Crenotherapy modulates the expression of proinflammatory cytokines and immunoregulatory peptides in nasal secretions of children with chronic rhinosinusitis

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ABSTRACT

Background: *The effect of crenotherapy on major mucosal markers of inflammation, TNF alpha, human beta-defensins 2 (hBD-2), and calprotectin, are largely unexplored in pediatric chronic rhinosinusitis (CRS). The aim of this study was to investigate the effects of crenotherapy with sulfate-sodium-chloride water on mucosal markers of inflammation in children with CRS.*

Methods: *Children with CRS received 15-day crenotherapy consisting of sulfate-sodium-chloride thermal water inhalations by nasal aerosol (15 minutes/day). Concentrations of nasal mucosal markers of inflammation (TNF alpha, hBD-2, and calprotectin) were measured before and after crenotherapy. Presence of specific symptoms (nasal obstruction, nasal discharge, facial pain, sense of smell, and cough), value of symptoms score sino-nasal 5 (SN5), quality of life (QoL) score (1 [worse] to 10 [optimal]) were also assessed.*

Results: *After crenotherapy a significant reduction was observed in TNF alpha (from 0.14 ± 0.02 to 0.08 ± 0.01; $p < 0.001$), calprotectin (from 2.9 ± 1.0 to 1.9 ± 0.5; $p < 9.001$), and hBD-2 (from 2.0 ± 0.1 to 0.9 ± 0.6; $p < 0.001$) concentrations. A significant ($p < 0.05$) reduction in number of subjects presenting symptoms of nasal obstruction (100% versus 40%), nasal discharge (33% versus 13%), facial pain (30% versus 10%), and sense of smell (60% versus 20%) was observed. A significant improvement of SN5 (from 3.07 ± 0.76 to 2.08 ± 0.42; $p < 0.001$) was observed after the crenotherapy. QoL also improved after crenotherapy (from 4.2 ± 1.1 to 6.6 ± 1.0; $p < 0.001$).*

Conclusion: *Crenotherapy induced a down-regulation of nasal mucosal inflammatory mediators in children with CRS.*

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Chronic rhinosinusitis (CRS) is one of the most common diseases in western countries. Although the exact pathogenesis of CRS remains unclear, the underlying mechanisms involve different inflammatory mediators, such as proinflammatory cytokines (TNF-α) and several antimicrobial and immunoregulatory peptides, including calprotectin and human β-defensins 2 (hBD-2).[1,2] Although the eosinophilic inflammation is typical of adult patients with CRS, the inflammatory response in children is characterized by a mixed lymphocyte population, macrophages and neutrophils, suggesting differences in the pathogenic mechanism.[3] Neutrophils play a fundamental role in the inflammatory process; they release inflammatory cytokines, such as TNF-α, and antimicrobial peptide, including defensins and calprotectin.[4–6] Calprotectin is among the most abundant cytoplasmic proteins in neutrophils and macrophages, and it is found in all body fluids in proportion to the degree of inflammation.[7] Elevated serum levels of calprotectin have been reported in several inflammatory conditions, including cystic fibrosis, rheumatoid arthritis, and systemic infections.[8–11] Human β-defensin 2 is an inducible antimicrobial peptide widely expressed by epithelial cells and involved most obviously in mucosal response in inflammatory and/or infectious conditions.[12,13] Human β-defensin 2 exerts chemotactic properties on dendritic cells and human neutrophils.[14,15] Elevated levels of hBD-2 have been founded in many human diseases characterized by chronic inflammation.[16–19]

Crenotherapy (from the Greek κρενη, spring fountain), is a complex of practices using water, with different mineral components and different physical properties, to cure many pathologies, mainly diseases of the respiratory, digestive and urinary tract, of the skin, and rheumatic diseases.[20] Crenotherapy has been approved as a drug in many countries, with specific therapeutic indications, targeted doses, and contraindications, but the exact mechanisms of action are still under active research. Clinical findings suggest an anti-inflammatory effect of crenotherapy in adult respiratory system diseases,[21–26] but data in the pediatric population are still very limited. The aim of this study was to investigate the effects of crenotherapy with sulfate-sodium-chloride water on mucosal markers of inflammation in children with CRS.

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METHODS

Study Design

The present investigation was a prospective study on pediatric patients with CRS. The study protocol was approved by the Ethics Committee of the University of Naples "Federico II." Written informed consent was obtained from all care givers of subjects before the procedures were performed.

Population

The trial was performed in collaboration with family pediatricians, who were in the Italian public health care system, for children up to 16 years of age. The family pediatricians were invited to contact the study coordinator at the Department of Pediatrics if a case of CRS was

suspected. Subjects considered eligible for the study were (1) aged 2–12 years; (2) had one or more of the following sinonasal symptoms: nasal discharge, nasal congestion, nasal obstruction, postnasal drip, daytime cough, or foul breath (fetor oris); (3) had active symptoms at the time of initial evaluation; (4) failed courses of antibiotics, saline irrigation, nasal steroids, or antihistamine; (5) and had persistent symptoms for ≥1 month. Eligible patients were evaluated by two otolaryngologists with great experience in the pediatric field that confirmed the diagnosis of CRS and invited the children and their families to participate in the study. The diagnosis of certainty of CRS was based on direct observation by nasal fibroendoscopy of nasal turbinates, middle meatus, and rhinopharynx, detecting mucopurulent discharge from the middle meatus and/or edema or mucosal obstruction.[27] All eligible subjects had full clinical evaluation and allergy and immunodeficiency workup. Staging of CRS was performed according to previous validated clinical score, *viz.*, sino-nasal 5 (SN5).[28] Children with indication of surgery (primary diagnosis of obstructive sleep apnea syndrome caused by tonsillar hyperplasia), chronic diseases, immunodeficiency, neurological impairment, topical or systemic therapies with drugs that interfere with the characteristics of nasal inflammation (steroids, nonsteroidal anti-inflammatory drugs, antihistamines, and vasoconstrictors) in the previous 4 weeks, varicose veins of the nasal septum, suspect of ciliary abnormalities, previous sinonasal surgery, malformations of the upper airway, sinonasal osteoneogenesis, sinonasal tumors or obstructive lesions, a history of facial trauma that distorted the sinus anatomy, or suspect of ciliary dysfunction requiring biopsy, and/or inability to perform a complete course of crenotherapy were excluded. Seventy-six consecutive patients with diagnosis of CRS were eligible and were enrolled in the study. Sixty subjects completed the study protocol. The results were compared with those obtained in a population of healthy children consecutively observed at our center because routine examination before vaccination program. The demographic characteristics of healthy children were comparable with children affected by CRS. These subjects were evaluated by otolaryngologists with experience in the pediatric field to exclude the presence of inflammation in the upper airway. Sixty-two children were observed, 12 were excluded because of mild upper respiratory infections, and 50 subjects were enrolled (28 boys; mean age, 3.4 ± 1.0 years).

Intervention and Data Collection

Enrolled patients with CRS received a 15-day course of sulfate-sodium-chloride thermal water inhalations by nasal aerosol (15 minutes/day). Therapy was performed in a thermal site in the island of Ischia approved by the Italian National Health System. The composition of the thermal water is reported in the Table 1. Demographic and clinical variables of all study subjects were collected in a specific chart. The severity of the symptoms and quality of life (QoL) were measured by SN5 and QoL score according to previous criteria[28] in each patient at the time of enrollment (T0), at the end of a 15-day course of crenotherapy (T1), and 4 weeks later (T2). SN5 and QoL scores were completed by patients in collaboration with caregivers unaware of the study aims. Before and immediately after crenotherapy a nasal mucous sample was collected by sterile cannula (8 French) through lavage of the nasal cavity using 5 mL of physiological saline, as previously reported.[29] Mucus samples were placed in 15-mL tubes containing 2 mL of buffer and then stored at −20°C. Data collection and nasal mucus samples, with an identification code, were sent to the Coordinator Center. A good compliance to crenotherapy was defined if the subject underwent at least of 90% of prescribed therapy.

Laboratory Measurements

Nasal mucus samples were thawed slowly at room temperature, diluted with distilled water, subjected to mild mechanical agitation for 60 minutes, and centrifuged at 3000 rpm for 20 minutes, and the supernatant was collected and frozen at −20°C for subsequent determinations. Quantitative determinations of TNF-α, calprotectin, and hBD-2 were performed using commercial ELISA kits (TNF alpha single analyte ELISA Kit; Orgenium, Vantaa, Finland; Calprest Eurospital Trieste, Italy; Human beta defensin 2 ELISA Kit Protocol; Phoenix Pharmaceuticals, Inc., Burlingame CA). In brief, for calprotectin determinations, 100 μL of each sample was added to the wells of a plate and incubated at room temperature for 45 minutes. The plate was then washed three times with diluted washing solution, and 100 μL of purified rabbit anti-calprotectin antibodies conjugated with alkaline phosphatase were added and incubated for 45 minutes at room temperature. A second washing procedure was performed; 100 μL of enzyme substrate solution was added to each well, and optical density was read at 405 nm. Nasal mucous calprotectin was calculated from the standards and expressed as micrograms per milliliter. For hBD-2 determinations,100 μL of each sample was added to the wells of a plate and incubated at room temperature for 120 minutes. The plate was then washed four times with diluted washing solution, and 100 μL of biotinylated anti-β-defensin 2 detection antibody was added and incubated for 120 minutes at room temperature. A second washing procedure was performed; 100 μL of streptavidin horseradish peroxidase solution was added to each well and incubated for 30 minutes at room temperature, and after addition of 100 μL of stop solution, optical density was read at 450 nm. Nasal mucous hBD-2 was calculated from the standards and expressed as nanograms per milliliter. For TNF-α determinations, 50 μL of each sample was added to the wells of a plate and incubated at room temperature for 60 minutes. The plate was then washed five times with diluted washing solution, and 50 μL of biotin antibody was added and incubated for 30 minutes at room temperature. A second washing procedure was performed; 50 μL of streptavidin horseradish peroxidase solution was added to each well and incubated for 30 minutes at room temperature. A third washing was performed. After addition of 50 μL of tetramethylbenzidine substrate solution, incubation for 20 minutes was performed. Optical density was read at 450 nm. Nasal mucous TNF-α was calculated from the standards and expressed as picograms per milliliter. Determinations were performed in triplicate and repeated on the samples collected before and after the cycle of crenotherapy.

Table 1 **Essential chemical composition of the sulphate-sodium-chloride water investigated in the study**

Parameter	Results
Temperature at source	45°C
Acidity (pH)	−7.12
Conductivity (at source; at 20°C)	8.30 μS/cm
Ammonium	0.62 mg/dL
Nitrites	0.06 mg/dL
Nitrate	41.00 mg/dL
Sodium	1700.00 mg/dL
Potassium	105.00 mg/dL
Calcium	100.20 mg/dL
Magnesium	82.15 mg/dL
Iron	0.01 mg/dL
Manganese	0.20 mg/dL
Lithium	0.50 mg/dL
Barium	1.00 mg/dL
Chloride	2800.79 mg/dL
Sulphate	473.20 mg/dL
Hydrogen carbonate	658.80 mg/dL
Silica	70.04 mg/dL

Note: The analysis was provided by Department of Pharmaceutical Chemistry and Toxicology, University of Naples Federico II, test report 41/2008, February 28, 2008.

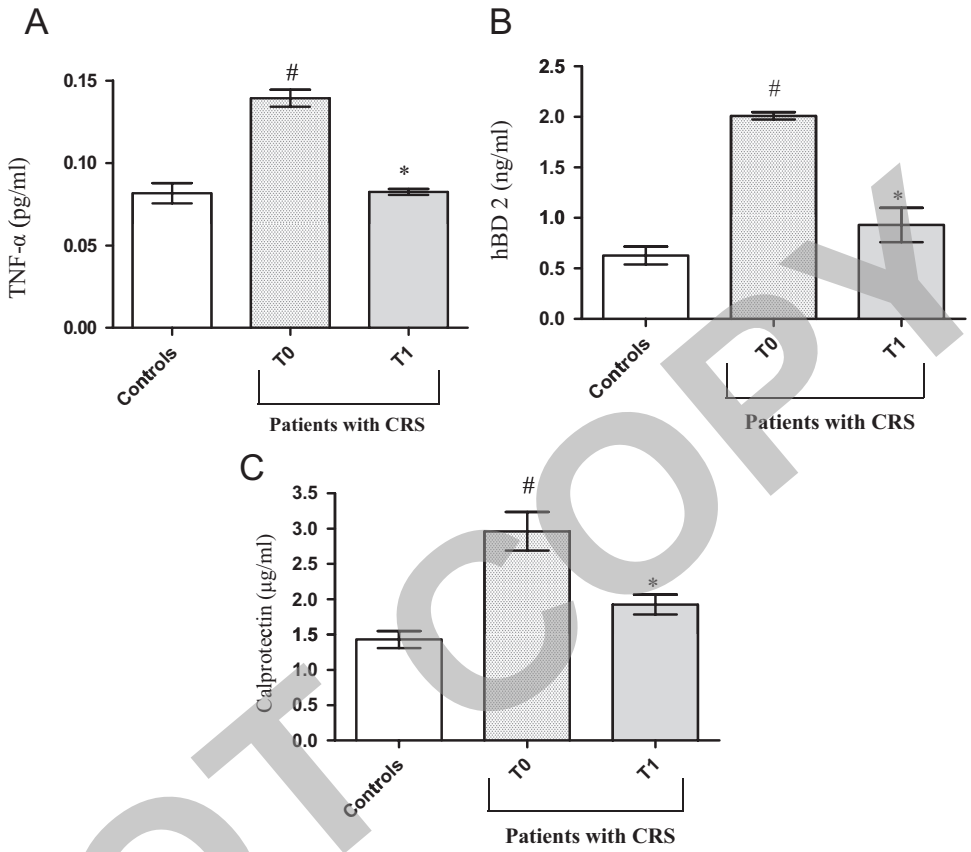

*Figure 1. Results of the determination of (A) TNF-α, (B) human β-defensin 2 (hBD-2), and (C) calprotectin in nasal secretions of children with chronic rhinosinusitis (CRS) before (T0) and after a 15-day course (T1) of crenotherapy. For comparison, results obtained in healthy children (controls) were also provided in the figure. * $p < 0.05$ T1 versus T0; #$p < 0.05$ T0 versus controls.*

Statistics

Statistical analysis was performed by a statistician blind to study aims. For categorical variables the Pearson chi-square test was performed, unless the exact method was required for frequency tables when >20% of the expected values were <5. Because of the Gaussian distribution of continuous variables assessed by Kolmogorov-Smirnov analysis, continuous variables were expressed as mean and 95% CI and analyzed by the one-way ANOVA procedure with the Bonferroni test for the *post hoc* analysis. A multivariate analysis was used to exclude the influence of demographic data on clinical and laboratory effects of crenotherapy. Spearman rank test was used to define coefficient of correlation (r) between SN5 score and levels of TNF-α, hBD-2, and calprotectin. Linear regression analysis was used to study the possible influence of such variables on the levels of TNF-α, hBD-2, and calprotectin. All tests of significance were two sided. A value of $p \leq 0.05$ was considered significant. Statistical analysis was performed with SPSS Version 16.0 for Windows (SPSS, Inc., Chicago, IL).

RESULTS

From January 2008 to April 2009, 76 consecutive patients with a positive diagnosis of CRS were evaluated. Thirteen patients were excluded because of topical or systemic therapy with drugs that interfere with the characteristics of nasal inflammation (steroids, nonsteroidal anti-inflammatory drugs, antihistamines, and vasoconstrictors) in the previous 4 weeks,[7] immunodeficiency,[2] diabetes,[2] malformations of the upper airways,[1] and varicose veins of the nasal septum.[1] Three patients were lost to follow-up. Sixty subjects (28 boys; mean age, 3.3 ± 0.9 years) completed the study protocol. Crenotherapy was well accepted by all study subjects, as suggested by the good compliance observed in all enrolled children. The TNF-α, calprotectin, and hBD-2 levels at baseline were significantly higher in children with CRS compared with healthy subjects. After a 15-day course of crenotherapy a significant reduction toward normal values was observed as shown in the Fig. 1. At the end of treatment with sulfate-sodium-chloride water a positive clinical response was observed in all patients with a significant reduction of the occurrence of nasal obstruction, nasal discharge, facial pain, and sense of smell (Table 2). A significant improvement in the SN5 score was observed after crenotherapy (Table 3) and at follow-up visit performed after 4 weeks. QoL also improved after crenotherapy (from 4.2 ± 1.1 to 6.6 ± 1.0; $p < 0.001$) and remained stable after 4 weeks (6.6 ± 1.0).

Table 2 **Clinical manifestations before (T0) and after (T1) crenotherapy in children with CRS**

Symptoms, *n* (%)	T0	T1	*p* value
Nasal obstruction	60 (100)	24 (40)	<0.001
Nasal discharge	20 (33)	8 (13)	0.010
Facial pain	18 (30)	6 (10)	0.006
Sense of smell	36 (60)	12 (20)	<0.001
Cough	11 (18)	5 (8.3)	0.107

CRS = chronic rhinosinusitis.

DISCUSSION

Although more basic and clinical data are still needed, crenotherapy is considered useful in the therapeutic approach for adult patients with CRS, reducing the use of other therapies and medical costs.[21–26] Crenotherapy is generally well accepted and tolerated by patients and in literature no cases of toxicity or neoplasm occurrence due to thermal water inhalation have been documented in the hundreds of thousands of patients treated.[20–26] The results of our study showed that even in pediatric patients with CRS, crenotherapy sig-

Table 3 **Symptomatic score (SN5) before treatment (T0), after 15 days of crenotherapy (T1), and at follow-up visit performed after 4 wk (T2) in children with CRS**

Item	T0	T1	T2	*p* Value T0 vs T1	*p* Value T1 vs T2
Sinus infection	2.5 ± 1.2	1.9 ± 0.6	1.8 ± 0.5	<0.0001	0.465
Nasal obstruction	4.4 ± 1.3	2.7 ± 0.9	2.5 ± 0.8	<0.0001	0.308
Allergy symptoms	2.2 ± 0.8	1.5 ± 0.5	1.5 ± 0.5	<0.0001	0.861
Emotional distress	3.1 ± 0.9	2.3 ± 0.7	2.2 ± 0.6	<0.0001	0.483
Activity limitations	3.0 ± 0.9	1.9 ± 0.5	1.9 ± 0.4	<0.0001	0.451
Total SN5 score	15.3 ± 3.8	10.4 ± 2.1	9.9 ± 1.8	<0.0001	0.253
Mean SN5	3.0 ± 0.7	2.0 ± 0.4	1.9 ± 0.4	<0.0001	0.253

Note: Data are reported as mean ± SD.
CRS = chronic rhinosinusitis.

nificantly improve nasal obstruction, nasal discharge, facial pain, and reduction of smell, all typical symptoms of CRS. Our goal was to evaluate the mechanisms behind the clinical effects of crenotherapy in pediatric patients with CRS. We observed that the positive effect exerted by crenotherapy on the persistent symptomatic inflammation of the nasal mucosa involves a modulation of proinflammatory cytokines and immunoregulatory peptides; in fact, in our study we observed a significant reduction toward normal values of TNF-α, calprotectin, and hBD-2 after a 15-day course of crenotherapy. The positive modulation of TNF-α is important because during inflammation this cytokine mediates the induction of peptides, such as hBD-2[13] and other proinflammatory cytokines involved in the alterations of the nasal mucosa, which usually occur in patients with CRS. In this light, crenotherapy can be regarded as an old therapy, which is now being reassessed and revived scientifically. In particular, our study is one of the first studies to investigate the efficacy of such therapy in the treatment of respiratory disorders of childhood and to assess the role of innate immunity in the mechanism of action of crenotherapy in childhood.

In a recent study Tieu *et al.*[30] examined the expression of calprotectin using ELISA in nasal lavage samples from 40 adult control subjects, patients with CRS with nasal polyps (CRSwNPs), and CRS without nasal polyps (CRSsNPs). Calprotectin levels were significantly decreased in nasal lavage samples of patients with CRSwNPs when compared with those seen in control subjects ($p < 0.05$). The authors suggest that reduced levels should be considered as a possible contributing factor in CRS pathogenesis. In another recent study, 32 adult patients with CRSwNPs and 10 control subjects were enrolled. In this study, the authors showed that epithelial cells derived from patients with recalcitrant CRSwNPs displayed decreased expression of hBD-2.[31] In our pediatric population, we found, apparently in contrast with those reported in adults, higher values of calprotectin and hBD-2 in patients with CRS compared with controls, whereas after crenotherapy these values tended to return to a normal level found in healthy subjects. However, one should consider that CRS is a heterogeneous group of inflammatory diseases of the nasal and paranasal cavities either accompanied by polyp formation or without polyps. The tissue inflammatory response in patients with CRSsNPs has been shown to be highly neutrophilic with a tendency toward TH1 polarization, whereas CRSwNP inflammatory responses are characterized by eosinophilia with a TH2 skewing.[32] Although the eosinophilic inflammation is typical of adult patients with CRS, the inflammatory response in children is characterized by a mixed lymphocyte population, macrophages, and neutrophils, suggesting differences in the pathogenic mechanism.[3] A diverse spectrum of alterations involving histopathology, inflammatory cell and T-cell patterns, remodeling parameters, IgE production, microorganisms, and epithelial barrier malfunctions is reported in the search to describe the pathogenesis of this heterogeneous group of upper airway diseases.[33,34] Distinct features of CRSwNPs and CRSsNPs are in line with the results obtained in our study group. The studies conducted in adult patients reported diminished levels of calprotectin and hBD-2 expression in patients with CRSwNP while we have enrolled children without nasal polyps, a form with distinct histopathological features and, consequently, with different expression of mucosal markers of inflammation.

CONCLUSION

The present study shows that crenotherapy with sulfate-sodium-chloride water modulates the expression of proinflammatory cytokines and immunoregulatory and antimicrobial peptides in nasal secretions of pediatric patients with CRS after a 15-day course of thermal water inhalations by nasal aerosol. At the end of the crenotherapy treatment a significant improvement of the persistent symptomatic inflammation of the nasal mucosa was observed. It is probably because of a positive effect exerted by the thermal water on the alterations of the epithelial immune barrier function, which usually occurs in patients with CRS. These evidences suggest that crenotherapy could be considered as an efficacious therapeutic tool in the management of these patients. In particular, the modulatory effect on TNF-α, calprotectin, and hBD-2 levels could be helpful for a better knowledge of the mechanisms of action of crenotherapy, which could open the way for new possible applications of this therapeutic strategy in other conditions characterized by inflammation. The therapeutic effects of the thermal water are probably because of its mechanical cleaning function and its physical and chemical composition, but other studies are needed to define the components of the thermal waters involved in these beneficial effects.

REFERENCES

1. Kuehnemund M, Ismail C, Brieger J, et al. Untreated chronic rhinosinusitis: A comparison of symptoms and mediator profiles. Laryngoscope 114:561–565, 2004.
2. Tieu DD, Kern RC, and Schleimer RP. Alterations in epithelial barrier function and host defense responses in chronic rhinosinusitis. J Allergy Clin Immunol 124:37–42, 2009.
3. Coffinet L, Chan KH, Abzug MJ, et al. Immunopathology of chronic rhinosinusitis in young children. J Pediatr 154:754–758, 2009.
4. Halayko AJ, and Ghavami S. S100A8/A9: A mediator of severe asthma pathogenesis and morbidity? Can J Physiol Pharmacol 87: 743–755, 2009.
5. Cheng P, Corzo CA, Luetteke N, et al. Inhibition of dendritic cell differentiation and accumulation of myeloid-derived suppressor cells in cancer is regulated by S100A9 protein. J Exp Med 205:2235–2249, 2008.
6. Foell D, Wittkowski H, Ren Z, et al. Phagocyte-specific S100 proteins are released from affected mucosa and promote immune responses during inflammatory bowel disease. J Pathol 216:183–192, 2008.
7. Berni Canani R, Terrin G, Rapacciuolo L, et al. Faecal calprotectin as reliable non-invasive marker to assess the severity of mucosal inflammation in children with inflammatory bowel disease. Dig Liver Dis 40:547–553, 2008.

8. Sutherland AD, Gearry RB, and Frizelle FA. Review of fecal biomarkers in inflammatory bowel disease. Dis Colon Rectum 51:1283–1291, 2008.
9. Golden BE, Clohessy PA, Russell G, et al. Calprotectin as a marker of inflammation in cystic fibrosis. Arch Dis Child 74:136–139, 1996.
10. Baillet A. S100A8, S100A9, and S100A12 proteins in rheumatoid arthritis. Rev Med Interne 31:458–461, 2010.
11. Berni Canani R, Rapacciuolo L, Romano MT, et al. Diagnostic value of fecal calprotectin in pediatric clinical practice. Dig Liver Dis 36: 467–470, 2004.
12. Doss M, White MR, Tecle T, et al. Human defensins and LL-37 in mucosal immunity. J Leukoc Biol 87:79–92, 2010.
13. Jäger S, Stange EF, and Wehkamp J. Antimicrobial peptides in gastrointestinal inflammation. Int J Inflam, 2010. (Epub ahead of print November 25, 2010.)
14. Yang D, Chertov O, Bykovskaia SN, et al. Beta-defensins: Linking innate and adaptive immunity through dendritic and T cell CCR6. Science 286:525–528, 1999.
15. Niyonsaba F, Ogawa H, and Nagaoka I. Human beta-defensin-2 functions as a chemotactic agent for tumour necrosis factor-alpha-treated human neutrophils. Immunology 111:273–281, 2004.
16. Kapel N, Benahmed N, Morali A, et al. Fecal beta-defensin-2 in children with inflammatory bowel diseases. J Pediatr Gastroenterol Nutr 48:117–120, 2009.
17. Hiratsuka T, Mukae H, Iiboshi H, et al. Increased concentrations of human beta-defensins in plasma and bronchoalveolar lavage fluid of patients with diffuse panbronchiolitis. Thorax 58:425–430, 2003.
18. Ross DJ, Cole AM, Yoshioka D, et al. Increased bronchoalveolar lavage human beta-defensin type 2 in bronchiolitis obliterans syndrome after lung transplantation. Transplantation 78:1222–1224, 2004.
19. Ishimoto H, Mukae H, Date Y, et al. Identification of hBD-3 in respiratory tract and serum: The increase in pneumonia. Eur. Respir. J. 27:253–260, 2006.
20. Vaccarezza M, and Vitale M. Crenotherapy: A neglected resource for human health now re-emerging on sound scientific concepts. Int J Biometeorol 54:491–493, 2010.
21. Staffieri A, and Abramo A. Sulphurous-arsenical-ferruginous (thermal) water inhalations reduce nasal respiratory resistance and improve mucociliary clearance in patients with chronic sinonasal disease: Preliminary outcomes. Acta Otolaryngol 127:613–617, 2007.
22. Staffieri A, Marino F, Staffieri C, et al. The effects of sulfurous-arsenical-ferruginous thermal water nasal irrigation in wound healing after functional endoscopic sinus surgery for chronic rhinosinusitis: A prospective randomized study. Am J Otolaryngol 29:223–229, 2008.
23. Passali D, Lauriello M, Passali GC, et al. Clinical evaluation of the efficacy of Salsomaggiore (Italy) thermal water in the treatment of rhinosinusal pathologies. Clin Ter 159:181–188, 2008.
24. Passali FM, Crisanti A, Passali GC, et al. Efficacy of inhalation therapy with water of Salsomaggiore (Italy) in chronic and recurrent nasosinusal inflammation treatment. Clin Ter 159:175–180, 2008.
25. Ottaviano G, Marioni G, Staffieri C, et al. Effects of sulfurous, salty, bromic, iodic thermal water nasal irrigations in nonallergic chronic rhinosinusitis: A prospective, randomized, double-blind, clinical, and cytological study. Am J Otolaryngol 32:235–239, 2011.
26. Salami A, Dellepiane M, Strinati F, et al. Sulphurous thermal water inhalations in the treatment of chronic rhinosinusitis. Rhinology 48: 71–76, 2010.
27. Leo G, Incorvaia C, Masieri S, et al. Imaging criteria for diagnosis of chronic rhinosinusitis in children. Eur Ann Allergy Clin Immunol 42:199–204, 2010.
28. Kay DJ, and Rosenfeld RM. Quality of life for children with persistent sinonasal symptoms. Otolaryngol Head Neck Surg 128:17–26, 2003.
29. Ciprandi G, Tosca MA, Milanese M, et al. Cytokines evaluation in nasal lavage of allergic children after *Bacillus clausii* administration: A pilot study. Pediatr Allergy Immunol 15:148–151, 2004.
30. Tieu DD, Peters AT, Carter RG, et al. Evidence for diminished levels of epithelial psoriasin and calprotectin in chronic rhinosinusitis. J Allergy Clin Immunol 125:667–675, 2010.
31. Ramanathan M Jr, Lee WK, Spannhake EW, et al. Th2 cytokines associated with chronic rhinosinusitis with polyps down-regulate the antimicrobial immune function of human sinonasal epithelial cells. Am J Rhinol 22:115–121, 2008.
32. Van Zele T, Claeys S, Gevaert P, et al. Differentiation of chronic sinus diseases by measurement of inflammatory mediators. Allergy 61: 1280–1289, 2006.
33. Van Crombruggen K, Zhang N, Gevaert P, et al. Pathogenesis of chronic rhinosinusitis: Inflammation. J Allergy Clin Immunol 128: 728–732, 2011.
34. Huvenne W, van Bruaene N, Zhang N, et al. Chronic rhinosinusitis with and without nasal polyps: What is the difference? Curr Allergy Asthma Rep 9:213–220, 2009. □