinase 3-like 1 (CHI3L1) in IBD, IgA could participate in CHI3L1-mediated improved adhesion and invasion of potentially pathogenic microorganisms. The tolerance brake to CHI3L1 and the occurrence of IgA autoantibodies to this particular target, the exact role and underlying mechanisms of CHI3L1 in the pathogenesis of IBD are still unclear.

AIM

To determine the predictive potential of Ig subtypes of a novel serological marker, anti-CHI3L1 autoantibodies (aCHI3L1) in determining the disease phenotype, therapeutic strategy and long-term disease course in a prospective referral cohort of adult IBD patients.

METHODS

Sera of 257 Crohn's disease (CD) and 180 ulcerative colitis (UC) patients from a tertiary IBD referral center of Hungary (Division of Gastroenterology, Department of Internal Medicine, Faculty of Medicine, University of Debrecen) were assayed for IgG, IgA, and secretory IgA (sIgA) type aCHI3L1 by enzyme-linked immunosorbent assay using recombinant CHI3L1, along with 86 healthy controls (HCONT).

RESULTS

The IgA type was more prevalent in CD than in UC (29.2% *vs* 11.1%) or HCONT (2.83%; $P < 0.0001$ for both). However, sIgA subtype aCHI3L1 positivity was higher in both CD and UC patients than in HCONT (39.3% and 32.8% *vs* 4.65%, respectively; $P < 0.0001$). The presence of both IgA and sIgA aCHI3L1 antibodies was associated with colonic involvement ($P < 0.0001$ and $P = 0.038$, respectively) in patients with CD. Complicated disease behaviour at sample procurement was associated with aCHI3L1 sIgA positivity (57.1% *vs* 36.0%, $P = 0.009$). IgA type aCH3L1 was more prevalent in patients with frequent relapse during the disease course in the CD group (46.9% *vs* 25.7%, $P = 0.005$). In a group of patients with concomitant presence of pure inflammatory luminal disease and colon involvement at the time of diagnosis, positivity for IgA or sIgA type aCH3L1 predicted faster progression towards a complicated disease course in time-dependent models. This association disappeared after merging subgroups of different disease locations.

CONCLUSION
CHI3L1 is a novel neutrophil autoantigenic target in IBD. The consideration of antibody classes along with location-based prediction may transform the future of serology in IBD.

Key Words: Chitinase 3-like 1 autoantibodies; Crohn's disease; Ulcerative colitis; Disease progression; Immunoglobulin subtypes; Enzyme-linked immunosorbent assay

Sipeki N, Kovats PJ, Deutschmann C, Schierack P, Roggenbuck D, Papp M. Location-based prediction model for Crohn's disease regarding a novel serological marker, anti-chitinase 3-like 1 autoantibodies. *World J Gastroenterol* 2023; In press

Core Tip: The tolerance brake to chitinase 3-like 1 (CHI3L1), a novel neutrophil autoantigenic target in inflammatory bowel disease (IBD), the presence of immunoglobulin A (IgA) autoantibodies against this specific target, as well as the

precise function and underlying processes of CHI3L1 in the development of IBD, continue to be uncertain. In the present prospective observational study, we first reported an enhanced formation of IgA and secretory IgA (sub)type against CHI3L1 in adult patients with Crohn's disease, which was associated with the clinical phenotype and development of a complicated disease course during follow-up in a tertiary referral IBD center in Hungary. By taking into account the classes of antibodies and utilizing location-based predictions, serology in IBD may undergo a significant transformation in the future.

INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic inflammatory condition involving all or parts of the gastrointestinal tract with a potential to present extraintestinal manifestations (EIM). The two main clinical entities are Crohn's disease (CD) and ulcerative colitis (UC), both of which have heterogeneous presentations and disease courses. Their significance lies in their increasing incidence, which mainly affects the young, still active, and working-age population[1].

The exact pathophysiology of the disease has not yet been fully elucidated. Partially unknown environmental factors are thought to trigger an uncontrolled immune-mediated inflammatory process in genetically predisposed individuals, in which altered microbiome, dysfunction of the intestinal mucosal barrier and dysregulation of the mucosal immune response to commensal or pathogenic microbes of the intestinal microbiota are key players[2-5]. This concept is well represented in the new immunological classification of autoimmune and autoinflammatory diseases, where mixed-pattern conditions such as IBD are acknowledged. The latter are considered to belong to the group of immune-mediated inflammatory disorders (IMIDs) in this autoimmune-autoinflammatory spectrum[6-9]. Like other IMIDs (*e.g.,* rheumatoid arthritis), IBD also exhibits features of autoimmunity with a dysregulated adaptive immune response (the breakdown of immune tolerance, the identification of self-antigens, and the activation of T cells and B cells, resulting in the creation of specific

autoantibodies) and autoinflammatory characteristics with the activation of the innate immune system (involvement of neutrophils, macrophages, as well as dendritic-, natural killer-, and mast cells, different granulocyte subsets, and the complement system as major players)[6,10,11].

Crosstalk between the gut microbiota and the host occurs at the intestinal mucosal level[12]. The mucosal immune system maintains the balance between tolerance to commensal bacteria and the immune response against pathogens and is responsible for mucosal homeostasis[13]. Mechanical and/or immunological disruption of the gut barrier can lead to the enhanced uptake of bacteria and bacterial products from the gut lumen [bacterial translocation (BT)], which can trigger and perpetuate chronic inflammation [14,15]. However, whether this phenomenon in IBD is a primary defect or consequence is still under debate[14,16]. Supported by robust scientific data, impaired sensing of intestinal bacteria by cytoplasmic and membrane-bound pattern recognition receptors (NOD-like and Toll-like receptors, respectively) is thought to play a role in IBD development[17]. Currently, evidence is accumulating, that the formation of auto- and antimicrobial antibodies in IBD appears to be the result of enhanced microbial load[18].

Several types of innate immune cells are involved in IBD immunopathogenesis[14]. Dysregulated neutrophil function can lead to damage to the intestinal mucosal barrier due to the infiltration of neutrophils into the inflamed mucosa and the accumulation of harmful pathogens[19,20]. In UC, the infiltration and accumulation of neutrophils were found to be elevated and associated with disease activity, whereas in CD, impaired recruitment of neutrophils, reduced production of cytokines and phagocytosis, and delayed/defective antimicrobial clearance were described[21,22]. Clinical utility of blood-based and faecal neutrophil-related biomarkers has been studied extensively in IBD. In the latter group, faecal calprotectin has been proven to be the most helpful in monitoring disease activity and is recommended by clinical practice guidelines in various clinical settings (initial diagnosis, diagnosis of relapse, and response to treatment)[22,23]. Other faecal neutrophil-derived biomarkers are lactoferrin, lysozyme,

polymorphonuclear neutrophil elastase, myeloperoxidase, human neutrophil peptides, neutrophil gelatinase-associated lipocalin, and chitinase 3-like 1 (CHI3L1)[24-26]. Similarly to FC, faecal CHI3L1 appears to correlate well with endoscopic activity in patients with CD[27,28].

Recently, loss of tolerance to a novel neutrophil autoantigenic glycoprotein target, CHI3L1 has been confirmed by our group in IBD patients[21,29,30]. Evaluation of autoantibodies against CHI3L1 (aCHI3L1) was performed in a paediatric IBD cohort[30]. Immunoglobulin A (IgA), a key player in acquired immune responses, contributes to the defense of the intestinal epithelium, similarly to how neutrophils are involved in innate immune responses. Under typical circumstances, IgA plays a crucial role in eliminating microbes through its antibacterial and antiviral activities. However, in the context of diminished tolerance to CHI3L1 in IBD, IgA may participate in the enhanced adhesion and invasion of potentially pathogenic microorganisms, as facilitated by CHI3L1. Given the current state of knowledge, the precise function and mechanism of CHI3L1 in the development of IBD, loss of tolerance to this antigen, formation of IgA autoantibodies against it, and their role in the pathogenesis of IBD remain elusive[21,30].

The main objective of this research was to identify the frequency and predictive value of Ig subtypes of a newly discovered serological marker, aCHI3L1, in relation to the development of disease-specific complications or the need for surgery in a large group of adult IBD patients with a prospective follow-up. Additionally, we aimed to investigate[1]: The long-term stability of aCHI3L1 autoantibodies[2]; the relationship between aCHI3L1 formation and the clinical, serological, and genetic characteristics of CD.

MATERIALS AND METHODS

Patient population

We performed a cohort study among adult CD and UC patients in a tertiary IBD referral center in Hungary (Department of Gastroenterology, Institute of Internal Medicine, University of Debrecen). The baseline clinical data regarding this cohort

overlap with those of our previous studies[29,31,32]; however, we present an extended follow-up time with nearly 2.5 years and re-evaluation of the outcomes. We used the same step-by-step thorough statistical evaluations; therefore, the text appeared to reproduce the information reported in detail elsewhere. The clinical characteristics of patients at diagnosis are presented in Table 1 in detail. The original CD cohort consisted of 271 patients, however 5 of them should have been excluded from the statistical analysis after performing total Ig measurements due to decreased or absent serum IgA levels equivalent of international consensus laboratory criteria of severe selective IgA deficiency (serum IgA level less than 7 mg/dL or 0.07 g/L - the lowest detectable limit established by most of the laboratories)[33-35].

The diagnosis of IBD was based on a combination of clinical, biochemical, stool, endoscopic, cross-sectional imaging, and histological investigations equivalent to the recently published European Crohn's and Colitis Organization (ECCO) and the European Society of Gastrointestinal and Abdominal Radiology guidelines[23,36]. Disease phenotype (age at onset, duration, location/extent, and behaviour including perianal involvement as a modifier) was determined according to the Montreal Classification[37]. Blood samples and detailed clinical phenotypes were captured at inclusion. Clinical data were determined by a thorough review of the patients' medical records, which were collected in a uniform format. Medical records documenting the disease phenotype, presence of EIM, frequency of flare-ups (frequent flare-up: > 1 clinical relapse/year)[38], medication use (e.g., steroid, immunosuppressive and/or biological use at any time), need for IBD-related surgery (resection and surgical intervention due to perianal disease complications in CD and colectomy in UC), and IBD-related risk factors (the presence of familial IBD, smoking habits and previous appendectomy) were retrospectively analysed for the period prior to prospective follow-up. At enrolment, clinical disease activity was calculated according to the Harvey-Bradshaw Index (HBI)[39] for CD and the Partial Mayo Score (PMS) for UC[40]. In this study, we followed the ECCO guidelines[41] and defined HBI ≤ 4 as a state of remission and ≥ 5 as a state of active disease. In case of UC, PMS ≤ 3 was defined as a state of remission and PMS > 4

as a state of active disease. Endoscopic activity was determined according to the Simple Endoscopic Score for Crohn's Disease (SES-CD) for CD[42] and the endoscopic component of the Mayo score for UC[43]. SES-CD defines endoscopic activity ≥ 3 points and inactive disease ≤ 2 in CD; meanwhile in UC, state of active disease is defined as an invasive PMS ≥ 1.

Phenotypical characterization of IBD patients during prospective follow-up
A total of 261 of 266 patients with CD and 183 of 187 patients with UC were enrolled in a prospective follow-up study. During regular and extraordinary outpatient follow-up visits and inpatient stays, the treating IBD physicians registered laboratory data, endoscopic and imaging results, disease activity, medical treatment regimens, and information on disease-specific complications and surgeries. In Hungary, a follow-up visit is typically scheduled every six months at a specialized gastroenterology center, which can vary between three and six months depending on the center. The medical and surgical treatment algorithms are aligned and conform to the ECCO guidelines[41,44-47]. The decision regarding the need for surgery and its timing involves a multidisciplinary approach, with the collaboration of gastroenterologists, radiologists, and surgeons. The data that was collected was transferred and saved in a database for the purpose of further evaluation. On October 11, 2016, all patient records and databases were revised and updated with the relevant data points. The patient's follow-up was discontinued if there were no additional records. The median duration of follow-up from diagnosis for CD patients was 143 mo [with an interquartile range (IQR) of 99 to 214], while for UC patients, it was 135 mo (with an IQR of 84 to 213). In CD, complicated disease behaviour is characterised by the presence of stenosis (stricture) or internal penetration (interintestinal fistula, inflammatory conglomerate, and/or abscess(es) formation). A distinction was made between perianal fistulizing disease and internal penetrating disease, and they were evaluated separately. The need for surgery in CD was defined as CD-related abdominal- (resection) or perianal surgery

(oncotomy). Complicated disease behavior in UC was defined as the progression of disease extent or the need for colectomy.

The healthy controls (HCONT) included 86 age- and sex-matched healthy individuals. Of these, 52 were male and 34 were female, with a median age of 28 years at disease onset (range: 24-41). The control group comprised of individuals who were free from gastrointestinal and liver diseases and were obtained from invent Diagnostica GmbH (Hennigsdorf, Germany).

Serological analysis

At enrollment, blood samples were obtained from each participant and were subsequently frozen at a temperature of -80 °C until further testing. All serological assays were conducted in a blinded manner, without prior knowledge of the patient's diagnosis or any other clinical information. To determine the stability of different serologic antibodies, we analyzed samples from the same patient taken at various arbitrary timepoints during the course of their disease. Most patients with CD (n = 165) had at least two serum samples taken and re-tested for all serological antibodies.

Detection of antibodies to CHI3L1 by enzyme-linked immunosorbent assays

Presence of IgG, IgA and secretory (sIgA) (sub)type aCHI3L1 in serum samples of IBD patients (n = 257 in CD and n = 180 in UC) and HCONT (n = 86) were determined by an "in house" enzyme-linked immunosorbent assay technique using recombinant human CHI3L1 as solid-phase antigen. A detailed description of the methodology is available in the authors' previous paper on paediatric IBD[30]. The levels of aCHI3L1 IgG, IgA, or sIgA were read as the ratio of OD_{sample}/OD_{cutoff} (OD_{cutoff}, mean + 3 SD of HCONT) for standardisation. Serum samples with a ratio of ≥ 1 were considered positive[30].

Detection of classic auto- and antimicrobial antibodies and NOD2/CARD15 SNP8, 12, 13 mutations

The detection of classic auto- [anti-neutrophil cytoplasmic antibodies (ANCA), anti-pancreatic autoantibodies (PAbs), antiphospholipid antibodies (APLAs)], and antimicrobial [anti-*Saccharomyces cerevisiae* antibodies (ASCA), anti-OMP PlusTM IgA] antibodies has been described in detail by the authors in previous publications[29,31,32]. NOD2/CARD15 SNP8, SNP12, and SNP13 genotyping was performed previously[48] in patients with CD (n = 235). A detailed description of the methodology has been provided in the cited manuscript.

Ethical permission

The study was granted ethical approval by the regional and national committees for research ethics (DE RKEB/IKEB: 4773-2017; DE OEC RKEB/IKEB: 3515-2011; TUKEB: ad.3880/2012-EKU). All patients were informed about the study's details and provided their informed consent.

Statistical analysis

We utilized GraphPad Prism 6 (San Diego,1 CA) and SPSS 22.0 (SPSS Inc., Chicago, IL) for statistical analysis. The study's statistical methods were reviewed by Elek Dinya, from Semmelweis University's Institute of Health Informatics, Development, and Further Training. The normality of variables was evaluated using the Shapiro-Wilk's W test. The continuous variables were presented in the form of means ± SD or medians and IQR based on their consistency. To determine distinctions between the IBD and HCONT groups, as well as among subgroups of patients with IBD, the following statistical methods were employed: Categorical variables were compared using Fisher's exact test or χ^2 test with Yates correction, depending on the situation. To compare continuous variables, we used Student's t-test, one-way analysis of variance (ANOVA), Mann-Whitney's U test, or Kruskal-Wallis H test with post hoc analysis (Dunn's multiple comparison test). The Spearman's nonparametric rank correlation test was used to determine correlations.

The Kaplan-Meier survival curves were used to examine the impact of categorical clinical variables or serologic antibodies on unfavourable disease outcomes during follow-up. To determine the association, the log-rank test or Cox regression analysis was conducted in time-dependent models. Cox regression analysis and a backward elimination method were used to investigate the predictive value of serological antibodies and all clinical variables as cofactors for the development of a complicated disease course in CD patients. The associations were expressed as odds ratio (OR) and hazard ratio (HR) accompanied by 95% confidence interval (CI). A probability value of less than 0.05, indicating a 2-tailed test, was deemed statistically significant.

RESULTS

Frequency of aCHI3L1 antibodies in IBD

The frequencies of different Ig (sub)types of aCHI3L1 antibodies in patients with IBD and controls are summarised in Table 2. The occurrence of IgA-type aCHI3L1 was higher in CD than in UC (29.2% vs 11.1%) or HCONT (2.83%; $P < 0.0001$ for both). The sIgA subtype aCHI3L1 was more prevalent among both CD and UC patients than in the HCONT group (39.3% and 32.8% vs 4.65%, respectively; $P < 0.0001$). No differences were found in IgG antibodies in either group. Information on the sensitivity and specificity of aCHI3L1 antibodies as diagnostic tests is available in Supplementary Table 1.

The stability of aCHI3L1 antibodies in CD and its correlation with the overall disease duration

In a subset of CD patients (n = 165), more than one serum sample per patient was available at different time points to assess the stability of antibody levels and statuses (positive or negative for the respective antibodies) over time. The median time between sample procurements was 44.4 mo (IQR: 23.3-66.6). Comparing the results of the first and last available serum samples using the Wilcoxon test, no significant differences in aCHI3L1 antibody levels were found for any Ig type. The aCHI3L1 antibody status was

equally stable for all Ig types, with an average of < 10% of patients showing a change in antibody status over time. The IgA type antibody status showed a slightly greater change (20.6%). The stability data for different serological antibodies are summarised in Table 3. There were no clinically significant differences in the evaluated study endpoints when considering stability data. Further studies with sequential sampling and serial measurements both before and after the study endpoint events are needed for a more detailed evaluation of aCHI3L1 antibody stability in CD, which is beyond the limits of the current work.

No correlation was found between antibody status and clinical or endoscopic disease activity (as measured by either HBI or SES-CD) or disease duration at the time of sample acquisition (Supplementary Table 2). aCHI3L1 IgG levels were not analysed further because of the low prevalence of antibody positivity in the CD cohort. IgA and sIgA aCHI3L1 antibody levels did not differ according to clinical activity (P = 0.385 and 0.6830, respectively). Nevertheless, the actual CDAI, HBI, and SES-CD indices were also not correlated with IgA and sIgA aCHI3L1 antibody levels, as determined by Spearman correlation analysis (Supplementary Table 3). The levels of aCHI3L1 antibodies were not associated with disease duration (Kruskal-Wallis test). Association of aCHI3L1 antibody formation with clinical, serological, and genetic characteristics of CD. A summary of the results is presented in Table 4.

Correlation with clinical features

The presence of both IgA and sIgA aCHI3L1 antibodies was associated with colonic involvement (IgA: 35.5% *vs* 7.7%, P < 0.0001; sIgA: 43.5% *vs* 26.9%, P < 0.038) in CD. The occurrence of aCHI3L1 sIgA was higher in patients with complicated disease behavior at diagnosis compared to those with uncomplicated disease behavior (57.1% *vs* 36.0%, P = 0.009). Additionally, a higher prevalence of IgA-type aCH3L1 was observed in patients with frequent relapses during the course of CD than in those without (46.9% *vs* 25.7%, P = 0.005).

Association with serological and genetic characteristics

Significant associations were found between the presence of certain IgA antimicrobial (ASCA, anti-OMP), antiphospholipid (anti-PS/PT), and autoantibody [anti-glycoprotein 2 (GP2)] types, and aCHI3L1 IgA and sIgA positivity. The following correlations were found when examining these antibodies. Anti-ASCA, anti-OMP, and anti-PS/PT IgA showed a significantly higher prevalence of anti-CHI3L1 antibody IgA and sIgA in patients positive for the above-mentioned antibodies. For anti-GP2 positive patients, this association was only seen for anti-CHI3L1 sIgA, with no significant difference for anti-CHI3L1 IgA. However, anti-GP2 positive patients had the highest prevalence of anti-CHI3L1 sIgA. In our study, the prevalence of different aCHI3L1 antibodies was not associated with the presence or absence of major NOD2/CARD15 mutations (data not shown).

Significance of aCHI3L1 antibodies in predicting the development of a complicated disease course in CD

In time-dependent univariate models, a trend was observed between the presence of IgA or sIgA aCHI3L1 and the time to development of a complicated disease behaviour with borderline significance (Figure 1).

Considering that the prevalence of antibodies against CHI3L1 was significantly higher in patients with colonic involvement (aCHI3L1 IgA positivity: L2/L3 35.5% *vs* L1 7.7%; sIgA: 43.5% *vs* 26.9%), we performed subgroup analysis. Kaplan-Meier analysis was used to analyze a group of patients with CD who had concomitant presence of pure inflammatory luminal disease (B1) and colon involvement at the time of diagnosis. The results showed that positivity for IgA or sIgA type aCH3L1 predicted faster progression towards a complicated disease course in time-dependent models (Figure 2). The association was no longer present after merging the subgroups of different disease locations (as shown in Figure 1). This relationship is shown in Tables 5-8, which summarizes the time-dependent univariate subgroup analyses for colon involvement.

The results of the Kaplan-Meier and univariate Cox regression analyses regarding associations between clinical factors and the development of complications are summarised in Tables 5-8. The risk of the development of internal penetrating and/or stenotic complications was associated with location, including extensive disease, which remained significant in the subgroup of patients with colonic involvement. In contrast, colon involvement was a protective factor (Tables 5-8). Based on this, the occurrence of perianal penetrating complications in P0 patients was associated with early onset disease, colon involvement, and frequent relapses. Within the subgroup of patients with colonic involvement, this association remained significant in those with frequent relapses (Tables 5-8).

Resective surgery was not associated with any of the clinical factors studied, neither in the B1 patient group nor in patients with colonic involvement. Patients with a previous surgery were more likely to undergo a new operation in patients who relapsed frequently, which was also true for the subgroup with colonic involvement (Tables 5-8).

In multivariate Cox regression analysis, the subgroup of B1 patients with colonic involvement, IgA-type aCHI3L1 positivity independently predicted a faster progression towards a complicated disease course in time-dependent models (HR = 1.67; 95%CI: 1.02-2.71; P = 0.041). Among the clinical factors, the same was observed for extensive disease (HR = 1.83; 95%CI: 1.12-2.99; P = 0.016). None of the other clinical factors and or serologic antibodies studied were independently associated with the development of unfavourable disease outcomes (Tables 5-8).

DISCUSSION

CHI3L1 (also known as human cartilage glycoprotein-39 or YKL-40 or 40-kDa mammary gland protein) is a 40 kDa heparin-, chitin-, hyaluronan-, and collagen-binding glycoprotein expressed by various cell types such as synovial cells, chondrocytes, endothelial cells, smooth muscle cells, hepatic stellate cells, cancer cells, fibroblast-like cells, macrophages, neutrophils, and colonic epithelial cells (CEC)[21,49]. It belongs to the 18-glycosylhydrolase family and lacks chitinase activity owing to an

amino acid change in the catalytic region[21,49-51]. Besides oncogenesis[49] previous studies proposed its role in numerous systemic-[52-57], respiratory-[58-69], digestive-[21,27,28,70-78], cardiovascular-[79-85], endocrine-[80,84,86,87], neurological-[88,89], urinary-[90-94], skeletal-[84,95], autoimmune-[96], and dermatological[97,98] conditions with features of either acute or chronic inflammation.

According to a comprehensive review by Tizaoui et al[99], 14 studies were conducted on the role of CHI3L1 in IBD. Our literature search identified only three additional papers from 2019 on the topic in PudMed[100-102]. Elevated serum CHI3L1 levels have been reported in most of those studies; however, there are controversial results regarding their correlation with disease activity[99]. In a study by Vind et al[103], serum CHI3L1 levels tended to be elevated in 30% of patients with CD, even when the disease became inactive. In UC, they correlated well with the laboratory and clinical markers of disease activity. These distinctions can be attributed to the different characteristics of inflammation in CD and UC (transmural vs sub]mucosal)[104].

Most of the studies were cross-sectional without a detailed prospective follow-up; therefore, it is very difficult to draw any conclusions regarding the usefulness of serum CHI3L1 in monitoring disease activity, even in UC alone. More promising information is available on the use of CHI3L1 as a fecal biomarker in paediatric[27] and adult[28] IBD cohorts as well, since it showed a good correlation with endoscopic activity in both CD and UC, with comparable performance to fecal calprotectin.

CHI3L1 was first described as an autoantigenic target in rheumatoid arthritis in 1997[105,106] and in IBD in 2019[30]. The latter was confirmed by the authors of this manuscript[30]. To date, autoantibodies against CHI3L1 have only been detected in these two entities[30, 107-110]. aCHI3L1 were evaluated in only one cross-sectional study in IBD on a paediatric population (CD: n = 110, UC: n = 95)[30]. To our knowledge, this is the first prospective study on aCHI3L1 as a biomarker with predictive potential in an adult cohort of IBD patients. Similar prevalence rates were reported by Deutschmann et al[30] for IgA and sIgA aCHI3L1 in our adult IBD cohort. IgG-type positivity was more prevalent in children. In the pediatric population, ileal-, whilst in adults colonic

involvement was associated with higher antibody positivity. The discrepancies between the two studies may be attributed to presumably different immune responses owing to the different maturity of the immune system, age-related changes in the microbiome, and differences in the onset and course of IBD in children[111].

It is suspected that, unlike UC, CD patients are more prone to developing a tolerance break against CHI3L1 due to persistent elevation of CHI3L1 independent of disease activity[30,103,112]. This hypothesis is also supported by our findings regarding the differences in aCHI3L1 autoantibody prevalence among paediatric[30] and adult IBD subgroups. In our study, similar to CHI3L1, no correlation was found between aCHI3L1 positivity and disease activity in CD patients. Regarding autoantibodies against CHI3L1, we did not find any association between aCHI3L1 formation and response to therapy, which is consistent with previous IBD studies. Unlike other disorders, the levels of serum auto- and antimicrobial antibodies in IBD remain largely unchanged regardless of the inflammatory burden or the effects of anti-inflammatory therapy or surgical resection. Unlike other autoimmune diseases such as ANCA-associated vasculitides, classic and newly discovered autoantibodies (*e.g.*, ANCA, PAbs, APLAs, aCHI3L1), and antimicrobial antibodies (ASCA, anti-OMP PlusTM IgA) have no established role in monitoring the efficacy of IBD treatment[18].

The prevalence of IgA-type aCHI3L1 antibodies was significantly higher in CD patients than in UC patients and HCONT. A similarly elevated prevalence was also observed for the sIgA subtype, with the difference that this was independent of IBD subtype. In our previous studies, significant differences in favour of IgA and sIgA (sub)types were also detected in the evaluated classic auto- (PAB, ACA, aPS/PT) and antimicrobial antibodies (ASCA, anti-OMP) in IBD[29,31,113,114]. In parallel, in the aforementioned studies, we found associations between complications in CD for IgA and not IgG types[29,31,113,114]. The mucosal immune system in the gut plays a central role in IgA antibody production. In previous studies, the increased IgA_2 subtype ratio and concomitant presence of the secretory component (SC) were considered as evidence for a mucosal origin of IgA secretion[115,116]. Our research group previously reported the

mucosal origin of ANCA IgA in a group of patients with liver cirrhosis indirectly by demonstrating the presence of SC[117]. Similarly, we were able to demonstrate the mucosal origin of ASCA IgA in CD using flow cytometry (unpublished results, first presented at the 12th Congress of the ECCO)[114]. In the present study, we also demonstrated the presence of aCHI3L1 antibodies of mucosal origin in IBD. The higher prevalence of aCHI3L1 IgA in CD than in UC may be due to the different nature of mucosal inflammation (degree of intestinal wall involvement more pronounced in CD - transmural *vs* mucosal)[104], as well as its localisation. In CD, serum total sIgA levels were also significantly higher (median: 51 *vs* 29 µg/mL; $P < 0.001$), which was more pronounced in the presence of certain antimicrobial antibody IgA types[114]. This phenomenon, namely the tendency for IgA-dominant antibody formation in IBD, is thus not specific for antibodies produced against CHI3L1 and may reflect an immune response to an enhanced microbial load. This hypothesis is also supported by the significant associations between the presence of certain IgA antimicrobial (ASCA, anti-OMP), antiphospholipid (anti-PS/PT), and autoantibody (anti-GP2) types and aCHI3L1 IgA and sIgA positivity found in our study. Meanwhile, IgA-type autoantibodies are viewed as an indication of an immune response to enteric antigens, which in other ailments has been linked to a rise in BT[29]. BT refers to the increased uptake of bacteria or bacterial products from the intestinal lumen into systemic circulation[118,119]. In IBD, the combination of structural and functional intestinal barrier damage due to chronic intestinal inflammation, reduced intestinal mucosal defences, altered microbiome, and dysregulated immune response to specific bacterial components is thought to lead to chronic BT. The uncontrolled uptake of various luminal bacteria and/or bacterial products exacerbates the local and systemic proinflammatory processes already underway, thereby contributing to disease exacerbation and complications[104,120-125]. However, it remains unclear whether these alterations can directly contribute to the development of IBD or whether they are just an epiphenomenon.

Roggenbuck *et al*[126] first hypothesised the pathophysiological role of anti-pancreatic antibodies in CD in inducing and maintaining increased BT[127]. Following loss of

tolerance to GP2, plasma cells synthesise anti-GP2 IgA, which is transported by intestinal epithelial cells into the intestinal lumen. Secreted anti-GP2 IgA can form a bridge between GP2-derived pancreatic GP2-opsonized FimH-positive bacteria and membrane-bound GP2 on the surface of M cells. These processes lead to microbial overload of the mucosa due to increased transcytosis in CD, which amplifies the inflammatory processes in the gut[29]. In the case of CHI3L1 and antibodies against it, very similar mechanisms can enhance BT if an increased tendency to form antibodies against this glycoprotein develops as a consequence of loss of CHI3L1 tolerance.

Under normal conditions, CHI3L1 levels are extremely low, but under inflammatory conditions, they are also expressed at elevated levels in CEC and macrophages[51,128]. Mizoguchi[129] demonstrated an upregulated expression of CHI3L1 in the lamina propria and CECs of IBD patients and animal models. This upregulation, along with the loss of tolerance to CHI3L1, enhances the adhesion and invasion of certain commensal and/or pathogenic bacteria *via* carbohydrate binding [chitin binding protein (CBP) 21] or homologous motifs (for example, ChiA)[21,30]. Significantly higher prevalence of IgA and sIgA (sub)type antibodies against CHI3L1 in patients with colonic involvement (aCHI3L1 IgA positivity: L2/L3 35.5% *vs* L1 7.7%; sIgA: 43.5% *vs* 26.9%) along with a positive predictive value for complicated disease course in only this subgroup of CD patients could be considered as an indirect confirmation of colonic origin of these antibodies. Further studies to assess the presence of aCHI3L1 antibodies in different parts of the gut mucosa of IBD patients are needed to confirm this hypothesis.

CHI3L1 is recognized as a biomarker of fibrosis due to its contribution to tissue remodeling and fibrosis by hindering the degradation of type I collagen and hyaluronic acid, and by regulating the enzymatic activity of matrix metalloproteinases, ultimately affecting cell adhesion and migration[39,40,101]. Previously it has been shown by Erzin *et al*[130] that increased serum CHI3L1 was associated with intestinal stricture formation in CD patients. Here, we present confirmatory results that autoantibodies against CHI3L1 may be involved in stricture and fistula formation in CD patients. In paediatric IBD patients with complicated disease behaviour, a trend toward higher prevalence of IgA

and sIgA (subtype) aCHI3L1 was observed (38.5% *vs* 22.0% and 53.8% *vs* 35.6%, respectively)[30]. The lack of statistical significance in this case can be explained by the relatively low number of patients in these subgroups of patients (aCHI3L1 IgA- and sIgA-positive patients with complicated disease behaviour: n = 5 and 7, respectively). In adults aCHI3L1 sIgA positivity was associated with complicated disease behaviour at diagnosis (OR = 2.37, 95%CI: 1.26-4.48). The results of cross-sectional single-time point studies should be interpreted with caution[18]. After more than sixty years longitudinal follow-up studies are still considered rare in the field of IBD serology[18]. This study offers, for the first time, longitudinal and prospective data regarding the predictive capabilities of aCHI3L1 antibodies in identifying disease-specific complications and surgical requirements. The presence of IgA or sIgA aCH3L1 antibodies in patients with both inflammatory luminal disease and colon involvement at the time of diagnosis indicates a faster progression towards a complicated disease course in time-dependent models. This association remained significant in multivariate models as well in case of IgA type. This observation, together with our previous results, highlights the need to consider location in addition to antibody class when predicting disease progression. Based on both pathogenic and clinical heterogeneity of IBD, instead of "the one size fits all" model, an increasing amount of data as this point towards the need for the development and application of prognostic matrix models[131]. As no data are available on the prognostic potential of aCHI3L1 antibodies in IBD, a literature search was performed in this regard in RA, the only other entity where the presence of aCHI3L1 was described. Based on these findings, CHI3L1 was not considered a long-term prognostic biomarker for RA[99]. As for autoantibodies against this particular target, no data are available on the predictive potential of RA[107-110].

Similar to other diseases, where CHI3L1 is involved in pathogenesis, CHI3L1 could also serve as a potential therapeutic target in IBD. Kawada *et al*[132] found that by inhibiting CHI3L1 with anti-CHI3L1 antibodies or CHI3L1-specific small interfering RNA, the adhesion of *E. coli* cells overexpressing CBP to CECs was reduced. Ongoing clinical trials are evaluating the use of antibodies against the active region of CHI3L1,

and recombinant nasal CHI3L1 (Org39141) to reach tolerance in RA[49]. In conclusion, the integration of IgA and sIgA against CHI3L1 with newly applied CD-specific antibodies (*e.g.,* anti-GP2) in laboratory screening of patients suspected of having CD may improve the sensitivity and specificity of the screening process. Further research is required to determine whether antibodies against CHI3L1 have a diagnostic potential and whether elevated aCHI3L1 levels are only a concomitant feature of CD or contribute to the development and presentation of IBD[30].

A drawback of our study was that the prevalence of antibodies against CHI3L1 was not high. Moreover, these antibodies do not play an exclusive role in prognostics. Although the number of cohorts was significant, we could detect significant differences only in a subgroup of patients, so it would be important to investigate these parameters in a larger group of patients. It would be necessary to establish a validation cohort, also of adults, with a large number of cases, followed prospectively, whose results could confirm or refute our findings.

CONCLUSION

In conclusion, we observed increased IgA autoantibody production in CD patients in an adult IBD cohort by testing autoantibodies against a novel neutrophil autoantigen target, CHI3L1. Antibodies against CHI3L1 showed long-term stability over the course of the disease. It was associated with the clinical phenotype of the disease and identified patients with colonic involvement, complicated disease course, or ASCA IgA and/or anti-OMP positivity. It was correlated with a more rapid onset of a complicated disease (B2 and/or B3) during disease progression in the subgroup of CD patients with no complications at diagnosis (B1) and with colonic involvement. In conclusion, the combined consideration of antibody classes and location in predicting disease progression may revolutionise IBD serology. IgA-type anti-CHI3L1 antibodies may interfere with innate immunity against intestinal bacteria or may reflect an immune response against microbial overload in the intestinal barrier.

ARTICLE HIGHLIGHTS

Research background

Chitinase 3-like 1 (CHI3L1) is a 40 kDa heparin-, chitin-, hyaluronan-, and collagen-binding glycoprotein expressed by various cell types, including fibroblast-like cells, macrophages, neutrophils, and colonic epithelial cells (CEC). Under normal conditions, CHI3L1 levels are extremely low; however, under inflammatory conditions, they are also expressed at elevated levels in CEC and macrophages. Upregulated expression of CHI3L1 in the lamina propria and CECs has been described in patients with inflammatory bowel disease (IBD). This upregulation, along with the loss of tolerance to CHI3L1, can enhance the adhesion and invasion of certain commensal and/or pathogenic bacteria *via* carbohydrate binding (chitin binding protein 21) or homologous motifs (for example, ChiA). Microbial overload, along with mechanical and/or functional derangement of the gut barrier, can lead to the uncontrolled uptake of various luminal bacteria and/or bacterial products. As a result, enhanced bacterial translocation exacerbates local and systemic proinflammatory processes already underway, thereby contributing to disease exacerbation and complications.

Research motivation

Given the current state of knowledge, the precise function and mechanism of CHI3L1 in the development of IBD, loss of tolerance to this antigen, formation of immunoglobulin A (IgA) autoantibodies against it, and their role in the pathogenesis of IBD remain elusive. Concomitantly, there is an unmet need to identify new biomarkers to identify patients with IBD with a complicated disease course.

Research objectives

To evaluate the predictive value of different immunoglobulin subtypes of the novel serological marker, anti-CHI3L autoantibodies (aCHI3L1), in terms of their ability to define the disease phenotype, therapeutic strategy, and long-term disease course in a group of adult IBD patients with prospective follow-up.

Research methods

A total of 257 patients with Crohn's disease (CD) and 180 patients with ulcerative colitis (UC) from a tertiary IBD referral center in Hungary (Division of Gastroenterology, Department of Internal Medicine, Faculty of Medicine, University of Debrecen) were tested for IgG, IgA, and secretory IgA (sIgA) type aCHI3L1 using enzyme-linked immunosorbent assay with recombinant CHI3L1, along with 86 healthy controls.

Research results

Enhanced formation of IgA and sIgA (sub)-type against CHI3L1 was first detected in adult patients with CD, which was associated with the clinical phenotype in a tertiary referral IBD centre in Hungary. This observational study presents, for the first time, longitudinal and prospective results on the predictive ability of aCHI3L1 antibodies to identify disease-specific complications and surgical requirements, which are critical for patient care. The presence of IgA or sIgA aCH3L1 antibodies in CD patients with both inflammatory luminal disease and colon involvement at the time of diagnosis indicates a faster progression towards a complicated disease course in time-dependent models.

Research conclusions

CHI3L1 is a newly discovered neutrophil autoantigenic target in IBD. By taking into account the classes of antibodies and utilizing location-based predictions, serology in IBD may undergo a significant transformation in the future.

Research perspectives

Based on both the pathogenic and clinical heterogeneity of IBD, instead of the "the one size fits all" model, an increasing amount of data points towards the need for the development and application of prognostic matrix models. Therefore, identifying new biomarkers for this purpose is of utmost importance. A significantly higher prevalence of IgA and sIgA (sub)type antibodies against CHI3L1 in patients with colonic

involvement, along with a positive predictive value for complicated disease course in only this subgroup of CD patients, could be considered as an indirect confirmation of colonic origin of these antibodies. Further studies to assess the presence of aCHI3L1 antibodies in different parts of the gut mucosa of IBD patients are needed to confirm this hypothesis. Unravelling the role of CHI3L1 and aCHI3L1 autoantibodies in IBD pathogenesis can help identify new potential therapeutic targets for IBD.

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