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ORIGINAL ARTICLE

Elevated Serum Total IgE Alter the Diagnostic Performance of Radio-Immune Assays for β-Lactam Specific Antibody Dosing

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SUMMARY

Background: ***In vivo*** **allergy tests and the detection of drug-specific antibodies are widely used in the diagnosis of β-lactam induced immediate-type hypersensitivity reactions. The diagnostic performance of immunoenzymatic tests for the demonstration of serum-specific IgE (IgE_s) antibodies is influenced by total IgE values (IgE_t). The aim of this study was to investigate whether the result obtained by radioimmunoassays (RIA) for β-lactams IgE_s antibodies is correlated to IgE_t.**

Methods: **68 paired *in vivo* and *in vitro* tests were performed for the culprit drugs in 49 patients with suspected previous hypersensitivity reactions to β-lactams. 14 controls who tolerated the tested antibiotics were similarly investigated. The dosing of IgE_t was performed using the Immulite Immunoassay (Siemens). We detected IgE_s using a sandwich-type RIA with sepharose as solid phase (Pathologie Cellulaire et Moléculaire en Nutrition, France) and anti-IgE_s I^{125}-labelled antibodies (Immunotech, Czech Republic).**

Results: **RIA-IgE_s sensitivity was 56.52% in patients with IgE_t < 120 IU/mL and 90.90% in patients with IgE_t > 120 IU/mL (p = 0.0052). All patients with IgE_t > 500 IU/mL had positive RIA results. RIA-IgE_s specificity was 90.90% for IgE_t < 120 IU/mL and 66.66% for IgE_t > 120 IU/mL. The linear equation that fits the relation between IgE_t and IgE_s is: $IgE_s = (IgE_t + 81.644)/137.94$, with a correlation coefficient of 0.4.**

Conclusions: **Serum total IgE alter the diagnostic performance of radioimmunoassays for β-lactam specific antibody dosing. Assays for the detection of both IgE_t and IgE_s need to be performed for each individual investigated, retrospectively, to confirm clinical immediate-type hypersensitivity reactions.**

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INTRODUCTION

Hypersensitivity reactions to β-lactams are the most frequent cause of adverse reactions mediated by specific immunological mechanisms and can be explored by *in vivo* and *in vitro* tests [1,2]. The retrospective diagnosis of suspected β-lactam immediate-type hypersensitivity reactions comprises a thorough allergological history, the allergologic skin tests, the potentially dangerous drug challenge tests, and the detection of drug-specific antibodies in the patients' sera. For β-lactams, current

guidelines recommend the use of *in vivo* diagnosis together with the detection of specific antibodies for the culprit drugs [3]. As all hypersensitivity reactions induced by drugs have the potential to represent a lethal event and *in vivo* studies may induce a clinical reaction, research has focused on the *in vitro* diagnosis. The *in vitro* diagnosis tests need to have optimal sensitivity and specificity in order to be clinically useful.

Measurement of total serum IgE (IgE_t) and allergen specific IgE (IgE_s) is often requested to assess possible allergy [4]. Several immunologic *in vitro* methods are currently available to detect IgE specific antibodies for drugs. The first assay designed for the detection of specific antibodies was the radioisotope-based radioallergosorbent test (RIA) [5], while the most frequently used are the newer immunoenzymatic tests. There have been previous reports regarding the diagnostic value of enzymatic assays, the specificity of which decreases with the increase in IgE_t levels [2,6,7]. The diagnostic performance of immunoenzymatic tests for the demonstration of serum-specific IgE antibodies is influenced by IgE_t values, both of the assays being needed in order to interpret individual results for patients [2].

As radio-immune assays were the first *in vitro* investigation tools for the detection of IgE_s for β-lactams and their methodology differs from immunoenzymatic tests, the aim of this study was to investigate whether the result obtained by radioimmunoassays (RIA) for β-lactam IgE_s antibodies is correlated to IgE_t values. Other possible modifiers we investigated were the presence of the atopic phenotype, major versus minor symptoms, and skin test positivity, testing the hypothesis that these might be predictors for IgE_s positivity.

MATERIALS AND METHODS

Patients

The enrollment of the patients was approved by the Research Ethics Committee of the Iuliu Hațieganu University of Cluj-Napoca. A total of 807 patients were referred to our department from January 1, 2008, until December 31, 2012. All patients and controls investigated for β-lactam immediate-type hypersensitivity reactions by the combined use of the allergological history, the skin tests results, the detections of IgE_s for the culprit drug, and the dosing of IgE_t levels were retrospectively included in this analysis. All patients signed the informed consent form for the performance of allergologic *in vivo* and *in vitro* tests and completed, guided by the allergologist, a structured questionnaire containing the history: the type and date of the previous reactions, culprit drugs, treatment, the presence of atopy, and other exposure to drugs. Minor symptoms were defined as urticaria and non-life threatening angioedema, while major symptoms were considered severe bronchospasm, hypotension, and cardio-vascular colapse (anaphylactic shock). The patients were tested for the culprit drug if they agreed. The exclusion criteria were history of steroid medication, treatment with antihistamines and pregnancy. From the initial 807 patients, we found 49 patients with previous suspected β-lactam induced immediate-type hypersensitivity reactions who had paired dosing of both IgE_t and IgE_s values from the immunological analysis.

In this analysis, we also included 14 healthy individuals who tolerated β-lactams well and were tested using drug-specific and total serum IgE dosing.

In vivo tests

Allergological *in vivo* tests were performed by two allergologists and included the skin prick test (SPT) and the intradermal test (IDT), according to international recommendations [8].

Total serum IgE dosing (IgE_t)

We obtained samples from the patients on the day of clinical evaluation and skin testing and the sera were frozen at -20°C. All the samples were run in parallel for the *in vitro* tests.

The detection of IgE_t was determined using the Immulite Immunoassay Systems (Siemens, New York, USA), with serum total IgE values below 120 IU/mL (OMS 75/502) being considered in the normal range [9]. The tests were performed simultaneously for all patients according to the manufacturer's instructions.

β-lactam specific IgE antibody dosing (IgE_s)

We detected IgE_s using a sandwich-type RIA with sepharose as the solid phase (Pathologie Cellulaire et Moléculaire en Nutrition, Université „H. Poincare", Nancy, France) and anti-IgE_s I^{125}-labelled antibodies (Immunotech, Czech Republic). 50 µL of each patient serum was centrifuged with the culprit antibiotic fixed in the sepharose gel for 3 hours at 24°C. After washing three times with phosphate buffer solution, 50 µL solution with anti-IgE_s I^{125}-labelled antibodies was added to each and the samples were incubated for 18 hours. Radioactivity was determined with an LKB gamma-counter (Clini-Gamma 1272-003, Wallac Oy, Finland). The final result was expressed as R, the ratio between the percentage of antibody fixed when using patient serum divided by the percentage of fixed antibody when using serum from healthy controls. A value of $R \geq 2$ was considered a positive result [10].

Statistical analysis

Linear logistic regression analysis was used to graphically display the relationship between IgE_t and IgE_s. We used the correlation coefficient to assess the strength of the relationship between IgE_t and IgE_s. Sensitivity was calculated as the number of patients with positive IgE_s divided by the total number of patients, while specificity was calculated as the number of controls with negative IgE_s divided by the total number of controls. Cohen kappa index (k) was used to assess the agreement between IgE_t results and the presence of the atopic phenotype. Cohen kappa index was calculated as $k = (a + b) x$

(a + c) + (c + d) x (b + d)/N x N, where: a = number of positive IgE_t tests in patients with atopy; b = number of positive IgE_t tests in patients with no atopy; c = number of negative IgE_t tests in patients with atopy; d = number of negative IgE_t tests in patients with no atopy; N = total number of tests. *Fisher's exact test* was used to assess the level of significance for the differences in the positivity rates for different patient groups (categorial variables).

RESULTS

A total of 49 patients were tested *in vivo* and *in vitro* for the culprit β-lactam antibiotics that caused previous suspected immediate-type hypersensitivity reactions. Of these, 34 patients were single-drug reactors, 22 patients presented previous reactions for two different β-lactam antibiotics, while 4 patients were tested for three culprit drugs. Thus, 68 tests were performed in patients with previous drug reactions (additional file 1).

From the 68 tests performed *in vivo* for the culprit drugs, there were 21 positive skin tests (8 positive skin prick tests and 13 positive intradermal tests). We found 46 positive RIA-IgE_s results from the 68 performed for the culprit β-lactam antibiotics (positivity rate 67.65%). 17 paired tests were performed in patients presenting atopy and 51 tests were performed in patients who declared themselves as not presenting an atopic phenotype. 37 paired tests were performed for minor clinical reactions (urticaria and angioedema) and 31 tests were performed for major clincial reactions (severe bronchospasm, hypotension or anaphylactic shock).

Fourteen controls who tolerated intravenous β-lactams in our department were similarly tested (suplemental material 1). None of the controls presented a positive skin test, one of them declared atopy and 2 presented positive IgE_s results with $R \geq 2$.

In order to investigate the relationship between IgE_t and IgE_s we divided IgE_t values into 4 categories (Table 1). The rate for IgE_s positivity increased progressively with the increase in total serum IgE_t, being 100% in patients with $IgE_t > 500$IU/mL (Table 1).

The positivity rate for RIA-IgE_s was 56.52% in patients with IgE_t below 120 IU/mL (26 positive IgE_s results from 46 tests) and increased with the increase in total serum IgE_t. The positivity rate for RIA-IgE_s was 90.90% in patients with $IgE_t > 120$ (20 positive IgE_s results from 22 tests). Thus, the calculated sensitivity for RIA-IgE_s was 56.52% in patients with $IgE_t < 120$ and 90.90% in patients with $IgE_t > 120$ (*Fisher's exact test*, $p = 0.0052$). In controls, there were 9 negative IgE_s results from the 11 tests performed in controls with $IgE_t < 120$ IU/mL (90.90% specificity) and 2 negative IgE_s results from the 3 performed in controls with $IgE_t > 120$ IU/mL (66.66% specificity). Thus, the specificity of RIA-IgE_s was higher when IgE_t was < 120 IU/mL.

Performing the linear regression analysis, we found the linear equation to fit the relation between IgE_t and IgE_s: $x = (y + 81.644)/137.94$, where $y = IgE_t$ and $x = IgE_s$ (R), with a correlation coefficient of 0.4 ($r^2 = 0.1551$) (Figure 1). According to this equation, we would find a positive IgE_s result ($R \geq 2$) for IgE_t values higher than 194.236 IU/mL.

From the 17 reactions investigated in patients presenting atopy, 11 presented positive IgE_s results (64.70%), while from the 51 reactions investigated in patients without atopy, 35 presented positive IgE_s results (68.62%), with no significant statistical difference (*Fisher's exact test*, $p = 0.77$).

We investigated 31 major clinical reactions, with 24 presenting positive IgE_s results (77.41%), and 37 minor clinical reactions, with 22 positive IgE_s results (59.45%). There was no statistical difference for IgE_s positivity rate between the major and minor clincial reactions we investigated (*Fisher's exact test*, $p = 0.128$).

There were 21 positive skin tests for the culprit drugs of which 17 presented a positive IgE_s result (80.95%) and 47 negative skin test results for the culprit drugs, of which 29 presented positive IgE_s results (61.70%). There was no significant statistical difference between reactions confirmed by a positive skin test result and those with a negative skin test result (*Fisher's exact test*, $p = 0.163$).

The concordance between the atopic phenotype and IgE_t values was 0.11 (95% CI: 0 - 0.34), as assessed using Cohen kappa index.

DISCUSSION

The retrospective diagnosis of immediate-type hypersensitivity reactions caused by different drugs is the result of a complex investigation that comprises the full allergological history, the allergy skin tests, the performance of challenge tests, and the detection of drug-specific antibodies. *In vivo* tests such as the drug challenge tests may cause severe clinical reactions [2]. Thus, reliable *in vitro* tests, in which the sensitised individuals are not exposed to the culprit drug, are necessary. Measurement of total serum IgE and allergen specific IgE is often requested to assess possible allergy [4]. Allergic sensitization mediated by IgE is the basis of allergic disease, and elevated total IgE is frequently included as diagnostic criterion for allergic disease, though its value is controversial [11]. The distribution of IgE values is extremely wide in patients with and without known allergic disease, and therefore defining an upper "limit of normal" for serum IgE is of doubtful clinical value [12]. However, reference limits have been established for several patient categories [13,14].

In vitro quantification of specific IgE combined with skin tests constitutes the cornerstone of the diagnosis of allergic diseases caused by a type I hypersensitivity reaction [5]. The detection of specific IgE is currently used to confirm sensitisation to a particular allergen. The use of *in vitro* tests for estimating IgE antibodies to antibiotics is important because of the existance of pa-

Table 1. Specific IgE dosing performance according to total serum IgE values.

Total IgE value (IU/mL)		< 100		100 - 250		250 - 500		> 500	
Reaction		Yes (N = 42)	No (N = 10)	Yes (N = 8)	No (N = 1)	Yes (N = 11)	No (N = 1)	Yes (N = 7)	No (N = 2)
IgE_s	Positive	21	0	6	1	10	1	7	0
	Negative	21	10	2	0	1	0	0	2
Sensitivity		50%		75%		91%		100%	

IgE_s - specific IgE for drugs, N - number.

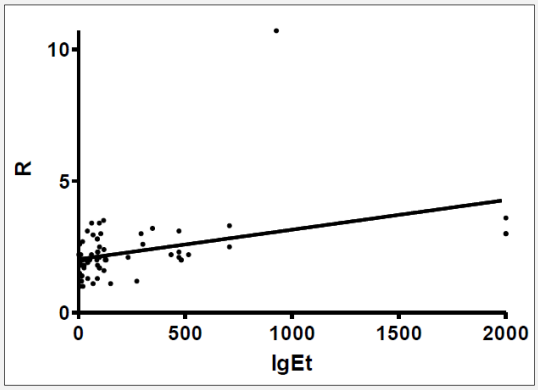

Figure 1. The linear relationship between specific IgE dosing and total serum IgE levels.

R - the ratio result for the radioimmunoassay, IgEt - total serum IgE.

tients with positive history, but negative skin tests [15]. IgE measurements can avoid potentially harmful drug challenge tests [1].

There are several methods for total and specific serum IgE dosing, including the radioisotope-based radioallergosorbent test and the immunoenzymatic tests: ImmunoCAP (Thermo Fisher, previously Phadia), Immulite (Siemens), and Hytec-288 (Hycor). There have been previous reports regarding the diagnostic value of enzymatic assays, the specificity of which decreases with the increase in total serum IgE levels [2,6,7]. Good correlations were found in previous studies between the values obtained using the different methods [5], though their results are not interchangeable [5,16]. As the results obtained with different methods for the measurement of serum specific IgE are not equivalent, we have investigated the hypothesis that specific β-lactam IgE antibody dosing using RIA is influenced by the values of IgE_t, similarly to the immunoenzymatic methods.

In this study, our aim was to investigate whether the IgE_s response in the sepharose radioimmunoassays is influenced by serum total IgE levels and to question whether IgE_s dosing alone can be used in the diagnostic algorithm of β-lactam induced immediate-type hypersensitivity reactions. We also investigated other additional predictors for positive IgE_s results, including atopy, the severity of the reaction, and the skin test results. We observed a moderate direct correlation between IgE_s and IgE_t values, the result for the radio-immune dosing increasing with the increase in total serum IgE_t. Increased serum IgE_t values can be considered a potential modifier of the diagnostic performance of immunoenzymatic IgE_s tests [2]. The sensitivity of the RIA was 56.52% when the patients had IgE_t in the normal range (< 120 IU/mL) and 90.90% in patients with elevated IgE_t (> 120 IU/mL). All patients with IgE_t > 500 IU/mL had positive RIA-IgE_s result. RIA has 90.90% specificity for IgE_t < 120 IU/mL.Thus, the absolute value of IgE_t

influences the sensitivity and the specificity of the RIA-IgE_s dosing result, similar to immunoenzymatic methods.

From our analysis of the effect of total serum IgE values, of the atopic phenotype, of the severity of the reaction, and of the skin tests' positivity on the diagnostic performance of the radio-immune assays for β-lactam specific IgE antibody dosing, the only predictor factor we found was the IgE_t value. We found a poor correlation between the presence of the atopic phenotype and IgE_t dosing, with Cohen kappa index being 0.11. Our results are in agreement with previous studies, where only 36.70% of the atopics could be correctly classified as allergic patients only on the basis of IgE_t dosing [17]. Because of the influence of IgE_t on the detection of β-lactam IgE_s antibodies, the combination of both tests is mandatory in the *in vitro* diagnostic approach of β-lactam allergy [2].

Reliable *in vitro* tests for drug allergy diagnosis are needed, with optimal sensitivity and specificity. A false positive result might not allow a patient to benefit from a necessary treatment and a false negative result might put the patient at risk if the individual was exposed to the drug. As all drug hypersensitivity reactions have the potential to lead to a fatal outcome, most sensitive approaches are needed.

In a patient with previous suspected drug-induced hypersensitivity reactions, the limitations of *in vitro* tests are that both false negative and false positive results can be encountered. A negative result in a patient could be explained by a low test sensitivity, the involvement of cellular mechanisms in the previous clinical reaction, or the loss of sensitivity with time. A negative IgE_s result does not rule out allergic disease [5]. A false positive result might be the result of elevated IgE_t level. Immunoenzymatic methods used for the detection of β-lactam specific antibodies give false positive results among healthy individuals who tolerate β-lactams well and have IgE_t values > 500 IU/mL, thus IgE antibodies are indicative but not conclusive proof of a β-lactam allergy [2,6,7]. We found the same relationship between IgE_t and the IgE_s using the radioimmunoassay. The false positive results observed with high IgE_t serum levels may be due to several factors including high stickiness with hapten-bound substrates [2].

Specificity is defined as the percentage of correct negative results in a non-diseased population [6]. The specificity of RIA-IgE_s declines with the increase in IgE_t because of the false positive results of RIA that appear at high IgE_t levels.

The results of specific antibody dosing may additionally be difficult to interpret. Positive IgE_s without symptoms must be carefully interpreted because they can be due to a low degree of sensitisation and unable to express clinical symptoms [18]. The mechanisms of the progression from sensitisation to the clinical reactions have not been fully elucidated.

Thus, the diagnostic value of IgE_s dosing is not absolute and the diagnostic performance, expressed in terms of sensitivity and specificity, is altered by the levels of the IgE_t antibodies in patients' sera. In the context of this relationship, the diagnostic reliability of the assays for specific IgE dosing might be questioned. As the IgE_t level is a modifier of the IgE_s result, the IgE_t value needs to be considered when interpreting the results for individual patients.

In immediate-type hypersensitivity reactions, IgE antibody concentration, affinity (tightness of binding), clonality (epitope specificity), and specific activity (the allergen specific IgE to total IgE ratio) influence the translation of IgE response into clinical symptoms following allergen exposure and the impact on effector cell activation [16]. The specific activity is less studied and little is known about the relative IgE-specific activity among drug allergy patients. The fraction of the total serum IgE that is specific for a given allergen increases as the total serum IgE levels decrease [19]. The limitation of our study is that we used two different methods to determine specific and total antibodies in sera. The assays' results being expressed in different units, we could not assess further the IgE immune response-specific activity. This needs to be investigated further among the variables that alter the efficacy of *in vitro* diagnostic methods for β-lactam hypersensitivity reactions.

Total IgE levels are needed to interpret the significance of specific IgE. The clinicians' intentions to limit the performance of potential harmful drug challenge tests are hampered by the complex interpretation of the β-lactam specific IgE dosing. The place for IgE measurements should be limited to situations of severe manifestations in order to avoid drug provocations [1].

In conclusion, elevated serum total IgE alters the diagnostic performance of radioimmunoassays for β-lactam specific antibody dosing. Assays for the detection of both total serum IgE and specific IgE need to be performed for each individual investigated retrospectively to diagnose clinical immediate-type hypersensitivity reactions and the results need to be interpreted carefully in conjunction with the clinical history.

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Declaration of Interest:

The authors declare that they have no competing interests.

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Supplemental Table 1. Patients' and controls' clinical data and test results.

No.	Substance	SPT	IDT	R	IgEt	Atopy	Clinical symptoms
Patients							
1	amoxicillin	-	-	2.6	5	-	urticaria
2	amoxicillin	-	+	1.8	9	-	urticaria
3	amoxicillin	-	-	2	11.4	-	angioedema
4	amoxicillin	-	+	1.2	13.1	-	angioedema
5	amoxicillin	-	-	1.2	13.7	+	angioedema
6	amoxicillin	-	-	2	16.2	-	bronchospasm
7	amoxicillin	-	-	1.7	25.6	+	urticaria
8	amoxicillin	-	-	1.8	26.2	-	urticaria
9	amoxicillin	-	-	2.95	68.4	-	urticaria
10	amoxicillin	+		2.3	90.7	-	shock
11	amoxicillin	-	-	2.1	97.9	+	bronchospasm
12	amoxicillin	-	-	3.2	346	+	hypotension
13	amoxicillin	+		2.3	470	-	urticaria
14	amoxicillin	+		2	481	-	shock
15	amoxicillin	-	+	2.2	515	-	urticaria
16	amoxicillin	-	-	2.5	706	+	angioedema
17	ampicillin	-	-	2.2	1.3	-	urticaria
18	ampicillin	-	-	2	5	-	urticaria
19	ampicillin	-	+	2	13.1	-	angioedema
20	ampicillin	-	-	1.4	15.8	-	urticaria
21	ampicillin	-	-	1.4	15.8	-	urticaria
22	ampicillin	-	+	2	18.9	-	shock
23	ampicillin	-	-	2.7	18.9	-	angioedema
24	ampicillin	-	-	2.2	60.9	+	angioedema
25	ampicillin	-	-	1.1	68.4	-	urticaria
26	ampicillin	-	-	1.8	88.9	-	urticaria
27	ampicillin	-	+	1.7	97.3	-	bronchospasm
28	ampicillin	-	-	1.7	97.9	+	bronchospasm
29	ampicillin	+		1.6	119	+	shock
30	ampicillin	-	-	2.1	125	+	hypotension
31	ampicillin	-	-	3	293	-	angioedema
32	ampicillin	-	+	2.2	433	-	angioedema
33	ampicillin	-	-	2	481	-	shock
34	ampicillin	-	-	3	2000	-	hypotension
35	ampicillin	-	-	3	2000	-	hypotension
36	ceftriaxone	-	-	2.2	10.4	-	hypotension
37	ceftriaxone	-	-	1.9	43.3	-	bronchospasm
38	cefuroxime	-	-	1.9	6	-	bronchospasm
39	cefuroxime	-	-	2	85.4	-	angioedema
40	oxacillin	-	-	1	20.3	+	urticaria
41	oxacillin	-	+	2.8	87.8	-	urticaria
42	oxacillin	-	-	2.8	87.8	-	urticaria
43	oxacillin	-	+	3.1	470	-	urticaria

44	**penicillin**	**-**	**-**	**1.5**	**5**	**-**	**urticaria**
45	**penicillin**	**-**	**-**	**1**	**7.6**	**-**	**angioedema**
46	**penicillin**	**-**	**-**	**1.8**	**17.7**	**-**	**bronchospasm**
47	**penicillin**	**-**	**-**	**2**	**35.1**	**-**	**angioedema**
48	**penicillin**	**-**	**-**	**3.1**	**41.4**	**+**	**bronchospasm**
49	**penicillin**	**-**	**-**	**1.3**	**43.3**	**-**	**bronchospasm**
50	**penicillin**	**-**	**+**	**2**	**51.8**	**-**	**shock**
51	**penicillin**	**-**	**-**	**3.4**	**61**	**-**	**hypotension**
52	**penicillin**	**-**	**-**	**1.3**	**87.8**	**-**	**urticaria**
53	**penicillin**	**-**	**-**	**2.3**	**88.9**	**-**	**urticaria**
54	**penicillin**	**-**	**+**	**3.4**	**97.3**	**-**	**bronchospasm**
55	**penicillin**	**-**	**-**	**2.5**	**97.9**	**+**	**bronchospasm**
56	**penicillin**	**-**	**-**	**3**	**104**	**-**	**shock**
57	**penicillin**	**+**		**3.5**	**117**	**-**	**shock**
58	**penicillin**	**+**		**2.4**	**119**	**+**	**shock**
59	**penicillin**	**-**	**+**	**2**	**125**	**+**	**hypotension**
60	**penicillin**	**-**	**+**	**2**	**129**	**+**	**urticaria**
61	**penicillin**	**-**	**-**	**1.1**	**150**	**+**	**urticaria**
62	**penicillin**	**-**	**-**	**2.1**	**232**	**-**	**shock**
63	**penicillin**	**-**	**-**	**1.2**	**273**	**-**	**angioedema**
64	**penicillin**	**-**	**-**	**2.6**	**301**	**-**	**shock**
65	**penicillin**	**+**		**2.1**	**470**	**-**	**urticaria**
66	**penicillin**	**-**	**-**	**3.3**	**706**	**+**	**angioedema**
67	**penicillin**	**+**		**10.7**	**926**	**-**	**shock**
68	**penicillin**	**-**	**-**	**3.6**	**2000**	**-**	**shock**
Controls							
1	**amoxicillin**	**-**	**-**	**1.7**	**1**	**-**	**-**
2	**amoxicillin**	**-**	**-**	**1.4**	**13.6**	**-**	**-**
3	**amoxicillin**	**-**	**-**	**1.8**	**76.2**	**-**	**-**
4	**amoxicillin**	**-**	**-**	**1.9**	**2000**	**-**	**-**
5	**ampicillin**	**-**	**-**	**1.4**	**1**	**-**	**-**
6	**ampicillin**	**-**	**-**	**1.6**	**10**	**-**	**-**
7	**ampicillin**	**-**	**-**	**1.3**	**13.6**	**-**	**-**
8	**ampicillin**	**-**	**-**	**2.2**	**117**	**-**	**-**
9	**ampicillin**	**-**	**-**	**2.1**	**330**	**+**	**-**
10	**cefuroxime**	**-**	**-**	**1.9**	**1**	**-**	**-**
11	**cefuroxime**	**-**	**-**	**1.9**	**39.3**	**-**	**-**
12	**cefuroxime**	**-**	**-**	**1.7**	**879**	**-**	**-**
13	**penicillin**	**-**	**-**	**1.5**	**13.6**	**-**	**-**
14	**penicillin**	**-**	**-**	**1.8**	**86.1**	**-**	**-**