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Granulocyte markers in hypertonic and isotonic saline-induced sputum of asthmatic subjects

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ABSTRACT: The aim of this study was to assess whether hyperosmolarity affects granulocyte mediator levels in induced sputum of asthmatic subjects.

A total of 32 mild-to-moderate asthmatics, who inhaled either hypertonic (HS; 4.5% NaCl) or isotonic (IS; 0.9% NaCl) solutions for 15 min, were studied. Selected sputum was used for analysis. Eosinophil cationic protein (ECP), eosinophil protein X (EPX), myeloperoxidase (MPO) and free neutrophil elastase (NE) were measured in sputum supernatant.

Sample weight, total and differential cell counts, as well as viability and squamous cell percentage were no different after the two tests. No significant differences in ECP, EPX, MPO or NE levels were observed between HS- and IS-induced sputum. Repeatability of the two tests was good for macrophages, neutrophils, eosinophils, ECP, EPX and NE, but not for lymphocytes and MPO.

In conclusion, hyperosmolarity does not affect sputum cell counts and the levels of most granulocyte degranulation markers examined in this study, confirming that both hypertonic and isotonic solutions can be reliably used to induce sputum in asthmatics. *Eur Respir J 2004; 24: 1018–1024.*

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Hypertonic saline (HS) inhalation is currently used to collect sputum from the airways of asthmatic subjects who do not produce sputum spontaneously. However, in high-risk subjects, inhalation with isotonic saline (IS) is recommended, at least at the beginning of sputum induction [1]. Thus, it is important that sputum samples collected after either induction method have a similar composition. Several studies have compared the cell composition of sputum obtained after the two different methods of induction and showed good concordance for inflammatory cell percentages [2–4]; however, few studies have compared the concentrations of soluble mediators in the sputum supernatant [5].

A large number of *in vivo* and *in vitro* studies have shown that hypertonic solutions modify mediator release from inflammatory cells by acting as a strong stimulus for cell activation [6–8], or, conversely, by inhibiting leukocyte degranulation [5, 9]. In sputum induction, the hypertonic solution might increase osmolarity of the airway lining fluid, thus influencing mediator release from granulocytes during inhalation or sputum processing, or both.

Therefore, the aim of this study was to compare preformed soluble mediator levels derived from eosinophils and neutrophils, such as eosinophil cationic protein (ECP), eosinophil protein X (EPX), myeloperoxidase (MPO) and neutrophil elastase (NE), in induced sputum obtained by means of either HS or IS inhalation in mild-to-moderate asthmatic subjects. In addition, the total and differential cell counts were also evaluated in the same sputum samples.

Subjects and methods

Subjects

A total of 44 mild-to-moderate asthmatic subjects, who were recruited from the authors' asthma clinic, were examined. Asthma was diagnosed at the time of the first examination, according to internationally accepted criteria [10], after assessing reversible airway obstruction and/or nonspecific bronchial hyperresponsiveness to methacoline. As an additional evaluation, all but 10 subjects also underwent skin-prick tests with 11 common airborne allergens (including house dust mites, pollens, moulds, animal danders). All subjects were examined in a stable period of the disease, at least 4 weeks after asthma exacerbation or change in regular pharmacological treatment.

Twelve subjects were unable to collect an adequate sputum sample on one of the two occasions and, therefore, were excluded from the study. The clinical characteristics of the 32 patients who completed the study are shown in table 1. Eleven subjects were ex-smokers, 19 were nonsmokers and two were current smokers. Mean pack-years of smokers and ex-smokers was 6.3±2.7. Screening forced expiratory volume in one second (FEV1) was >80% predicted in most subjects. All but five subjects had been regularly treated with anti-inflammatory drugs and long-acting inhaled β_2-agonists.

Table 1. – Characteristics of asthmatic subjects

Total n	32
Age yrs	51.3±16.9
Screening FEV1[#]	91.2±15.5
Atopy[¶] Y/N/ND	12/13/7
Rescue SABA	5
ICS	4
LTRA	1
ICS+LABA	17
ICS+LTRA	3
ICS+LABA+LTRA	2

Data are presented as n or mean±SD. FEV1: forced expiratory volume in one second; Y: yes; N: no; ND: not done; SABA: short-acting β_2-agonists; ICS: inhaled corticosteroids; LTRA: leukotriene receptor antagonists; LABA: long-acting β_2-agonists. [#]: FEV1 is expressed as per cent predicted; [¶]: positive skin-prick test to one or more inhalant allergens.

Study design

After screening examination, all subjects randomly underwent sputum induction by means of inhalation of either HS or IS, on 2 different days, separated by 1 week, at the same hour of the day. Pharmacological treatment was withdrawn 24 h before each induction, except for rescue salbutamol, which was withdrawn 6 h before each induction. Sputum volume, cell viability, total and differential cell counts, as well as ECP, EPX, MPO and NE concentrations in the supernatant were measured in sputum samples obtained after either HS or IS inhalation.

The protocol was approved by the University of Pisa Ethical Committee (Pisa, Italy) and informed consent was obtained from each patient.

Sputum induction

Sputum was induced according to European Respiratory Society Task Force recommendations [1], except that no β_2-agonist was administered as pretreatment. HS (NaCl 4.5% w/v) or IS (NaCl 0.9% w/v) were nebulised by means of an ultrasonic nebuliser (Sirius; Technomed, Florence, Italy), with 2.8 $mL \cdot min^{-1}$ output and were inhaled for three 5-min periods for up to 15 min. Every 5 min, after the start of nebulisation, subjects were asked to rinse their mouth and throat carefully, to discard saliva, and to try to cough sputum into a container; FEV1 was then measured. Nebulisation was stopped after 15 min or when FEV1 fell by $\geqslant$20% from baseline value. Saline-induced bronchoconstriction was promptly relieved by short-acting β_2-agonist inhalation.

Sputum processing

Sputum samples were processed within 1 h after collection and all dense portions were selected by means of an inverted microscope. The sample was then processed as previously reported [11]. After appropriate homogenisation, dilution and centrifugation, the cell pellet was resuspended in PBS for total cell count and cell viability assessment, by means of Trypan blue exclusion in a haemocytometer. The supernatants were stored at -80°C for further analysis. Samples with cell viability <50% were discarded. Aliquots (70–150 μL) of cell suspension at a concentration of $0.4–0.8 \times 10^3 \cdot \mu L^{-1}$ were then cytocentrifuged (Cytospin; Shandon Scientific, Sewickley, PA, USA) at 25×*g* for 5 min and cytospin slides were stained with Diff-Quik (Baxter Scientific Products, Miami, FL, USA) for differential cell counts on at least 300 nonsquamous cells. Two investigators, blinded to the subject's code and with good inter-observer repeatability [12], counted at least 500 cells on each sputum slide, so as to obtain the inflammatory cell count and also squamous cell percentage as an indicator of saliva contamination. Cytospin slides with an amount of squamous cells such that 300 inflammatory cells could not be counted were considered unsatisfactory and discarded. Macrophages, lymphocytes, neutrophils and eosinophils were then expressed as a percentage of total inflammatory cells, excluding squamous cells. In the current authors' laboratory, reproducibility of sputum cell counts, performed by the same laboratory staff involved in the present study (S. Cianchetti and M.L. Bartoli), has been shown to be good, as evaluated by intraclass correlation coefficients for macrophages (RI 0.80), neutrophils (RI 0.85) and eosinophils (RI 0.87), with the exception of lymphocytes (RI 0.15) [13].

Biochemical analysis of sputum supernatant

Sputum ECP levels. Sputum ECP levels were measured by means of UniCAP fluoroenzymeimmunoassay (Pharmacia Upjohn AB, Uppsala, Sweden). The intra-assay coefficient of variation was 4.5% and the inter-assay coefficient of variation was 8.2%. The detection limit of the assay was 0.5 $\mu g \cdot L^{-1}$.

Sputum EPX and MPO concentrations. Sputum EPX and MPO concentrations were determined using a double antibody radioimmunoassay (Pharmacia Upjohn AB, Uppsala, Sweden). The EPX intra-assay coefficient of variation was 5.3% and the inter-assay coefficient of variation was 10.1%. The detection limit of the EPX assay was 3 $\mu g \cdot L^{-1}$. The MPO intra-assay coefficient of variation was 7.6% and the inter-assay coefficient of variation was 14.5%. The detection limit of the MPO assay is 8 $\mu g \cdot L^{-1}$.

Sputum NE concentrations. Sputum NE concentrations were determined by measuring NE enzymatic activity in the samples. The NE activity was assessed by means of a chromogenic substrate specific for human NE (methoxy-succinyl-ala-ala-pro-val-paranitroanilide (MEOSAAPVNA); Sigma Chemical Co., St Louis, MO, USA), according to the slightly modified technique reported by FAHY *et al.* [14]. Supernatants from PBS-diluted sputum samples or purified NE (Sigma) were incubated (24 h, 25°C) with 0.1 M 4-(2-hydroxyethyl)-1-*N*′-piperazine-HEPES, 0.5 M NaCl, 0.1% Brij and 2% DMSO, pH 7.5, in the presence of the specific substrate MEOSAAPVNA (1.5 mM). Activity was measured by determining the change in absorbance at 410 nm on a microplate reader (Flow Laboratories, McLean, VA, USA) and was quantified by extrapolation from a standard curve performed with increasing concentrations (0.62–80 $mU \cdot mL^{-1}$) of purified NE (specific activity 99 $U \cdot mg^{-1}$). The change in absorbance was found to be linear over a 48 h incubation period at 25°C. The detection limit of the method was 6 $ng \cdot mL^{-1}$. Single samples were assayed in duplicate for each assay, and on two different occasions, in order to obtain the intra- and inter-assay coefficients of variation. The mean intra-assay and inter-assay coefficients of variation were 5.6% and 12%, respectively. The specificity of NE activity in all sputum samples, as well as in standard samples, was evaluated by measuring the activity after 30 min of sample preincubation with methoxy-succinyl-ala-ala-pro-val-chloromethyl ketone (CMK; Sigma) or ethylenediamine tetraacetic acid (EDTA) [15]. At the end of the preincubation period, the specific substrate was added and the NE activity was measured as described previously. This activity was almost fully inhibited (>95%) by preincubation with the specific inhibitor CMK but

not with EDTA. As a matter of fact, sputum NE activity correlated with that measured in the same samples pretreated with EDTA (rho=0.9, p=0.0001).

Standard curve of mediators. Standard ECP, EPX, MPO and NE were diluted in HS or IS solution and in assay buffer (control standard); a standard curve of increasing concentrations of each mediator was performed and the recovery of each standard in HS or IS was calculated as percentage of the control standard.

Spiking recovery of mediators in sputum samples. In order to assess the spiking recovery of ECP, EPX, MPO and NE in HS- and IS-induced sputum, a known quantity of purified mediator was added to different aliquots of sputum samples (ECP n=12; EPX n=14, MPO n=13, NE n=21). The expected concentration of the spiked mediator, assuming 100% recovery, was in the mid-part of the standard curve. The spiking recovery of each mediator was calculated as percentage of the expected value, which was the sum of the spiked purified mediator and mediator concentration in the original sputum sample.

Osmolality. Osmolality was evaluated *via* the freezing-point depression method using an ARKRAY Osmostat OM6030-cryogenic osmometer (Menarini, Firenze, Italy), where the freezing point depression of the sample was converted to osmotic concentration.

Sodium concentrations in sputum supernatants. Sodium concentrations in sputum supernatants were measured using a potentiometric method with a Vitros 950/950 AT analyser (Ortho Clinical Diagnostic, New Jersey, NJ, USA).

Statistical analysis

Sputum volumes, cell viability, total cell count, cell percentages and biochemical mediators are all expressed as median (range). The screening FEV1, the fall in FEV1 (ΔFEV1%) after saline solution inhalation and the duration of saline inhalation are all expressed as mean$\pm$SD. Wilcoxon's signed rank test was used to compare sputum cell percentages and biochemical mediator levels in HS and IS-induced sputum. The paired t-test was used to compare ΔFEV1% and duration of saline inhalation after HS or IS sputum induction. Concordance of sputum cell percentages and soluble mediator levels between the two tests was evaluated by intraclass correlation coefficients; RI values $\geqslant$0.70 were considered satisfactory [16]. Differences in mediator levels between the two tests were shown graphically, by plotting the differences between the values of each pair of observations against the mean value of the same pair of observations [17]. Spearman's rank correlation was calculated to assess the correlation between absolute numbers or percentages of inflammatory cells and biochemical mediators. Significance was accepted at the 95% level.

Results

Sputum induction

There was no significant difference between FEV1 measured before HS and before IS inhalation (FEV1%; 90.6$\pm$13.4 and 88.3$\pm$16.9, respectively). The fall in FEV1 after sputum induction was greater and the duration of the test was shorter after HS than after IS inhalation (ΔFEV1%; -9.7$\pm$9.9% (-32–5) after HS and -2$\pm$6.6% (-21–7) after IS, p=0.003; duration 12.6$\pm$3.7 min after HS and 15$\pm$0 min after IS; p=0.028).

Sputum weight, total and differential cell counts

An inadequate sputum sample was obtained in four subjects after IS inhalation, in two subjects after HS inhalation and in six subjects after both inhalations. Thus, 32 subjects were evaluated in this study.

No significant difference was observed between sample weights, total cell counts, cell viability, squamous cell percentages or inflammatory cells measured after the two tests (p>0.17 all comparisons) (table 1).

The intraclass correlation coefficient between the two methods was high for macrophages, neutrophils and eosinophils (>0.7) (fig. 1). Reproducibility was best for eosinophils, as confirmed by the confidence interval of repeatability coefficients. When cell counts were expressed as absolute numbers, intraclass correlation coefficients were low for all inflammatory cells (RI <0.3).

Sputum soluble mediators

For some mediators, the number of measurements was lower than 32, because of an insufficient amount of supernatant. There were no significant differences in ECP, EPX and NE levels in sputum samples induced by either HS or IS inhalation (p>0.05) (table 2). RI values were high for ECP, EPX and NE levels (fig. 2), thus demonstrating good reproducibility of the two methods of sputum induction. By contrast, RI for MPO was poor (fig. 2), despite the lack of difference between MPO levels measured in sputum after either method of induction. Good reproducibility was confirmed by the low confidence interval of the repeatability coefficients obtained for these mediators, except for MPO.

When only the subjects with no difference in the duration of HS and IS inhalations and all mediators measured (n=16) were considered, the RI were similar to those obtained in all subjects (ECP 0.79, EPX 0.83, MPO 0.53, NE 0.74). When cell percentages in the same group of subjects were considered, RI values were still high for macrophages, neutrophils, eosinophils (RI 0.72, 0.71 and 0.84, respectively) and poor for lymphocytes (RI 0.12). Again, when cell counts were expressed as absolute numbers, intraclass correlation coefficients were low for all inflammatory cells (RI <0.25).

ECP and EPX values significantly correlated with the absolute number of eosinophils after both tests (HS: ECP Rho=0.65, p<0.01 and EPX Rho=0.55, p<0.05, n=24; IS, ECP Rho=0.50, p<0.05 and EPX Rho=0.50, p<0.05, n=24). Moreover, ECP levels were found to be highly correlated with EPX after both HS and IS inhalation (Rho=0.8, p<0.001 in both, n=22). There was a significant correlation between NE levels and neutrophil absolute number after both HS (Rho=0.51, p<0.01, n=23) and IS inhalation (Rho=0.47, p<0.05, n=23); by contrast, MPO levels measured after IS (Rho=0.43, p<0.05, n=23) but not after HS inhalation (Rho=0.28, p>0.05, n=23) significantly correlated with neutrophil absolute number. MPO significantly correlated with levels of NE after IS (Rho=0.43, p<0.05, n=23) but not after HS inhalation (Rho=0.20, p>0.05, n=22). These correlations were confirmed when cell percentages were considered and when only the subjects with all mediators measured (n=19) were included for analysis.

Osmolality of sputum supernatant samples was significantly higher in HS than in IS samples (283.5 (250–324) *versus*

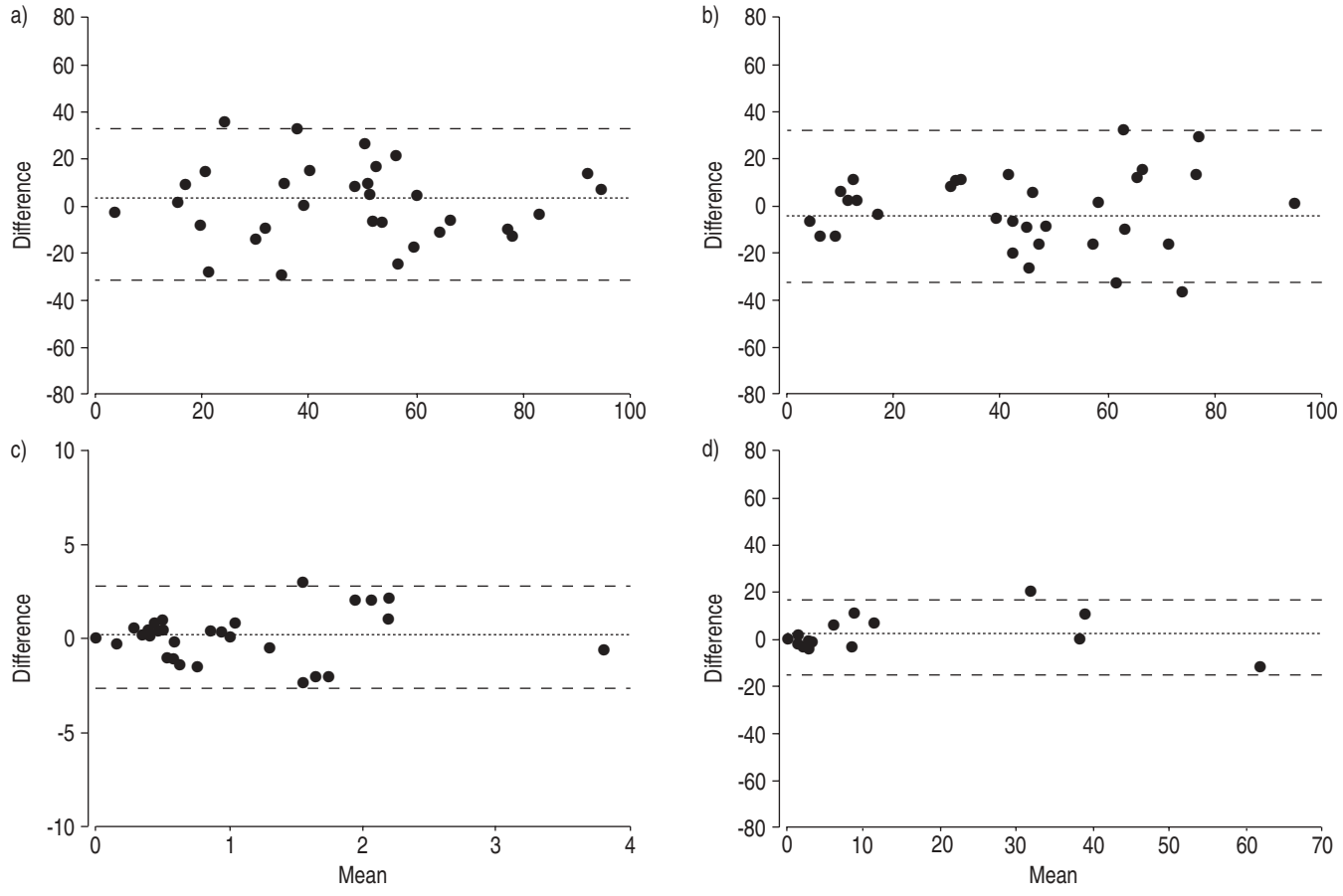

Fig. 1. – Plots of differences in a) macrophages, b) neutrophils, c) lymphocytes and d) eosinophils between sputum induction by hypertonic or isotonic saline inhalation (test 1–test 2) *versus* mean values of the two measurements, according to Bland and Altman. ·····: mean of the differences for the cell percentages; - - -: coefficient of repeatability (2SD of the mean difference). It is expected that 95% of the differences are between mean±2SD. RI: a) 0.74; b) 0.72; c) 0.14; d) 0.89. Confidence intervals of the coefficients of repeatability: a) 22–41; b) 22–41; c) 1.8–3.4; d) 7.4–12.8.

271 (150–309) mOsm·kg^{-1}, p=0.01), as well as sodium concentrations (153 (135–176) *versus* 146 (107–164) mEq·L^{-1}, p=0.01).

Table 2. – Cellular and biochemical findings in induced sputum after hypertonic (HS) or isotonic saline (IS) inhalation

	HS inhalation	IS inhalation
Selected sputum weight g	0.17 (0.045–0.97)	0.24 (0.03–1.2)
Viability %	71 (57–94)	75 (55.3–90)
Squamous cells %	8.9 (0.1–19.5)	10.6 (0.7–20)
Total inflammatory cells $\times 10^6 \cdot g^{-1}$	1.66 (0.49–19.2)	2.02 (0.39–19.2)
Macrophages %	50.7 (3.1–99.6)	45.2 (4.9–91.3)
Macrophages $\times 10^6 \cdot g^{-1}$	0.79 (0.05–3.9)	0.82 (0.08–13.3)
Lymphocytes %	0.7 (0–3.5)	0.7 (0–4.1)
Lymphocytes $\times 10^6 \cdot g^{-1}$	0.087 (0–0.1)	0.01 (0–0.2)
Neutrophils %	39.5 (0–96.1)	48.8 (6.9–94.5)
Neutrophils $\times 10^6 \cdot g^{-1}$	0.68 (0–12.5)	0.84 (0.1–5.9)
Eosinophils %	1.1 (0–56)	1.7 (0–67.9)
Eosinophils $\times 10^6 \cdot g^{-1}$	0.02 (0–7.2)	0.03 (0–4.2)
ECP $\mu g \cdot mL^{-1}$	0.85 (0.2–20)	0.97 (0.2–20)
EPX $\mu g \cdot mL^{-1}$	0.72 (0.03–14.3)	0.57 (0.02–13.2)
NE $\mu g \cdot mL^{-1}$	3.16 (0.1–20.6)	3.42 (0.15–23.5)
MPO $\mu g \cdot mL^{-1}$	0.18 (0.04–47.36)	0.66 (0.04–22.6)

Data are presented as median (range). ECP: eosinophil cationic protein; EPX: eosinophil protein X; NE: neutrophil elastase; MPO: myeloperoxidase.

The standard curve of ECP, EPX, MPO and NE in the assay was not affected by the dilution of the standard in HS or IS solution; recovery of the different concentrations of the standard in HS or IS solution was >94% for all mediators studied. Recovery in sputum induced by HS was 96% (90–104) for ECP, 102% (96–113) for EPX, 95% (89–100) for MPO and 101% (100–108) for NE, and in sputum induced by IS was 98% (93–110) for ECP, 99% (91–101) for EPX, 97% (95–105) for MPO and 99% (90–100) for NE.

Spiking experiments showed good recovery for all mediators in both HS- and IS-induced sputum (table 3). Although MPO recovery was lower in sputum after HS inhalation than after IS, this difference was not significant.

Discussion

This study shows that soluble mediators derived from eosinophils and neutrophils (ECP, EPX and NE) are measurable in the supernatants of sputum collected from proximal airways of asthmatic subjects, and that their concentrations are not affected by the type of saline solution (HS or IS) used to induce sputum. Thus, both HS and IS inhalation can be reliably used to collect sputum supernatant and to measure soluble markers of granulocyte activation.

Cytoplasmic eosinophilic and neutrophilic granules contain toxic proteins, including ECP, EPX, NE and MPO, which are released into the extracellular space after cell activation and

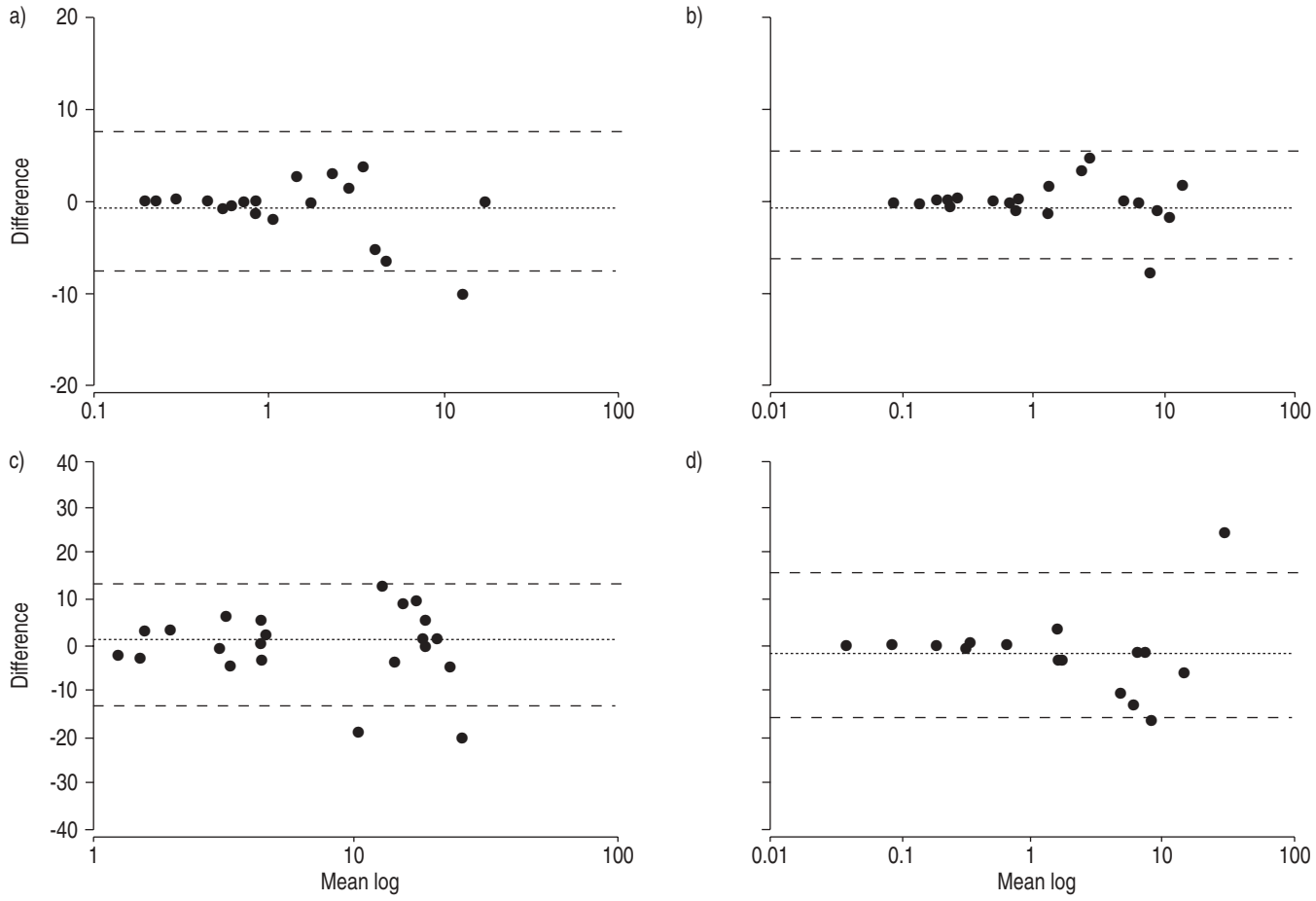

Fig. 2. – Plots of differences in a) eosinophil cationic protein, b) eosinophil protein X, c) neutrophil elastase and d) myeloperoxidase between sputum induction by hypertonic (HS) or isotonic saline (IS) inhalation (test 1–test 2) *versus* mean values of the two measurements, according to Bland and Altman. ·····: mean difference for the mediator levels; - - -: coefficient of repeatability (2SD of the mean difference). It is expected that 95% of the differences are between mean±2SD. a) RI 0.78, n=21; b) RI 0.88, n=20; c) RI 0.76, n=27; d) RI 0.51, n=19. Confidence intervals of the coefficients of repeatability: a) 3.8–8.6; b) 2.8–6.7; c) 7.1–13.9; d) 9.7–23.1.

degranulation; these markers have been measured in induced sputum of asthmatic subjects [18] and some of them correlate with the severity of asthma [19, 20]. Positive correlations between soluble markers of cell activation and counts of neutrophils and eosinophils in sputum have been observed in several studies [19, 21]. The relationship between cell counts and soluble markers might be affected by several factors, including nonspecific cell degranulation induced by increased osmolarity of sputum supernatant during HS inhalation. Some authors have reported that HS may also directly affect some leukocyte function and mediator release through hypothetical osmoreceptors, triggering specific signals on neutrophils [22, 23]. Thus, it is important to know whether hyperosmolarity of induced sputum can artificially modulate the concentrations of soluble markers of neutrophil and eosinophil activation.

Table 3. – Spiking recovery of biochemical mediators in induced sputum after hypertonic (HS) or isotonic saline (IS) inhalation

	HS inhalation	IS inhalation
ECP %	87.7 (69.1–96.6)	89.2 (73.7–99.7)
EPX %	88.2 (71.3–99.4)	85.1 (63.4–98.3)
NE %	82.4 (65.3–98.2)	80.8 (53.7–95.6)
MPO %	72.7 (46.6–96.2)	84.3 (48.5–92.6)

Data are presented as median (range). ECP: eosinophil cationic protein; EPX: eosinophil protein X; NE: neutrophil elastase; MPO: myeloperoxidase.

This study shows that ECP, EPX and NE concentrations are similar in sputum supernatants obtained after HS and IS inhalation, since the concordance indices were >0.7 and the confidence interval of the repeatability coefficients was low for all these soluble mediators. In addition, ECP and EPX correlated with each other as well as with eosinophils, and NE with neutrophils in both HS- and IS-induced sputum.

Only MPO showed poor concordance, perhaps because of different factors. Compared to other mediators, MPO concentration is greatly affected by the method used for its detection or for sputum sample processing [24–26], and sputum MPO concentrations have been reported to be extremely variable in different studies, with a large standard deviation [27]. In the samples in this study, an acceptable interassay coefficient of variation and a fairly good recovery of MPO were found, which, however, was lower in sputum induced by HS than in sputum induced by IS inhalation.

In this study, hyperosmolarity seems to differently affect NE and MPO release; MPO was indeed lower, even though not significantly, in sputum samples induced by HS than in those induced by IS inhalation, while NE levels were no

different in the two tests and showed a high intraclass correlation coefficient; furthermore, NE correlated with neutrophils in induced sputum after both HS and IS inhalation, while MPO correlated with neutrophils and NE after IS but not after HS inhalation. Although both NE and MPO are contained in the primary granules of neutrophils, neutrophil enzymes have shown to be associated with subpopulations of different density primary granules which might be under separate secretory control [28]; furthermore, different stimuli or ion concentrations might modify granule mobilisation [29, 30].

In this study, there was no pretreatment with β_2-agonists before saline inhalation, in order to elucidate a possible pathophysiologic mechanism of hypertonic inhalation on airway inflammatory cells. Considering that β_2-agonists may affect the degranulation of inflammatory cells, as demonstrated by *in vitro* and *in vivo* studies [31, 32], it was thought that this effect might mask a possible difference between HS and IS solution mediator release. Since *in vitro* studies have demonstrated that HS solution can activate inflammatory cells [6–9], and there is no demonstration from *in vivo* studies that MPO, EPX and NE levels are similar in HS- and IS-induced sputum supernatant [4], β_2-agonist pretreatment was avoided in the present comparison between HS- and IS-induced sputum. However, this fact is not relevant in practice, because guidelines recommend β_2-pretreatment before both HS and IS inhalation [1].

The lack of β_2-agonist pretreatment may differently affect the duration of saline inhalation, thus influencing the levels of soluble mediators [33]. However, when subjects with the same duration of HS and IS inhalation were considered, the agreement between the two methods was confirmed for both cells and mediators.

Pharmacological treatment was withdrawn 24 h before each challenge in order to avoid a direct effect of long-acting β_2-agonists and leukotriene antagonists on mediator release from sputum inflammatory cells. The duration of the effect of inhaled corticosteroids is difficult to assess and only a long-term withdrawal (*e.g.* 1 month) may warrant no interference with mediator release. This long withdrawal period may be unethical and not easily feasible in these moderate asthmatic subjects.

This study is the first evaluating the hypertonic effect on granulocyte soluble mediators in selected plugs of induced sputum. Although it has been widely demonstrated that total and differential cell counts are not affected by tonicity of saline solutions [2–4], only a previous study has reported data concerning the concentration of albumin, ECP and pro-matrix metalloproteinase-9 activity measured in whole sputum samples obtained after HS or IS inhalation [4]. This work provides additional information to the previous observations and extends the results to a greater number of asthmatic subjects. Considering that international guidelines [1] suggest the use of IS as first induction in severe patients or during exacerbation, followed eventually by HS inhalation, the present study shows that using two different saline solutions does not cause a different cell and mediator composition of induced sputum.

In conclusion, the analysis of sputum induced by hypertonic or isotonic saline inhalation showed that hyperosmolarity has no effect on sputum cell counts and granulocyte degranulation. Thus, this study confirms that both hypertonic and isotonic saline can be reliably used to induce sputum in asthmatic subjects, at least in the evaluation of inflammatory cells and soluble mediators from granulocytes. Only myeloperoxidase showed a poor concordance, possibly due to a variable interference with some sputum components at higher ion concentrations.

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