Eur Arch Otorhinolaryngol (2009) 266:847–855
DOI 10.1007/s00405-008-0836-0

RHINOLOGY

Postoperative application of amphotericin B nasal spray in chronic rhinosinusitis with nasal polyposis, with a review of the antifungal therapy

I. Gerlinger · A. Fittler · F. Fónai · A. Patzkó · A. Mayer · L. Botz

Received: 29 June 2008 / Accepted: 1 October 2008 / Published online: 25 October 2008

Abstract Chronic rhinosinusitis (CRS) affects 1–4% of the adult population. The etiology of this multifactorial, chronic disease, which leads to a significant impairment of the quality of life, often accompanied by nasal polyposis, is not fully understood. In the past decade, it was presumed that the disease, which causes characteristic eosinophilic infiltration of the nasal mucosa, is triggered by an enhanced (but not classical allergic IgE-type) immune response against fungal organisms in the nasal mucus. If this supposition is correct, then it appears obvious that the administration of amphotericin B nasal spray in adequate concentration following endoscopic polypectomy should be advantageous for these patients, and might even reduce the number of recurrent cases. To check on this assumption, we conducted a prospective randomized placebo-controlled trial involving 33 patients, 30 of whom remained in the study throughout. Patients with nasal polyposis were operated on with an endoscopic technique between 1 November 2005 and 1 October 2006; group A (14 randomly selected patients) were treated with a nasal spray containing 5 mg/ml amphotericin B, while the placebo group B (16 randomly selected patients) received a nasal spray lacking amphotericin B. We evaluated our results with the aid of a modified Lund–Mackay CT score, the SNAQ-11 test (which assesses changes in the symptoms), a quality of life test and endoscopy. The SPSS 14.0 for Windows program was utilized to process the data of examinations performed preoperatively and 1 year postoperatively. The CT scores of the group A patients 1 year after the operation exhibited wide scattering, without signs of recovery. The CT scores of the group B patients indicated a slight improvement, though this did not prove significant relative to group A. Both the SNAQ-11 test and the quality of life test revealed a significant improvement in each group, but the degrees of change in these tests did not differ significantly between the two groups of patients. The endoscopic findings indicated a slight improvement to the advantage of the amphotericin B-treated group 12 months after the operation. These results lead to the conclusion that the administration of amphotericin B nasal spray to patients operated on for nasal polyposis does not give rise to a significant alteration in either CT score, clinical symptoms, or quality of life. The more favorable clinical aspects observed in the amphotericin B-treated group during the endoscopic follow-up did not correspond to an improvement in the symptoms. In connection with the conclusions drawn from this study, the authors discuss the controversial data available on the fungal etiology of CRS. They critically analyze the contradictory observations and conclusions of seven recent clinical studies.

Keywords Amphotericin B · Chronic rhinosinusitis · Nasal irrigation · Nasal polyposis · Nasal spray

I. Gerlinger (✉) · F. Fónai · A. Patzkó
Department of Otorhinolaryngology and Head and Neck Surgery, Medical School, University of Pécs, Munkácsy Mihály utca 2, 7621 Pecs, Hungary
e-mail: i.gerlinger@freemail.hu

A. Fittler · A. Mayer · L. Botz
Department of Pharmaceutics, University Pharmacy, University of Pécs, Pecs, Hungary

Introduction

Chronic rhinosinusitis (CRS) is an inflammatory disease of the nasal and paranasal sinus mucosa that has existed for more than 3 months, with typical leading symptoms such as nasal obstruction, thick nasal discharge, a reduction/loss of

the ability to smell, facial pressure and/or pain, in some cases accompanied by an extreme degree of nasal polyposis [1]. As concomitant symptoms, CRS patients may complain of headaches, fever, halitosis, fatigue, dental pain and ear fullness. Current medical research suggests that CRS can be referred to as a multifactorial disease, which may be associated with asthma, cystic fibrosis, primary ciliary dyskinesia, aspirin intolerance and allergy [2–5]. Around 4% of the adult population suffer from a compromised quality of life in consequence of the disease [3]. The etiology and pathogenesis of CRS are neither completely known nor understood. One of the most popular theories, which is a subject of intensive research, postulates that the causal factors are morphological variations on the lateral wall of the nasal cavity [3]: other hypotheses include the biofilm theory [6], and the role of superantigens [7]. The basic standard treatment is surgical intervention and the use of corticosteroids. It should be emphasized that even the most modern antibiotics are effective only during the acute exacerbations of CRS, making a bacterial etiology doubtful. The fact that antiallergic medication results in a symptomatic improvement merely in the event of a proved concomitant allergy suggests that an IgE-mediated allergy is not an etiological factor of CRS [8].

The role of fungal organisms in the development of some rare forms of CRS has long been known. Allergic fungal rhinosinusitis (AFRS) was first described by Katzenstein et al. in 1980 [9]. DeShazo and Swain [10], and later on Bent and Kuhn [4] proposed the criteria of this previously rarely diagnosed disease.

Ponikau et al. [11] recently developed new mucus-collecting and culturing methods with which they were able to demonstrate the presence of mucin containing hyphae and clusters of degenerating eosinophils (Charcot–Leyden crystals) referring to allergy in 96% of CRS patients with polyposis undergoing endoscopic sinus surgery. With the diagnostic criteria of DeShazo and Swain, the previously rarely diagnosed AFRS was seen in 93% of their patients. To their surprise, with this new method they cultured at least two fungal species individually in the nasal mucus of a control group consisting of 14 healthy adults, but without detection of the presence of an IgE-mediated type I hypersensitivity reaction. In the opinion of Ponikau et al. "allergic mucin" exists independently from an IgE-mediated type I hypersensitivity reaction. For this reason, they proposed a change in the terminology; they prefer the term "eosinophilic mucin" (EM) instead of "allergic mucin", and suggest that "allergic fungal rhinosinusitis" be changed to "eosinophilic fungal rhinosinusitis". Thus, the eosinophilic granulocytes within the nasal mucus of CRS patients do not play a part in allergic reactions, but are transitory components of the nasal secretion. After destroying the hyphae, they fall apart and the major basic protein released from them exerts an extremely toxic effect on nasal mucosa [8, 11–13]. As a consequence of secondary superinfection of the nasal mucosa via an epithelial lesion, biofilm colonization and the appearance of superantigens CRS may develop. All these facts indicate the multifactorial nature of the disease.

Pant et al. [14] recently proved the absence of fungi in the EM of some CRS patients. They also demonstrated the similarity in clinicopathologic features between EM CRS subgroups, despite the occurence of positive fungal cultures and hypersensitivity against fungi. Their observations indicated that the presence of EM always reflects a more severe grade of CRS than that in its absence. Erbek et al. [15] showed that the grade of eosinophilia is more influential as regards the severity of CRS than the presence of a positive fungal culture. If the fungal theory holds true, it seems obvious that, through a reduction of the amount of antigen, or its total eradication, thereby influencing the triggers of CRS, the symptoms of the patients can be relieved. Treatment of CRS patients with intranasal lavage or a spray containing amphotericin B has so far proved controversial [11, 13, 16–20]. In the present study, we investigated whether the 1-year postoperative usage of a nasal spray containing amphotericin B developed by our Department of Pharmaceutics decreases the tendency of nasal polyposis to recur.

Patients and methods

Patients

In a double-blind, randomized, placebo-controlled study, patients received amphotericin B (A group) or placebo (B group) nasal sprays for 12 months after endoscopic polypectomy. Our aim was to determine whether any difference could be observed between the two groups in the rates of recurrence of nasal polyposis, in the symptoms, in the quality of life or in the endoscopic findings. The protocol of the study was approved by the Regional Ethics Committee of the University of Pécs. All the patients received concomitant nasal steroid sprays and were allowed to use their regular medication. The study protocol is presented in Fig. 1. Thirty-three patients with chronic rhinosinusitis with nasal polyps (CRSwNP) were recruited from among the patients presenting at our clinic for endoscopic sinus surgery between November 2005 and October 2006. Thirty patients completed the study; three patients were excluded (group A, 2; group B, 1) because of noncompliant behavior. The patient demographics are shown in Table 1.

The diagnosis for CRS was set up according to the criteria laid down by the "EAACI position paper on rhinosinusitis and nasal polyps, executive summary" [3]. The diagnosis was confirmed by the presence of symptoms that

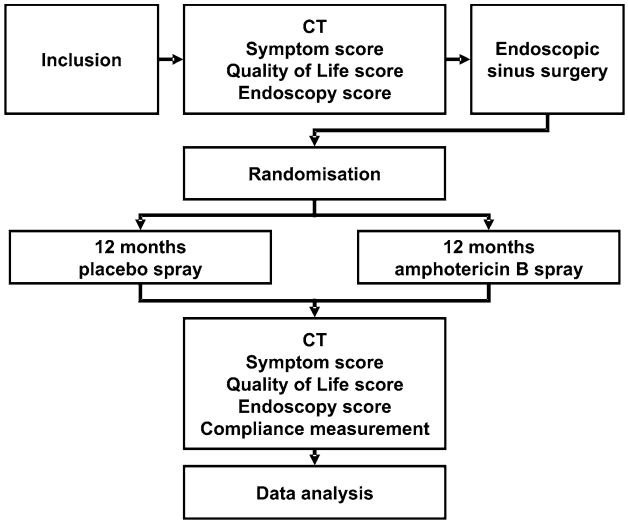

Fig. 1 Scheme of double-blind, placebo-controlled, prospective, randomized clinical study

Table 1 Demographic data on patients participating in the clinical study

	Amphotericin B group (n = 14)	Placebo group (n = 16)
Mean age (SD)	51 (10.32)	56 (9.81)
Male/female	9/5	10/6
Asthma	3	5
ASA intolerance (n)	1	1
Allergy (general) (n)	6	8
Allergy (antifungal) (n)	0	0
Smoking (n)		
Yes	3	3
No	11	13
Previous procedures		
FESS (1 op)	3	3
FESS (2 op)	0	1

has existed for 3 months and the swelling of the nasal mucosa by 5 mm in at least two sinuses according to the CT scans and endoscopic observations. Exclusion criteria are listed in Table 2.

Active and placebo sprays

The amphotericin B and placebo nasal sprays were compounded in the Pécs University Pharmacy. The two solutions were indistinguishable by color, smell or taste. The active spray made from Fungizone (Bristol-Myers Squibb, Epernon, France) contained 5 mg/ml amphotericin B, 4.1 mg/ml sodium deoxycholate and 20.2 mg/ml sodium phosphate in sterile distilled water, while the placebo was an aqueous 0.2 μg/ml acriflavin chloride solution. The sprays were manufactured under aseptic conditions and

Table 2 Criteria from exclusion from the clinical study

- Known hypersensitivity to amphotericin B
- Pregnancy or lactation
- Age <18 years
- Suspicion of allergic fungal rhinosinusitis
- Mental disease, cystic fibrosis
- Osteoporosis, chronic liver or renal disease
- Immune-compromised state (HIV, transplantation, diabetes)
- Acute upper airway infection (within a week)
- Complication of CRS (e.g., abscess)
- Acute bacterial exacerbation of CRS (acute pain, pressure feeling, high temperature, mucopurulent discharge)
- Orbital or intracranial complication of CRS
- Antibiotic and/or antihistamine therapy within 3 weeks
- Application of oral steroid within 3 weeks
- Systemic antifungal therapy within 1 week

were measured out in brown, light-rejecting glass containers with dosing spray caps. The patients received seven spray containers of 3.3 ml solutions monthly and were instructed to apply two times two doses (100 μl) daily into each nostril, i.e., the total daily dose of amphotericin B was 4 mg/day. We earlier tested the stability of the solutions at 4°; the 5 mg/ml amphotericin B solutions proved stable for 30 days.

Primary outcome measure

Modified Lund–Mackay CT score

The scoring system as modified by Juniper [21, 22] was used to evaluate the CT scans of the patients prior to endoscopic sinus surgery and also after the 12-month treatment period (Table 3).

Secondary outcome measures

1. Sinonasal assessment questionnaire (SNAQ-11): Compared with similar tools (the Sino Nasal Outcome Test 20, SNOT-20; or the General Nasal Patient Inventory,

Table 3 Modified Lund–Mackay CT evaluation score system

Scoring
0 = Not opacified
1 = Less than 1/3 opacified
2 = Between 1/3 and 2/3 opacified
3 = More than 2/3 opacified, but still air-containing
4 = Complete opacification (no air)
Maximum available score: 5 × 4×2 = 40

Each side was separately evaluated for opacification of the maxillary, anterior and posterior ethmoideal, sphenoidal and frontal sinus

GNPI) the 11-item SNAQ-11 [23] allows a more adequate evaluation of the main symptoms of CRS (e.g., nasal congestion or loss of the sense of smell), without a deep analysis of the nonspecific symptoms (such as a feeling of aural pressure) (Table 4). The questionnaires were completed by all the patients both before surgery and 1 year later, at the end of the study period.

2. Quality of life test: The questionnaires were filled out prior to surgery and after 12 months (Table 5). The patients answered the 6 questions on a 7-grade scale [24]; the maximum score was 36.
3. Endoscopic assessment: Following nasal mucus membrane congestion, all the patients were graded preoperatively and 1 year later, at the end of the therapy, according to the scoring system of Malm [25]. All gradings were performed by the same author (I.G.). The scores ranged from 0 (no polyp) to 3 (total obstruction).

Statistical analysis

For the analysis of the primary outcome measure (the CT score), the SNAQ-11 and the quality of life scores, independent sample *t* tests were used. The applicability of this was checked with the Levene test, and the normality of the variables was demonstrated with Kolgomorov–Smirnov test. The procession and evaluation of the data were performed with the SPSS 14.0 for Windows (SPSS Inc., Chicago, IL, USA). The results are expressed as medians ± interquartile ranges.

Table 4 SNAQ-11 questionnaire relating to sinus symptoms

Questions
(1) Nasal obstruction
(2) Feeling of nasal fullness, snuffling
(3) Facial pain, pressure feeling
(4) Anterior nasal discharge
(5) Posterior nasal discharge
(6) Sneezing
(7) Cough
(8) Diminished smell
(9) Headache
(10) Ear pain, pressure feeling in the ear
(11) Sleeping difficulties, daytime sleepiness
Evaluation
0 = No symptom
1 = Very mild symptom
2 = Mild symptom
3 = Moderate symptom
4 = Serious symptom
5 = Extremely serious symptom
Scores to questions 1 and 2 should be multiplied by 3, and to question 3 by 2; the maximum available score is 80

Table 5 Quality of life evaluation score system

Subjective evaluation on 7-point scale. (0: not troubled by nasal symptoms 6: extremely troubled by nasal symptoms)
Questions
(1) Regular activities at home and at work?
(2) Recreational activities?
(3) Sleep?
(4) Tiredness and/or fatigue?
(5) Thirst?
(6) Feeling irritable?
Maximum score: 36 points

Results

Fourteen of the 16 patients in group A, and 16 of the 17 patients in group B completed the study.

Modified Lund–Mackay CT score

During the evaluation of the preoperative and postoperative CT scans, we observed a high standard deviation in the scores without any relevant improvement. Though a slight improvement was seen in the placebo group (Fig. 2), the comparison of the two patient groups did not indicate a significant change ($P = 0.052$).

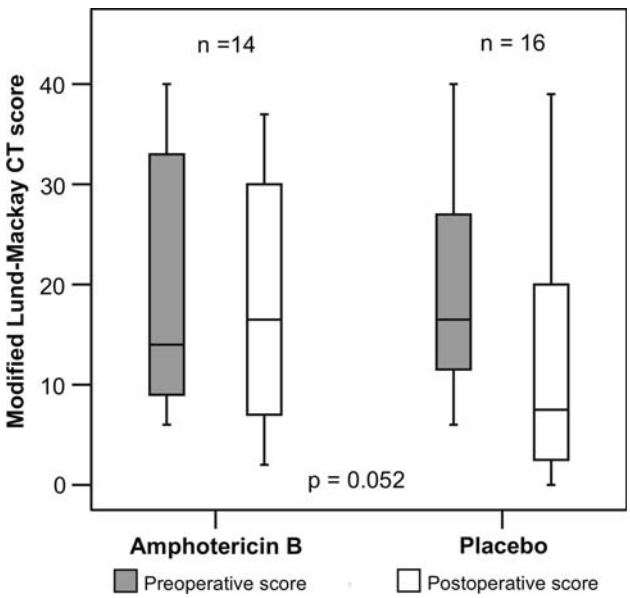

Fig. 2 Preoperative and 12-month postoperative CT scores (modified Lund–Mackay test) in the two groups of patients. Means are indicated by *horizontal lines*, the *rectangle* denotes the mean ± 25% values

Sinonasal symptom score (SNAQ-11)

A relevant improvement in the symptoms was observed in both medication groups (Fig. 3), but a statistically significant difference was not observed when the changes in the two groups were compared ($P > 0.1$).

Quality of life test

A definite improvement was observed in both groups after 12 months (Fig. 4), but the rates of improvement in the two groups did not differ statistically significantly ($P > 0.1$). Overall, the changes in the sinonasal symptoms and quality of life scores correlated with each other, but a statistically significant change was not observed in the rate of improvement between the two groups.

Endoscopic assessment

Table 6 shows the endoscopic status of the patients at the beginning and at the end of the study, based on the system of Malm. Although the study population was relatively small, it can be clearly seen that there were more patients in the placebo group whose endoscopic status did not change. On the other hand, 1, 2 and 3-level changes were observed in more cases in the amphotericin B group.

Side-effects

Headache, sleep disorders, nasal congestion, fatigue, phlegm/catarrh in the back of the throat and cough are common symptoms of CRS, and thus they cannot be considered side-effects of the therapy. Short-term nasal burning was reported by six patients in the amphotericin B group, and only one patient mentioned dryness of the nasal mucus and bleeding in the placebo group. The therapy did not have to be interrupted because of side-effects in any of the cases.

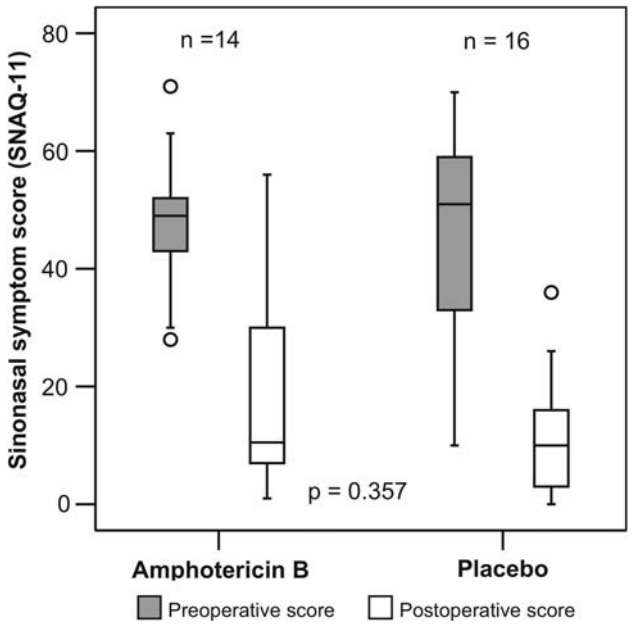

Fig. 3 Preoperative and 12-month postoperative symptomatic scores (SNAQ-11 test) in the two groups of patients

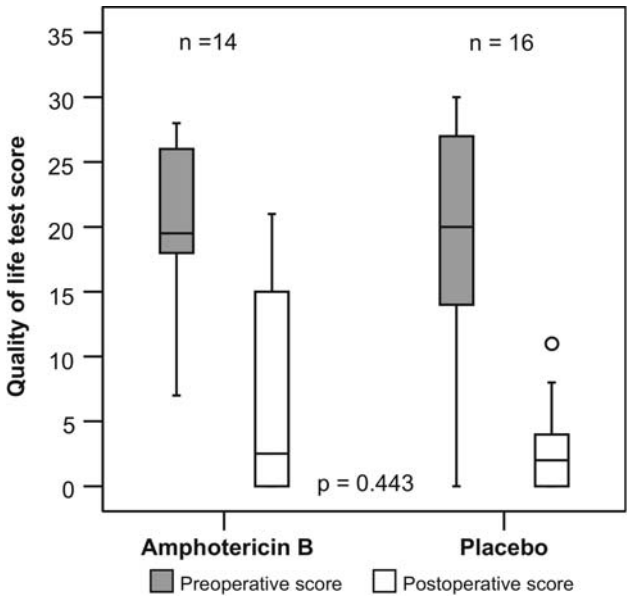

Fig. 4 Preoperative and 12-month postoperative quality of life test data in the two groups of patients

Table 6 Preoperative and 12-month postoperative endoscopic stages in the two groups of patients

Endoscopic stages (Malm [25])		Amphotericin B. group (*n*)	Placebo group (*n*)
Preop.	Postop. 12 months		
St. III	St. III	0	0
St. III	St. II	1	2
St. III	St. I	2	2
St. III	St. 0	4	2
St. II	St. II	1	1
St. II	St. I	2	2
St. II	St. 0	2	2
St. I	St. I	0	4
St. I	St. 0	1	1
Total		14	16
Change in stage		*n* (%)	*n* (%)
0		1 (8)	5 (31)
1		5 (36)	5 (31)
2		4 (28)	4 (25)
3		4 (28)	2 (13)
Total		14 (100)	16 (100)

Summary

Fungal organisms can give rise to extremely serious acute and chronic sinonasal infections in immune-compromised hosts [4, 8–10]. The fungal etiology of CRS in immune-competent patients is still a topic of considerable debate and needs further clarification [4, 5, 8, 18, 19, 26].

AFRS is a well-known, but relatively rare form of CRS, which is characterized by IgE-type immune reactions against fungi cultured from the sinuses. Collins et al. [27] reported the presence of local specific IgE to fungi in the EM of some CRS patients, but even in those cases no systemic IgE to fungi was detected. They also noted the presence of local specific IgE to fungi in all the CRS patients, with positive fungal cultures.

However, there are at least three reasons why some investigators doubt the relevance of fungi in CRS. First, the incidence of fungal colonization in patients with CRS is similar to that in healthy individuals [3, 18, 19, 26]. Second, the presence of EM is not always associated with a positive fungal culture, and moreover the group of CRS patients with EM includes subgroups based on the presence of fungi in the EM and on IgE-mediated fungal hypersensitivity that share clinical characteristics [15]. The third important fact is that bacteria have also been isolated in the sinus cavities of patients with positive fungal cultures; it therefore remains unclear whether intranasal fungal antigens exacerbate the chronic inflammatory response or are simply present coincidentally in patients with CRS [3].

There are important clinical observations too, which cannot be ignored and which point to the possible etiological role of fungi in CRS. These are as follows: (1) Gosepath et al. [28] recently successfully proved the presence of DNA fragments of *Alternaria* in the sinonasal mucous membranes of patients with CRS, concluding that the fungal antigen is transported from the EM to the small vessels of the nasal mucous membranes by the antigen presenting cells (macrophages), triggering eosinophil infiltration. (2) Shin [26] demonstrated that the levels of serum IgG to *Alternaria* and *Cladosporium* were higher in patients with CRS than in healthy individuals. (3) The presence of specific IgG antibodies correlated with increased IL-5 and IL-13 levels during exposure to *Alternaria* in vitro, but this did not hold controls [26]. 4. Ponikau et al. [13] reported that treatment for 6 months, with regular application of a nasal lavage containing amphotericin B resulted in decreased concentrations of both neurotoxin and IL-5 originating from eosinophilic cells. In contrast, the concentrations of both mediators increased in the placebo group, though the changes were not significant. The observations of other researchers on the inflammatory mediators did not confirm these results [29, 30]. If the fungal etiology is true, it would be reasonable to recommend antifungal treatment to patients with CRS in order to decrease or eliminate the fungal load and achieve a symptomatic improvement.

During the past 6 years, seven clinical studies have been published in which experience with local antifungal treatment regimes in CRS was discussed [11, 13, 16–20]. The controversial characteristics of these studies are summarized in Table 7. Only three of the papers were based on double-blind, placebo-controlled studies [13, 17, 20], and of these three studies only one was multicentric [17], making the interpretation of the results rather difficult. One of the studies excluded patients with AFRS completely [20]. In some of the studies, the number of recruited patients was extremely low [13, 18]. The treatment period ranged between 4 and 80 weeks [16, 19], and the form of drug/placebo application also varied: nasal lavage [11, 13, 17, 19], nasal spray [18, 20] or nasal inhalation [16]. The concentration and the daily amount of amphotericin B recommended also varied in these studies. In one study the nasal spray even contained sugar, which could facilitate the growths of fungi [20].

The conditions of drug storage and checking the stability of the amphotericin B solutions was completely ignored in most of these studies. Due to the fact that the patient compliance was examined in only one study [17], it is extremely difficult to draw conclusions concerning the effectiveness [11, 13, 16, 19] or ineffectiveness [17, 18, 20] of amphotericin B treatment.

In an attempt to clarify the situation, we conducted a double-blind, prospective, randomized, placebo-controlled clinical study. This differed from the previous ones in that, the active drug or placebo was administered postoperatively in the form of a nasal spray. We preferred the use of a nasal spray to nasal lavage because of the well-known, favorable effect of hypertonic saline solution on the symptoms of CRS [31, 32]. As a result of nasal lavage, the mucociliary activity improves, the degree of mucous membrane edema and the concentrations of inflammatory mediators decrease and the inspissated mucous can be cleared mechanically [33].

It is important from the aspect of compliance that application of a nasal spray is more convenient for the patient than either nasal lavage or inhalation. A significant amount of nasal lavage is frequently swallowed, questioning its therapeutic effectiveness in the depths of polyp-filled sinuses, especially if it is used preoperatively. The application of amphotericin B is favored by the facts, that the drug is not absorbed through the gastrointestinal tract, and it is highly effective (90%) against the majority of fungi cultured from the sinonasal tract. In our study, the concentration of the drug was 5 mg/ml, which is at least 1,600–5,000 times higher than the minimal antifungal concentration [34]. We considered it reasonable to apply the amphotericin B in the

Table 7 Analysis of clinical studies dealing with antifungal treatment of CRS

Authors	Ricchetti et al. [19]	Ponikau et al. [11]	Ponikau et al. [13]	Weschta et al. [20]	Helbling et al. [18]	Corradini et al. [16]	Ebbens et al. [17]
Year	2002	2002	2005	2004	2006	2006	2006
Journal	J Laryng Otol	J Allergy Clin Immunol	J Allergy Clin Immunol	J Allergy Clin Immunol	J Laryng Otol	J Investig Allergol Clin Immunol	J Allergy Clin Immunol
Type of study	Open	Open	Double-blinded, placebo-controlled	Double-blinded, placebo-controlled	Open	Open	Multicentric, double- blinded, placebo-controlled
Number of patients involved	74	51	30	78	21	89	116
Amphotericin B treatment	74	51	10	28	21	39	59
Length of treatment (weeks)	4	12	24	8	12	80	12
Application	Lavage	Lavage	Lavage	Spray	Spray	Inhalation	Lavage
Amphotericin B concentration (mg/ml)	0.1	0.1	0.25	3	10	3.33	0.1
Daily amount of Amphotericin B (mg)	8	8	20	4.8	3	0.8 and 0.5	10
Daily application (ml)	2 × 20	2 × 20	2 × 20	4 × 0.2	3 × 0.1	1 × 0.24/0.16	2 × 25
Storage mentioned	No	No	No	Refrigerator	Refrigerator	No	Refrigerator
Stability mentioned	No	No	No	No	No	No	Yes
Compliance mentioned	No	No	No	No	No	No	Yes
Final outcome	Effective	Effective	Effective	Ineffective	Ineffective	Effective	Ineffective

postoperative period, when the drug could pass easily and effectively into the EM-free sinuses. Our experience relating to the examination of the stability, storage and compliance of amphotericin B sprays, features that can seriously affect the final outcome of any clinical study [35], will be published elsewhere.

Our evaluation of the preoperative and postoperative CT scans in the amphotericin B group did not reveal an improvement in the scores; the data were scattered significantly. However, an improvement was noted in the placebo group. The comparison of the extents of change in the two groups did not indicate a significant difference ($P = 0.052$). In this respect, mention should be made of the well-known clinical experience that the symptomatic recovery following endoscopic sinus surgery does not correlate significantly with the extent of improvement seen on the CT scans [36, 37].

Our patients displayed marked improvements in the sinus complaints by the end of the 1-year course of treatment in both the groups, though without a significant difference in the extent of change in the two groups. During the evaluation of the results, it should be borne in mind that amphotericin B also exerts a direct toxic effect on the epithelial cells of nasal polyps, but does not damage the integrity of turbinate epithelial cells. Many authors explain the successful amphotericin B treatment of nasal polyposis through this effect [38]. It should be noted that no polyp tissue was present in our study population at the beginning of antifungal treatment which rules out any possible anti-polyp tissue effect of amphotericin B. During the surgical procedures, the mucus in the sinuses, potentially containing fungal antigens, was thoroughly eliminated. These two facts may have contributed to the final outcome that the two groups did not exhibit a significant difference as concerns the changes in the symptoms even after 1 year. A significant difference was not observed in the quality of life test either; the explanation of this might be similar to that described in connection with the evaluation of the sinus complaints.

The assessment of the preoperative and the 12-month postoperative findings clearly shows that 1, 2 or 3-stage changes occured more often in the amphotericin B group, while an unchanged endoscopic finding was observed more frequently in the placebo group. However, in view of the relatively small number of patients, caution is advisable in the interpretation of this finding. Nonetheless, it is a fact that the endoscopic finding did not correlate with the changes observed in either the primary or the secondary outcome measures.

The question arises as to what other causes could have led to the ineffectiveness of amphotericin B treatment in almost half of the previously published (methodologically not always correct) clinical studies (Table 7). There are a number of possibilities: (1) CRS is a multifactorial disease: immune deficiency, genetic factors, anatomic variations, atopy and environmental factors such as air pollution and smoke are predisposing factors [3]; (2) the compliance may not be satisfactory during the course of long-lasting examination protocols, which is usually not taken into consideration in clinical studies [35]; (3) differences in sensitivity of the various fungi to amphotericin B [34]; (4) differences in effectiveness of the mechanisms repairing the direct membrane damage and the consequences of oxidative stress [39]; (5) in patients with advanced CRSwNP, the amount of drug that penetrates to the bottom of sinuses filled with EM and polyps may be insufficient.

The experience gained so far indicates that the results of oral antifungal treatment are rather confusing [40, 41]. Our clinical study does not justify the need for the administration of amphotericin B nasal spray for 12 months following endoscopic nasal polypectomy. The methodological errors involved in the studies conducted so far lead us to think that it is an oversimplified explanation that fungi are "innocent bystanders". Our results and the data available draw attention to the necessity of integrated further clinical studies, with improved methodology. Treatment modalities should be applied on a case-by-case basis. Future research should focus on the selection of the CRS patients who will certainly benefit from antifungal therapy [14, 15]. Particular attention should be paid to the diagnosis and treatment of patients with aspirin intolerance among those who require revision surgery, and to the recognition of those patients who will benefit from specific immunotherapy or prolonged low-dose macrolide therapy [3].

References

1. Lanza DC, Kennedy DW (1997) Adult rhinosinusitis defined. Otolaryngol Head Neck Surg 117:S1–S7. doi:10.1016/S0194-5998(97)70001-9
2. Hedman J, Kaprio J, Poussa T, Nieminen MM (1999) Prevalence of asthma, aspirin intolerance, nasal polyposis and chronic obstructive pulmonary disease in a population-based study. Int J Epidemiol 28:717–722. doi:10.1093/ije/28.4.717
3. Fokkens W, Lund V, Mullol J et al (2007) European position paper (EPP) on rhinosinusitis and nasal polyps 2007. Rhinol Suppl 20:1–136
4. Bent JP, Kuhn FA (1994) Diagnosis of allergic fungal rhinosinusitis. Otolaryngol Head Neck Surg 111:580–588. doi:10.1016/S0194-5998(94)70525-9
5. Hirschberg A, Jókúti A, Darvas Z, Almay K, Répássy G, Falus A (2003) The pathogenesis of nasal polyposis by immunoglobulin E and interleukin-5 is completed by transforming growth factor-beta 1. Laryngoscope 113:120–124. doi:10.1097/00005537-200301000-00022
6. Sanclement JA, Webster P, Thomas J, Ramadan HH (2005) Bacterial biofilms in surgical specimens of patients with chronic rhinosinusitis. Laryngoscope 115:578–582
7. Douglas R, Bruhn M, Tan LW, Ooi E, Psaltis A, Wormald PJ (2007) Response of peripheral blood lymphocytes to fungal extracts

and staphylococcal superantigen B in chronic rhinosinusitis. Laryngoscope 117:411–414. doi:10.1097/MLG.0b013e31802c0707
8. Braun H, Buzina W, Freudenschuss K, Beham A, Stammberger H (2003) "Eosinophilic fungal rhinosinusitis": a common disorder in Europe? Laryngoscope 113:264–269. doi:10.1097/00005537-200302000-00013
9. Katzenstein A, Sale G, Greenberger P (1982) Pathologic findings in allergic aspergillus sinusitis. J Allergy Clin Immunol 72:89–93. doi:10.1016/0091-6749(83)90057-X
10. DeShazo RD, Swain RE (1995) Diagnostic criteria for allergic fungal sinusitis. J Allergy Clin Immunol 96:24–35. doi:10.1016/S0091-6749(95)70029-3
11. Ponikau JU, Sherris DA, Kita H, Kern EB (2002) Intranasal antifungal treatment in 51 patients with chronic rhinosinusitis. Allergy Clin Immun 110:862–866. doi:10.1067/mai.2002.130051
12. Ponikau JU, Sherris DA, Kephart EB, Humburger HA, Frigas E, Gaffey TA et al (1999) The diagnosis and incidence of allergic fungal sinusitis. Mayo Clin Proc 28:1145–1152
13. Ponikau JU, Sherris JA, Weaver A, Kita H (2005) Treatment of chronic rhinosinusitis with intranasal amphotericin B: a randomised, placebo-controlled, double-blind pilot trial. J Allergy Clin Immunol 115:125–131. doi:10.1016/j.jaci.2004.09.037
14. Pant H, Kette FE, Smiths WB, Macardle PJ, Wormald PJ (2006) Eosinophil mucus chronic rhinosinusitis: clinical subgroups or a homogenous pathogenetic entity? Laryngoscope 116:1241–1247. doi:10.1097/01.mlg.0000224547.14519.ad
15. Erbek SS, Serefhanoglu K, Erbek S, Demirbilek M, Can F, Tarhan E et al (2008) Clinical subgroups and antifungal susceptibilities in fungal-culture positive patients with chronic rhinosinusitis. Eur Arch Otorhinolaryngol 265(7):775–780
16. Corradini C, Del Ninno M, Buonomo A, Nucera E, Paludetti G, Alonzi C et al (2006) (Amphotericin B and lysine acetylsalicylate in the combined treatment of nasal polyposis associated with mycotic infection. J Investig Allergol Clin Immunol 16:188–193
17. Ebbens FA, Scadding GK, Badia L, Hellings PW, Jorissen M, Mullol J, Cardesin A, Bachert C, van Zele TP, Dijkgrawf MG, Lund V, Fokkens WJ (2006) Amphotericin B nasal lavages: not a solution for patient with chronic rhinosinusitis. J Allergy Clin Immunol 118:1149–1156. doi:10.1016/j.jaci.2006.07.058
18. Helbling A, Baumann A, Hänni C, Caversaccio M (2006) Amphotericin B nasal spray has no effect on nasal polyps. J Laryngol Otol 120:1023–1025. doi:10.1017/S0022215106002167
19. Ricchetti A, Landis BN, Maffioli A, Giger R, Zeng C, Lacroix JS (2002) Effect of anti-fungal nasal lavage with amphotericin B on nasal polyposis. J Laryngol Otol 116:261–263
20. Weschta M, Rimek D, Formanek M, Polzehl D, Podbielski A, Reichelmann H (2004) Topical antifungal treatment of chronic rhinosinusitis with nasal polyps: a randomized, double-blind clinical trial. J Allergy Clin Immunol 113:1122–1128. doi:10.1016/j.jaci.2004.03.038
21. Juniper EF, Thompson AK, Ferrie PJ, Roberts JN (2000) Development and validation of the mini Rhinoconjunctivitis Quality of Life Questionnaire. Clin Exp Allergy 30:132–140. doi:10.1046/j.1365-2222.2000.00668.x
22. Lund VJ, Mackay IS (1993) Staging in rhinosinusitis. Rhinology 31:183–184
23. Fahmy FF, McCombe A, Mckiernan DC (2002) Sino-nasal outcome questionnaire, a patient focussed, rhinosinusitis specific outcome measure. Rhinology 40:195–197
24. Basu S, Georgalas C, Kumar BN, Desai S (2005) Correlation between symptoms and radiological findings in patients with chronic rhinosinusitis: an evaluation study using the Sinonasal Assessment Questionnaire and Lund–Mackay grading system. Eur Arch Otorhinolaryngol 262:751–754. doi:10.1007/s00405-004-0891-0
25. Malm L (1997) Assessment and staging of nasal polyposis. Acta Otolaryngol 117:465–467. doi:10.3109/00016489709113422
26. Shin S, Ponikau JU, Sherris DA, Congdon D, Frigas E, Homburger HA et al (2004) Chronic rhinosinusitis: an enhanced immune response to ubiquitous airborne fungi. J Allergy Clin Immunol 114:1369–1375. doi:10.1016/j.jaci.2004.08.012
27. Collins M, Nair S, Smiths W, Kette F, Gillis D, Wormald PJ (2004) Role of local immunoglobulin E production in the pathophysiology of non-invasive fungal sinusitis. Laryngoscope 114:1242–1246. doi:10.1097/00005537-200407000-00019
28. Gosepath J, Brieger J, Vlachtsis K, Mann WJ (2004) Fungal DNA is present in tissue specimens of patients with chronic rhinosinusitis. Am J Rhinol 18:9–13
29. Seung-Heon S, Mi-Kiung Y (2004) Effect of topical amphotericin B on expression of cytokines in nasal polyps. Acta Otolaryngol 124:1174–1177. doi:10.1080/00016480410017404
30. Weschta M, Rimek D, Formanek M, Podbielski A, Riechelmann H (2006) Effect of nasal antifungal therapy on nasal cell activation markers in chronic rhinosinusitis. Arch Otolaryngol Head Neck Surg 132:743–747. doi:10.1001/archotol.132.7.743
31. Heatley DG, McConnell KE, Kille TL, Leverson GE (2001) Nasal irrigation for the alleviation of sinonasal symptoms. Otolaryngol Head Neck Surg 125:44–48. doi:10.1067/mhn.2001.115909
32. Wormald PJ, Cain T, Oates L, Hawke L, Wong I (2004) A comparative study of three methods of nasal irrigation. Laryngoscope 114:2224–2227. doi:10.1097/01.mlg.0000149463.95950.c5
33. Tomooka LT, Murphy C, Davidson TM (2000) Clinical study and literature review of nasal irrigation. Laryngoscope 110:1189–1193. doi:10.1097/00005537-200007000-00023
34. Wildfeuer A, Seidl HP, Paule I, Haberreiter A (1997) In vitro evaluation of variconazole against clinical isolates of yeasts, moulds and dermatophytes in comparison with itraconazole, ketoconazole, amphotericin B and griseofulvin. Mycoses 41:309–319
35. Fittler A, Mayer A, Kocsis B, Gerlinger I, Fónay F, Botz L (2007) Stability testing of amphotericin B nasal spray solutions with chemical and biological analysis. Acta Pharm Hung 77:159–164
36. Bhattacharyya T, Piccirillo J, Wippold FJ (1997) Relationship between patient based descriptions of sinusitis and paranasal sinus computed tomographic findings. Arch Otolaryngol Head Neck Surg 123:1189–1192
37. Kenny TJ, Duncavage J, Bracikowski J, Yildirim A, Murray JJ, Tanner SB (2001) Prospective analysis of sinus symptoms and correlation with paranasal computed tomography scan. Otolaryngol Head Neck Surg 125:40–43. doi:10.1067/mhn.2001.116779
38. Jornot L, Rochat T, Caruso A, Lacroix J (2005) Effects of amphotericin B on ion transport proteins in airway epithelial cells. J Cell Physiol 204:859–870. doi:10.1002/jcp.20347
39. Sokol-Anderson ML, Brajtburg J, Medoff G (1986) Amphotericin B-induced oxidative damage and killing of *Candida albicans*. J Infect Dis 154:76–83
40. Kennedy DW, Kuhn FA, Hamilos DI (2005) Treatment of chronic rhinosinusitis with high-dose oral terbinafine: a double blind placebo-controlled study. Laryngoscope 115:1793–1799. doi:10.1097/01.mlg.0000175683.81260.26
41. Rains BM, Mineck CW (2003) Treatment of allergic fungal sinusitis with high-dose itraconazole. Am J Rhinol 17:1–8