

The basophil activation test (BAT) in bakers with obstructive allergic airway disease

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Key words
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test – BAT – flow
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Abstract

Background: The basophil activation test (BAT) represents a sensitive test method that is available for the detection of sensitization to rye and wheat flour in bakers with obstructive allergic airway disease. Since ImmunoCAP® fails to detect specific immunoglobulin E (IgE) in a part of the investigated bakers with airway disease, BAT should be used as an additional research method.

Methods: A collective of 248 bakers taking part in the prevention program of the German Social Accident Insurance Institution for the foodstuffs and catering industry (Berufsgenossenschaft Nahrungsmittel und Gastgewerbe, BGN), as well as 31 non-allergic BGN employees as control subjects, were investigated. BAT was performed according to the manufacturers' instruction and reagents (Beckman Coulter, Krefeld, Germany). Activated basophils were stained with CD203c-PE and visualized using flow cytometry. The percentage of basophils that show significantly higher CD203c expression following allergen-specific stimulation with rye and wheat flour compared with unstimulated basophils yields the stimulation index (SI), which is consid-

ered as an positive allergic reaction from a value of ≥ 2.0 . Total IgE and specific IgE to rye and wheat flour were detected using the ImmunoCAP® FEIA system (Thermo Fisher Scientific).

Results: In total, 7 % of affected bakers and 10 % of controls were non-responders. Using BAT, 83 % of „responder bakers“ were positive for either wheat or rye flour. Control subjects were negative for both wheat and rye flour. This yielded a sensitivity of 83 % and a specificity of 100 % for BAT stimulation related to the control group. Of the flour-negative bakers in the CAP-FEIA, 38 % tested positive to flour in the BAT.

Conclusions: BAT is a method well suited as an adjunct to the serological diagnosis of allergic baker's asthma and rhinitis, particularly if the results of serological specific IgE detection are inconclusive in relation to the patient history.

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Introduction

The involvement of basophils in the pathogenesis of allergic disease was discovered back in the 1990s. These granulocytes can be detected in (lung) tissue in allergic inflammation, for example, as seen in asthma. Activated basophils can also be found in nasal lavage fluids following allergen provocation during the late phase of the allergic response. They produce and store a number of vasoactive and inflammatory mediators that are released during an

allergic reaction, thereby contributing the clinical picture of allergic symptoms. Inflammatory mediators such as histamine and leukotriene are released via the binding of immunoglobulin E (IgE) to the high-affinity IgE receptor FcεRI on basophils and the cross-linking of IgE by allergens. In addition, basophils synthesize the interleukins IL-3, IL-4, and IL-13, proteins essential for T helper cell (Th)-2 polarization and IgE synthesis, which can be characteristically detected in, e. g., allergic disease [1].

Basophils express the activation molecule CD203c (ecto-nucleotide pyrophosphate/phosphodiesterase, ENPP), among others. This ectoenzyme sits on the plasma membrane and in the cytoplasm; however, its biological significance is as yet unknown. CD203c is an activation molecule that is expressed exclusively on resting and activated basophils in peripheral blood. Cross-linking of the IgE-binding receptor FcεRI by an allergen or an anti-IgE antibody results in a rapid (maximum 5 min) upregulation of intracellular CD203c to the cell surface. This activation, via a complex cascade, causes basophil degranulation, mediator release, and cytokine synthesis, resulting ultimately in the clinical picture of allergic symptoms [2, 3].

In general, both specific and total IgE are determined in serum in order to identify IgE-mediated sensitization, whereby total IgE determination is relevant in the assessment and evaluation of specific IgE. The basophil activation test (BAT) is not a primary routine diagnostic tool as such, but rather an important adjunctive method in allergen-specific IgE diagnostics [4]. Nevertheless, BAT represents a sensitive test which, despite being a complex method, is available for certain indications such as sera with low total IgE and failure to detect specific IgE despite probable sensitization [5]. According to recent assessments [6], BAT is on the way to becoming an important additional test for a variety of allergological diagnostic questions and, as a reproducible *ex vivo* test that is less stressful for the patient, can be considered as an alternative to challenge testing.

The aim of the present study was to investigate the suitability of BAT to detect sensitization to rye and wheat flour in bakers with obstructive allergic airway disease. A further aim was to establish whether BAT can be used as an additional diagnostic assay in asthmatic and rhinitic bakers where no specific IgE could be detected using the ImmunoCAP® method.

Method

BAT was performed according to the manufacturer's instructions (Beckman Coulter, Krefeld, Germany) and using their reagents. Heparin-stabilized whole blood was stimulated with various concentrations of wheat (f4) and rye (f5) flour allergen extracts (Dr. Fooke GmbH, Neuss, Germany) within 2 h following blood sampling. Basophil stimulation was stopped after 15 min at 37°C with phosphate-buffered saline solution (PBS)/20 mM ethylenediaminetetraacetic acid (EDTA). The activated basophils were stained with CD203c-PE (antibody clone 97A6, Immunotech, Beckman Coulter GmbH, Krefeld, Germany). An unlabeled monoclonal anti-IgE antibody (anti-IgE antibody clone E124.2.8/De2, Immunotech, Beckman Coulter GmbH, Krefeld, Germany) was used as a positive control.

The difference between stimulated and unstimulated cells was at least 5%. Since the basophil population can be well visualized and differentiated from lymphocytes and monocytes in the side-scatter signal (SSC), all basophils in gate B and at least 500 activated basophils in gate A were analyzed.

Wheat and rye flour allergen extracts were tested in concentrations (sample weight) of 5 and 0.5 µg/ml. Extract protein concentrations varied between 1.2 and 1.5 mg/ml (f4) and 1.5 and 2.0 mg/ml (f5) (data from Dr. Fooke GmbH). Following allergen-specific stimulation, the expression of CD203c on the cell surface rose so sharply that the fluorescence intensity of the stained basophils increased by a logarithmic step and could be visualized in flow cytometry (EPICS XL™, Beckman-Coulter GmbH, Krefeld, Germany). The ratio of the mean intensity of the entire basophil population following stimulation to the mean intensity prior to stimulation yielded the stimulation index (SI), i.e., the percentage of basophils with significantly higher CD203c expression following allergen-specific stimulation compared

Abbreviations

AB	Antibodies
AUC	Area under the curve
BAT	Basophil activation test
BATneg	BAT-negative
BATpos	BAT-positive
BGN	German Social Accident Insurance Institution for the foodstuffs and catering industry (Berufsgenossenschaft Nahrungsmittel und Gastgewerbe)
BKV	German Ordinance on Occupational Diseases (Berufskrankheiten-Verordnung)
EDTA	Ethylenediaminetetraacetic acid
ENPP	ectonucleotide pyrophosphatase/phosphodiesterase
FEIA	Fluorescence enzyme immunoassay
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IL	Interleukin
PBS	Phosphate buffered saline solution
PE	Phycoerythrin
PC	Positive control
ROC	Receiver operating characteristic
SI	Stimulation index
sIgE	Specific immunoglobulin E
SSC	Side-scatter signal
Th	T helper cells

with unstimulated basophils. The SI was generally considered to be positive for an allergic reaction from a value of ≥ 2.0 [7].

Total IgE and specific IgE (sIgE) to rye and wheat flour were determined using the ImmunoCAP® system (CAP-FEIA, fluorescence enzyme immunoassay; Thermo Fisher Scientific GmbH, Dreieich, Germany). Values ≤ 120 kU/l were specified as the normal range of total IgE. Sensitization was assumed from CAP class 1 (≥ 0.35 kU/l).

Fig. 1 shows stimulation in a test subject with rye and wheat flour in a double determination: side-scatter signal vs. phycoerythrin (PE) fluorescence. The increased expression of CD203c on the stained basophils, both in the positive control and in the flour-stimulated samples in relation to the unstimulated negative control was apparent.

Collective

The collective comprised 248 bakers participating in the baker's asthma prevention program of the German Social Accident Insurance Institution for the foodstuffs and catering industry (Berufsgenossenschaft Nahrungsmittel und Gastgewerbe, BGN), and 31 non-allergic BGN employees as controls. The prevention program is offered within the framework of § 3 of the German Ordinance on Occupational Diseases (Berufskrankheiten-Verordnung, BKV) for insured individuals with allergic respiratory disease (including rhinopathy) and is made up of three modules: risk analysis, an interdisciplinary

health seminar, as well as continuous occupational medical and technical follow-up.

BAT investigations took place in the context of the continuous occupational medical follow-up in the BGN's health protection department. Bakers with airway disease were examined by occupational physicians of the BGN in an extended occupational medical screening program according to policy G23 of the Guided Care Process of the German Employer's Liability Insurance Association.

The mean age of bakers was 42.8 (23–70) years; 21 (9 %) of the test subjects were female. The average age of the 24 female and seven male controls was 44.6 years. In all, 81 % of the bakers were affected by allergic asthma and/or 86 % by allergic rhinitis, whereby 40 % of the asthmatics were classified into asthma stages 2–4 and 34 % into asthma stage 1. Grading was carried out according to German asthma management guidelines [8]. Lung function was normal in 59 %, mildly impaired in 31 %, and moderately to severely impaired in 10 %. Of the test subjects, 16 % required no medication, 27 % were classified into drug levels 1 and 2, 57 % into drug levels 3 and 4, and only one subject required drug level 5. Inhaled corticosteroids are taken from drug level 2. In total, 43 % of bakers stated that they did not use medication on demand, 27 % rarely, and 30 % more frequently. The grading of airway medication was carried out according to the „Global Strategy for Asthma Management and Prevention“ [9]. At the time, 24 % of bakers were smokers. Bakers reported the time interval since their previous exposure to flour as being 48 h (median). The occupational medical screening program did not include skin testing.

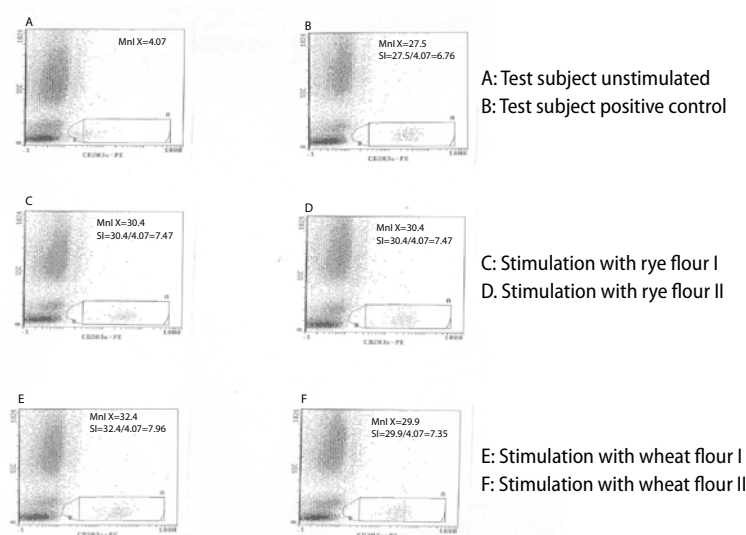
Statistical analysis

The statistical analysis of data was performed using SPSS statistical software, version 21. Mean $\pm$ standard deviation, median and value range, or the absolute frequency and percentages are given in the tabular descriptive statistics. Difference hypotheses between two groups were tested, depending on the level of measurement, using the χ^2 test or the Mann-Whitney U test. Correlation hypotheses were calculated using the non-parametric Spearman correlation. An error probability of ≤ 0.05 was assumed for all statistical tests.

Results

In a first step, IgE-dependent stimulation in the test system [positive control (PC) of the test system – anti-IgE AB clone E124.2.8/D ϵ 2] was investigated in all participants (SI-PC ≥ 2.0). Results are shown in **Tab. 1**.

Altogether, 93 % of bakers and 90 % of controls tested positive to IgE-dependent stimulation. The following calculations were made with the responders (230 responding bakers and 28 responding controls).



MnI X, mean intensity; SI, stimulation index

Fig. 1: Stimulation in a subject with rye and wheat flour (0.5 μ g/ml each) in a double determination and controls. Side-scatter signal vs. phycoerythrin (PE) fluorescence.

Tab. 2 shows total IgE, CAP-*FEIA*, as well as BAT results for responders.

SI-PC values in bakers and controls scarcely differ (4.9 and 5.0, respectively).

In the CAP-*FEIA*, 182 of the 230 bakers (79 %) were sensitized to wheat flour and 184 (80 %) to rye flour (CAP class ≥ 1). In all, 190 (83 %) were sensitized to at least one of the two flours.

None of the controls were sensitized to either wheat or rye flour. Total IgE was < 120 kU/l in 30 controls. At 137 kU/l, total IgE was elevated to above the normal value in one control subject with CAP class 0 to both flours.

Total IgE values were significantly higher among bakers compared with controls.

Tab. 3 gives the SI wheat flour and SI rye flour values for two flour allergen extract concentrations.

CD203c expression was lower for both wheat and rye flour when the lower flour allergen extract concentration was used. **Tab. 4** shows frequencies of wheat and rye flour BAT-positive bakers.

BAT using either wheat or rye flour was positive in 191 (83 %) of the 230 bakers, 164 of which tested positive to both. Altogether, 13 tested positive for only rye flour and nine for only wheat flour. BAT induced no activation with neither of the tested flours in 39 test subjects (SI < 2.0 in all concentrations tested). The 28 control subjects were negative for both wheat and rye flour. This yielded a high sensitivity of 83 % and an ideal specificity of 100 % for BAT stimulation in the control group.

The 191 bakers that tested positive in BAT for wheat flour or rye flour will be referred to below as BATpos (BAT-positive). The 39 participants that tested negative are referred to as BATneg (BAT-negative).

There is no significant difference between BATneg and BATpos in the data on rhinitis, asthma, asthma stage, lung function, medication level, as-needed medication, smoking status, and the interval since the last exposure to flour (data not shown). The positive controls (SI-PC values) of the BATpos bakers were significantly higher (5.0 ± 1.8) compared with the BATneg bakers (3.9 ± 1.4).

The characteristic values for the ImmunoCAP® results in BATpos and BATneg bakers are given in **Tab. 5**.

BATpos bakers had significantly higher kU/l values and CAP classes for wheat and rye flour compared with BATneg bakers. The total IgE concentration was significantly lower in BATneg bakers ($p = 0.02$) compared with BATpos bakers.

The correlation between BAT and CAP-*FEIA* results is illustrated in the following using the example of rye flour (**Fig. 2**).

The Spearman rank correlation between SI values at a concentration of 5 µg/ml and the CAP-*FEIA*

Tab.1: Frequency of positive control (PC) in bakers and controls (Stimulation Index-PC ≥ 20)

	Bakers n = 248	Controls n = 31
Responders	230 (93 %)	28 (90 %)
Non-responders	18 (7 %)	3 (10 %)

Tab. 2: Basophil activation test (BAT), specific IgE, and total IgE values in responders

	Bakers n = 230	Controls n = 28
BAT: SI-PC values, positive controls	4.9 ± 1.8 4.7 (2.0–13.0)	5.0 ± 2.0 5.0 (2.0–8.4)
CAP- <i>FEIA</i> : slgE wheat (kU/l)*	8.4 ± 17.9 2.3 (0–100)	0.02 ± 0.01 0.02 (0.01–0.05)
CAP- <i>FEIA</i> : slgE rye (kU/l)*	10.4 ± 19.9 3.2 (0–100)	0.02 ± 0.02 0.02 (0.01–0.11)
Wheat flour CAP class $1 \geq 1$	182 (79 %)	0 (0 %)
Rye flour CAP class ≥ 1	184 (80 %)	0 (0 %)
Wheat or rye: CAP class ≥ 1	190 (83 %)	0 (0 %)
Total IgE (kU/l)*	415 ± 745 140 (6–5,000)	33 ± 32 25 (2–137)
Total IgE ≥ 120	127 (55 %)	1 (4 %)

IgE, immunoglobulin E; PC, positive control; SI, stimulation index; slgE, specific immunoglobulin E

*Values ≥ 100 kU/l or $\geq 5,000$ kU/l were included in the calculation as 100 or 5,000

Tab. 3: Stimulation index (SI) wheat flour and SI rye flour values for two flour allergen extract concentrations in 230 bakers

	Wheat flour		Rye flour	
	5 µg/ml n = 224	0.5 µg/ml n = 190	5 µg/ml n = 227	0.5 µg/ml n = 186
SI values	4.3 ± 2.4 4.5 (0.7–13.2)	2.2 ± 1.9 1.2 (0.6–8.8)	4.3 ± 2.2 4.6 (0.7–11.8)	2.4 ± 2.0 1.5 (0.6–9.8)

Tab. 4: Activation upon stimulation with wheat, rye, and both flours in 230 bakers in the basophil activation test (BAT)

	Wheat flour	Rye flour	Wheat or rye flour
Activation upon stimulation (SI-PC ≥ 2.0)	175 (77 %)	180 (79 %)	191 (83 %)
No activation upon stimulation (SI-PC < 2.0)	52 (23 %)	48 (21 %)	39 (17 %)

PC, positive control; SI, stimulation index

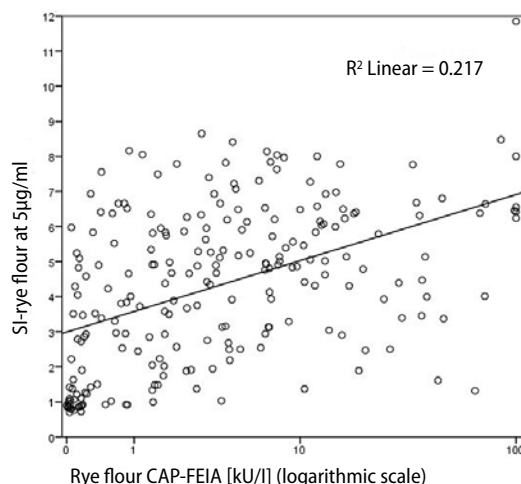
Tab. 5: Comparison of CAP-FEIA results in bakers that tested positive in the basophil activation test (BATpos) or negative (BATneg)

		BATpos bakers n = 191	BATneg bakers n = 39	U-Test (p)
Wheat flour: CAP values (kU/l)		10.0 ± 19.3 3.3 (0–100)	0.5 ± 1.2 0.1 (0–6)	< 0.01
Rye flour: CAP values (kU/l)		12.3 ± 21.2 4.3 (0–100)	0.7 ± 1.8 0.2 (0–10)	< 0.01
CAP classes wheat flour	CAP 0 CAP 1 CAP 2 CAP ≥ 3	20 (11 %) 16 (8 %) 60 (31 %) 95 (50 %)	28 (72 %) 5 (13 %) 5 (13 %) 1 (3 %)	< 0,01
CAP classes rye flour	CAP 0 CAP 1 CAP 2 CAP ≥ 3	19 (10 %) 8 (4 %) 55 (29 %) 109 (57 %)	28 (72 %) 3 (8 %) 6 (15 %) 2 (5 %)	< 0,01
Total IgE (kU/l) ^a		427 ± 725 151 (7–5,000)	355 ± 844 96 (6–5,000)	0,02
Total IgE ≥ 120		111 (58 %)	16 (41 %)	0,05 ^b

^aValues ≥ 5,000 kU/l were included in the calculation as 5,000^bχ² Test

values in 230 bakers was $r = 0.50$ ($p > 0.01$) for rye flour and $r = 0.56$ ($p > 0.01$) for wheat flour.

To assess diagnostic quality, **Fig. 3** shows a receiver operating characteristic (ROC) curve with SI rye flour values for the 5 µg/ml flour allergen extract concentration and the criterion of a rye flour CAP class ≥ 1 (191/227 bakers).

**Fig. 2:** Correlation diagram between stimulation index (SI) rye flour values at a concentration of 5 µg/ml and CAP-FEIA rye flour kU/l values in 227 bakers

The ROC curve has an area under the curve (AUC) of 0.844. Following a uniformly sharp rise, the ROC curve achieves an extremely high sensitivity of 0.95 at a specificity of around 0.6.

Of the flour-negative bakers in the CAP-FEIA, 15 (37.5 %) tested positive to flour in the BAT. Total IgE in these 15 test subjects was 61.9 kU/l on average. Of these subjects, 14 tested positive to rye flour (5 µg/ml) and three subjects additionally to 0.5 µg/ml) and eight to wheat flour (5 µg/ml). One subject responded positively to BAT using wheat flour, one to rye flour.

Discussion

The present paper shows that the BAT is generally suitable to determining IgE-mediated sensitizations, such as can be found in allergic baker's asthma and rhinitis. It shows a high sensitivity of 83 % and specificity of 100 % for wheat and rye flour compared with a control group of non-allergic individuals. More specifically, 77 % of bakers tested positive to wheat flour allergen extract and 79 % to rye flour allergen extract. In an investigation of wheat-allergic children, Tokuda [10] demonstrated a sensitivity of 85 % and 77 % with fractionated wheat extract, whereby none of the wheat-tolerant patients responded in the BAT. It should be borne in mind that no challenge findings were available for the participants in the BGN prevention program at the time of the BAT investigations; however, all bakers underwent an on-site examination under real exposure conditions as part of their risk analysis.

According to the literature, CD203c upregulation is recorded in virtually all CAP-positive allergy sufferers upon stimulation with the relevant allergen. Although it was not possible to achieve allergen-induced upregulation of CD203c in non-allergic subjects, such as our control collective, this was possible using anti-IgE antibodies (positive control). BAT was unable to achieve a positive result (BATneg) in a small percentage of sensitized patients, which can possibly be attributed to previous allergen contact and basophil desensitization. Blocking immunoglobulin G (IgG) antibodies is one conceivable explanation, or the level of allergen-specific basophil-bound IgE was too low to elicit an allergic reaction [7, 11]. These BATneg bakers had significantly lower SI-PC values in the positive controls compared with BATpos bakers (although above the cut-off value of $SI \geq 2$), as well as significantly lower CAP classes to wheat and rye flour and a median total IgE level in the normal range. There were no differences between groups in terms of the following parameters: rhinitis, asthma, drug level, and as-needed drugs. These were in large part mild disease processes.

BAT pointed to sensitization in a high number (37.5 %) of the ImmunoCAP®-negative bakers affected by airway disease. Due to non-reactivity in the control collective, a single BAT was considered positive in terms of sensitization. The BAT permits the sensitive and specific measurement of an IgE-mediated immune response on an effector cell level and thereby a functional response of basophils. Thus, this ex vivo test, unlike specific IgE determination, primarily reflects a clinical response to inhaled allergens [3, 6]. As such, the BAT is not necessarily suitable for basic routine diagnostics, as reported by Renz et al. [5]; however, it can provide additional information, as shown here, in the case of a discrepancy between sensitization identified in the patient history – the collective was made up of insured individuals affected by obstructive airway disease of allergic etiology – and sensitization not detectable in serological tests [12].

One drawback of BAT is that not all investigated subjects exhibit activation upon IgE-dependent stimulation. In line with the literature (5 %–10 % non-responders), the investigated collective had 7 % non-responders [11, 13]. This limits BAT's suitability as a routine diagnostic method. Nevertheless, it appears suited to answering special diagnostic questions, despite being more methodologically demanding.

Testing for a suitable allergen extract concentration is a further critical point relating to BAT. Each allergen extract needs to be tested in a variety of concentrations, which increases experimental complexity. The time-consuming and crucial pre-analytic processes involved in BAT have hitherto limited its application as a routine diagnostic instrument; however, its use in diagnostics could be broadened by a move toward greater standardization [6].

More research is needed, particularly with regard to the investigation of a flour-exposed control group of healthy bakers and a larger control collective. Investigating a group with comparable atopy status, e. g., grass pollen-allergic individuals that respond in part to rye and wheat flour, would be helpful in the further assessment of BAT.

BAT is a method well suited as an adjunct to the serological diagnosis of allergic baker's asthma and rhinitis, particularly if the results of serological specific IgE detection are inconclusive in relation to patient history.

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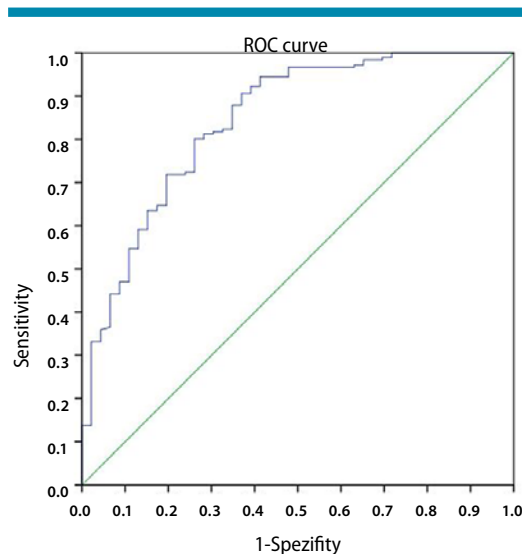


Fig. 3: Receiver operating characteristic (ROC) curve with 227 stimulation index (SI) rye flour values for the 5-µg/ml flour allergen extract concentration and 191/227 rye flour CAP class ≥ 1

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Conflict of interest

The author states that there are no conflicts of interest.

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