

Amphotericin B and Lysine Acetylsalicylate in the Combined Treatment of Nasal Polyposis Associated With Mycotic Infection

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Abstract. *Background:* Fungal infection may be secondary to nasal polyposis or represent a real etiopathogenic factor in the infection itself.

Objective: The aim of this study was to evaluate the effectiveness of a combined treatment with lysine acetylsalicylate (LAS) and amphotericin B in preventing recurrence in patients with nasal polyposis with accompanying mycotic infection in comparison with a control group with nasal polyposis and fungal infection who did not receive antifungal therapy.

Patients and Methods: A total of 115 patients with nasal polyposis were randomly assigned to 4 different groups and treated as follows: 1) group A, 25 patients were first surgically treated and then treated with LAS; 2) group B, 25 patients received 40 mg of triamcinolone retard intramuscularly 3 times every 10 days (total dose 120 mg) and then they were treated with LAS; 3) group C, 16 patients were surgically treated and then treated with LAS and amphotericin B; 4) group D: 23 patients were treated with a medical polypectomy and steroids (as in the group B) and then with LAS and amphotericin B.

Results: We found no significant differences between groups C and D, groups C and A, or groups B and D. However, the recurrence of nasal polyps in the groups treated with amphotericin B plus LAS (C and D) was significantly lower ($P = .018$) than in the 2 groups treated only with LAS (A and B).

Conclusion: Our results indicate that long term topical treatment with LAS and amphotericin B may be clinically effective in the treatment of patients with nasal polyposis associated with fungal infection.

Key words: Nasal polyposis. Fungal infection. Amphotericin B. Lysine acetylsalicylate.

Resumen. *Antecedentes:* Según los datos de la literatura, la infección fúngica podría ser secundaria a la poliposis nasal o representar un factor etiopatogénico real de ésta.

Objetivo: El objetivo de este estudio fue evaluar la eficacia de un tratamiento combinado con acetilsalicilato de lisina (ASL) y amfotericina B en la prevención de recidivas en pacientes con poliposis nasal acompañada de infección micótica en comparación con un grupo de control con poliposis nasal e infección fúngica no sometido a tratamiento antifúngico.

Pacientes y Métodos: Se incluyeron 115 pacientes con poliposis nasal y que acudieron a los departamentos de Otorrinolaringología y Alergia de la Universidad Católica de Roma. Los pacientes sin infección fúngica fueron excluidos del estudio. El resto de pacientes fueron asignados aleatoriamente a cuatro grupos distintos: 1) grupo A: 25 pacientes fueron sometidos primero a cirugía y luego tratados con ASL; 2) grupo B: 25 pacientes recibieron 40 mg de triamcinolona retard por vía intramuscular 3 veces cada 10 días (dosis total de 120 mg) y luego fueron tratados con ASL; 3) grupo C: 16 pacientes fueron sometidos primero a cirugía y luego tratados con ASL y amfotericina B; 4) grupo D: 23 pacientes fueron sometidos a polipectomía médica con esteroides (igual que en el grupo B anterior) y luego tratados con ASL y amfotericina B.

Resultados: No se observó significación estadística al comparar el grupo C con el grupo D, el grupo C con el grupo A y el grupo B con el grupo D, mientras que por el contrario las recidivas de pólipos nasales en los grupos tratados con amfotericina B + ASL (C + D) fueron inferiores y estadísticamente significativas ($P = 0,018$) en comparación con los dos grupos tratados sólo con ASL (A + B).

Conclusión: Estos resultados indican que el tratamiento tópico a largo plazo con ASL y amfotericina B puede ser clínicamente eficaz en el tratamiento de pacientes con poliposis nasal asociada a infección fúngica.

Palabras clave: Poliposis nasal. Infección fúngica. Amfotericina B. Acetilsalicilato de lisina.

Introduction

Nasal polyps are nonneoplastic swellings of edematous tissue that usually arise from the ethmoid sinuses, pass through the middle meatus and prolapse into the nasal cavity. Recurrence comes within a period ranging from 6 months to 3 years [1, 2]. Conditions that are particularly associated with nasal polyposis are bronchial asthma (in 20%-40% of cases) [3-5], aspirin hypersensitivity (in 35% of cases) [6, 7], cystic fibrosis (in 3%-48% of cases) [8, 9], as well as other respiratory diseases.

The last decade has seen more interest taken in the association between nasal polyps and fungal infection of the nose and sinuses. This is not a new concept since it was first suggested in 1981 by Millar et al [10], who identified an *Aspergillus* species as a cause of sinusitis. Currently, 3 different types of fungal sinusitis are recognized [11]: a) chronic noninvasive disease, b) invasive disease that may have either an acute or chronic presentation depending on host factors, and c) allergic fungal sinusitis (AFS), the most recently described form. *Aspergillus* and other dematiaceous fungi (eg, *Alternaria*, *Curvularia*, *Rhizopus*, and *Fusarium* species) are the primary etiologic agents [12-14]. Some authors think that patients affected by allergic fungal sinusitis usually have a long-standing history of atopy, with peripheral blood eosinophilia and raised IgE levels; the unique feature of this type of sinusitis however is the presence of the so called allergic mucin, which contains eosinophils, Charcot-Leyden crystals and fungal hyphae [15, 16]. The pathogenesis of AFS is incompletely understood, and there is controversy as to what role type I and type III hypersensitivity mechanisms play. It is widely accepted that fungi become entrapped in the sinuses of allergic patients with ostiomeatal complex obstruction, extremely thick mucus and/or mucociliary clearance disorders. The ensuing immune response exacerbates the disease. It is also likely that simple saprophytic fungi play a role. When Corradini et al [17] studied the allergic profile of 24 patients affected by chronic rhinosinusitis with fungi in nasal secretions, they found that the presence of fungi in the nasal secretions of patients affected by nasal polyposis did not seem to correlate with an IgE-mediated response to the isolated fungus. Fungi may be the primary infecting agents or they may be secondary opportunistic invaders in an underlying chronic infection of the sinuses. In the latter case the secondary infection will worsen the clinical picture.

As regards the medical treatment of nasal polyposis, it has been demonstrated that lysine acetylsalicylate (LAS) has a dose-dependent antiproliferative effect on fibroblasts

of both nasal polyps and normal skin [18] in vitro. The effectiveness of LAS treatment in preventing the relapse of nasal polyposis both in patients treated by surgical polypectomy or with intramuscular corticosteroids has been studied in comparison with matched controls (patients who underwent simple polypectomy with no further treatment) [19-21]. We compared 76 patients affected by nasal polyposis treated with surgery and LAS to 191 patients who had been treated only with surgery [20]. Patients treated with surgery and LAS showed a significant reduction in recurrence of nasal polyposis. Conversely, when we compared 49 patients treated with steroids and LAS to the 191 patients treated with surgery, we also found a reduction of recurrence of nasal polyposis for the LAS-treated patients. The efficacy of an intranasal fungal treatment with amphotericin B in patients with chronic rhinosinusitis has also been reported [22, 23].

The aim of this study was to evaluate the effectiveness of a combined treatment with LAS and amphotericin B in preventing recurrence in patients affected by nasal polyposis with concomitant mycotic infection.

Materials and Methods

One hundred fifteen patients with nasal polyposis who were treated at the ear-nose-throat and allergy departments of Catholic University in Rome were recruited. The Ethics Committee of the Catholic University approved the study and an informed consent was obtained from all patients.

All 115 patients underwent a complete allergy and ear, nose and throat evaluation: a) medical history and physical examination; b) skin prick tests (SPT) with the most common respiratory allergens (Merck SpA, Milan, Italy) including *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Phleum pratense*, *Parietaria officinalis*, *Olea europea*, cat and dog dander as well as with the following fungi (Merck SpA): *Aspergillus niger*, *Alternaria tenuis*, *Botrytis cinerea*, *Candida albicans*, *Cladosporium herbarum*, *Chaetomium olivaceum*, *Epicoccum purpurascens*, *Helminthosporium halodes*, *Mucor mucedo*, *Penicillium notatum*, *Rhizopus nigricans* and *Stemphylium botryosum*; c) measurement of specific IgE in sera (UniCAP, Pharmacia, Uppsala, Sweden) to the following fungi: *Aspergillus fumigatus*, *A tenuis*, *B cinerea*, *C albicans*, *C herbarum*, *E purpurascens*, *H halodes*, *Mucor racemosus*, *P notatum*, *R nigricans* and *S botryosum*; and d) a nasal lavage. Using the Ponikau technique [24], 2 puffs of phenylephrine hydrochloride 1% were sprayed into each nostril to induce vasoconstriction. The spray also increased the nasal lumen and consequently the amount of secretion collected. After

Table. Fungi Isolated in 89 Patients With Nasal Polyposis and Fungal Infection.

Fungi Isolated	Cases
<i>Alternaria tenuis</i>	24
<i>Candida albicans</i>	24
<i>Aspergillus niger</i>	18
<i>Penicillium notatum</i>	10
<i>Cladosporium herbarum</i>	8
<i>Rhizopus nigricans</i>	2
<i>P Notatum, A niger</i>	3
Total	89

3 minutes, each nostril was flushed with 20 mL of sterile saline, using a sterile syringe with a sterile curved blunt needle. Patients took a deep breath before injection of saline and then forcefully exhaled through the nose during flushing. The return was collected in a sterile pan. The fluid was then placed in centrifuge tubes and the specimens were processed under a laminar flow hood to prevent contamination. The resultant sediment was inoculated on a Sabouraud Dextrose agar slant, with or without antibiotics, on a brain-heart infusion agar slant, and on a Bacto agar BCG plate for the development of yeasts. The slants were incubated at 30°C for a period of 30 days and the plates at 37°C for a week.

Patients without fungal infection were excluded from the study. The remaining ones were randomly assigned to 4 different groups as follows:

– Group A: 25 patients were first surgically treated with an endoscopic transnasal ethmoidectomy. One month after surgery they underwent a nasal provocation test with LAS, followed by a topical endonasal treatment with LAS.

– Group B: 25 patients received 40 mg of triamcinolone retard intramuscularly 3 times every 10 days (total dose 120 mg). Triamcinolone treatment achieves a decrease in polyp volume and facilitates penetration of LAS into the nasal and sinus cavities. The patients then underwent a nasal provocation test with LAS followed by a topical endonasal treatment with LAS.

– Group C: 16 patients were surgically treated with an endoscopic transnasal ethmoidectomy. One month after surgery they underwent a nasal provocation test with LAS [25] and then started a double topical endonasal inhalation treatment with LAS (4 mg a day 6 times a week) [19, 20] and amphotericin B. The treatment with amphotericin B (50 mg × 15 mL of 5% glucose solution) was started with an initial dose of 0.24 mL/day (equal to 0.8 mg of amphotericin B) 6 times/week for 1 month followed by 0.16 mL/day (equal to 0.5 mg of amphotericin B) 6 times/week as the maintenance dose.

– Group D: 23 patients were treated with a medical polypectomy with steroids (as in group B above); after the nasal provocation test with LAS, this last drug and amphotericin B were administered by inhalation according to the protocol described above.

All treated patients were followed-up for 20 months with regular endoscopic and imaging examinations.

A Kaplan-Meier curve and log rank test were used to evaluate and compare the effectiveness of the treatment with LAS (groups A and B) vs treatment with LAS and amphotericin B (groups C and D). Analyses were performed using the SPSS statistical software package (release 13.0 for Windows, SPSS, Chicago, USA).

Prevalence of sensitization to common aeroallergens was evaluated by means of the Pearson χ^2 and the Fisher exact tests.

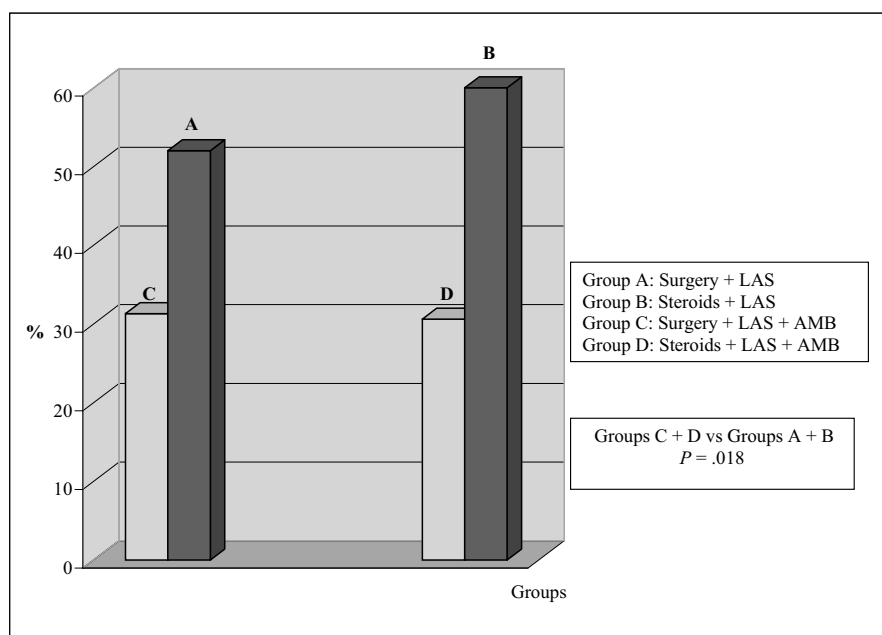


Figure 1. Incidence of recurrence of nasal polyposis in patients with nasal polyposis and fungal infection treated with lysine acetylsalicylate (LAS) vs LAS plus amphotericin B (AMB).

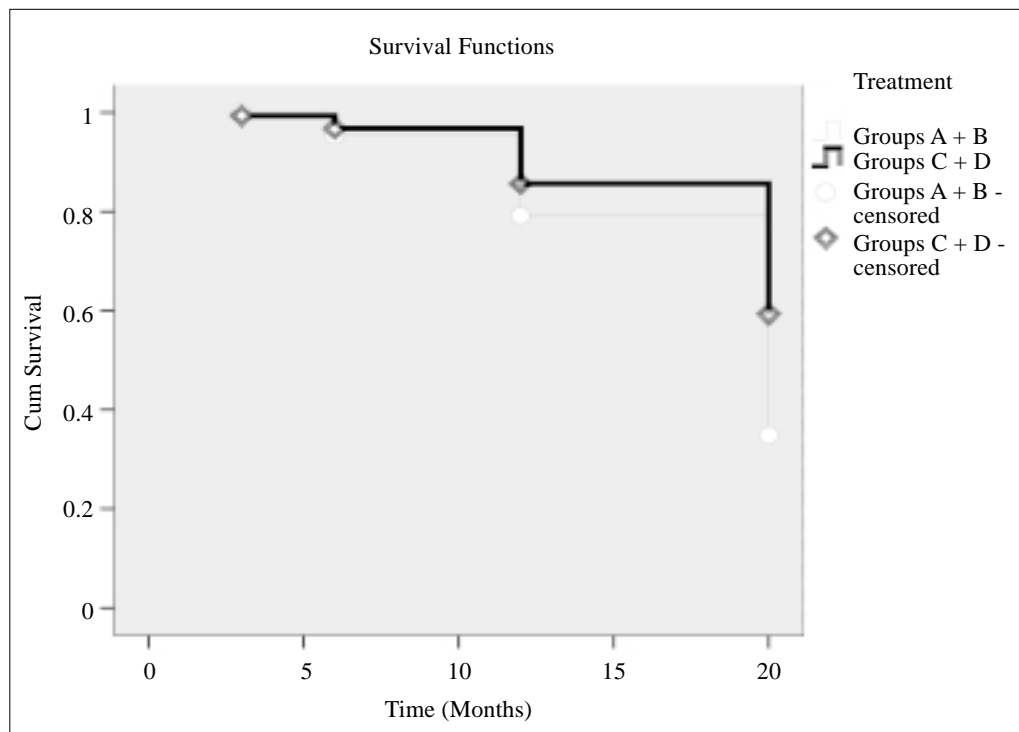


Figure 2. Kaplan-Meier curves showing incidence of relapse in patients affected by nasal polyposis and mycotic infection treated with lysine acetylsalicylate (LAS) vs patients treated with LAS plus amphotericin B. Cum indicates cumulative.

Results

In 89 patients (77%) we found evidence of fungal infection, with no signs of allergic fungal sinusitis. In this group 15 (17%) had aspirin sensitivity (mainly with asthmatic symptoms), 18 subjects (20%) had the complete aspirin triad syndrome, and 56 (63%) had only nasal polyposis with fungal infection. The types of fungi isolated are shown in the Table. Twenty-six patients with nasal polyposis but without fungal infection were excluded from the study.

Fifty patients underwent medical or surgical polypectomy followed by topical endonasal therapy with LAS without amphotericin B (Figure 1). In group A, 12 (48%) out of the 25 patients treated with surgery and LAS were recurrence-free at 20 months, while 13 (52%) showed evidence of recurrence. In group B, 10 (40%) out of the 25 patients treated with steroids and LAS showed no progression of the nasal polyposis, while in 15 (60%) the disease worsened. In these groups SPT for seasonal and perennial allergens were positive in just 4 patients (8%); 1 patient was positive to *P pratense*, 1 to *D pteronyssinus*, and 2 to *P pratense*, *D pteronyssinus*, and *O europeae*. SPT and tests for specific IgE to fungi were negative in all patients.

Thirty-nine patients underwent medical or surgical polypectomy followed by topical endonasal therapy with LAS and amphotericin B (Figure 1). In group C, 11 (68.7%) out of 16 patients treated with surgery, LAS, and amphotericin B were recurrence-free at 20 months. In this group the nasal lavage showed fungal positivity only in 4 patients (36.4%). Five patients out of the 16 (31.3%) showed evidence of recurrence and 4 patients had positive

nasal lavage for fungi. In group D, 16 (69.6%) out of the 23 medically treated patients (triamcinolone, LAS, amphotericin B) showed no recurrence after 20 months. In this group the nasal lavage showed a fungal positivity only in 3 patients (18.7%). In 7 of the group D patients (30.4%) the disease worsened and surgical removal had to be carried out; 4 patients (57.1%) had a positive nasal lavage for fungi. In these groups, SPT for seasonal and perennial allergens were positive in 5 patients (12.8%); 2 were positive to *D pteronyssinus*, 2 to *P pratense* and *O europeae* and 1 to *P officinalis*. SPT and tests for specific IgE to fungi were negative in all patients.

There was no difference in the prevalence of sensitization to allergens among patients who received amphotericin B and the ones who did not ($\chi^2 = 2.00$; $P = .15$). We did not find any statistical significance in therapeutic effectiveness when comparing groups C and D, groups C and A, or groups B and D. However, the rate of recurrence of nasal polyps in the groups treated with amphotericin B plus LAS (C and D) was significantly lower ($\chi^2 = 5.617$; $P = .018$) when compared to the 2 groups treated only with LAS (A and B) (Figures 1 and 2).

LAS and amphotericin B treatment was well tolerated by all patients and no adverse reactions were observed.

Discussion

Intranasal amphotericin B has been used for the treatment of chronic rhinosinusitis and mycotic infection, but with conflicting results. Ponikau et al [23, 24] showed that intranasal amphotericin B reduces inflammatory mucosal thickening found on both computed tomography

scans and by nasal endoscopy and decreases the levels of intranasal markers for eosinophilic inflammation in patients with chronic rhinosinusitis and mycotic infection. Weschta et al [26] found that topical amphotericin B is ineffective and worsens symptoms of patients with chronic rhinosinusitis, mycotic infection, and nasal polyps. Those authors also doubted that fungal elements had a role as essential causative agents of chronic rhinosinusitis.

We have already demonstrated that LAS has an antiproliferative effect on fibroblasts of nasal polyps [18] and that it is useful in reducing recurrence [19, 20]; moreover, we have provided evidence that IgE-mediated allergy to molds is not involved in the pathogenesis of allergic fungal sinusitis [17]. However, fungal infection could worsen the course of nasal polyposis since it could be responsible for a chronic inflammatory stimulus that induces the production of cytokines and drives eosinophilic inflammation. Thus, on the basis of our previous experience regarding the treatment of nasal polyposis with LAS, we decided to investigate the efficacy of topical amphotericin B and LAS in the treatment of patients with nasal polyps and mycotic infection.

Our results indicate that long term topical treatment with LAS and amphotericin B may be clinically effective in the treatment of patients with nasal polyposis associated with fungal infection. This finding underlines the importance of searching for superimposed fungal infection by sinus and nasal lavage microbiology prior to scheduling surgery. Such microbiological investigation is preferable to histology because, even if hematoxylin and eosin and periodic acid-Schiff staining techniques are used, fungal infection may be missed. If there is fungal superinfection in nasal polyposis then the condition is unlikely to resolve unless there is postoperative treatment for the superadded fungal invasion. This association is more frequent than expected, found with a prevalence of 77% in our study and up to 96% of all cases of nasal polyposis for some authors [24].

Since the prevalence of allergy was similar in patients who received amphotericin B and the ones who did not, it does not seem likely that sensitivity to common aeroallergens influences recurrence of nasal polyps.

Our results also confirm our previous study in which we considered the presence of fungi as a probable secondary cofactor in nasal polyposis, acting by way of enhancing the inflammatory response of the nasal polyps and thus worsening the clinical signs and symptoms [17]. This could explain the fact that the rate of relapse after 20 months' follow-up was lower in patients with nasal polyposis without fungal infection treated only with LAS (35%) [20] than in patients in the present study with nasal polyposis associated with a fungal infection (52%). This evidence is probably due to a worse clinical course of nasal polyposis when complicated by fungal infection.

Many fungi may be simple saprophytes of the nasal-sinus complex without primarily causing nasal polyps, but some fungi may potentiate recurrence of existing polyps.

Long-term topical treatment with LAS and amphotericin B is almost free of side effects and it may

therefore be used in subjects known to be at risk, such as those suffering from the aspirin triad.

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