

In Vivo Cysteinyl Leukotriene Release in Allergic and Nonallergic Immediate Hypersensitivity Reactions during Anesthesia

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ABSTRACT

Background: Immediate hypersensitivity reactions occurring during anesthesia are classified as allergic when skin tests and mast cell tryptase are positive and as nonallergic when negative results are obtained. Cysteinyl leukotrienes (cysLTs) are potent mediators synthesized by mast cell and eosinophil that induce bronchial constriction. They could play a role in hypersensitivity reactions.

Methods: cysLT C4, D4, and E4 concentrations were measured by a competition immunoassay in serial plasma samples obtained prospectively from 21 anesthetized controls and retrospectively from 34 patients who reacted at induction of anesthesia (24 with allergic and 10 with nonallergic reactions).

Results: In controls, the median (interquartile range) cysLT concentration was 0.83 (0.69 to 1.02) $\mu\text{g/l}$ before anesthesia and was unchanged 30 min, 6 h, and 24 h afterward. In the patients with allergic reactions, the values were highly increased 30 to 60 min after the reaction (17.9 [7.8 to 36.0] $\mu\text{g/l}$), while the patients with nonallergic reactions had less increased values (7.3 [3.0 to 11.5] $\mu\text{g/l}$). The difference between the three groups was significant ($P < 0.0001$). Increased values persisted during the 24 h of observation. Concentrations were significantly higher in patients with bronchospasm ($P = 0.016$).

Conclusions: cysLTs appear to be an important mediator of allergic and nonallergic immediate hypersensitivity reactions. These findings might open a new field for management of patients with hypersensitivity reactions, especially nonallergic ones. (**ANESTHESIOLOGY 2017; 126:834-41**)

IMMEDIATE hypersensitivity reactions to drugs proceed through allergic or nonallergic mechanisms.¹ Allergic hypersensitivity is supported by clinical history, positive skin tests, and/or other immunologic testing to a suspected agent. Allergic hypersensitivity is generally associated with increased plasma concentrations of mast cell degranulation markers such as histamine and tryptase^{2,3} although tryptase may appear in the reference range in the less severe reactions and histamine disappears rapidly from plasma due to its short half-life.² Nonallergic hypersensitivity is considered when clinical history is suggestive, with negative allergy testing. Tryptase is released along with histamine, as shown *in vitro* for reactions to vancomycin, but concentrations are only occasionally increased in plasma.³⁻⁵ Seventy percent

What We Already Know about This Topic

- Immediate hypersensitivity reactions are characterized by release of multiple mediators from inflammatory cells, but are classified as allergic based on immunologic testing and mast cell tryptase release. The role of specific mediators for hypersensitivity reactions is not well defined.

What This Article Tells Us That Is New

- Cysteinyl leukotrienes appear to be important mediators of both allergic and nonallergic immediate hypersensitivity reactions. These findings may be important for managing patients with hypersensitivity reactions.

of immediate hypersensitivity reactions occurring during anesthesia in France are classified as allergic at the end of

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the diagnostic procedure, most of them to a neuromuscular blocking agent (NMBA), while 30% appear nonallergic.⁶ Frequencies appear different in the United States, with only 47% allergic, mostly to antibiotics.⁷ Cysteinyl leukotrienes (cysLT; initially called slow-reacting substance of anaphylaxis) are a series of three molecules, leukotrienes C₄, D₄, and E₄, with rapid conversion from C₄ to D₄ and E₄ forms in extracellular fluids.^{8,9} CysLT are generated together with histamine in animal and human lung tissues and to a lesser extent in arterial and venous tissues after *ex vivo* antigen challenge.⁸ CysLT elicit airway smooth muscle constriction after inhalation.¹⁰ Moreover, a cutaneous effect has been demonstrated by cutaneous vasodilatation and edema formation when cysLT were injected into skin.¹⁰ Increased urinary concentrations were demonstrated in patients with pathologies possibly related to allergy, such as nocturnal asthma¹¹ or severe atopic eczema/dermatitis syndrome in children.¹² Urinary cysLT concentrations measured during reactions to food or antibiotics appeared significantly higher in patients with anaphylactic shock, *i.e.*, hypotension, than in patients without anaphylactic shock.¹³ Increases were also described in nonallergic hypersensitivity to aspirin,^{14,15} as well as during acute viral wheeze in preschool children¹⁶ or in bronchiolitis,¹⁷ events that are not related to hypersensitivity.

Thus, cysLT most probably participate in allergic immediate hypersensitivity reactions during anesthesia, together with histamine, tryptase, and other allergy mediators. In contrast, the pathophysiologic pathways or mediators associated with nonallergic hypersensitivity reactions have not been studied thoroughly *in vivo*.

The aim of the current study was to evaluate the *in vivo* release of cysLT in plasma during the course of allergic and nonallergic immediate hypersensitivity reactions during anesthesia. We hypothesized that cysLT concentrations would be higher in allergic patients than in nonallergic patients and controls.

Materials and Methods

The study was approved by the local Ethics Committee (Comité Consultatif de Protection des Personnes se prêtant à la Recherche Biomédicale [CCPPRB], Caen, Basse-Normandie, France, protocol 2004-32). Written informed consent was obtained from consecutive patients included in the study.

Patients

Patients with hypersensitivity reactions were selected retrospectively from a cohort of patients who presented symptoms of an allergic reaction within minutes after the induction of anesthesia during years 2001 to 2009 in different surgical centers in Basse-Normandie, France. The severity of the reactions was graded according to the classification of Ring and Messmer¹⁸: grade 1 consists in cutaneous or mucosal signs (extended erythema or urticaria and/or facial or extended edema); grade 2 in mild systemic reaction (moderate tachycardia and/or hypotension and/or coughing or dyspnea),

usually with cutaneous signs; grade 3 in cardiovascular shock (systolic blood pressure less than 60 mmHg was retained in the current study) and/or severe bronchospasm, and grade 4 in cardiac arrest. The inclusion criteria were the availability of stored plasma samples collected within the first hours after the reaction and the results of allergy tests. To ensure diagnosis consistency, patients with allergic hypersensitivity were selected on the basis of positive skin tests to NMBA and increased tryptase and/or histamine concentrations and patients with nonallergic hypersensitivity on negative skin tests, together with normal tryptase concentrations.

Controls

Control patients were recruited consecutively from February 2007 to June 2007 in the surgery units of the University Hospital of Caen, Caen, France, after signed informed consent before scheduled surgical procedures. The inclusion criteria were the administration of general anesthesia including NMBA injection, followed by surgery, without occurrence of hypersensitivity reactions or complications within 24 h. Children and pregnant women were excluded. Moreover, plasma samples from two patients who presented in other hospital units with cardiac arrest unrelated to hypersensitivity reactions were also tested, to check the effect of cardiac arrest and resuscitation procedures on cysLT concentrations.

Serum samples from the control group and from several patients of the current study previously served, among others, to quantify NMBA-specific IgE in a bicenter study, the results of which have been published elsewhere.¹⁹

Samples

Sequential blood samples were obtained from reacting patients after the beginning of the resuscitation procedures, and up to 24 h afterward. Blood samples from controls were obtained before the anesthetic procedure and 30 min, 6 h, and 24 h after the induction of anesthesia. Blood samples were withdrawn in EDTA-containing tubes, and plasma was stored frozen in aliquots at -20°C. The exact times of induction of anesthesia, of reaction if appropriate, and of blood collection were obtained for patients and controls.

Soluble Mediator Assays

Leukotriene C₄, D₄, and E₄ combined concentrations were measured in duplicate by a competition enzyme immunoassay (Neogen, USA), following the instructions of the manufacturer. We first compared the concentrations measured after extraction (performed according to the manufacturer's instructions) and from direct measurements in plasma. As the values appeared correlated ($y = 1.457x - 70$; $r^2 = 0.54$, data not shown) and sufficiently high not to need to concentrate the plasma samples, we further assayed plasma without extraction. Plasma from controls was assayed after 1:2 dilution in a kit buffer, and plasma from patients was diluted 1:2 up to 1:50, in order to obtain values standing in the linear portion of the calibration curve.

Plasma histamine concentrations were measured in patients in duplicate by a radioimmunoassay (RIA Histamine; Beckman Coulter, Immunotech, France) and mast cell total tryptase concentrations by an automated fluoroimmunoassay (Tryptase UniCAP; Thermo Fisher Scientific, Phadia, France). The reference values determined in our laboratory from anesthetized controls are less than 6 nmol/l for histamine concentrations and less than 12.5 µg/l for tryptase.

Allergy Work-up

Skin tests were performed in patients with hypersensitivity reactions at least 4 weeks after the reaction with all the administered drugs (intradermal tests) and with latex extracts (prick tests).²⁰ NMBA-specific IgE concentrations were evaluated with the quaternary ammonium-specific radioimmunoassay, as described previously.²¹ Results were expressed as the percent of radioactivity bound to the solid phase, with reference values less than 2% and equivocal values between 2 and 3%.

Statistics

No statistical power calculation was conducted before the study. The sample size was based on the available data. Continuous variables were presented as median and interquartile range, excepted for age where mean (SD) was used. Categorical variables were presented as numbers. Statistical analysis was performed with SAS software version 9.4 (SAS Institute, USA). The Wilcoxon two-sided test, the ANOVA, and the Spearman correlation test were used as appropriate. We modeled the dynamics of cysLT concentrations overtime within groups by linear mixed regression accounting for repeated measurements.

Receiver operating characteristics (ROC) curves were generated with Stata version 13 (StataCorp, USA). The area under the ROC curve was calculated, and the optimal threshold value was determined to maximize sensitivity and specificity. The Clopper–Pearson exact method was used to compute 95% CIs for the sensitivity and specificity. All hypotheses were two tailed. The main hypothesis was a difference between groups regarding cysLT concentrations. To test this hypothesis, we applied Bonferroni correction to account for multiple testing at three time points. Therefore, $P < 0.0167$ was considered to denote statistical significance. For all other hypotheses, $P < 0.05$ indicated statistical significance.

Results

This is the primary analysis of these data, and therefore they have not been published in any previous form.

Characteristics of the Subjects

Patients with Immediate Hypersensitivity Reactions.

Thirty-four patients who presented symptoms of an allergic reaction within the minutes after induction of anesthesia and fulfilled our inclusion criteria were included, and none were excluded from the analysis. Four successive plasma samples were available for 28 patients, three samples for 5, and one sample for 1. Four time points were defined: time point 1:

less than 30 min after the reaction; time point 2: 30 min to 1 h; time point 3: 2 to 6 h; and time point 4: 8 to 24 h.

Patients were classified into two groups according to the results of skin tests: group A for positive tests (allergic hypersensitivity) and group B for negative tests (nonallergic hypersensitivity). The main characteristics of the patients are reported in table 1, and the individual characteristics are detailed in Supplemental Digital Content 1, table (<http://links.lww.com/ALN/B389>), together with the results of biochemical and allergy tests.

Allergic Hypersensitivity Reactions (Group A). Group A consisted of 24 adult patients (13 men) who reacted at median 2.0 min (interquartile range [IQR], 0.3 to 5.0 min) after induction of anesthesia. Twenty-two patients presented with cutaneous signs (erythema, 22; and face and mucosal edema, 1), 16 had respiratory symptoms (bronchospasm, 14; oxygen desaturation, 1; and hypocapnia, 1), and 24 had cardiovascular signs (shock, 18; tachycardia, 19; and cardiac arrest, 4). Twenty-two reactions (92%) were life-threatening ones (severity grades, 3 to 4; table 1). Plasma histamine and tryptase concentrations were highly increased (table 2). All the patients had positive intradermal tests to NMBA and had negative skin tests to the other administered agents and to latex. NMBA-specific IgE was increased in 19 patients, equivocal in 3, and normal in 2.

Nonallergic Hypersensitivity Reactions (Group B). Group B consisted of 10 patients (2 men) with negative skin tests to all the administered drugs and to latex and negative or equivocal NMBA-specific IgE (table 1). The distribution of the administered NMBAs was different between groups A and B, with mostly atracurium in group B and suxamethonium in group A. The median time delay between induction of anesthesia and reaction was not significantly different from group A: 1.5 min (0.5 to 5.0 min). Nine patients had cutaneous signs (erythema, 6; urticaria, 4; diffuse edema, 1; and lips edema, 1), seven had cardiovascular signs (shock, 2; hypotension, 2; tachycardia, 4; and cardiac arrest, 1), and one had bronchospasm. Four patients (40%) had life-threatening reactions (table 1). The severity grade of the reaction was significantly lower in group B than in group A ($P < 0.01$). Plasma tryptase concentrations were in the reference range for all the patients of group B, and histamine concentrations were normal in eight patients and were marginally increased in two (Supplemental Digital Content 1, table, <http://links.lww.com/ALN/B389>). Tryptase and histamine concentrations were significantly lower in group B than in group A ($P < 0.001$ for both; table 2). NMBA-specific IgE was normal in nine patients and equivocal in one. The median value in group B was 1.2% (0.6 to 1.6), significantly lower than in group A (8.7%; 3.9 to 16.5; $P < 0.001$).

Controls (Group C). Twenty-one anesthetized control patients (12 men) were included (table 1), and none were excluded from the analysis. Administered NMBA was *cis*-atracurium in 95% of them. There was no significant difference between controls and reactors for age or gender.

Table 1. Characteristics of Patients with Allergic or Nonallergic Immediate Hypersensitivity Reactions and of Controls

	Immediate Hypersensitivity n = 34		Controls n = 21	P Value
	Group A: Allergic Hypersensitivity n = 24	Group B: Nonallergic Hypersensitivity n = 10	Group C: Controls n = 21	
Mean age, yr (SD; range)	53 (17; 28–82)	55 (15; 25–78)	62 (13; 28–86)	0.17
Female/male ratio	11/13	8/2	9/12	0.19
Clinical signs (n)			No reaction	
Cutaneous	22	9		
Cardiovascular	24	7		
Respiratory	16	1		
Severity grade* (n)				0.006
Grade 1	0	3		
Grade 2	2	3		
Grade 3	18	3		
Grade 4	4	1		
NMBA† (n)	Administered NMBA	Positive skin test	Administered NMBA	
Suxamethonium	20	20	4	
Atracurium	5	3	6	
Pancuronium	1	1	0	
Cis-atracurium	0	0	0	

*Grade 1: cutaneous or mucosal signs; grade 2: mild systemic reaction; grade 3: cardiovascular shock and/or bronchospasm; and grade 4: cardiac arrest.¹⁸

†Some subjects received two different neuromuscular blocking agents (NMBAs).

n = number of cases.

Table 2. Evolution with Time of Plasma Cysteinyl Leukotriene Concentrations in Patients with Allergic (Group A) or Nonallergic (Group B) Hypersensitivity Reactions and in Controls (Group C) and of Histamine and Tryptase in Groups A and B

	Before Induction of Anesthesia		< 30 min after	30 min to 1 h	2–6 h	8–24 h
	Group A	Group B	Group C	P value		
Leukotriene, µg/l; median [IQR]	ND	ND	0.83 [0.69–1.02]	<0.0001		
	15.9 [7.2–32.4]	11.1 [2.0–12.8]	ND	<0.0001		
	17.9 [7.8–36.0]	7.3 [3.0–11.5]	0.80 [0.64–1.43]	<0.0001		
	16.3 [6.6–28.1]	6.7 [1.6–11.6]	0.72 [0.62–0.91]	<0.0001		
	19.9 [4.5–27.8]	6.6 [1.9–11.7]	0.78 [0.59–1.07]	<0.0001		
Histamine, nmol/l; median [IQR]	ND	ND	372 [77–1197]			
	372 [77–1197]	4.6 [3.0–6.3]	58 [18.5–137]			
	4.6 [3.0–6.3]	3.3 [2.5–5.0]	18.5 [6.6–59]			
	2.1 [1.5–4.8]	1.9 [1.4–2.4]	38.7 [13.1–167]			
	5.9 [2.9–13.1]	3.2 [2.6–3.9]	5.9 [2.9–13.1]			
Tryptase, µg/l; median [IQR]	ND	ND	48.9 [15.6–167]			
	48.9 [15.6–167]	4.2 [3.8–5.5]	60.5 [34.5–313]			
	4.2 [3.8–5.5]	4.3 [3.6–5.6]	38.7 [13.1–167]			
	3.6 [3.1–4.8]	3.2 [2.6–3.9]	5.9 [2.9–13.1]			

Normal values for histamine, less than 6 nmol/l; for tryptase, less than 12.5 µg/l.

IQR = interquartile range, ND = not done.

The two supplementary control patients were women, aged 54 and 86 yr, respectively, who presented with cardiac arrest due to acute hypoxemia unrelated to hypersensitivity, anesthesia, or surgery. They were treated with adrenaline and had external cardiac massage.

Leukotriene C₄, D₄, and E₄ Concentrations

Controls. In group C, the median (IQR) cysLT concentrations were 0.83 (0.69 to 1.02) µg/l before induction of anesthesia, 0.80 (0.64 to 1.43) µg/l 30 min after induction of anesthesia, 0.72 (0.62 to 0.91) µg/l 6 h after, and 0.78 (0.59 to 1.07) µg/l 24 h after (table 2). CysLT concentrations were stable over time (slope = $-0.0028 \pm 0.0037 \mu\text{g} \cdot \text{l}^{-1} \cdot \text{min}^{-1}$;

$P = 0.44$). There was no relationship between cysLT concentrations and patient's age ($P = 0.57$) or gender ($P = 0.54$).

The cysLT concentrations in the two unanesthetized patients resuscitated from cardiac arrest were in the same range as in the other controls, at 0.95 and 0.44 µg/l, respectively.

Patients with Hypersensitivity Reactions. CysLT concentrations in reactors were highly increased compared with controls, at all the corresponding time points ($P < 0.0001$; table II). Group A (allergic hypersensitivity) showed the highest concentrations: 17.9 (7.8 to 36.0) µg/l 30 to 60 min after the reaction, and group B (nonallergic hypersensitivity) showed less increased concentrations: 7.3 (3.0 to 11.5) µg/l

(fig. 1). Concentrations remained increased during the 24 h of observation (table 2). A slow, but significant decrease with time was observed in group A (slope = $-0.196 \pm 0.095 \mu\text{g} \cdot \text{l}^{-1} \cdot \text{min}^{-1}$; $P = 0.044$). Assuming an exponential decrease, the mean half-life of cysLT in plasma was calculated as 58 h in group A. In group B, no decrease was observed ($0.034 \pm 0.036 \mu\text{g} \cdot \text{l}^{-1} \cdot \text{min}^{-1}$; $P = 0.35$).

CysLT concentrations were not significantly related to the severity grade of the reaction, but appeared significantly higher in patients with bronchospasm than in patients without bronchospasm ($P = 0.016$). In contrast, there was no difference in patients with or without cardiovascular ($P = 0.20$) or cutaneous ($P = 0.97$) signs, but the statistical power was low, as cases without these signs were few.

ROC curves were generated from the concentrations of reactors at time point 2 (30 min to 1 h) and those of controls at 30 min (fig. 2, upper). The optimal cutoff differentiating reactors from controls was $2.0 \mu\text{g/l}$ (area under ROC curve = 0.94; 95% CI, 0.85 to 1.00), allowing 91% (76 to 98%) sensitivity and 100% (84 to 100%) specificity in the studied population. The optimal cutoff between allergic and nonallergic hypersensitivity reactions was $15.2 \mu\text{g/l}$ (area under ROC curve = 0.79; 95% CI, 0.63 to 0.94), with 65% (41 to 81%) sensitivity and 100% (69 to 100%) specificity (fig. 2, lower).

Discussion

The current study demonstrated a large increase of cysLT concentrations during allergic immediate hypersensitivity reactions as well as during nonallergic ones, compared with controls, suggesting that cysLT is an important mediator of immediate hypersensitivity. No increase was observed in patients resuscitated from cardiac arrest unrelated to

hypersensitivity. The increase was observed within the first 30 min of the reaction and was sustained during at least 24 h. Higher cysLT concentrations were observed where bronchospasm was present, which is in agreement with the respiratory effect of cysLT evidenced by *ex vivo* and *in vivo* studies of inhaled allergen challenge⁸ or synthetic leukotrienes.¹⁰ Many cofactors such as underlying uncontrolled asthma, insufficient depth of anesthesia, or heavy smoking may also participate in per-anesthetic bronchospasm, but no such data were available to us, and bronchospasm was associated with cutaneous and/or cardiovascular signs in all the patients. Several reactors without bronchospasm also had very high cysLT concentrations, indicating that the respiratory tract was not the only organ concerned.

Apart from the pathophysiologic interest of these findings, the diagnostic value of cysLT needs discussion. As from ROC curves, sensitivity (90.9%; 95% CI, 76 to 98%) and specificity (100%; 84 to 100%) of a threshold of $2.0 \mu\text{g/l}$ appeared high enough for differentiating immediate hypersensitivity from other possible mechanisms. The diagnostic interest was lower for differentiating allergic from nonallergic hypersensitivity, with high specificity (100%; 69 to 100%) but modest sensitivity (65.2%; 41 to 81%) using a threshold of $15.2 \mu\text{g/l}$. As cysLT concentrations appeared unrelated to the severity of the reaction, measurements could be interesting in grade 1 to 2 reactions, where histamine and tryptase concentrations are frequently not or mildly increased.

To our knowledge, this study is the first that investigates cysLT concentrations in the plasma during drug hypersensitivity reactions, according to the mechanism involved. The model chosen (reactions to NMBAs) was of particular interest because of the high severity of the reactions, the large

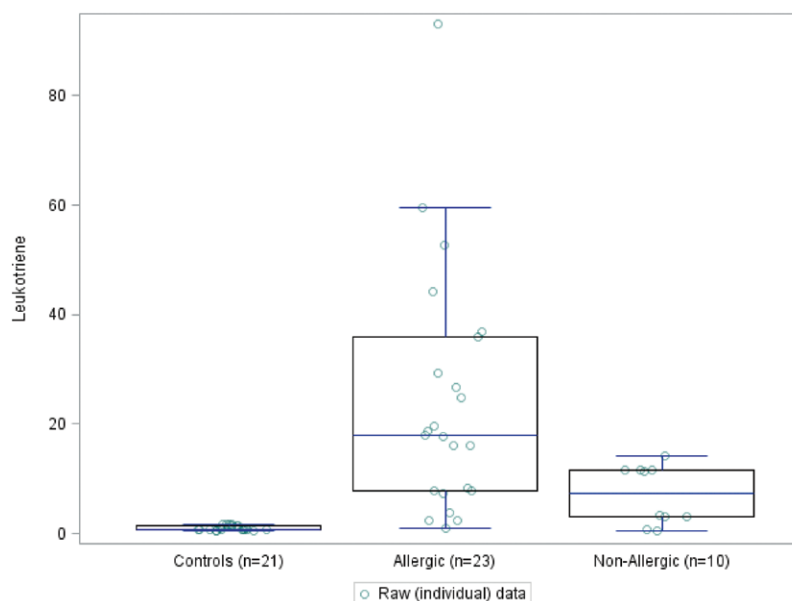


Fig. 1. Cysteinyl leukotriene concentrations ($\mu\text{g/l}$) measured 30 min after induction of anesthesia in controls, or 30 to 60 min after reaction in patients with allergic (missing data: 1) or nonallergic reactions. Individual data and box plot. Values were significantly different between the three groups ($P < 0.001$).

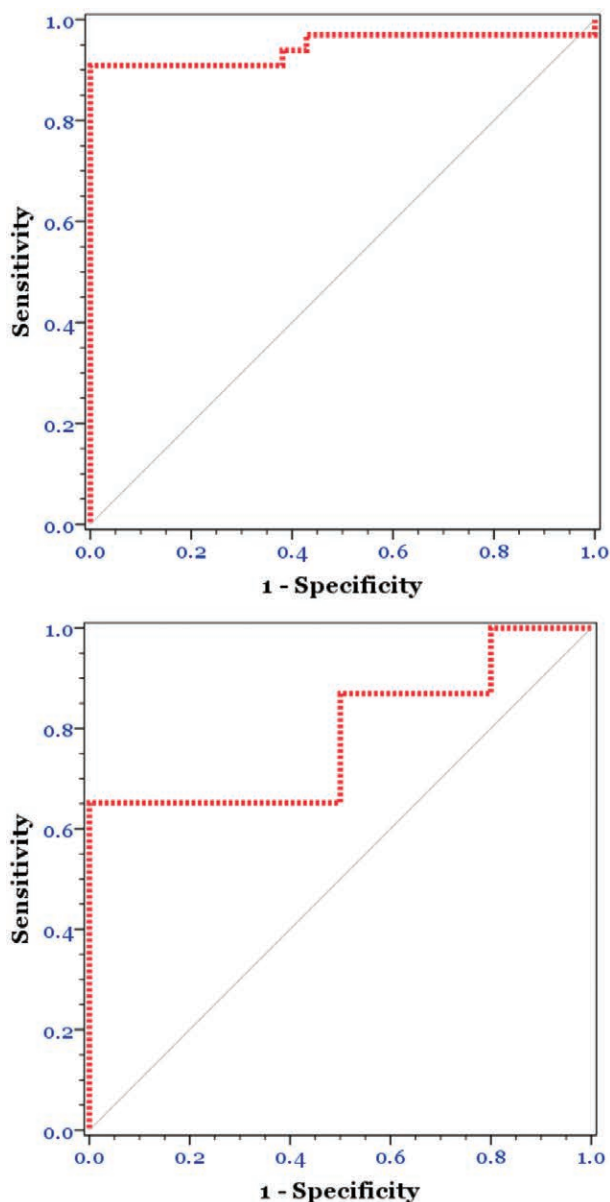


Fig. 2. Receiver operating characteristics (ROC) curves. *Upper:* ROC curve generated from cysteinyl leukotriene concentrations of reacting patients ($n = 33^*$) measured 30 to 60 min after reaction *versus* controls ($n = 21$) 30 min after induction of anesthesia. Area under ROC curve = 0.945 (95% CI, 0.847 to 1.000). *Lower:* ROC curve generated from cysteinyl leukotriene concentrations measured 30 to 60 min after reaction in allergic hypersensitivity patients ($n = 23^*$) *versus* nonallergic ($n = 10$). Area under ROC curve = 0.787 (0.63 to 0.94). * Sample was missing for one patient of the allergic hypersensitivity group.

release of plasma histamine and tryptase in most allergic reactions, and the reliability of allergy testing to discriminate between allergic and nonallergic reactions in this setting.⁶ Thus, the allergic and nonallergic groups were characterized with high accuracy. However, the retrospective choice of patients with clear-cut diagnosis can introduce a possible pitfall in the study, the studied population possibly reflecting

not all cases seen by anesthesiologists. Another pitfall could be the marked difference between the three groups with respect to the administered NMBA, mainly suxamethonium in NMBA-allergic reactors, atracurium in nonallergic reactors, and *cis*-atracurium in controls. Suxamethonium is associated with a higher frequency of anaphylaxis,⁶ due to linear and symmetrical steric conformation. Atracurium is known to induce nonspecific histamine release, which can explain its high frequency in nonallergic reactions. On the contrary, *cis*-atracurium has minimal histamine-releasing property and rarely elicits allergic reactions.⁶ When we recruited the controls, the ongoing public procurement contract with our institution had selected *cis*-atracurium, whereas reactions occurred in different surgical centers and for a longer time period.

A large release of cysLT was anticipated during allergic reactions, due to synthesis of cysLT from mast cell membrane lipids together with cell degranulation as a response to IgE bridging by allergen. A large increase was confirmed in allergic reactions but was also seen in nonallergic ones, which was not expected. The increase was sustained during the 24 h of observation, which is unusual for allergy mediators. We did not measure cysLT several days after the reaction, thus we cannot rule out high basal values in reactors. However, this seems improbable, as values reached 10 to 100 times the normal levels, and no control patient, even resuscitated, presented similar values.

Previous *in vivo* studies are rare. The majority focused on cysLT urinary excretion as a reflection of local or systemic release. However, urinary concentrations may not reflect accurately mediator release. Especially renal filtration may be impaired in reactions with profound hypotension, which could lead to reduced or absent mediator excretion in severe reactions. This is why we preferably measured cysLT concentrations in plasma. Increased serum levels of cysLT and prostaglandin F₂ were demonstrated in 18 patients brought to emergency departments within 2 h after anaphylactic reactions to insect sting, food, or drug compared with 39 controls.²² Our results are consistent with this study concerning controls, but appear much higher in our reactors, possibly due to the higher frequency of grade 3 to 4 reactions in our study or to the intravenous administration route, compared with oral or transcutaneous ones.

To our knowledge, the current study is the first that demonstrates increased plasma concentrations of a specific prostanoid mediator in nonallergic hypersensitivity reactions during anesthesia. Nonallergic hypersensitivity reactions to drugs cover a wide range of possible triggers.²³ They are generally considered as due to nonspecific histamine and tryptase release at the cellular level as shown with vancomycin.⁵ This local release can induce clinical effects but may be insufficient to increase concentrations in plasma after mediator diffusion into blood vessels, especially for tryptase due to its high molecular weight. Thus, moderately increased plasma histamine concentrations and rarely increased tryptase are

measured in most nonallergic reactions.^{3,4} The current finding of a large increase of cysLT concentrations in plasma from patients with documented nonallergic reactions suggests that cysLT could play a major part in acute symptoms and raises the issue of cells and pathways leading to such reactions. The main sources of cysLT are mast cell/basophil and eosinophil. A concomitant increase of prostaglandin D₂ (a mast cell mediator), without increase of eosinophil-derived neurotoxin (a protein of the eosinophil granules), has been observed after anaphylactic reactions, suggesting that eosinophil is not the responsible cell.¹³ Some neoplastic drugs can induce leukotriene production in mast cell *in vitro* and in patients *in vivo*.²⁴ Mast cell can be activated independently of IgE antibodies through Mrg receptor.²⁵ The murine mast cell receptor MrgprB2 has been shown to induce histamine release *in vitro* and pseudoallergic reactions in mice when targeted by some small molecules.²⁶ According to this study, members of all NMBA families except succinylcholine activated murine mast cell in an MrgprB2-dependent manner. *In vitro* experiments with atracurium in mice showed histamine release and activation from MrgprB2+ cells but not from cells lacking the receptor. Experiments with a human mast cell line gave similar results for MrgprX2 receptor.²⁶ This finding needs *in vivo* confirmation in humans. However, the increase of CysLT in nonallergic immediate hypersensitivity evidenced in the current study could be hypothesized as a consequence of mast cell activation through receptor MrgprX2.

Our results suggest that cysLT is an important mediator of the acute phase of immediate hypersensitivity (allergic or nonallergic). Therefore, patients might benefit from antileukotriene medication during reaction, especially patients with nonallergic reactions who do not release substantial amounts of histamine, but release large amounts of cysLT. Leukotriene antagonists combined with antihistamines have shown additive effect on bronchial constriction during not only allergen challenge *in vitro* and *in vivo*, but also nonimmunologic mast cell activation *in vitro*.²⁷ The current findings provide a rationale to investigate the possible benefits of additional treatment by antileukotriene drugs for patients reacting during anesthesia, as well as for pretreatment of patients with a history of reaction diagnosed as nonallergic hypersensitivity.

Specific limitations of our study include a small sample size that provided weak statistical precision of the estimates, in particular regarding the sensitivity and specificity of cysLT concentrations. In addition, the cases and controls were observed using very different, retrospective and mostly non-overlapping, time periods. Further studies using improved sampling methods may be required to confirm our findings.

In conclusion, cysLT concentrations are highly increased during at least 24h in allergic as well as nonallergic immediate hypersensitivity reactions during anesthesia. CysLT might play a role in nonallergic reactions.

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Competing Interests

The authors declare no competing interests.

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References

- Johansson SG, Hourihane JO, Bousquet J, Bruijnzeel-Koomen C, Dreborg S, Haahtela T, Kowalski ML, Mygind N, Ring J, van Cauwenberge P, van Hage-Hamsten M, Wüthrich B; EAACI (the European Academy of Allergology and Clinical Immunology) Nomenclature Task Force: A revised nomenclature for allergy. An EAACI position statement from the EAACI nomenclature task force. *Allergy* 2001; 56:813–24
- Laroche D, Vergnaud MC, Sillard B, Soufarapis H, Bricard H: Biochemical markers of anaphylactoid reactions to drugs. Comparison of plasma histamine and tryptase. *ANESTHESIOLOGY* 1991; 75:945–9
- Fisher MM, Baldo BA: Mast cell tryptase in anaesthetic anaphylactoid reactions. *Br J Anaesth* 1998; 80:26–9
- Renz CL, Laroche D, Thurn JD, Finn HA, Lynch JP, Thisted R, Moss J: Tryptase levels are not increased during vancomycin-induced anaphylactoid reactions. *ANESTHESIOLOGY* 1998; 89:620–5
- Veien M, Szlam F, Holden JT, Yamaguchi K, Denson DD, Levy JH: Mechanisms of nonimmunological histamine and tryptase release from human cutaneous mast cells. *ANESTHESIOLOGY* 2000; 92:1074–81
- Mertes PM, Alla F, Tréchet P, Auroy Y, Jouglé E; Groupe d'Etudes des Réactions Anaphylactoides Peranesthésiques: Anaphylaxis during anesthesia in France: An 8-year national survey. *J Allergy Clin Immunol* 2011; 128:366–73
- Gurrieri C, Weingarten TN, Martin DP, Babovic N, Narr BJ, Sprung J, Volcheck GW: Allergic reactions during anesthesia at a large United States referral center. *Anesth Analg* 2011; 113:1202–12
- Brocklehurst WE: The release of histamine and formation of a slow-reacting substance (SRS-A) during anaphylactic shock. *J Physiol* 1960; 151:416–35
- Peters-Golden M, Henderson WR Jr: Leukotrienes. *N Engl J Med* 2007; 357:1841–54
- Lee TH, Woszczek G, Farooque SP: Leukotriene E₄: Perspective on the forgotten mediator. *J Allergy Clin Immunol* 2009; 124:417–21
- Bellia V, Bonanno A, Cibella F, Cuttitta G, Mirabella A, Profita M, Vignola AM, Bonsignore G: Urinary leukotriene E₄ in the assessment of nocturnal asthma. *J Allergy Clin Immunol* 1996; 97:735–41
- Øymar K, Aksnes L: Increased levels of urinary leukotriene E₄ in children with severe atopic eczema/dermatitis syndrome. *Allergy* 2005; 60:86–9
- Ono E, Taniguchi M, Mita H, Fukutomi Y, Higashi N, Miyazaki E, Kumamoto T, Akiyama K: Increased production of

- cysteinyl leukotrienes and prostaglandin D2 during human anaphylaxis. *Clin Exp Allergy* 2009; 39:72–80
14. Higashi N, Mita H, Ono E, Fukutomi Y, Yamaguchi H, Kajiwaru K, Tanimoto H, Sekiya K, Akiyama K, Taniguchi M: Profile of eicosanoid generation in aspirin-intolerant asthma and anaphylaxis assessed by new biomarkers. *J Allergy Clin Immunol* 2010; 125:1084–1091
 15. Celejewska –Wojcik N, Mastalerz L, Wojcik K, Nieckarz R, Januszek R, Hartwich P, Szaleniec J, Hydzik-Sobocinska K, Oles K, Cybulska A, Strek P, Sanak M. Incidence of aspirin hypersensitivity in patients with chronic rhinosinusitis and diagnostic value of urinary leukotriene E4. *Pol Arch Med Wewn* 2012;122:422–7
 16. Oommen A, Grigg J: Urinary leukotriene E4 in preschool children with acute clinical viral wheeze. *Eur Respir J* 2003; 21:149–54
 17. Sznajder Y, Westcott JY, Wenzel SE, Mazer B, Tucci M, Toledano BJ: Airway eicosanoids in acute severe respiratory syncytial virus bronchiolitis. *J Pediatr* 2004; 145:115–8
 18. Ring J, Messmer K: Incidence and severity of anaphylactoid reactions to colloid volume substitutes. *Lancet* 1977; 1:466–9
 19. Laroche D, Chollet-Martin S, Léturgie P, Malzac L, Vergnaud MC, Neukirch C, Venemalm L, Guéant JL, Nicaise Roland P: Evaluation of a new routine diagnostic test for immunoglobulin E sensitization to neuromuscular blocking agents. *ANESTHESIOLOGY* 2011; 114:91–7
 20. Mertes PM, Laxenaire MC, Lienhart A, Aberer W, Ring J, Pichler WJ, Demoly P; Working Group for the SFAR; ENDA; EAACI Interest Group on Drug Hypersensitivity: Reducing the risk of anaphylaxis during anaesthesia: Guidelines for clinical practice. *J Investig Allergol Clin Immunol* 2005; 15:91–101
 21. Gueant JL, Mata E, Monin B, Moneret-Vautrin DA, Kamel L, Nicolas JP, Laxenaire MC: Evaluation of a new reactive solid phase for radioimmunoassay of serum specific IgE against muscle relaxant drugs. *Allergy* 1991; 46:452–8
 22. Nassiri M, Eckermann O, Babina M, Edenharter G, Worm M: Serum levels of 9 α ,11 β -PGF2 and cysteinyl leukotrienes are useful biomarkers of anaphylaxis. *J Allergy Clin Immunol* 2016; 137:312–4
 23. Farnam K, Chang C, Teuber S, Gershwin ME: Nonallergic drug hypersensitivity reactions. *Int Arch Allergy Immunol* 2012; 159:327–45
 24. Geuenich S, Haberl C, Egger D, Kaspers U, Hültner L, Wilmanns W, Denzlinger C: Induction of leukotriene production by bleomycin and asparaginase in mast cells *in vitro* and in patients *in vivo*. *Biochem Pharmacol* 1998; 55:447–53
 25. Tatemoto K, Nozaki Y, Tsuda R, Konno S, Tomura K, Furuno M, Ogasawara H, Edamura K, Takagi H, Iwamura H, Noguchi M, Naito T: Immunoglobulin E-independent activation of mast cell is mediated by Mrg receptors. *Biochem Biophys Res Commun* 2006; 349:1322–8
 26. McNeil BD, Pundir P, Meeker S, Han L, Udem BJ, Kulka M, Dong X: Identification of a mast-cell-specific receptor crucial for pseudo-allergic drug reactions. *Nature* 2015; 519:237–41
 27. Bartho L, Benko R: Should antihistamines be re-considered as antiasthmatic drugs as adjuvants to anti-leukotrienes? *Eur J Pharmacol* 2013; 701:181–4