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ORIGINAL RESEARCH

Prevalence and Clinical Relevance of Anti-FcεRI Autoantibody in Crohn's Disease

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Background: Mast cells can be activated in various ways and were shown to be involved in the development of Crohn's disease (CD). The diagnosis of CD is still challenging, and seeking novel biomarkers is a worthwhile endeavor.

Methods: An indirect enzyme-linked immunosorbent assay (ELISA) was successfully established for semi-quantitative detection of IgG anti-FcεRI in serum using human FcεRIα coated microplates and an enzyme-labeled anti-human IgG as secondary antibodies. The optimal working conditions were explored, followed by conducting the method evaluation. The serum samples and clinical data of 117 CD patients and 75 healthy controls were collected. IgE was measured by the rate turbidity turbidimetry; IgG anti-IgE and IgG anti-FcεRI were detected by ELISA. IgG anti-pancreatic antibody (PAB) and anti-Saccharomyces cerevisiae antibody (ASCA) were determined by indirect immunofluorescence assay. Data were analyzed concerning the clinical characteristics.

Results: IgG anti-FcεRI was an effective marker for CD ($P < 0.001$), but IgE and IgG anti-IgE ($P = 0.089$, 0.219, respectively) were not. There was a positive correlation between anti-IgE and anti-FcεRI ($R = 0.380$, $P < 0.001$). Anti-FcεRI positive patients behaved with higher disease activity [OR: 1.478 (1.200~1.821), $P < 0.001$], but were less likely to be located in L4 among Montreal classification [OR: 0.253 (0.077~0.837), $P = 0.024$]. Existing indicators, PAB and ASCA, behaved with high specificity (both > 95%) with low sensitivity (both < 30%). The combination of anti-FcεRI with existing markers significantly improved the diagnostic efficiency [AUC: 0.879 (0.831~0.928)].

Conclusion: An ELISA for the detection of anti-FcεRI was established and validated, which may contribute to facilitating research on Crohn's diseases. Anti-FcεRI positive CD patients were associated with higher disease activity indices, suggesting its potential value in the diagnosis and management of CD.

Keywords: Crohn's disease, anti-FcεRI, biomarkers, autoantibodies, mast cells

Introduction

More than 140 years have passed since the first description of mast cells (MCs) by Professor Paul Ehrlich in 1878, and the understanding of MCs has become increasingly comprehensive. As a kind of multifunctional cells mainly located in the mucous membrane and connective tissue, MCs can respond to various stimuli under different physiological and pathological conditions, which play an important role in immune defense, allergy, and various diseases.[1] It is well-known that stimulants are allergens, which bind to specific IgE and cross-link with the high-affinity IgE receptor (FcεRI) on MCs to trigger intracellular signal transduction, causing MCs to degranulate and release a large number of inflammatory mediators.[2] Moreover, there are some autoantibodies in serum, such as anti-FcεRI and anti-IgE antibodies, which can directly or indirectly cross-link FcεRI to activate cells like allergens.[3] This mechanism may explain patients with similar allergic symptoms but no exact allergen, or those with suspected autoimmune diseases but no serological evidence. Chronic urticaria (CU) is the first disease associated with it, and relevant research is also the most in-depth.[4,5]

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Some patients with Crohn's disease (CD) fit the description above. High serum IgE levels are common in CD patients despite the absence of atopic symptoms.[6,7] However, whether the allergy is involved in the pathogenesis of CD is still controversial.[8] In addition to abdominal pain, diarrhea, and weight loss, patients may also develop complications, such as abscess, anal fistula, and intestinal obstruction, and unexplained extraintestinal manifestations, such as rash and arthritis. The above results indicate that MCs may be involved in the pathogenesis of CD, which is also confirmed by other reports. For instance, studies have shown that the number of MCs in the intestine increased remarkably in CD, especially in the active inflammatory region. They can be inappropriately activated, leading to the release of multiple proinflammatory mediators.[9,10]

Recent studies have highlighted emerging pathogenetic models of CD that focus on the interactions between genetic factors, immune dysregulation, and gut microbiota imbalance. Certain pathogenic bacteria including adherent-invasive Escherichia coli (AIEC) may trigger immune responses in the progress of CD.[11,12] Mutations in genes such as NOD2, ATG16L1, and IRGM can affect immune function and autophagy.[13–15] MCs may play a role in these immune responses, potentially interacting with genetic and microbial factors to influence inflammation in CD. Besides, recent studies have highlighted the melanocortin system as a potential modulator of immune responses in CD.[16] Although the melanocortin system and MCs are not directly related, they may have some interaction in the context of inflammation and immune responses in CD patients.

As mentioned, CD's etiology involves the disequilibrium of intestinal microbiota and autoimmune, evidenced by serum-specific antibodies like anti-pancreatic antibodies (PAB) and anti-Saccharomyces cerevisiae antibodies (ASCA).[17] They have been proven to be reproducible tools to assist clinicians in CD diagnosis, but efficiency varies with race[18] and insufficiency still exists in the Chinese crowd. It is of great significance to study the diagnostic value, and pathogenic importance of existing serological markers and to discover novel and better serological markers.

The detection method of IgE is relatively mature, and that of anti-IgE autoantibody has been reported.[19] Therefore, the present study will report an enzyme-linked immunosorbent assay (ELISA) for detecting anti-FcεRI autoantibody and evaluate the levels of serum IgE, anti-IgE, anti-FcεRI autoantibodies in CD patients from China, and analyze their performance alone or in combination with conventional autoantibodies for CD diagnosis, as well as the association with disease phenotypes.

Materials and Methods

Reagents

Recombinant human FcεRIα protein (Cat#6678-FC-050) from R&D systems; Goat anti-human IgG Fc horseradish peroxidase (HRP) conjugated antibody (Cat#ab97225), HRP anti-human IgE (Cat#ab73901) and native Human IgG protein (Cat#ab91102) from Abcam; Native human IgE from myeloma plasma (Cat#401152) from Merck Millipore; Coating buffer (Cat#00-0044-59) from eBioscience; 10 mM phosphate buffer saline (PBS), bovine serum albumin (BSA, Cat#4240) from BioFroxx; Tween-20 (Cat#P7949) and (3,3',5,5')-tetramethylbenzidine (TMB, Cat#T0440) from Sigma-Aldrich; 2N Sulfuric acid (Cat#A300SI-212) and total IgE kit (Cat#14-4509-08) from Fisher Scientific; Human IgE kit (Cat#OQTG15) from Siemens; Chronic inflammatory bowel disease antibody (PAB and ASCA) test kit from Euroimmun (Cat#1380-1005-4).

Equipment

Immuno 96 microwell solid plates (Cat#42592) from Corning; Full wavelength microplate reader from Molecular Devices; Electro-thermal incubator from Shanghai Jing Hong Laboratory Instrument Co. Ltd; ImmunoCAP 250 from Phadia; BNII automatic immuno scatter turbidimeter from Siemens; E502 fluorescence microscope from Nikon.

Sample Types

Used for homemade quality control products: a total of 75 CU patients, 33 males and 42 females, aged 18–77 years, with an average age of (42.5 ± 17.5) years, were selected from the Department of Dermatology.

Testing objects: Seven antibodies were tested, which required at least 70 samples in the case group according to the "10 events per variable" (10 EPV) rule.[20] The CD group was prioritized because the antibodies might act as disease triggers. Although the size of the control group was limited by resource and time constraints, data reliability was maintained through strict quality control. Ultimately, serum samples were obtained from 117 CD patients, and 75 healthy controls (HC, with normal examination items randomly selected from the physical examination center). The CD group was defined by: 1) using standard laboratory tests, radiological findings, and endoscopic criteria according to the 2016 Third European Evidence-based Consensus on the Diagnosis and Management of Crohn's Disease;[21] and 2) excluding patients with severe organic diseases such as malignancies, acute myocardial infarction, or extensive cerebral infarction. The control group excluded individuals with any of the following: 1) a history of gastrointestinal diseases; or 2) kidney disease, liver disease, pregnancy, or autoimmune diseases. Besides, CD was further categorized according to the Montreal classification.[22] All samples were collected from Shanghai General Hospital between February and December 2020. The serum was frozen at −80°C until testing. The clinical records of each patient were reviewed by one member of the experimental group and two clinicians to confirm a diagnosis of CD and document the clinical data. For CD, the Montreal classification was used as previously described.[22] The disease activity was calculated according to the Harvey-Bradshaw Index (HBI).[23] The study was approved by the Medical Ethics Council of Shanghai General Hospital (No: 2020SQ027). All participants were informed about the purpose of the study and provided written informed consent in accordance with the Declaration of Helsinki. Individual medical histories, laboratory and clinical data at the time of blood sampling were recorded in database files and were gathered anonymously for research purpose.

Establishment of Indirect ELISA Method for Detection of FcεRI

Effect of Heating on IgE and Anti-FcεRI

It is worth noting that the presence of free IgE in serum may interfere with the reaction as it is also capable of binding to FcεRI, ultimately resulting in false positive results. Therefore, before investigation, serum was heated at 56°C for 30 min to denature IgE and inactivate complement. To clarify the effect of heating on IgE and anti-FcεRI, the serum with known IgE concentration detected by ImmunoCAP was divided into two parts, one of which was heated at 56°C for 30 min. Experiments were performed on the same microplate coated with human FcεRI. Heated or unheated serum was added and incubated at 37°C for 1 h. After washing the plates, HRP anti-human IgG was added to detect IgG anti-FcεRI, or HRP-anti-human IgE was added to detect IgE.

Assay Procedure

Plates were coated with recombinant human FcεRIα protein in coating buffer (2.5 μg/mL, 100 μL/well) and incubated overnight at 4°C, followed by washing away excess antibodies. Then, microplates were rinsed 6 times with washing buffer (PBS/0.05% Tween-20, 300 μL/well), and plates were washed and blocked with PBS/3% BSA (200 μL/well) at 37°C for 2 h. Serum samples were pretreated with heat to inactivate IgE. During heating, the samples were shaken and mixed every 10 min to avoid the formation of protein condensates. Plates were washed and incubated with the serum above (diluted with PBS/1% BSA, 100 μL/well) at 37°C for 1 h. Then, plates were washed and incubated with the goat anti-human IgE HRP-conjugated antibody (diluted with PBS/1% BSA, 100 μL/well) at room temperature for 45 min. After washing, the TMB substrate solution (100 μL/well) was added. After 10 min, 2N H_2SO_4 (50 μL/well) was used to stop the HRP reaction, followed by measuring the optical density (OD) at 450 nm using a 96-well microplate reader.

Prepare Quality Control and Reference Products

The serum from CU patients was preliminarily screened by self-built ELISA. According to the test results, the serum samples were divided into three levels: low, medium, and high. 10 samples from each level were randomly selected for mixing to obtain low, medium, and high quality controls or positive controls, respectively. The serum from healthy subjects was preliminarily screened to obtain 10 cases of low anti-FcεRI serum, which were mixed in equal volume and used as negative controls after verification by the above method. The quality control and control products were packed in 25 copies each, frozen at −80°C, and remelted at room temperature before testing.

Optimization of Detection Conditions

The quality control serum, negative control serum, and HRP anti-human IgG were diluted at different concentrations, and the optimal dilution ratio of serum and enzyme-labeled antibody was selected using the checkerboard titration.

Validation of Anti-FcεRI ELISA

The new anti-FcεRI assay is a semi-quantitative ELISA. Inter- and intra-assay variability tests were used to determine the accuracy of the assay. Detecting human IgG which has a structure similar to the target molecule to evaluate specificity. Multiple dilutions of quality control serum were taken, and sensitivity was defined as the maximum dilution distinguishable from negative control serum.

Clinical Specimen Detection

Detection of IgE, IgG Anti-IgE and IgG Anti-FcεRI

Total serum IgE was determined by immunoscattering turbidimeter (BN II system, Siemens, USA) and matching reagents. IgG anti-IgE autoantibodies were tested by ELISA according to previous reports.[19] In brief, human IgE myeloma was used as the capture antibody, and goat anti-human IgG-HRP was used as the secondary antibody. Anti-FcεRI was detected using the new method described above. Results were given as optical density (OD). To control for inter-assay variation, blank, positive, and negative controls were included on each plate as a sample solution, positive patient serum, and healthy serum, respectively.

Detection of PAB and ASCA Antibodies

Indirect immunofluorescence was used to detect serum IgG levels of PAB and ASCA antibodies, and the reagents were provided by EUROIMMUN. The substrates in the biochips consisted of the ethanol-fixed pancreas and Saccharomyces cerevisiae. PAB antibody test samples were diluted at 1:10, while ASCA antibody test samples were diluted at 1:1000. All operations were performed strictly following the reagent instructions. The testing results were reported as positive or negative using a fluorescence microscope.

Statistical Analysis

Count data were reported as numbers and percentages (%) and compared using $\chi 2$ tests. Quantitative data were reported as median (25th, 75th percentile). Mann–Whitney *U*-tests were used for group comparison as most variables were not normally distributed. $\chi 2$ test or logistic regression analysis was used to examine the relationship between marker positivity and clinical phenotypes. Univariate analysis was employed to identify potential independent antibodies for diseases. In the univariate association test, variables at $P \leq 0.1$ were all included in the binary logistic regression model. Results were indicated as the odds ratio with 95% confidence intervals [OR (95% CI)]. Receiver operating characteristic (ROC) curves were generated to detect the diagnostic power of each marker. Data were processed and analyzed by SPSS IBM (version 21) or GraphPad Prism (version 5.0). $P<0.05$ was considered statistically significant.

Results

Clinical Characteristics of Study Subjects

There was no significant difference in age and gender between CD patients and healthy controls. At the time of study inclusion, the median duration of disease was 9 (1.0, 41.5) months for CD. More than one-third of CD patients had a terminal ileum (L1) or an ileocolonic disease location (L3). In total, 28.2% of CD patients had at least one extraintestinal manifestation (EIM). The three most common types were arthritis, hepatobiliary manifestation, and an oral ulcer (12.0%, 6.8%, and 5.1%, respectively), and others included skin (1.7%), urinary system (1.7%), and ocular manifestation (0.9%). 47.9% of CD patients developed complications, mainly including intestinal stenosis, obstruction, and perianal lesions, with one case of massive gastrointestinal bleeding. 30 of 117 CD patients (25.6%) had a previous history of related surgery (abdominal resection). Table 1 summarizes the clinical characteristics of patients and controls.

Table 1 Clinical Characteristics of Patients and Controls

	CD n=117	HC n=75	*P* value
Age (yr)[a]	32(25.0, 43.0)	35(26.0, 47.0)	0.111[c]
Female[b]	38(32.5)	31(41.3)	0.212[d]
Duration (mo)[a]	9(1.0, 41.5)		
Location[b]	L1 47(40.2)		
	L2 18(15.4)		
	L3 52(44.4)		
	L4 only 0(0.0)		
	All L4 13		
EIM[b]	33(28.2)		
Complications[b]	56(47.9)		
Surgery[b]	30(25.6)		
Active disease[b]	79(67.5)		

Notes: [a]Median (25th, 75th percentile); [b]N (%). [c]Using Mann–Whitney *U*-test. [d]Using $\chi 2$ Test. Surgery: bowel resection. According to the Montreal classification, HBI ≥5 in CD is defined as a state of active disease. **Abbreviations**: CD, Crohn's disease; HC, healthy control; Location: L1: terminal ileum; L2: colon; L3: ileocolon; L4 only: upper digestive tract only, without involvement of L1, L2 or L3; All L4: upper digestive tract with involvement of L1, L2 or L3; EIM, extraintestinal manifestation.

Establishment of Indirect ELISA for Anti-FcεRI Autoantibody

Effect of Heating on Serum IgE and Anti-FcεRI

Total IgE detected by ImmunoCAP was: normal (2 kU/L), low (45 kU/L), medium (303 kU/L), and high (600 kU/mL). OD values of total IgE (Figure 1A) and anti-FcεRI (Figure 1B) before and after heating were compared. The results showed that IgE lost the biological activity of binding to the receptor after heating, and effectively avoided the false negative of anti-FcεRI detection caused by high IgE levels.

Conditions Optimization

The concentration of recombinant FcεRIα was determined to be 0.5 μg/mL in the pre-experiment. The dilutions of serum were 1:200, 1:300, and 1:400, and the dilutions of HRP anti-human IgG antibody were 1:60,000, 1:80,000, and 1:100,000. The checkerboard method was used for detection. The positive/negative (P/N) ratio of detection signals between low, medium, and high quality control serum and negative control serum was calculated, as shown in Table 2. The P/N ratio was larger, and there was a significant difference between low, medium, and high values. After comprehensive consideration, the optimal dilution of serum and secondary antibody were 1:200 and 1:100,000. At this time, the OD values of the blank well and the highest point were 0.100 and 1.304, respectively.

Precision

The quality control serum (low, medium, and high value) was detected in the same experiment, with each sample 10 times in parallel, and the coefficient of variation (*CV*) within-run was calculated. On different experimental days, the content of anti-FcεRI was detected under the same conditions, and the measurement of each concentration was repeated 3 times for consecutive 7 days. Afterward, the *CV* between batches was calculated. The results of precision measurement are shown in Table 3. The corresponding *CV* of all repeatability experiments was less than 10%, which met the verification standard of immunoassay.

Specificity

Redissolve human IgG according to the instructions and configure it at 10mg/mL. With 10 as the dilution factor, dilute 7 times until the final concentration is 1ng/mL. Human IgG samples with different concentrations were prepared as the samples to be tested according to this ELISA method. The results show that the signal value of the whole detection range is close to the background and continues to be low.

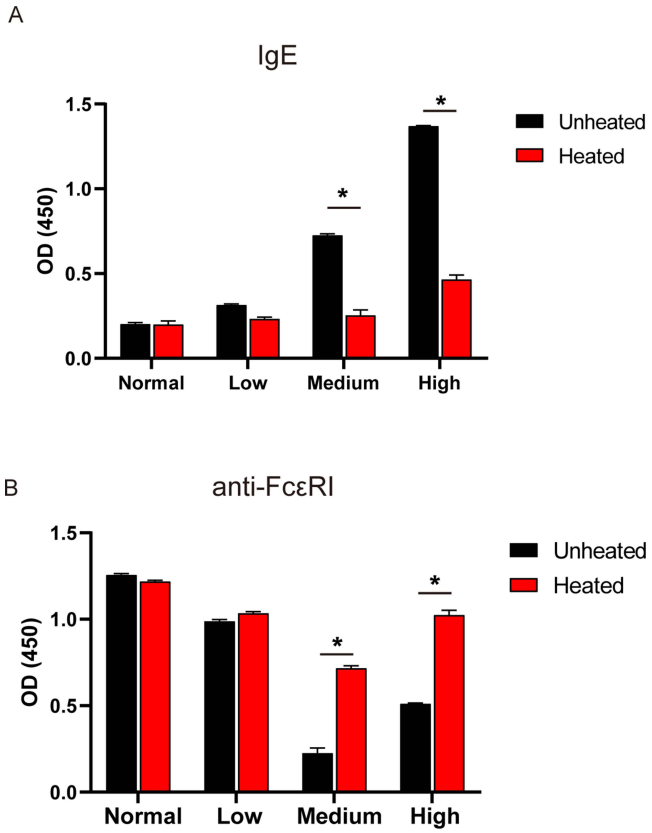

Figure 1 Effect of heating on IgE and anti-FcεRI. (**A**): Four serum samples containing normal, low, medium, and high levels of IgE were taken. The serum was divided into two parts, one of which was pretreated by heating at 56°C for 30 min. IgE was measured by ELISA, coating the microplate with human FcεRIα and using HRP-anti-human IgE as the second antibody. IgE levels before and after heating were compared; (**B**): Serum containing four different IgE concentrations was subjected to the same treatment. Anti-FcεRI was measured by another ELISA, coating the microplate with human FcεRIα and using HRP-anti-human IgG as the second antibody. Anti-FcεRI levels before and after heating were compared. The *T*-test was used for group comparison, $^*P < 0.05$.

Sensitivity and Hook Effect

The results obtained after multiple dilutions of quality control serum and negative control serum were plotted, as shown in Figure 2. The detection value of the highest dilution (1:4800) of quality control serum was distinguished from that of negative control serum, with a sensitivity of >1:4800. No hook effect was found when OD = 1.304.

Serum Levels of Immunoglobulin E, Anti-Immunoglobulin E Antibody, and Anti-FcεRI Antibody

Total serum IgE was found to be normal in the majority of CD patients (78.6%) as compared to the reference value (100 IU/mL). The median serum concentration of total IgE in CD (28.3 IU/mL) increased compared with HC (18.8 IU/mL), but there was no significant difference between patients and controls ($P = 0.089>0.05$).

Table 2 Checkerboard Titration Results (P/N Ratio)

2nd Antibody	1:60,000			1:80,000			1:100000		
Serum	**1:200**	**1:300**	**1:400**	**1:200**	**1:300**	**1:400**	**1:200**	**1:300**	**1:400**
Low	1.42	1.50	1.50	1.42	1.39	1.50	1.39	1.23	1.37
Median	1.89	2.10	2.04	1.86	1.92	2.07	1.94	2.06	1.86
High	2.37	2.52	2.60	2.51	2.39	1.50	2.57	2.59	2.32

Notes: The checkerboard method was used to determine the optimal dilution of serum and secondary antibody. The dilutions of serum were 1:200, 1:300 and 1:400, and the dilutions of HRP-anti-human IgG antibody were 1:60,000, 1:80,000 and 1:100,000.
Abbreviation: P/N: positive/negative.

Table 3 Precision of Indirect ELISA Method for Anti-FcεRI in Serum

Quality Control	Within-Run			Between-Run		
	$\bar{x}$	SD	CV (%)	$\bar{x}$	SD	CV (%)
Low	0.41	0.011	2.68	0.39	0.026	6.67
Medium	0.78	0.0080	1.02	0.76	0.040	5.26
High	1.11	0.015	1.35	1.11	0.037	3.33

Notes: Within-run precision is indicated by ten replicates of test samples including low, medium and high concentrations of anti-FcεRI on a single plate. Between-run precision is conducted as follows: the same three levels of anti-FcεRI were repeated three times for consecutive 7 days. The mean calculated concentration and percentage of coefficient of variation (CV%) are shown for each concentration (n=10).
Abbreviation: SD, standard deviation.

Serum anti-IgE levels (OD values) in each group were CD 0.46 (0.30, 0.63), and HC 0.39 (0.29, 0.58). As shown in Figure 3A, anti-IgE in CD tended to increase compared with that in HC, despite the lack of statistical significance ($P = 0.219 > 0.05$). In Figure 3B, the anti-FcεRI level in CD [0.74 (0.56, 0.95)] was significantly higher than that in HC [0.51 (0.38, 0.60)], $P < 0.001$. Furthermore, the correlation between IgE, anti-IgE, and anti-FcεRI was investigated by the Spearman test, and no correlation was found between total IgE and anti-IgE ($P = 0.132 > 0.05$) or anti-FcεRI ($P = 0.107 > 0.05$). There was a positive correlation between anti-IgE and anti-FcεRI ($R = 0.380$, $P < 0.001$). The ROC curve was plotted, and the cut-off value was taken as the corresponding point at the maximum of the Youden index (sensitivity + specificity −1). Finally, the cut-off values of anti-IgE and anti-FcεRI were 0.41 and 0.65, respectively. The measurement results above the cut-off value were judged as positive, and those below were judged as negative.

Correlation Between Mast Cell-Related Autoantibodies and Clinical Characteristics

Only the anti-FcεRI was markedly increased in CD and thus the characteristics of CD patients were described according to the anti-FcεRI status in Table 4. After Mann–Whitney *U*-test or chi-square test, compared with anti-FcεRI negative CD patients, those with positive anti-FcεRI were mostly female with higher activity and less involvement of the upper digestive tract. Otherwise, none of the other clinical parameters was significantly different among anti-FcεRI positive patients. As shown in Table 5, the logistic regression analysis found that female patients

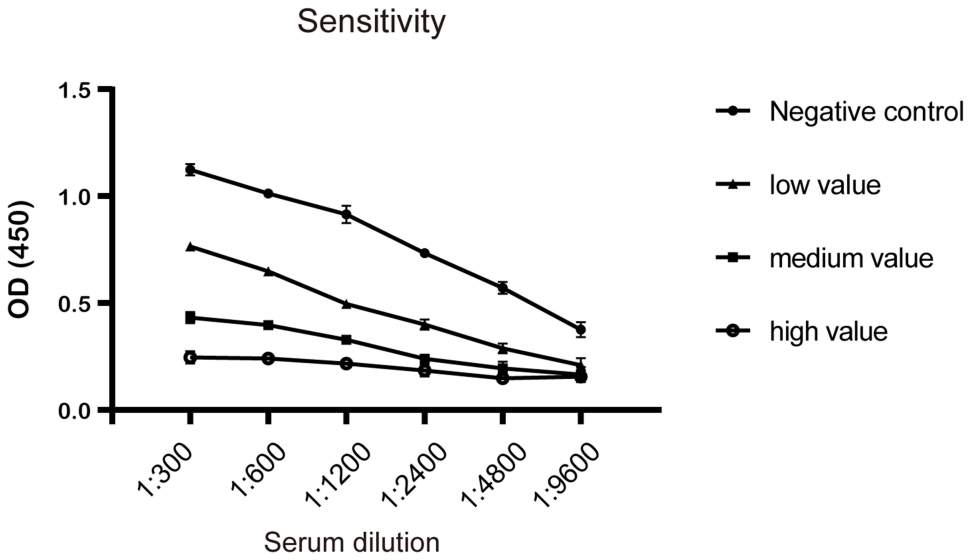

Figure 2 Determination of anti-FcεRI sensitivity. The multiple proportion dilution of serum was used to determine the sensitivity of this method. 1:4800 is the highest dilution for distinguishing the positive control from the negative control.

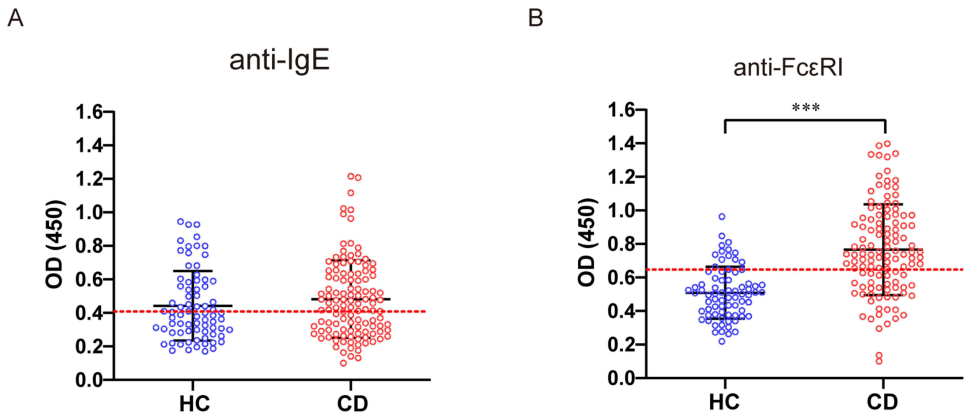

Figure 3 Serum anti-IgE and anti-FcεRI levels in Crohn's disease and healthy control **(A)**: IgG anti-IgE was tested by ELISA, using human IgE myeloma as the capture antibody; and HRP-anti-human IgG as the secondary antibody. (**B**): IgG anti-FcεRI was tested by self-developed ELISA: samples were pretreated by heating; human FcεRIα was used as the coating antigen; HRP anti-human IgG was used as the tracer. Results are given as optical density (OD). The red dashed line represents the cut-off value, 0.41 for anti-IgE and 0.65 for anti-FcεRI. The Mann–Whitney *U*-test was used for group comparison, ***$P < 0.001$.

were more likely to be anti-FcεRI positive [OR: 2.860 (1.121~7.295), $P = 0.028$]. Patients with positive anti-FcεRI were also associated with higher disease activity [OR: 1.478 (1.200~1.821), $P < 0.001$], but less likely to be located in L4 among Montreal classification [OR: 0.253 (0.077~0.837), $P = 0.024$]. As shown in Table 6, there was no significant difference in the concentration of anti-FcεRI antibodies between the remission group (median of 0.57) and the HC group (median of 0.51) ($P > 0.017$). However, a significant difference was observed between the remission group and the active group (median of 0.79) ($P < 0.017$). Additionally, differences in positivity rates were observed among all three groups.

Prevalence of PAB and ASCA Antibodies

It was found that PAB and ASCA antibodies were significantly positive more often in CD when compared with those detected in HC (17.9% vs 0.0% for PAB or 23.9% vs 0.9% for ASCA).

Table 4 Clinical Characteristics of Anti-FcεRI-Positive and Anti-FcεRI-Negative CD Patients

Characteristic	CD		
	Anti-FcεRI+ n=79(67.5%)	**Anti-FcεRI- n=38(32.5%)**	***P* value**
Age[a]	32(26.0, 44.0)	31(23.0, 37.3)	0.339[c]
Female[b]	31(39.2)	7(18.4)	0.024[d]
Duration[a]	6(1.0, 36.0)	12(1.0, 51.0)	0.620[c]
EIM[b]	22 (27.8)	11(28.9)	0.902[d]
Complications [b]	39(49.4)	17(44.7)	0.639[d]
Surgery[b]	20(25.3)	10(26.3)	0.9084[d]
Activity[a]	6(5.0,8.0)	4(3.0,6.0)	0.0003[c]
Location			
L1[b]	31(39.2)	16(42.1)	0.767[d]
L2[b]	12(15.2)	6(15.8)	0.933[d]
L3[b]	36(45.6)	16(42.1)	0.724[d]
All L4[b]	5(6.3%)	8(21.1)	0.039[d]

Notes: [a]Median (25th, 75th percentile); [b]N (%). [c]Using Mann–Whitney *U*-test. [d]Using $\chi 2$ Test.

Abbreviations: CD Crohn's disease; EIM, extraintestinal manifestation; Location: L1, terminal ileum; L2, colon; L3, ileocolon; All L4, upper digestive tract with involvement of L1, L2 or L3.

Table 5 Logistic Regression: Predictive Factors for Anti-FcεRI-Positive in Patients with CD

Factor	OR	95% CI	*P* value
Female	2.860	1.121~7.295	0.028
Activity	1.478	1.200~1.821	< 0.001
All L4	0.253	0.077~0.837	0.024

Abbreviations: OR, odds ratio; 95% CI, 95% confidence interval; All L4, upper digestive tract with involvement of L1, L2 or L3.

Table 6 Anti-FcεRI Levels in Crohn's Disease Patients at Different Stages and Healthy Controls

	N	Anti-FcεRI	
		Concentration[i,ii]	Positive Rate[iii,iv]
CD in active	79	0.79 (0.68, 0.98)[ab]	63(79.8%)[cd]
CD in remission	38	0.57(0.48, 0.78)	16(42.1%)[c]
HC	75	0.51(0.38, 0.60)	13(17.3%)

Notes: [i]Median (25th, 75th percentile); [ii]Using Kruskal–Wallis *H*-test, [a]$P < 0.017$ compared with HC, [b]$P < 0.017$ compared with CD in remission; [iii]N (%); [iv]Using $\chi 2$ Test, [c]$P < 0.017$ compared with HC, [d]$P < 0.017$ compared with CD in remission. CD in active, define Harvey-Bradshaw Index ≥ 5 as the active stage of CD; CD in remission, define Harvey-Bradshaw Index ≤ 4 as astate of remission.
Abbreviations: CD, Crohn's disease; HC, healthy control.

Clinical Value of Single or Combined Antibodies for Diagnosis of Patients with CD

According to the above-mentioned antibody positive evaluation criteria, the specificity, sensitivity, and negative and positive predictive values of anti-FcεRI, PAB, and ASCA antibody tests for diagnosis of CD are shown in Table 7. The sensitivity of anti-FcεRI was the highest, up to 67.5%. The other antibodies had high specificity and positive predictive value (PPV) but had low sensitivity and negative predictive value (NPV) in the diagnosis of CD. The combined application of three autoantibodies greatly improved the sensitivity and NPV. Clinically, the female was considered to be a possible risk factor for CD.[22] As a result, gender and the three autoantibodies were included in binary logistic regression analysis to obtain the predictive variable PRE. The ROC curve analysis showed that the AUC of PRE was 0.879 (0.831~0.928), which was higher than the diagnostic value of anti-FcεRI alone and three jointly (Figure 4).

Table 7 Diagnostic Values of Three Autoantibodies in Patients with CD

	Sens, %	95% CI	Spec, %	95% CI	PPV, %	95% CI	NPV, %	95% CI
Anti-FcεRI	67.5	58.9~76.1	82.7	73.9~91.4	85.9	78.6~93.1	62.0	52.3~71.7
PAB	17.9	10.9~25.0	100.0		100.0		43.9	36.3~51.4
ASCA	23.9	16.1~31.8	98.7	96.0~100.0	96.6	89.5~100.0	45.4	37.7~53.1
3 joint	80.3	73.0~87.7	82.7	73.9~91.4	87.9	81.6~94.1	72.9	63.3~82.6
PRE	72.6	64.5~80.8	92.0	85.7~98.3	93.4	88.2~98.6	68.3	59.1~77.5

Abbreviations: CD, Crohn's disease; PAB, anti-pancreatic antibody; ASCA, anti-Saccharomyces cerevisiae antibody; 3 joint, anti-FcεRI+/PAB+/ASCA+; PRE, predictive variable obtained by binary logistic regression analysis, including anti-FcεRI, PAB, ASCA and gender; Sens, Sensitivity; Spec, Specificity; PPV, positive predictive value; NPV, negative predictive value; 95% CI, 95% confidence interval.

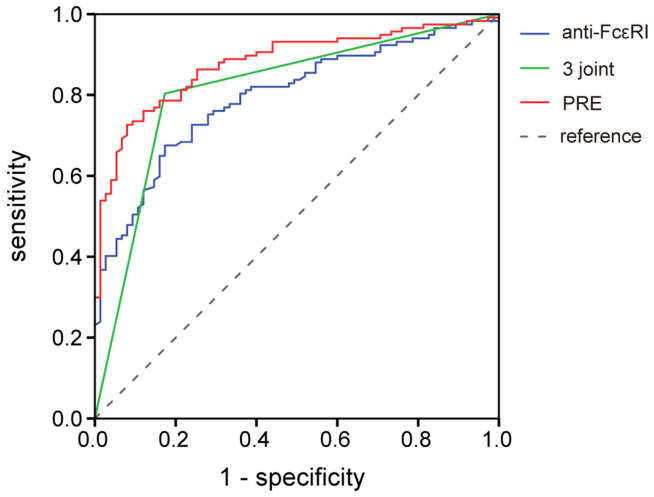

Figure 4 ROC curves of anti-IgE, PRE, 3 joint for the diagnosis of CD. Receiver operating characteristic (ROC) curves were generated by SPSS IBM (version 21). 3 joint: anti-FcεRI+/PAB+/ASCA+; PRE: predictive variable obtained by binary logistic regression analysis, including anti-FcεRI, PAB, ASCA, and gender.

Discussion

In addition to the allergen-IgE activation pathway, some autoantibodies such as anti-IgE, anti-FcεRI can also bridge FcεRI, induce mast cell activation, and trigger the release of pro-inflammatory and immunomodulatory mediators.[24] Mast cell activation-related molecules are consistently found in patients with autoimmune diseases who have allergic symptoms. Thus, the relationship between these antibodies and diseases has also received attention.

The establishment of methodology is the basis of this study. While human serum IgE detection is widely used for diagnosing allergic patients, the detection of anti-IgE antibodies has also been reported.[19] However, there is no stable commercial kit available for the detection of anti-FcεRI so far. Therefore, the first step is to establish a detection method for this antibody. Our preliminary performance verification found that the self-developed ELISA method for IgG anti-FcεRI has good specificity and precision, ensuring the correctness and repeatability of the results. Even if the dilution of the positive control serum reaches 1:4800, the detection value can be distinguished from the negative control, which suggests that the sensitivity is acceptable Moreover, there is no hook effect detected in the high-value serum (OD = 1.127). This novel method allows for a comparative analysis of anti-FcεRI levels in health and disease.

Considering the activation of MCs in CD pathogenesis, the serum levels of IgE, IgG anti-IgE, and IgG anti-FcεRI autoantibodies were investigated together with existing markers, PAB, and ASCA in 117 CD patients from China. Then, the correlation between these markers and disease phenotypes was analyzed. Our findings showed a significant increase in serum anti-FcεRI levels in CD patients, with a positivity rate of 67.5%. Moreover, positive anti-FcεRI patients were mostly female with higher disease activity but less likely to invade the upper digestive tract. Elevated anti-FcεRI levels may be associated with increased disease activity. The combined detection of anti-FcεRI and existing CD-related indicators can greatly improve the sensitivity of diagnosis.

It has been reported that intestinal MCs from CD patients release histamine in response to anti-IgE, indicating that the presence of functional IgE in intestinal mast cells and anti-IgE can activate MCs.[25,26] In our study, although total IgE levels were elevated in CD patients compared to healthy controls, the change was not statistically significant. This suggests that the actual role of IgE in CD requires further investigation, as MC activation can occur through other mechanisms.

Some studies have demonstrated the presence of anti-IgE and anti-FcεRI autoantibodies in autoimmune diseases, allergic disorders, atopic dermatitis,[27] asthma,[28,29] and systemic lupus erythematosus.[30] This is the first report investigating the serum levels of IgG anti-IgE and IgG anti-FcεRI in CD. In this study, anti-FcεRI antibodies were indeed found to be significantly elevated in the serum of CD patients compared with healthy controls, indicating that anti-FcεRI may play a more crucial role in CD. On the other hand, neither serum IgE nor anti-IgE levels were significantly increased in CD

patients in the present study. This discrepancy may be due to IgE and anti-IgE forming immune complexes, which could interfere with test results.[31] However, this possibility cannot be ruled out.

The clinical characteristics of anti-FcεRI positive and negative patients were compared for further analysis. The results revealed that high levels of anti-FcεRI antibodies were related to higher disease activity and were less likely to invade the upper digestive tract. Sabroe et al identified functional autoantibodies in chronic urticaria patients using the autologous serum skin test or histamine release from basophils, revealing a subset of patients with more severe symptoms.[32,33] Although the functional detection of antibodies is important, these assessment methods take the serum with complex components as irritants and test the comprehensive effect of mixed components such as allergens and autoantibodies, which cannot measure the content of individual components. Moreover, these methods are complicated to operate and even have certain risks. In contrast, the detection of specific autoantibody levels is targeted and more practical for clinical use.

The study demonstrated that the concentration of anti-FcεRI antibodies was significantly lower in the remission phase than in the active phase, with no significant difference compared to the HC group. This indicates that anti-FcεRI antibodies may be useful in distinguishing the severity of CD. Additionally, there was a difference in positive rates between the remission group and the HC group. It is likely due to the levels in both groups being close to the cut-off value (0.65), placing them in a detection gray area. This phenomenon requires further verification with an increased sample size.

Existing indicators, PAB and ASCA, are highly specific for CD, but with low sensitivity.[34] Regarding the positive rate of PAB, the results (17.9%) were similar to those of previous studies (15–30%).[35,36] As for the positive rate of ASCA, the results (23.9%) were much lower than those reported in European countries (39–76%).[37] Nevertheless, the frequency of ASCA IgG in CD was similar to that of other studies based on Chinese cohorts.[38,39] Lawrence et al[40] directly compared CD patients from Hong Kong and Australia, suggesting that in Chinese cohorts, ASCA IgG detection was similar to Australian Caucasian IBD cohorts (50–60%), but ASCA IgA detection was significantly lower. Population characteristics, including genetic, environmental, dietary differences, and detection methodology variations, may explain these discrepancies. Further investigation is needed to evaluate these biomarkers comprehensively.

All of the above markers showed limited diagnostic sensitivity and NPV in the diagnosis of CD alone, especially existing indicators. Through binary logistic regression, the combination of three antibodies (anti-FcεRI, PAB, ASCA) and gender was able to obtain a reasonable sensitivity of 72.6%, while maintaining a high specificity of 92.0%. However, the joint detection of the above markers in the practical clinical application needs to be further investigated and confirmed.

This study demonstrates strong internal validity through rigorous experimental design and control measures, including random sampling and blind testing, which minimize potential biases. The accuracy of detecting anti-FcεRI autoantibodies was enhanced by implementing heat treatment to eliminate IgE interference. For external validity, the diverse Chinese sample supports the generalizability of findings within this demographic. However, further research is needed to validate these results across different ethnic and geographic groups, given the potential variability of immune responses.

Besides, this study is limited by the small sample size from a single center, which may introduce selection bias and affect the definition of cut-off values and associations with clinical phenotypes. Despite the limitations, our findings provide a foundation for larger confirmatory studies.

The increase of molecules associated with mast cell activation in CD indirectly reaffirms the involvement of MCs in the disease and allows for the speculation that IgG anti-IgE or IgG anti-FcεRI autoantibodies may represent a mechanism of allergen-independent disease deterioration in CD, which needs to be further explored in the future. The connection between disease activity or lesion location in CD and anti-FcεRI antibody levels suggests that it can be used for the diagnosis or monitoring of the disease.

Conclusion

Detecting anti-FcεRI autoantibodies using ELISA could facilitate research on Crohn's diseases. In CD patients, the IgG anti-FcεRI autoantibody is significantly elevated, highlighting it as a key marker among molecules related to mast cell activation. Anti-FcεRI positivity is correlated with higher disease activity indices. When combined with established markers such as PAB and ASCA, IgG anti-FcεRI provides a more comprehensive understanding of CD's etiology and may significantly enhances diagnostic sensitivity. Nonetheless, further research is necessary to investigate the specific mechanisms involved and the broader clinical implications of these findings.

Abbreviations

ASCA, Anti-Saccharomyces cerevisiae antibody; BSA, Bovine serum albumin; CD, Crohn's disease; CU, Chronic urticaria; ELISA, Enzyme-linked immunosorbent assay; FcεRI, The high affinity IgE receptor; HBI, Harvey-Bradshaw Index; HC, Healthy controls; HRP, Horseradish peroxidase; MCs, Mast cells; PAB, Anti-pancreatic antibody; PBS, Phosphate buffer saline; P/N, Positive/Negative; ROC, Receiver operating characteristic; TMB, (3,3′,5,5′)-tetramethylbenzidine.

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Author Contributions

Yue Yin, Yusen Hu should be regarded as equivalent first authors. All authors made a significant contribution to the work reported, whether in the conception, study design, execution, acquisition of data, analysis, and interpretation, or in all these areas; took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work. All authors have read and approved the final version of the manuscript.

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Disclosure

The authors declare that they have no conflicts of interest.

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