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Mediators of anaphylactic reactions: tryptase and histamine stability in whole blood.

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To the Editor,

Immediate hypersensitivity reactions are related to mast cell and/or basophil activation. The mediators released such as tryptase and histamine are involved in clinical symptoms and are key parameters that contribute to diagnosis. Serum tryptase exists as two main isoforms (alpha and beta), their total concentrations peak between 30 minutes and 4 hours following the reaction. Tryptase release is considered a robust marker of mast cell degranulation but is not informative in mild reactions and often normal during food-induced allergic reactions.¹ Histamine is released at the early beginning of the reaction and may be useful when tryptase levels are normal or not informative. The concentration value can be altered by pre-analytic conditions and due to a short half-life.² Anaphylactic reactions can occur during night and weekend when laboratories can't take charge of samples. To our knowledge, no thorough study of the stability in whole blood of these markers in anaphylaxis has been published. The aim of our study was to evaluate the impact of whole blood sample storage conditions (temperature and delay before centrifugation and plasma collection) on the reliability of tryptase and histamine measurements.

Blood samples from 14 patients with no history of mast cell diseases and presenting hypersensitivity reactions (grade 2 to 4 of the Ring and Messmer scale)³ and from 10 healthy volunteers were collected on EDTA after signed informed consent (CCPPRB Caen Basse-Normandie protocol 2004-32). Patients included 6 women and 8 men, their median age was 63.5 y [35 - 89 y]. The description of the patient anaphylactic episodes appears in Table 1. The healthy volunteer group included 9 women and 1 man. The median age was 38.5 y [25 - 56 y].

When received in the laboratory, an aliquot of whole blood was processed for diagnostic (reference measurement) and the remaining was divided in aliquots stored at room temperature (RT) or at +4°C for 24, 72 hours or 7 days (patients) or 2, 6, 24 or 72 hours for controls before centrifugation and plasma collection.

Total tryptase concentrations were measured by an automated fluoroimmunoassay (ThermoFisher, Phadia SAS, Uppsala, Sweden). Increased tryptase is defined as $\geq 1.2 \times$ basal value + 2 $\mu\text{g.L}^{-1}$.⁴ In our hands, tryptase uncertainties of measurement for low and high concentrations

(9 $\mu\text{g.L}^{-1}$ and 38.2 $\mu\text{g.L}^{-1}$) are 17% and 16% respectively, in accordance with published results.⁵ Plasma histamine concentrations were measured by a radioimmunoassay (Beckman Coulter, Immunotech, France). Increased values defined by the manufacturer are $>10 \text{ nmol.L}^{-1}$, in accordance with published data.⁶ In our hands, histamine uncertainties of measurement for low and moderate concentrations (4.7 nmol.L^{-1} and 12.9 nmol.L^{-1}) were 22% and 25%, respectively. The differences between the concentrations measured before and after storage were compared by paired two-tailed t-tests using SAS software. Results were considered significant for $p < 0.05$. For the 10 healthy volunteers, the median tryptase baseline concentration was 3.7 $\mu\text{g.L}^{-1}$ [1.0 - 5.1 $\mu\text{g.L}^{-1}$]. For 12 of 14 patients, we observed a significant tryptase release (Table 1) and a significant *in vivo* decrease with time (Figure 1A). As shown in Figure 1B, storage conditions did not modify tryptase concentrations (linear regression: slope=1.079, $R^2=0.9675$). Tryptase concentrations appeared stable in whole blood left at $+4^\circ\text{C}$ for 7 days or 72 hours at RT. Histamine concentrations in patient samples were not modified during 72h at $+4^\circ\text{C}$ (Figure 1C) or at RT (Figure 1D). In the control group at RT, histamine concentrations were significantly increased at 6 hours ($p=0.005$) although moderately increased and staying within the limits of uncertainty measurement and never reaching the positivity threshold (Figure 1F). After 24 hours at RT a false positivity was observed for 8 of 10 samples ($p<0.0001$) (Figure 1F). At $+4^\circ\text{C}$, histamine concentrations were significantly increased after 24 hours ($p<0.0001$) but remained in the limits of uncertainty measurement and under the threshold of positivity (Figure 1E). After 72h at $+4^\circ\text{C}$, histamine concentrations exceeded the limits of uncertainty measurement and the positivity threshold for 4 samples of 10 (Figure 1E).

Anaphylactic reactions occur unexpectedly. Tryptase and histamine measurements are recommended to prove degranulation in anaphylaxis.⁷ It is thus important to master sample shipment and processing before mediator measurement.

The knowledge of possible artifacts modifying the measured values is necessary for the biochemist to address accreditation criteria of pre-analytic requirements (ISO 15189 standard) and for the physician to rely on trustable diagnostic data.

Tryptase stability in whole blood had not been described previously. Our data has shown no impact on results after 72h at RT or 7 days at $+4^\circ\text{C}$. Tryptase stability in plasma or serum has been evaluated by the manufacturer who ensured stability for 48h at RT or 5 days at $+2^\circ\text{C}$ to $+8^\circ\text{C}$

(Thermofisher).⁸ Thus, measured values of tryptase appear highly reliable.

Histamine stability in whole blood had only been evaluated in controls and false positive results may be attributed to passive release from basophil during prolonged storage.² We observed no impact for patient blood samples after 72h at +4°C or at RT. In contrast, false positive results were observed in controls after storage at RT during 24h or at +4°C during 72h. Histamine is known to be stable in the plasma obtained after centrifugation up to 4 days at RT for patients and controls.⁹

According to these results, we suggest that whole blood samples can be stored at +4°C up to 72h for histamine and 7 days for tryptase when the laboratory is not available immediately. In any case, the biochemist must accept all these valuable samples. It is his role to take into account the pre-analytical conditions to interpret the results and provide helpful information to the physician.

Keywords: pre-analytic; tryptase; histamine; whole blood.

Conflicts of interest:

The authors have no conflicts of interest relevant to this article to disclose.

Author contributions:

DL and DM designed the research study, performed the study, analysed the data and reviewed the manuscript. BLM interpreted the data and reviewed the manuscript. JJP did the statistical analysis. KK performed the study and designed the figures. GP reviewed the manuscript. JS wrote the manuscript.

References:

1. Vadas P, Perelman B, Liss G. Platelet-activating factor, histamine, and tryptase levels in human anaphylaxis. *J Allergy Clin Immunol* 2013;131(1):144-149.
2. Laroche D, Dubois F, Gérard JL et al. Radioimmunoassay for plasma histamine: a study of false positive and false negative values. *Br J Anaesth* 1995;74(4):430-437.
3. Ring J, Messmer K. Incidence and severity of anaphylactoid reactions to colloid volume substitutes. *Lancet* 1977;309(8009):466-469.
4. Valent P, Akin C, Arock M et al. Definitions, criteria and global classification of mast cell disorders with special reference to mast cell activation syndromes: a consensus proposal. *Int Arch Allergy Immunol* 2012;157:215–225.
5. Sarrazat A, Couderc R, Alyanakian MA et al. Performance criteria for the verification of IgE and tryptase assay methods: recommendations from the AllergoBioNet network. *Ann Biol Clin* 2020;78(3):329-342. (*French*)
6. Poli C, Laurichesse M, Rostan O et al. Comparison of two enzymatic immunoassays, high resolution mass spectrometry method and radioimmunoassay for the quantification of human plasma histamine. *J Pharm Biomed Anal* 2016;118:307-314.
7. Laroche D, Debaene B. How to relate the observed event to anaphylaxis? Practice of diagnostic investigations. *Ann Fr Anesth Reanim* 2011;30(3):280-293. (*French*)
8. Thermo Fisher diagnostics. ImmunoCAP™ Tryptase - Phadia. Fluoroenzymeimmunoassay. Directions for Use 52-5467-EN/03.
9. Laroche D, Mayo P, Lefrançois C et al. Exploration in vitro de l'anaphylaxie médicamenteuse. *Immunoanal Biol Spec.* 1998;13(2):63-73.

Table 1: Clinical characteristics and histamine and tryptase concentrations in patients (P1-P14) with hypersensitivity reactions.

Case	Gender	Age	Circumstances	Clinical symptoms	Severity grade*	Suspected agent	Time of sampling	Histamine (nmol.L ⁻¹)	Tryptase (µg.L ⁻¹)	Increased tryptase**	Skin tests results
P1	F	89	Anaesthesia	Hypotension	3	Suxamethoni	20min	574	104	Yes	Positive:
			Induction	Urticaria		um	2h20	42	79.3		Suxamethonium
			(NMBA)				>24h	-	4.5		
P2	F	60	Desensitization	Tachycardia	2	Bee venom	5min	44.1	22.1	Yes	Positive: Bee
				Erythema		(Apis spp.)	2h05min	5.5	15.7		venom (Apis
				Urticaria			>24h	-	12.0		spp.)
P3	F	48	Sting	Tachycardia	2	Wasp venom	30m	9.4	9.1	Yes	Positive: Wasp
				Face oedema		(Vespula	2h30	5.1	5.2		venom (Vespula
						spp.)	>24h	-	ND		spp.)
P4	M	79	Anaesthesia	Circulatory	3	Atracurium	10min	22.2	6.9	Yes	ND
			Induction	shock			2h20	< 1.1	5.5		
			(NMBA, cefazolin)				>24h	-	2.5		
P5	F	57	Thyroid	Hypotension	3	Technetium	30min	5.1	6.2	Yes	ND
			scintigraphy	Vomiting			2h30	3.2	4.9		
							21h	-	2.7		
P6	F	58	Anaesthesia	Bronchial	3	Latex	3h15	5.8	5.8	No	Negative with

			1h delay (without NMBA)	wheeze			5h10 >24h	5.0 -	5.7 6.2		latex and anaesthetic drugs
P7	M	70	Anaesthesia Induction (NMBA)	Hypotension	3	Atracurium	1h30 3h30 >24h	16.6 1.2 -	5.0 3.7 <1	Yes	ND
P8	M	64	Gelatin infusion	Hypotension Desaturation	3	Gelatin	45min 2h45 >24h	34.6 3.5 -	5.1 5.3 2.0	Yes	ND
P9	F	76	Patent blue dye injection	Circulatory shock Urticaria	3	Patent blue dye	55min 2h50 >24h	159 70.3 -	4.0 4.8 2.7	No	ND
P10	M	67	Anaesthesia (without NMBA)	Hypotension Erythema Urticaria	2	Iobitridol	5min 2h05 >24h	4.5 9.6 -	IQ 16.5 4.0	Yes	ND
P11	M	65	Anaesthesia Induction (NMBA)	Not stated	3	Atracurium	15min 2h15 >24h	ND ND -	8.6 13.3 4.6	Yes	ND
P12	M	35	Anesthesia Induction (NMBA, cefazolin)	Circulatory shock Bronchial wheeze	3	Atracurium Cefazolin	3min 2h48 22h	297 48.4 -	21.1 22.7 3.7	Yes	Positive: Cefazolin

P13	M	63	Anaesthesia Induction (NMBA)	Erythema	4	Suxamethoni um	45m	17.7	13.1	Yes	Negative
				Circulatory arrest			1h45	12.5	16.8		
							>24h	-	5.8		
P14	M	61	Amoxicilline, clavulanic acid	Hypotension	3	Amoxicilline Clavulanic acid	1h25	239	25.1	Yes	ND
				Bronchial wheeze			3h15	43.1	18.2		
				Erythema			>24h	-	1.7		

*Ring and Messmer scale³

** Increased tryptase is defined as $\geq 1.2 \times \text{basal value} + 2 \mu\text{g.L}^{-1}$.⁴

Abbreviations: IQ, insufficient quantity; ND, not done; NMBA, neuromuscular blocking agents.

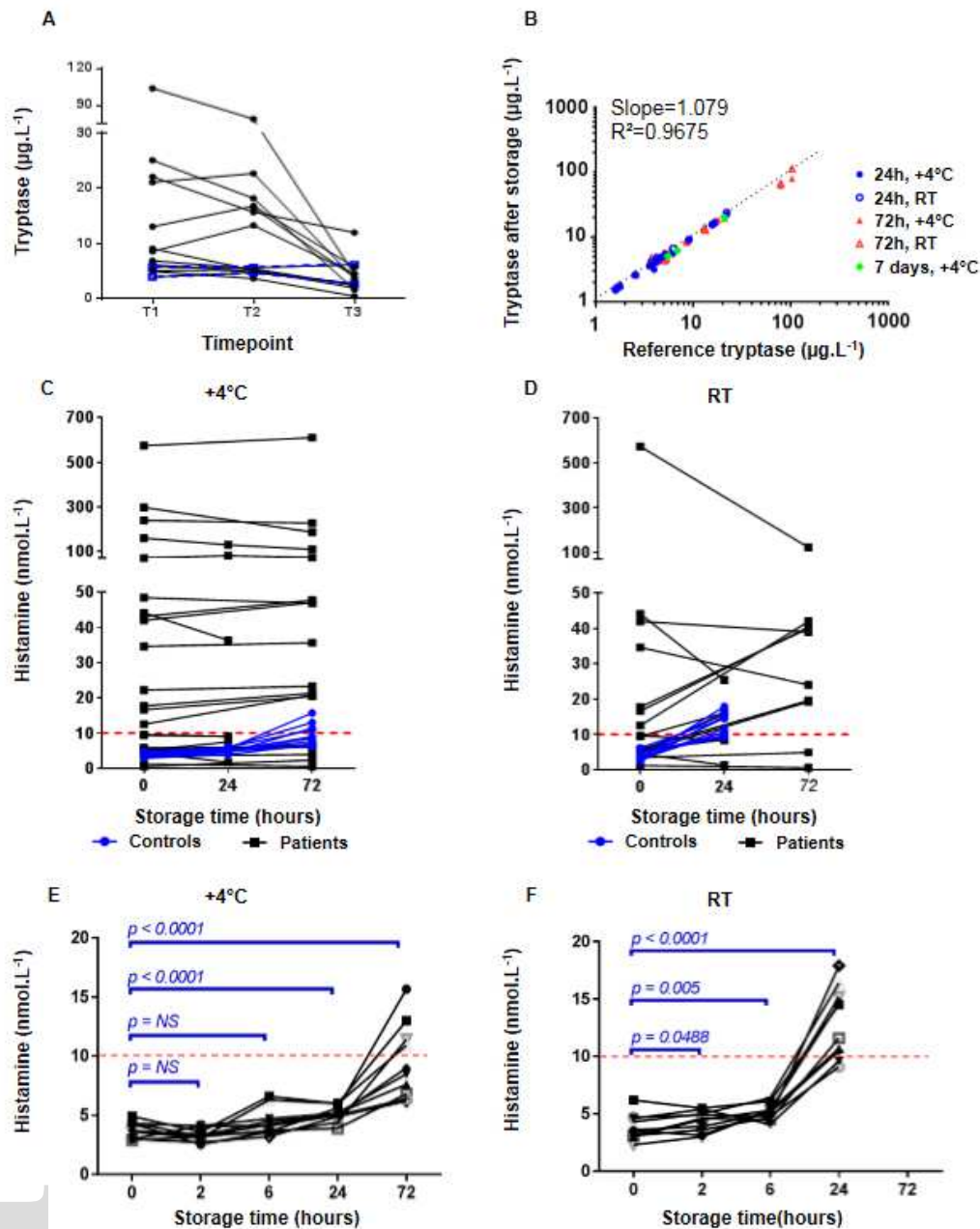


Figure 1:

Panel A: *In vivo* tryptase concentration variations with time in patients after their reaction. In dashed blue, patients with a non-significant tryptase degranulation ($< 1.2 \times \text{basal value} + 2 \mu\text{g.L}^{-1}$).⁴

Panel B: Linear regression of tryptase concentrations measured in different storage conditions compared with reference.

Panel C: Individual variations of histamine concentrations in patients (n=14) and controls (n=10) during storage at +4°C up to 72h.

Panel D: Individual variations of histamine concentrations in patients (n=14) and controls (n=10) during storage at room temperature up to 72h.

Panel E: Individual variations of histamine concentrations in controls (n=10) during storage at +4°C during 2h; 6h; 24h; 72h.

Panel F: Individual variations of histamine concentrations in controls (n=10) during storage at room

temperature during 2h; 6h; 24h.

Abbreviations: RT, room temperature; NS, not significant; T1, first tryptase time point after the reaction; T2, second tryptase time point ; T3, baseline tryptase