LETTER TO THE EDITOR

Efficacy of a nasal spray containing N-acetylcysteine in hypertonic solution in the treatment of non-allergic chronic rhinitis with goblet cell metaplasia

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To the Editor,

Continued exposure to exogenous irritants, such as tobacco smoke, air pollutants, substances dispersed in the workplace, and endogenous irritants (hydrochloric acid, pepsin), as in gastroesophageal and laryngopharyngeal reflux, initiates inflammatory processes in the mucous membranes of the upper respiratory tract, leading to their chronic status. The chronic inflammatory process that characterizes chronic rhinitis results in a progressive remodelling of the nasal mucosa. This can lead to goblet cell metaplasia, a condition in which there is an increase in distribution of goblet cells in the nasal cavity at nasal cytology. The altered integrity of the nasal mucosa leads to a progressive impairment of mucociliary clearance which favours bacterial colonization and the onset of infections, which further support the inflammatory status. This generates a vicious circle with mutual reinforcement of inflammation and metaplasia. Chronic rhinitis with goblet cell metaplasia is characterized by nasal obstruction, rhinorrhoea, sneezing, itching, post-nasal drip, facial pain and anosmia or hyposmia.

Nasal cytology is a useful, cost-effective and easily applicable diagnostic method to better classify the various rhinitis phenotypes. It makes it possible to quantify cell populations in the nasal mucosa to better discriminate pathological conditions and to assess the effect of the therapeutic strategy undertaken (1, 2). The clinical trial started in early May 2019 and was completed in mid-December 2019. Its purpose was to evaluate the efficacy of a medical device containing 3% buffered hypertonic saline and 6% N-acetylcysteine (NAC) compared to the efficacy of a nasal spray containing only 3% hypertonic saline in the treatment of patients who have non-allergic chronic rhinitis with cytological diagnosis of goblet cell metaplasia.

MATERIALS AND METHODS

Tested medical device

Viscoflu® Nasal Spray (VNS - Pharma Line S.r.l. Milan, on the market since July 2018) is a medical device containing 3% hypertonic saline solution (NaCl), with controlled pH, and 6% N-acetylcysteine. The product is indicated to facilitate fluidification and removal of stagnant mucous secretions or purulent mucus in nasal cavities and paranasal sinuses, improving the symptoms and course of acute, subacute and chronic inflammation of the airways. The effectiveness of this product was compared to the effectiveness of a nasal spray containing only 3% hypertonic saline solution (NaCl) (HSS). The two products were made available free of charge by Pharma Line (Milan).

Key words: chronic rhinitis; goblet cell metaplasia; nasal cytology; N-acetylcysteine; hypertonic saline solution; topical therapy

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Assessed subjects

Eighty patients (41 men, 39 women), with an average age of 43.3 years (minimum age 18, maximum age 76), with chronic rhinitis or rhinosinusitis with goblet cell metaplasia were evaluated. Patients were divided into two groups of 40 patients each: a group treated with VNS (19 men, 21 women) and a group treated with HSS (22 men, 18 women). The demographic and medical history data of the patients enrolled in the study are shown in Table I.

Adult male and female subjects suffering from chronic non-allergic rhinitis with cytological diagnosis of goblet cell metaplasia were included in the study. Subjects with proven sensitivity to one or more components of VNS or HSS, with active infections, malignant diseases and/or nasal polyposis were excluded from the study. Those who had taken drugs for the treatment of rhinopathy in the four weeks prior to the start of the application of the two nasal sprays were also excluded. Patients were enlisted with their personal identification data and signed regular informed consent to both the proposed therapy and the processing of personal data. At the time of enrolment, a medical history form was completed for each patient with all the data collected and a form to be completed at the next check-up was attached.

Study design

This is a prospective and comparative clinical study. Two treatment arms were created to which patients were randomly assigned. The subjects belonging to the VNS group and the subjects belonging to the HSS group were

Table I. *The two groups of patients treated were homogeneous in demographic and medical history characteristics as well as in regard to the signs and symptoms of the disease and the outcome of the rhinocytogram in basal conditions.*

Variable	VNS (n=40)	HSS (n=40)	p-value
Age in years, average±SD	42.7 ± 13.9	44.1 ± 15.8	0.669
Sex, %F (n)	52.5 (21)	45.0 (18)	0.655
Chronic rhinitis diagnosis*, % (n)	90.0 (36)	92.5 (37)	1.000
Smoking, % yes (n)	12.5 (5)	15.0 (6)	1.000
Familiarity**, % yes (n)	50.0 (10)	40.0 (8)	0.751
Rhinocytogram, median [25° - 75°]			
Neutrophils	2 [0 – 3]	1 [0 – 3]	0.566
Lymphocytes	1 [0 – 2]	1 [0 – 1]	0.386
Goblet cells	3 [3 – 4]	3 [3 – 3]	0.260
*Bacteria***	2 [0 – 2.3]	0 [0 – 2]	0.075
Turbinate hypertrophy, median [25° - 75°]	3 [3 – 3]	3 [3 – 3]	0.936
TNSS**, median [25° - 75°]	7 [6.8 – 9]	6 [5 – 8]	0.271
Fibrorhinoscopy**, % (n)			
Serous catarrhal rhinorrhoea	15 (3)	40 (8)	0.133
Serum-mucosal rhinorrhoea	20 (4)	25 (5)	
Serum-purulent rhinorrhoea	65 (13)	35 (7)	

**The remaining subjects suffered from chronic rhinosinusitis: 10% in the VNS group and 7.5% in the HSS group. ** For familiarity rate, bacterial count, TNSS and fibrorhinoscopy, data are only available for 20 patients in each treatment group.*

checked on two occasions: at the start of the application of VNS or HSS (T0) and after 10 days of application of VNS or HSS (T1). From the moment of the start of the application of the assigned product (T0) and in the following days (T0 to T1), the subjects applied the assigned product in the measure of 2 deliveries per nostril 3 times a day, for 10 consecutive days.

Assessment

At the time of enrolment, subjects underwent an accurate medical history with evaluation of allergies, symptoms, smoking, occupation, familiarity with allergic and non-allergic nasal diseases, operations undertaken and ongoing therapies. Data were also collected by means of a physical examination: deviation of the nasal septum, lower turbinate hypertrophy, presence or absence of polyps, presence of catarrhal, serous or purulent rhinorrhoea. Finally, data were collected through nasal cytological examination: confirmation of goblet cell metaplasia, presence of bacterial biofilm, nasal epithelium appearance. At the time of enrolment, the two groups of patients were homogeneous in terms of signs and symptoms (Table I).

All patients underwent a sample collection through scraping technique both at T0 and T1. Each sample underwent cytological examination, which was carried out by spreading the sample over a glass slide and staining it with the May Grunwald - Giemsa method. All the slides were observed under 100, 400, 1000 magnifications to count the cellular inflammatory (neutrophils and lymphocytes) and mucin-producing elements and the presence of any bacteria. The outcome of the nasal cytological examination was evaluated by using the classification shown in Table II (3). In addition, all patients were assessed for turbinate hypertrophy by scoring from 0 to 3 in accordance with the Mladina classification, depending on the presence and severity of this sign (4).

A sample of 20 patients treated with VNS and 20 patients treated with HSS was evaluated by using, as a method of symptom detection, the Total Nasal Symptom

Table II. *Quantitative classification of nasal cytological examination results.*

Cell type	Description	Quantity	Classification
Neutrophils and lymphocytes	None	0	0
	Sporadic	0.1 – 1%	½+
	A Few scattered cells, small groups	1.1 – 5%	1+
	In discreet numbers, large groups	5 – 15%	2+
	Large cellular clusters that do not occupy the entire field	15 – 20%	3+
	Large cellular clusters occupying the entire field	>20%	4+
Goblet cells	None	0	0
	From rare to few cells	1 -24%	1+
	In a significant number	25 - 49%	2+
	In large numbers	50 - 74%	3+
	Lots of cells scattered all over the field	75 – 100%	4+
Bacteria	None	Not standardized	0
	Sporadic cell cluster		1+
	In a significant number		2+
	Many easily visible cells		3+
	Bacteria in the entire field		4+

Score (TNSS), which assigns a 4 point rating (from 0 to 3), depending on the presence and intensity of the symptoms the patient is experiencing, among the following 4 nasal symptoms: nasal obstruction, rhinorrhoea, nasal itching and sneezing (5). The remaining sample of 20 patients treated with VNS and 20 patients treated with HSS, which was not evaluated by using TNSS, underwent fibrorhinoscopy to assess rhinorrhoea. A scale commenting the increasing severity of the rhinorrhoea detected was used: absence of rhinorrhoea, catarrhal rhinorrhoea, serum-mucosal rhinorrhoea, serum-purulent rhinorrhoea or purulent rhinorrhoea.

All investigations were conducted both at the start of application (T0) and after 10 days of regular application of the nasal spray assigned to each patient (T1). Patients were also asked to express an opinion on the tolerability and degree of satisfaction with the therapy they underwent, scoring from 0 to 4, with the emoticons system: worsening (0), unchanged situation (1), moderate improvement (2), good improvement (3), great improvement (4). Finally, reports of any adverse effects attributable to the application of the nasal sprays tested were collected.

Statistical analysis

The descriptive statistics were used for the summary presentation of patient cohort characteristics in terms of median and percentiles [25° - 75°] or frequencies, when deemed appropriate. The differences in baseline conditions between the two treatment groups were assessed by *t*-test or the corresponding non-parametric test in the case of continuous variables and by Fisher's exact test in the case of frequencies. The effect of the treatment was evaluated against the variation in the result between the T1 and T0 visits and the significance of the differences was determined by applying Mann-Whitney's non-parametric test for paired data in the case of the

Table III. *Medians of cell count, of the score attributed to turbinate hypertrophy according to the Mladina classification and of the score given to the TNSS at the time of initiation of therapy (T0) and after 10 days of nasal spray application (T1), median of variations for each parameter investigated and statistical significance of the variations.*

Rhinocytogram	Treatment	T0 Median [25° - 75°]	T1 Median [25° - 75°]	p-value	T1-T0 Median [25° - 75°]
Neutrophils	VNS	2 [0; 3]	1 [0; 2]	p < 0.0001	-1 [-1.3; 0]
	HSS	1 [0; 3]	1 [0; 2]	p = 0.009	0 [-1; 0]
Lymphocytes	VNS	1 [0; 2]	0.5 [0; 1]	p < 0.0001	-0.5 [-1; 0]
	HSS	1 [0; 1]	1 [0; 1]	p = 0.773	0 [0; 0]
Goblet cells	VNS	3 [3; 4]	1 [1; 2]	p < 0.0001	-2 [-2; -1.5]
	HSS	3 [3; 3]	3 [2; 3]	p < 0.0001	-1 [-1; 0]
Bacteria*	VNS	2 [0; 2.3]	1 [0; 1]	p = 0.002	-1 [-1; 0]
	HSS	0 [0; 2]	0 [0; 1.3]	p = 0.346	0 [0; 0]
Turbinate hypertrophy	VNS	3 [3; 3]	2 [1; 2]	p < 0.0001	-1 [-2; -1]
	HSS	3 [3; 3]	2 [2; 3]	p < 0.0001	-1 [-1; 0]
TNSS	VNS	7 [6.8; 9]	3 [2; 4]	p < 0.0001	-4 [-5.3; -3]
	HSS	6 [5; 8]	4 [3; 5.3]	p = 0.0001	-2 [-4; -1]

The values shown in the table are expressed as medians of the measures collected, based on the classification shown in Table II, for all subjects at T0 and T1. The square brackets indicate the values of the 25th and 75th percentile. (*) Bacterial cells were present at T0 in 8 patients treated with HSS and 12 patients treated with VNS.

comparison between T1 and T0 and for unpaired data in the case of the comparison of the variations between the two treatment groups. In all the analyses carried out the results are considered statistically significant for $p < 0.05$.

Statistical analysis was performed by using the R software version 3.6.1 for Windows (R Core Team; 2013. A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Cytological examination

Treatment with VNS and treatment with HSS resulted in a significant reduction in neutrophils ($p < 0.0001$ and $p = 0.009$ respectively). Treatment with VNS resulted in significantly greater neutrophil reduction than that induced by HSS (-1 [-1.3; 0] *vs*

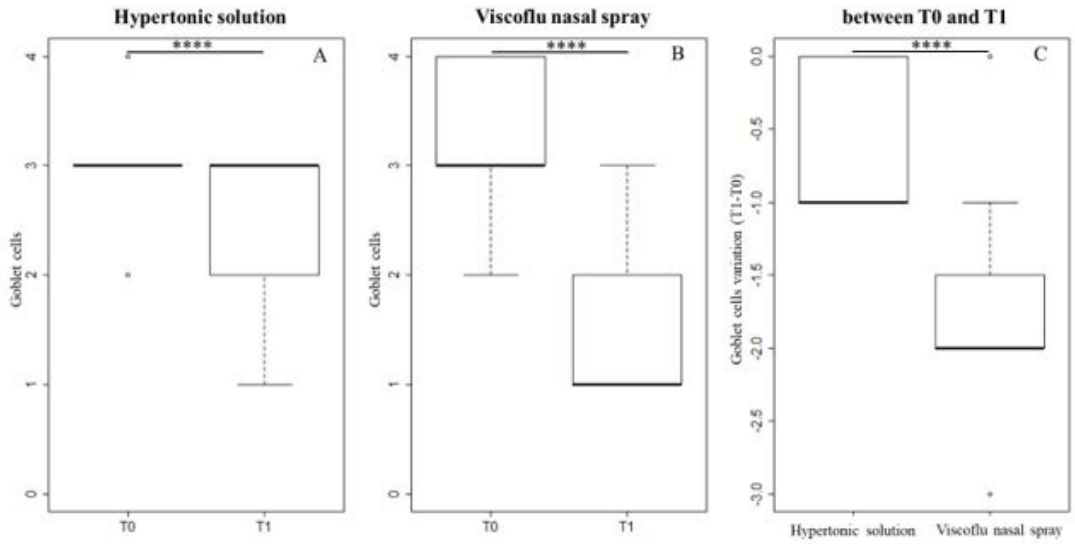

Fig. 1. *Both treatments resulted in significant neutrophil reduction (**A, B**). Treatment with VNS resulted in a significantly greater reduction in neutrophils than that induced by HSS (**C**). All the graphs use Tukey's representation; * $p < 0.05$; ** $p < 0.01$; **** $p < 0.0001$.*

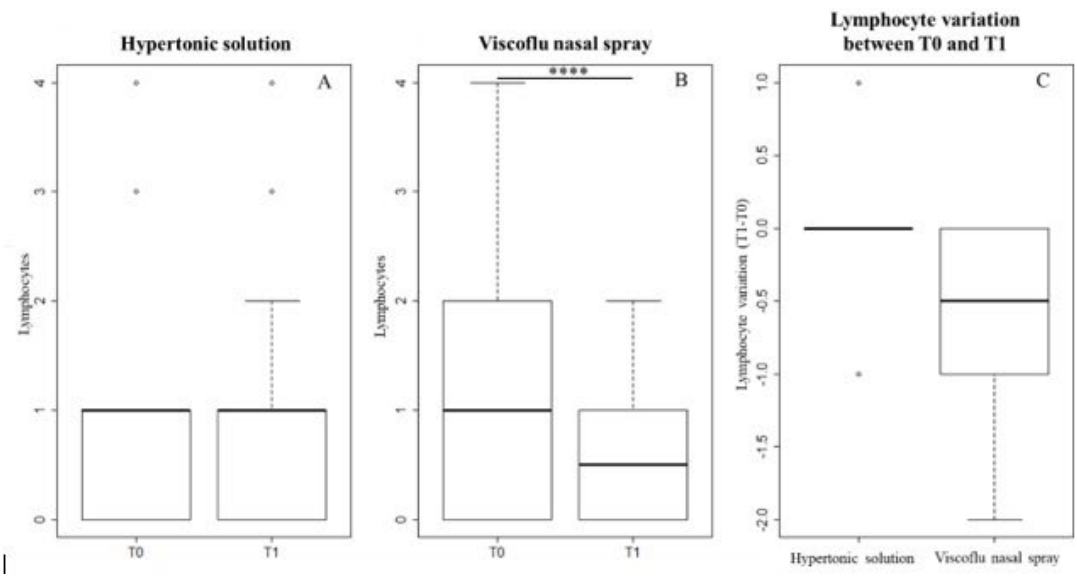

Fig. 2. *Lymphocyte reduction was significant only in subjects treated with VNS (**B**) and averaged -0.5 [-1; 0]. Treatment with HSS did not result in a statistically significant reduction in lymphocytes (**A**). Treatment with VNS resulted in a significantly greater reduction in lymphocytes than that induced by HSS (**C**). All the graphs use Tukey's representation; **** $p < 0.0001$.*

0 [-1; 0]; p = 0.01) (Table III, Fig. 1 A, B, C). The reduction in lymphocytes was significant only in subjects treated with VNS (p < 0.0001) and on average was -0.5 [-1; 0]. Treatment with HSS did not result in a statistically significant reduction in lymphocytes (Table III, Fig. 2 A, B, C). Both treatments induced a significant reduction in the number of goblet cells (p < 0.0001). Treatment with VNS resulted in a significantly greater reduction in the number of goblet cells than that induced by HSS (-2 [-2; -1.5] vs -1 [-1; 0]; p < 0.0001) (Table III, Fig. 3 A, B, C). Bacterial reduction was significant only in subjects treated with VNS (p = 0.002) and on average was -1 [-1; 0]. Treatment with HSS did not result in a statistically significant bacterial reduction (Table III).

Turbinate hypertrophy

Both treatments induced a statistically significant reduction in turbinate hypertrophy (p < 0.0001). In patients treated with VNS the reduction was greater than that induced by HSS and the difference between the two results is statistically significant (-1 [-2; -1] vs -1 [-1; 0]; p = 0.0001; Table III).

Total Nasal Symptom Score

Treatment with VNS and treatment with HSS resulted in a statistically significant reduction in the TNSS score (p < 0.0001 and p = 0.0001 respectively), but in subjects treated with VNS the effect was significantly greater than that measured in subjects treated with HSS (-4 [-5.3; -3] *vs* -2 [-4; -1]; p = 0.029; Table III)

Fibrorhinoscopy

Treatment with VNS resulted in rhinorrhoea improvement in 95% of patients under treatment, while HSS resulted in rhinorrhoea improvement in 40% of patients under treatment. The effect of VNS on the rhinorrhoea profile, evaluated by fibrorhinoscopy, was significantly greater than that induced by treatment with HSS alone (p < 0.001).

Satisfaction

Subjects in both treatment groups were satisfied (p = 0.435) and reported moderate to good improvement (VNS: good improvement 40%; moderate improvement 45%; unchanged 15%. HSS: good improvement 35%; moderate improvement 30%; unchanged 35%). The data on patient satisfaction is in line with what was observed in the evaluation of the other parameters.

Reported adverse effects

None of the patients in the two groups complained

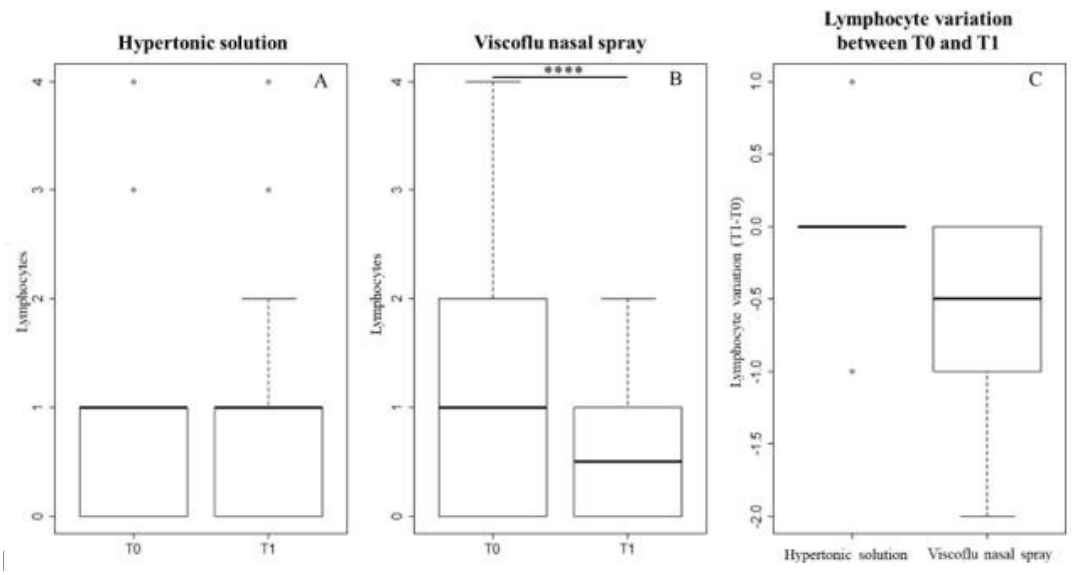

Fig. 3. *Both treatments induced a significant reduction in goblet cells (**A, B**). Treatment with VNS resulted in a significantly greater reduction in the number of goblet cells than that induced by HSS (**C**). All the graphs use Tukey's representation; **** p < 0.0001.*

of adverse effects attributable to the application of nasal sprays during the 10-day treatment. No patient stopped applying nasal spray.

DISCUSSION

In addition to basic research literature, there is extensive literature available on the clinical use of saline solution: it cleanses the mucous membranes, removes secretions, physiologically mobilizes the surface gel layer, increases hydration in the sol layer, improves mucociliary function and removes inflammatory mediators. It is recommended as an adjuvant treatment in the management of rhinitis and rhinosinusitis (6), as it is effective, safe, cost-effective and easy to use. NAC has a proven mucolytic action, stimulates glutathione biosynthesis and has antioxidant activity (7). Studies show that NAC has anti-inflammatory activity and the ability to counteract the formation of bacterial biofilms (8, 9).

No clinical studies were identified in the literature in which a combination of hypertonic saline solution and NAC was tested for topical use in the treatment of non-allergic chronic rhinitis with goblet cell metaplasia. A recent retrospective clinical study demonstrated the efficacy of this combination in the topical treatment of chronic rhinosinusitis without polyposis, with early recurrence after Functional Endoscopic Sinus Surgery (FESS) (10).

The present clinical study shows a significant improvement in the rhino-cytological profile of the nasal mucosa of patients who have non-allergic chronic rhinitis with a cytological diagnosis of goblet cell metaplasia and who applied a nasal spray containing HSS or VNS for 10 consecutive days. VNS has been shown to be significantly more effective than HSS in reducing the number of goblet cells, neutrophils, lymphocytes and bacteria, and in reducing turbinate hypertrophy, TNSS score and rhinorrhoea. This result can be supposedly attributed to the synergistic action between the 3% hypertonic saline solution and 6% NAC. VNS proved safe and well tolerated: in the 10 days of product intake, no patient reported adverse events and no patient discontinued therapy.

VNS can therefore be considered a valid aid in the treatment of the most frequent nasal-sinus diseases, especially in those with a high irritative component accompanied by a strong mucous reaction. In conclusion, the use of VNS can be recommended for its significant mucolytic activity, for its ability to restore surface ciliary activity at the epithelium level and for the modulation of inflammation, which are essential processes for the optimisation of nasal-sinus defensive functions.

CONFLICT OF INTEREST

Enrico Maffezzoni, Mario Notargiacomo and Matteo Gelardi declares that they have no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article. Stefano Agostini is an employee of Pharma Line.

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