
The diagnostic value of basophil activation test in patients with an immediate hypersensitivity reaction to radiocontrast media

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Background: No available test diagnoses allergic reactions to radiocontrast media (RCM). The basophil activation test (BAT) has been introduced for the diagnosis of both immunoglobulin (Ig) E and non-IgE-dependent mast cell degranulation, but its value to diagnose immediate RCM reactions is still unknown.

Objective: This study aims to evaluate the diagnostic value of BAT in immediate RCM hypersensitivity.

Methods: The BATs were performed in 26 patients with immediate RCM reactions and in 43 specimens from healthy volunteers. The sample's whole blood was incubated with the responsible RCM and % activated (CD63+/CCR3+) basophils were analyzed by flow cytometry. Receiver operating characteristics (ROC) curve analysis was performed to calculate the optimal cutoff value of activated basophils to diagnose patients with RCM hypersensitivity.

Results: The incubation of blood with RCM yielded significantly higher activated basophil percentages in patients with a history of immediate RCM reactions than in normal controls with both 1:100 and 1:10 dilutions (13.11% vs. 2.71%, P value = .01; and 19.23% vs. 3.73%, P = .001, respectively). Both % activated basophils and stimulation index (SI) had acceptable discrimination powers to diagnose RCM hypersensitivity. The area under the curve was 0.79 (95% CI 0.67–0.91, P = .000) by using SI as the diagnostic criteria with 1:100 dilution of RCM. The specificity of the test ranged from 88.4% to 100%.

Conclusion: Our study demonstrated the potential of BAT as a diagnostic tool for an immediate RCM hypersensitivity, particularly as a confirmation test. Further studies are required to confirm the test accuracy and identify a patient's predisposing factors.

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INTRODUCTION

Hypersensitivity reactions to radiocontrast media (RCM) are part of the immediate adverse reactions associated with diagnostic and interventional procedures.¹ The diagnosis of hypersensitivity reactions from RCM is still a problem in allergy practice, and the exact pathogenesis remains unknown. Although the incidence of immediate reactions is as low as 0.7% to 3.1% after the introduction of nonionic low-osmolarity contrast media, severe reactions have been reported.² No diagnostic tests currently predict or confirm this hypersensitivity reaction. Drug provocation testing is dangerous and generally not recommended.

Mechanisms of immediate RCM reaction are unclear. Previously, it was believed that non-immunoglobulin (Ig) E-me-

diated mechanisms, such as complement activation and direct mast cell stimulation, were mainly responsible for the reaction.³ Recent studies, however, demonstrated that 23% to 73% of these patients had positive skin reactivity to culprit RCM, and authors usually suggested an IgE-mediated mechanism as a cause of this skin test positivity.^{4–6}

The basophil activation test (BAT) has been introduced in the diagnosis of immediate hypersensitivity reactions to several drugs such as beta-lactam antibiotics, nonsteroidal anti-inflammatory drugs, and latex allergy.^{7,8} CD63 and CD203c are surface markers of activated basophils corresponding to basophil degranulation and are useful in the diagnosis of immediate drug reactions.⁹ Theoretically, BAT is useful to evaluate patients with an immediate RCM reaction regardless of whether it is IgE or non-IgE mediated, if the ultimate pathway leads to mast cell/basophil activation and degranulation. Its value to diagnose RCM hypersensitivity and correlation with skin testing results has yet to be determined. Only a few reports show the value of BAT in the diagnosis of immediate RCM reactions.¹⁰

Positive skin test reactivity with an immediate reading is believed to represent skin mast cell degranulation and to be closely related to an IgE-mediated mechanism. However, no systematic study regarding the correlation between skin test results and basophil activation testing has previously been reported. We hypothesized that BAT should be able to detect a portion of patients with a history of immediate RCM

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reaction, particularly in patients with a positive skin test result. The purpose of this study was to investigate the potential role of BAT for the diagnosis of immediate RCM hypersensitivity and its correlation to the skin test result.

METHODS

Sixty-three patients with a history of immediate RCM reactions in King Chulalongkorn Memorial Hospital were invited for skin testing. These patients experienced the symptom onset within 1 hour after intravenous RCM injection. The severity grading according to Ring and Messmer's classification was then determined.¹¹ Skin prick tests with undiluted RCM were negative in all patients; therefore, the intradermal tests were subsequently performed. Fifteen of them tested positive on the skin test with the corresponding RCM (wheal diameter at least 3 mm larger than the initial wheal by intradermal test with 0.02 mL of 1:10 diluted RCMs). This concentration was confirmed by us and others⁶ to have no irritant effect in subjects who had never been exposed to RCM. Eleven skin test–positive patients gave consent for further BAT study, and 15 skin test–negative patients were randomly selected for comparative BAT analysis. Blood from 14 healthy blood donors who had no previous history of RCM reactions was used as negative controls.

Five different kinds of RCMs (Ioxithalamate [Telebrix, Guerbet, Villepinte, France], Iopromide [Ultravist, Bayer Schering Pharma, Berlin, Germany], Iohexol [Omnipaque, GE Healthcare Ireland, Cork, Ireland], Iobitridol [Xenetix, Guerbet, Villepinte, France], and Iopamidol [Iopamiro, BRACCO S.p.A., Milan, Italy]) from 10^0 to 10^{-5} dilutions were incubated with 3×10^5 cells of peripheral blood mononuclear cells to determine cellular toxicity by using propidium iodide and annexin-V staining (FITC Annexin V apoptosis detection kit II, BD Biosciences, San Jose, California). The viability of leukocytes after incubation with RCMs for 20 minutes in all specimens were comparable to

control RPMI media (95%–97% living cells) except with the undiluted ioithalamate that cell viability decreased to 82%. The concentrations of 1:10 and 1:100 of RCMs were then selected for BAT analysis.

A commercial kit (Flow2-CAST, Bühlmann Laboratories, Schönenbuch, Switzerland) was used for BAT according to the previously described protocol.^{12,13} In brief, 50 μ L of a patient's venous blood was drawn into the ethylenediamine-tetra-acetic acid tube and then incubated at 37°C for 15 minutes with 100 μ L stimulation buffer, 50 μ L 1:10 and 1:100 dilutions responsible RCM, and 50 μ L staining reagent (anti-CD63-PE and CCR3-FITC antibodies). Erythrocytes were lysed for 5 minutes before centrifugation at 500g. Flowcytometric analysis was performed at 488 nm on a FACScan flowcytometer (Becton Dickinson, Franklin Lakes, New Jersey) and analyzed by CellQuest software. Basophils were gated around the lymphocyte area, and the second gate was defined around anti-CCR3–positive cells. Double-positive CCR3⁺ and CD63⁺ cells were defined as activated basophils as shown in Figure 1. Data acquisition was performed on at least 500 basophils per sample, and the stimulation buffer and anti-Fc ϵ RI monoclonal antibody were used as a negative and a positive control. The results are presented as the percentages of activated basophils per total basophils and the stimulation index (the ratio between percent activated basophils after stimulation to percent activated basophils at baseline).

The study was approved by the Ethics and Research Committee of the Faculty of Medicine, Chulalongkorn University, and all subjects gave written informed consent. All statistical calculations were performed using SPSS 17.0 for Windows (SPSS, Chicago, Illinois). Because the data were not normally distributed, the Mann-Whitney *U*-test was used to compare the difference between percent activated basophils and the stimulation index between patients and normal controls, and Fisher's exact test was used to compare the cate-

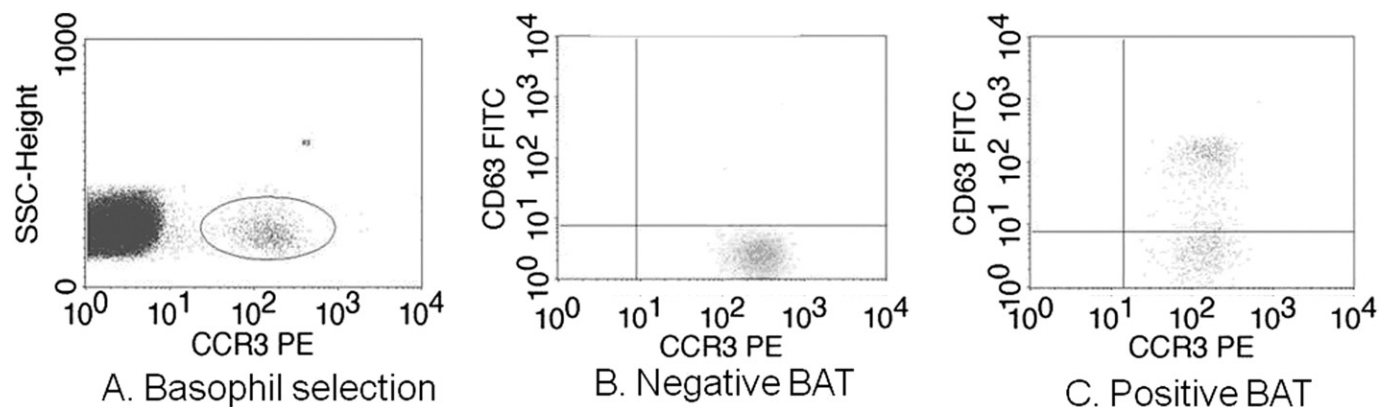


Figure 1. Basophil activation test evaluated by flow cytometry technique. Figures of flow cytometry demonstrate increased activated (CD63+/CCR3+) basophils after the incubation with RCM in patients with a history of an immediate RCM reaction. The selection of basophils was based on CCR3 positivity and side scatter characteristics (A). The examples of a specimen with negative BAT (B) and a specimen with positive BAT after incubated with culprit RCM (C) are provided.

Table 1. The results of basophil activation test in patients with a history of immediate RCM reactions and normal healthy controls

1A. Basophil Activation Test in 26 Patients with a History of Immediate RCM Reaction														
No.	Gender	Age	RCM	Adverse Reactions	Severity Grading	% Activated Basophils with Different Stimulations				Skin Test Result	Time Delay (months)			
						Baseline	FcεRI	1:10	1:100					
1	F	34	loxithalamate	Urticaria	1	0.35	62.32	1.1	1.91	+ve	27			
2	F	56	loxithalamate	Urticaria	1	4.17	88.45	20.1	14.16	+ve	45			
3	M	38	lopromide	Urticaria	1	0.92	90.78	4.31	1.53	+ve	16			
4	M	70	lopromide	Urticaria	1	2.99	70	12.24	4.23	+ve	18			
5	F	65	lohexol	Urticaria	1	0.57	51.43	2.17	3.21	+ve	18			
6	F	26	loxithalamate	Urticaria, upper airway edema, hypotension	3	0.56	88.22	60	18.53	+ve	36			
7	M	68	lopromide	Urticaria	1	0.53	89.87	26.96	34.53	+ve	39			
8	M	73	lopromide	Anaphylactic shock	3	1.53	61.61	3.46	2.08	+ve	25			
9	F	41	lohexol	Generalized erythema	1	3.77	43.78	47.52	33.74	+ve	15			
10	F	54	lopamidol	Facial rash	1	0.32	60.06	27.9	8.08	+ve	37			
11	M	44	lobitridol	Urticaria	1	3.27	69.41	16.67	13.65	+ve	25			
12	F	39	lopromide	Facial rash	1	0.94	86.82	5.61	3.18	−ve	16			
13	F	27	lopromide	Facial rash	1	0.09	92.19	3.47	3.78	−ve	15			
14	F	56	lopamidol	Pruritic rash	1	0.25	78.37	0.91	0.7	−ve	21			
15	F	65	lopromide	Pruritic rash	1	0.22	79.32	24.07	14.61	−ve	33			
16	F	71	lopromide	Pruritic rash	1	0.28	55.18	19.96	7.01	−ve	19			
17	M	65	lopromide	Pruritic rash	1	0.27	49.28	13.12	7.5	−ve	36			
18	F	39	lopromide	Hypotension, cyanosis	3	0.59	25.54	1.68	0.96	−ve	37			
19	M	37	lopromide	Generalized erythema	1	0.42	92.19	20.45	4.87	−ve	19			
20	M	64	lopromide	Generalized erythema	1	0.97	92.19	1.04	1.18	−ve	14			
21	F	43	lopromide	Facial rash	1	3.73	83.6	32.74	21.81	−ve	19			
22	M	55	lopromide	Generalized erythema	1	1.38	15.75	12.8	14.16	−ve	38			
23	M	60	lopromide	Generalized erythema	1	1.09	87.99	93.91	93.31	−ve	36			
24	F	33	lopromide	Pruritic rash	1	0.82	12.96	0.76	1.34	−ve	21			
25	F	41	lopromide	Urticaria	1	0.5	72.41	43.17	28.46	−ve	37			
26	F	63	lopromide	Periorbital edema	1	0.91	49.59	3.79	2.31	−ve	39			
1B. The Results of Basophil Activation Test in 14 Healthy Blood Donors without a History of RCM Reaction														
No.	Gender	Age	Basal	FcεR	% Activated Basophils									
					lopromide		loxithalamate		lohexol		lopamidol		lobitridol	
					1:10	1:100	1:10	1:100	1:10	1:100	1:10	1:100	1:10	1:100
1	F	33	0.56	85.96	0.40	0.56	0.93	0.83	1.06	0.5	2.01	1.49	ND	ND
2	M	48	0.42	54.09	2.44	1.44	14.35	1.04	5.38	3.43	2.74	3.53	3.87	2.32
3	F	65	1.21	63.14	3.81	1.44	2.13	0.73	2.58	1.56	2.06	1.41	4.26	4.81
4	F	45	1.87	85.45	3.49	2.05	4.04	6.11	1.46	1.94	4.04	6.32	3.94	4.8
5	M	45	1.73	83.07	5.53	4.88	4.97	3.76	5.28	4.16	4.04	4.29	5.15	4.14
6	M	49	1.7	53.95	5.17	3.49	5.1	5.38	ND	ND	ND	ND	ND	ND
7	F	34	1.46	86.12	4.82	1.71	5.16	1.9	ND	ND	ND	ND	ND	ND
8	F	36	1.92	23.17	3.41	5.2	6.39	3.53	ND	ND	ND	ND	3.7	2.5
9	M	42	0.2	82.94	1.39	1.13	3.07	1.97	ND	ND	ND	ND	ND	ND
10	M	28	2.12	75.35	1.60	1.07	5.76	1.55	ND	ND	ND	ND	ND	ND
11	M	38	3.97	9.3	2.91	2.89	3.81	2.37	ND	ND	ND	ND	ND	ND
12	F	36	2.3	37.09	2.24	2.25	1.49	2.54	ND	ND	ND	ND	ND	ND
13	F	50	5.25	32.78	6.06	3.74	5.35	4.1	ND	ND	ND	ND	ND	ND
14	M	43	3.2	47.53	1.18	0.91	1.9	0.86	ND	ND	ND	ND	ND	ND

Abbreviation: ND, not done.

Difference (%) between activated basophils and stimulation index in patients and healthy controls

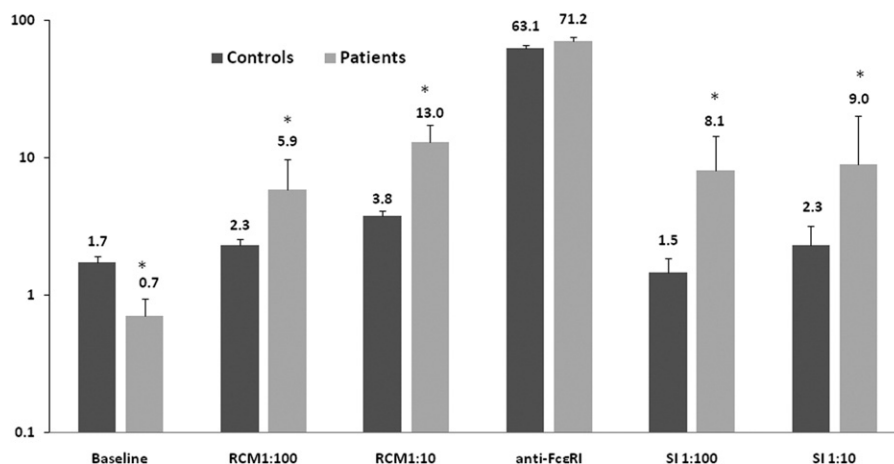


Figure 2. Results of basophil activation tests in patients with a history of immediate reaction with RCM ($n = 26$) compared with healthy controls ($n = 43$). Patients with a history of an immediate RCM reaction had significantly increased activated basophil percentages from baseline after incubated with the responsible RCM in a dose-dependent manner. Contrastingly, the percentages of activated basophils in normal controls were minimally affected. Data are expressed as medians $\pm$ standard errors, and * represents $P < .05$ compared with normal controls.

gorical data between BAT-positive and BAT-negative subjects. Receiver operating characteristics (ROC) curve analysis was performed to determine the diagnostic values of BAT in an immediate RCM reaction. $P < .05$ was considered statistically significant.

RESULTS

Patients with an immediate RCM reaction had higher basophil activation on RCM stimulation compared with normal controls

Basophil activation tests were performed on 26 patients with a history of an immediate RCM reaction with culprit RCM. Forty-three basophil activation tests were performed with 5 different RCMs on 14 healthy individuals who had no history of RCM hypersensitivity to serve as normal controls. The details of BAT results and patients' characteristics are shown in Table 1.

Although the percentages of activated basophils at baseline were lower in the patient group than in the control group (0.71% vs. 1.73%, $P = .017$), they were at comparable levels after stimulation with anti-FCεRI monoclonal antibody (63.14% vs. 71.21%, $P = .281$). After incubation with RCM for 15 minutes, the percentages of activated basophils in healthy controls increased slightly from 1.73% at baseline to 2.32% with 1:100 diluted RCM and 3.81% with 1:10 diluted RCM. In contrast, the degrees of basophil activation markedly increased from baseline in the patient group and were much higher than those in healthy controls, in terms of both activated basophil percentages and the stimulation index. With 1:100 dilution of RCM, the percentage of activated basophils and the stimulation index were 5.94% and 8.06 in

the patient group, as compared with 2.32% and 1.48 in the control group ($P = .002$ and $P = .000$, respectively). With 1:10 dilution of RCM, percent activated basophils and the stimulation index were 12.96% and 9.03 in the patient group, compared with 3.81% and 2.34 in the control group ($P = .003$ and $P = .000$, respectively) (Fig 2).

The measurements of activated basophil percentages and stimulation index showed moderate sensitivity and very good specificity to discriminate patients with a history of an immediate RCM reaction from normal individuals

Currently, no consensus or recommendations have been proposed on the cutoff level for BAT to diagnose RCM hypersensitivity. A case report used percent activated basophils greater than 5% and the stimulation index 2 or greater as the cutoff value.¹⁰ BAT would have the sensitivity of 61.5% and the specificity of 76.7% with 1:10 RCM, whereas the sensitivity of 50% and the specificity of 90.7% with 1:100 RCM would be achieved based on these arbitrary criteria.

We decided to perform ROC curve analysis to evaluate the diagnostic value of BAT. The calculated areas under the curves were 0.712 to 0.727 using activated basophil percentages and 0.787 to 0.792 using stimulation index as the diagnostic criteria. Data from 1:100 RCM had slightly better discrimination powers compared with those from 1:10 RCM for the diagnosis of an immediate RCM reaction. In our study, the basophil activation test has a sensitivity of 46.2% to 61.5% and a specificity of 88.4% to 100%, depending on the cutoff values. The sensitivities and specificities of some selected cutoff points using percent activated basophils are demonstrated in Figure 3A and Table 2. Figure 3B dem-

The diagnostic values of BAT based on different parameters

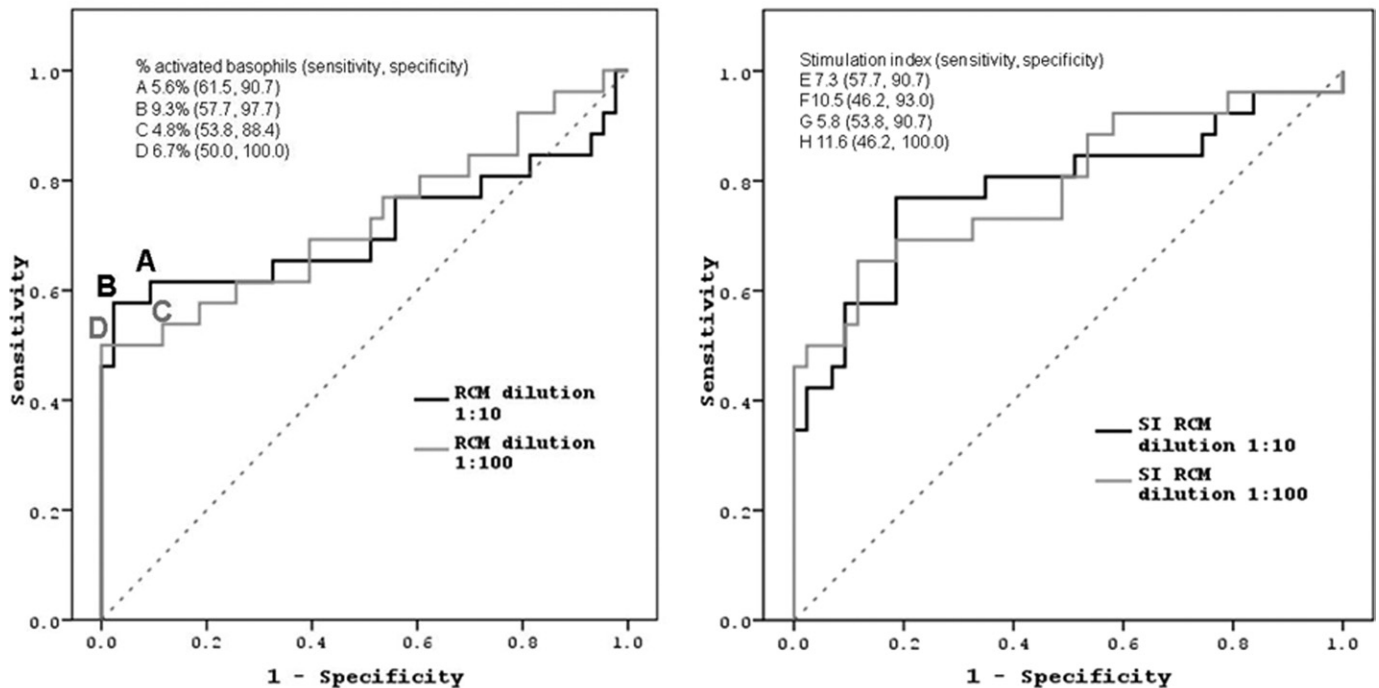


Figure 3. The analysis of receiver operating characteristics (ROC) of basophil activation test results in patients with immediate RCM reactions and healthy controls (Table 2). The ROC curve analysis shows the sensitivity and specificity of BAT using different concentrations of RCM to diagnose an immediate RCM hypersensitivity.

onstrates the sensitivities and specificities related to stimulation index.

Comparative characteristics between patients with a history of an immediate RCM reaction with different results of basophil activation test

To identify the prognostic factors for BAT positivity in patients with an immediate RCM reaction, the clinical data between patients with positive and negative basophil activation test results were comparatively analyzed. The result showed no significant differences between BAT-positive and BAT-negative individuals who had an immediate reaction to RCM in terms of age and sex predilection, underlying allergic

diseases, RCM types, previous sensitization, duration after RCM exposure to BAT, or clinical severity. Interestingly, no correlation was found between the results of skin testing and BAT in patients with RCM hypersensitivity as well (Table 3). The BAT-positive rate and percent activated basophils after incubation with responsible RCM between the skin test-positive and skin test-negative patients were also comparable.

DISCUSSION

The mechanism of an immediate reaction to radiocontrast media is currently unknown. No standard test to diagnose RCM-induced immediate hypersensitivity reactions is

Table 2. Area Under the Curve

Test Result Variable (s)	Area	Standard Error	Asymptotic Sig.	Asymptotic 95% CI	
				Lower Bound	Upper Bound
Dilution 1:10	0.712	0.076	0.003	0.562	0.862
Dilution 1:100	0.727	0.069	0.002	0.592	0.863
SI 1:10	0.787	0.063	0.000	0.664	0.910
SI 1:100	0.792	0.061	0.000	0.674	0.911

Table 3. Comparison Between BAT-Positive and BAT-Negative Subjects

	Positive BAT (N = 15)	Negative BAT (N = 11)	P Values
Female sex	8 (53.3%)	8 (72.7%)	0.33
Mean age (years)	53.3	47.5	0.30
Having drug or food allergy	1 (6.7%)	3 (27.3%)	0.16
RCM type: Iopromide	10 (66.7%)	8 (72.7%)	0.74
Previous exposure to ICM	3 (20.0%)	2 (18.2%)	0.91
Positive skin test reactivity	7 (46.7%)	4 (36.4%)	0.61
Median time from reaction to BAT (months)	29.7	22.2	0.08
Severe reactions (grade III–IV)	1 (6.7%)	2 (18.2%)	0.37

Data from patients with positive and negative BAT (using 9.3% activated basophils with 1:10 RCM as a cutoff value) show similar clinical characteristics between both groups.

available. This syndrome is the so-called “anaphylactoid” reaction, because the specific IgE to RCM has rarely been demonstrated, although its clinical manifestations mimic IgE-mediated pathogenesis. Recently, the idea of IgE involvement in RCM reaction was revisited, because some studies reported that approximately 20% of patients showed positive skin test results, suggesting that IgE-mediated pathogenesis may be possible.^{4–6,10} Most hypersensitive patients, however, remain undiagnosed before exposure that could lead to possible deleterious effects on RCM administration. The correct diagnosis would be beneficial for the selection of appropriate RCM, because anaphylactic reactions can still occur despite appropriate pretreatment in patients with a previous reaction to RCM.¹⁴

Basophils and mast cells share several similar characteristics in releasing several mediators on activation from drugs by either IgE or non-IgE-dependent pathways. Therefore, the BAT has been introduced in the evaluation of immediate hypersensitivity reaction to drugs.¹⁵ The BAT is potentially a helpful test to diagnose an immediate RCM reaction, because the existence of specific IgE is not necessary. Case reports show that BAT could have a role in an immediate RCM reaction,^{9,16} but no clinical data on the predictive ability of BAT in this syndrome have been published.

Our study aimed to investigate the potential role of BAT in the diagnosis of an immediate RCM hypersensitivity. The results indicated that, after stimulation with RCM, the degree of basophil activation increased significantly in a dose-dependent fashion in approximately half of the patients with a history of an immediate RCM reaction, whereas the degree of basophil activation in the control group was barely affected. The statistical analysis indicated that BAT could be a useful test in the diagnosis of RCM reactions by using percent activated basophils or the stimulation index as indicators. The stimulation index seems to have better predictive ability than percent activated basophils to distinguish between RCM-sensitive patients and normal controls. However, no predisposing factors for BAT positivity in these patients have been found.

According to the classification of the accuracy of the diagnostic system, the BAT is considered to have moderate accuracy (area under the curve > 0.70 and < 0.90).¹⁷ A good

specificity of BAT (88.4%–100%) makes it a valuable tool to confirm an immediate hypersensitivity reaction to RCM.

Although BAT can detect only half of the patients with a previous history of immediate reactions of RCM in this study, it is still considered a promising diagnostic method. Certain confounding factors possibly affect the BAT result. The duration after RCM reaction to BAT in our study was rather long (1–4 years), and this lag time possibly lowered the BAT reactivity. Most of the patients in this study experienced only mild skin symptoms, which may explain a sensitivity of 46% to 61.5% in our report. Some of the adverse reactions in these patients may not have been mediated by basophil/mast cell degranulation and therefore could not have been detected using the BAT technique.

This is the first study systematically analyzing the accuracy of BAT in an immediate RCM reaction. Our data provide evidence that BAT could be a promising test, although the optimal cutoff values still need further exploration. Further studies with larger sample sizes should be conducted to find out the diagnostic values of BAT in RCM hypersensitivity in terms of sensitivity and specificity.

According to conventional beliefs, skin test positivity implies the presence of specific IgE and theoretically should be detectable using the BAT technique. Our study failed to show the correlation between skin test results and basophil activation test. The relationship between skin test results and BAT should be repeated by performing both tests in a similar time frame. This could help determine whether the discrepancy between skin testing and BAT was attributable to different subgroups of RCM-sensitive patients or whether it was attributable to the waning of BAT reactivity over time. The stripped basophils histamine release bioassay or passive sensitization of basophils could be performed to verify the role of IgE in immediate RCM sensitivity.^{18,19} The predictive value of the combination of a skin test and BAT is worth further study as well, because this skin test/BAT combination could be another option to screen RCM hypersensitivity in high-risk patients before RCM administration and to select a safe RCM in patients with a previous severe immediate reaction.

In conclusion, our study demonstrated the potential of BAT as a diagnostic tool for an immediate RCM hypersensitivity, particularly as a confirmation test. Further study with

a larger sample size is required to confirm the test accuracy and to identify patients' predisposing factors.

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