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 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.[1] Histamine is released at the early beginning of the reaction but has a short half-life. The concentration value can be altered by pre-analytic conditions.[2] Anaphylactic reactions can occur during night and week-end 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 suspected of anaphylactic reactions (grade 2 to 4 of the Ring and Messmer scale)[3] and from 10 volunteers were collected on EDTA after signed informed consent (CCPPRB Caen Basse-Normandie protocol 2004-32). The description of the patient anaphylactic episodes appears in Table 1.

When received in the lab, 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,). Increased tryptase is defined as [?] 1.2 x basal value + 2 $\mu g.L^{-1}$.[4] In our hands, tryptase uncertainties of measurement for low and high concentrations (9 $\mu g.L^{-1}$ and 38.2 $\mu g.L^{-1}$) 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 $nmol.L^{-1}$, 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 $p < 0.05$.

As shown in Figure 1A, 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°C for 7 days or 72 hours at RT.

Histamine concentrations in patient samples were not modified during 72h at +4°C (Figure 1B) or at RT (Figure 1C). 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 1E). After 24 hours at RT a false positivity was observed for 8 of 10 samples (p<0.0001) (Figure 1E). At +4°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 1D). After 72h at +4°C, histamine concentrations exceeded the limits of uncertainty measurement and the positivity threshold for 4 samples of 10 (Figure 1D).

Tryptase and histamine measurements are recommended to prove degranulation in anaphylaxis.[7] Anaphylactic reactions occur unexpectedly. 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. Our data has shown no impact on results after 72h at RT or 7 days at +4°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°C to +8°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 unrenewable 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.

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