Azithromycin for the treatment of eosinophilic nasal polyposis: Clinical and histologic analysis

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ABSTRACT

Introduction: *Macrolides used as immunomodulators are a promising tool for chronic inflammatory airway diseases. Eosinophilic nasal polyposis (ENP) is still considered a disease that is difficult to control with the currently standardized treatments.*

Objectives: *To evaluate prolonged treatment with low-dose azithromycin for ENP based on clinical and histopathologic variables.*

Methods: *The present investigation was a self-paired case study of 33 patients with ENP. A comparison was performed between patients before and after treatment with azithromycin for 8 weeks. The patients were subjected to clinical examinations, staging (three-dimensional imaging by endoscopy), application of the questionnaire, and biopsy of nasal polyps at the beginning and at the end of the treatment.*

Results: *The treatment yielded a clinical improvement regarding the two variables studied: polyposis staging (69.7%) and questionnaire (57.6%). We did not find significant differences in the inflammatory pattern and in the percentage or absolute number of eosinophils per field between samples obtained before and after the treatment ($p > 0.05$). There was no difference between the answers obtained from groups with and without asthma and/or aspirin intolerance ($p > 0.3$). The patients with advanced initial staging exhibited lower subjective improvement index and staging reduction ($p = 0.031$ and $p = 0.012$, respectively).*

Conclusion: *Based on this study, azithromycin may be considered as another therapeutic option for ENP. However, further studies are necessary to define the real mechanism of action involved.*

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The immunomodulatory effect of macrolides, widely published after 1987 with the study by Kudoh *et al.*,[1] has been used to control several chronic inflammatory airway diseases.[2] In some pulmonary diseases, such as cystic fibrosis and diffuse panbronchiolitis, the use of macrolides is well established and has exhibited satisfactory symptom control.[3,4] In sinonasal disease is still needed more study.

Although several studies have been conducted, eosinophilic nasal polyposis (ENP) is still considered a disease that is difficult to control and is an important candidate for the study of alternative therapies.[4–6] Most of the time, standardized clinical treatments yield a limited and/or temporary improvement of symptoms. In addition, there is a risk of adverse effects with their prolonged use, as is the case for oral corticosteroid therapy.[5] Nasal endoscopy surgery is an alternative when drugs fail. However, even broad approaches by expert surgeons are unable to guarantee a cure or symptomatic improvement for long periods.[4] The recurrence of ENP remains high and may reach 50%.[7] Among macrolides, azithromycin is the drug with the greatest intracellular permanence time,[8] which makes dosing easier and minimizes adverse effects.

The present study aimed to evaluate the action of low-dose azithromycin used for a prolonged period in patients with ENP, based on polyposis staging and on the quality-of-life questionnaire, the 22-item Sino-Nasal Outcome Test (SNOT-22). Another objective was to characterize the inflammatory behavior and the percentage of eosinophils, before and after the treatment, found in biopsy specimens of eosinophilic nasal polyps of treated patients, to correlate the possible clinical improvement in a given patient with changes in the inflammatory infiltrate and/or in the percentage of eosinophils from biopsy specimens of the eosinophilic nasal polyps.

METHOD

The present study was subjected to the evaluation of the ethics and research committee of the Federal University of Minas Gerais (Universidade Federal de Minas Gerais [UFMG]) (approval opinion no. 234.835).

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The sample was composed of 41 patients with ENP, ages 18 to 70 years, referred by the Unified Health System (Sistema Único de Saúde) to receive a surgical treatment in the Teaching Hospital of the Federal University of Minas Gerais (Hospital das Clínicas UFMG [HC-UFMG]). The study was conducted at the São Geraldo Hospital (Hospital São Geraldo), annex to the Teaching Hospital, the School of Medicine of the Federal University of Minas Gerais. The calculated sample size was 32 patients. A difference higher than 14 units in the evaluation of the SNOT-22 questionnaire before and after an intervention was considered significant, based on the formula $n = \sigma^2(z_{\alpha/2} + z_{\beta})/\epsilon^2$.[9]

Patients who met the following criteria were selected:

- Inclusion criteria: Patients with ENP with a percentage of eosinophils of ≥20% in the polyp biopsy specimen who did not exhibit evidence of active nasal infection (*e.g.,* purulent secretion in the nasal cavity) in the clinical and endoscopic examination; patients who had already been subjected to a standard clinical treatment (oral and topical corticosteroids) with no satisfactory improvement and formal recommendation for endoscopic nasal surgery; and patients ages between 18 and 70 years.
- Exclusion criteria: Patients with noneosinophilic types of polyposis, *e.g.,* cystic fibrosis, Kartagener syndrome, antrochoanal polyp, and/or ENP with active infection; patients who had used corticosteroids or antihistamines within the 30 days preceding the beginning of the study; patients who had used any antibiotics for a short period within the 30 days preceding the beginning of the study or during the study; patients with established cardiovascular and/or hepatic disease; and patients with changes in their electrocardiograms (*e.g.,* prolonged QT interval).

General Design

The present investigation was a self-paired case study of patients with ENP. A comparison was performed between patients before and after treatment with azithromycin. The ENP diagnosis was based on the clinical history, nasal endoscopy, computed tomography, and biopsy of the nasal polyp according to criteria from the European Position Paper on Rhinosinusitis and Nasal Polyps 2012.[10] The research began with a complete otolaryngologic evaluation, ENP staging,[11] and biopsy of the polyps. After anesthesia of the nasal cavity with cotton soaked in 2% neotutocaine, poly with 5 × 2 mm was removed by using EXPLORENT (Olympus America Inc., Center Valley, PA) forceps, avoiding maceration of the tissue. The small size of the removed fragment did not change the assessed staging. Complementary tests were requested, including an electrocardiogram, complete blood cell count, and hepatic function tests. The hepatic function tests were requested because azithromycin is metabolized in the liver.

Patients were informed about the study and, after agreeing to participate, were asked to sign an informed consent form after orientation and joint reading. The patients were also assisted by the researcher (I.S.O.) when completing the SNOT-22 questionnaire.[9] Subsequently, AZI (azithromycin dihydrate, 500-mg coated tablets; EMS S/A, Hortolândia, São Paulo, Brazil) was prescribed, orally, in the dosage of 1 tablet (500 mg) three times a week (Monday, Wednesday, and Friday)[12–14] for 8 weeks.[15–17] The medication was donated to the Rhinology Outpatient Clinic, HC-UFMG, and the 24 tablets required for the complete treatment were provided to the patients. In the ninth week, the patients returned to the outpatient clinic for a new clinical and endoscopic evaluation and staging; a new biopsy of the nasal polyp was performed, and a new SNOT-22 questionnaire was completed. At that point, the patients were also asked about adverse effects and appropriate use of the medication and regarding possible delays or omissions of doses. All the patients reported the complete and correct use of the medication provided.

Variables Analyzed

Staging. In the literature, several methods of ENP staging are described, and there is not a universal consensus on the method. The staging method chosen in the present study has been used in the Otolaryngology Service, HC-UFMG, for several years. The method consists of three-dimensional staging that has the advantage of identifying the location of the polyps in the three spatial planes and that classifies polyps that are in regions other than the middle meatus (Table 1).[11] The method is based only on the nasal endoscopy (nasofibroscopy). Each nasal cavity is staged separately.

Quality-of-life questionnaire. The SNOT-22 questionnaire was translated, validated, and adapted to Portuguese in 2011.[9] The questionnaire consists of 22 questions and/or symptoms that can be scored by the patients from 0 (no problem) to 5 (worst possible problem). Patients must answer the questions based on symptoms from the two previous weeks. The normality limit for the Brazilian SNOT-22 is 10 points, and a variation of >14 points among the SNOT-22 indexes of the same patient is considered significant.[9] The patients completed the SNOT-22 questionnaire before the beginning of the treatment and when they returned after 8 weeks of treatment.

Table 1 **Three-dimensional staging***

Staging	Characteristics
Horizontal	
H0	No polyps
H1	Polyps restricted to the middle meatus
H2	Polyps expand beyond the middle meatus, without touching the nasal septum
HT	Polyps expand beyond the middle meatus and touch the septum
Vertical	
V0	No polyps
V1	Polyps in the middle meatus only
VI	Polyps extending inferiorly to the middle meatus, going beyond the upper border of the inferior turbinate
VS	Polyps extending superiorly to the middle meatus, between the septum and the middle turbinate
VT	Polyps occupying the entire vertical aspect of the nasal cavity
Anteroposterior	
P0	No polyps
P1	Polyps in the middle meatus only
PA	Polyps extending anteriorly to the middle meatus, reaching the head of the inferior turbinate
PP	Polyps extending posterior to the middle meatus, reaching the tail of the inferior and middle turbinate
PT	Polyps occupying the entire anteroposterior aspect of the nasal cavity

**From Ref. 11.*

Histologic Evaluation. The slides stained with hematoxylin and eosin were evaluated on an Olympus BX-40 microscope (×10 ocular and ×40 objective) (Olympus America Inc., Melville, NY). The images were captured with a Spot Insight Color microcamera (Diagnostic Instruments, Inc., Sterling Heights, MI) adapted to the microscope by using the SPOT Basic 3.4.5 software (Diagnostic Instruments, Inc.) and analyzed by using Corel Draw version 7.468 (Corel Corporation, Ottawa, Canada). The cellularity was analyzed by exploring five fields of the optical microscope with ×400 magnification, as suggested by Ingels *et al.*[18]

The semiquantitative evaluation of the inflammatory infiltrate followed a well-defined score.[19] The inflammatory infiltrate was classified according to its distribution, intensity, and predominant cell type. Regarding the distribution, the classification was as follows: (1) focal: the presence of one to three inflammatory foci, (2) multifocal: the presence of more than three inflammatory foci, and (3) diffuse: the presence of uniformly distributed inflammatory cells. The inflammatory reaction intensity was categorized into three subgroups: mild (+), moderate (++), and intense (+++), based on the morphologic analysis of the total inflammatory infiltrate. The predominant cell pattern was also evaluated and classified as mononuclear, mixed, or polymorphonuclear. For the statistical analysis, the patterns were graded as numbers (1, 2, and 3) according to worsening in the inflammation distribution, increased infiltrate intensity, and increased polymorphonuclear pattern.

To evaluate the percentage of eosinophils, the field with the largest inflammatory infiltrate (more representative) among the five captured fields was chosen, and 100 leukocytes were counted in each sample (before and after treatment) by using ×400 magnification.[20,21] We stained the slides with Cromotrope 2R to better identify the eosinophils and to confirm the observations. By using the ImageJ software, the absolute number of eosinophils was counted in five captured fields in slides stained with Cromotrope 2R. All the samples were analyzed by a double-blinded pathologist (D.C.R.). Two patients did not participate in the histopathologic evaluation. One patient did not authorize the biopsy after the treatment, despite having displayed clinical improvement, and the sample of one biopsy from before the treatment was not representative. One patient exhibited complete regression of the polyps, and the biopsy was performed in the middle meatus mucosa after the treatment.

Statistical Analysis

The paired *t*-test was used to compare the means before and after the treatment. The confidence intervals for the percentages were obtained by using the Clopper-Pearson method. The frequency of binary variables in different subgroups was compared by using the χ^2 test. The statistical analyses were performed by using the public domain software R x64 version 2.15.2, and the conclusions extracted from the results were obtained by considering a significance level of 5% and a confidence interval of 95%. The information collected was entered into a data base developed in Microsoft Excel (Microsoft, Redmond, WA).

RESULTS

General

Four patients were excluded from the study for using antibiotics (prescribed by a different physician during

Table 2 **Results of the analysis of subjective improvement, the staging, the SNOT-22 questionnaire, the percentage of eosinophils, and counting the absolute number of eosinophils of patients with ENP after treatment with azithromycin**

Variable	No. Patients	Patients with Improvement in the Variable, no. (%)	95% Confidence Interval (Clopper-Pearson), %
Improvement in staging	33	23 (69.7)*	54.5–84.4
Reduced SNOT-22 of >14	33	19 (57.6)*	42.2–74.5
Reduction of eosinophils, %	31	18 (58.1)#	42.3–75.5
Reduction of eosinophils, no.	20	10 (50.0)#	31.6–72.8

SNOT-22 = 22-item Sino-Nasal Outcome Test; ENP = eosinophilic nasal polyposis.
**$p < 0.001$.*
 # $p > 0.05$.

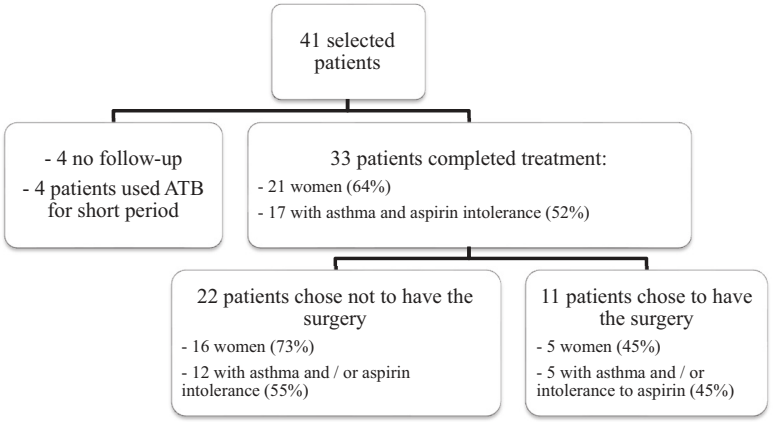

Figure 1. *Diagram of cases. Diagram cases of patients with eosinophilic nasal polyposis (ENP) treated with azithromycin in this study.*

the treatment) for a short period. Another four patients missed the follow-up. Thus, 33 patients completed the study (results are presented in Table 2). The patient ages ranged from 18 to 69 years, with a mean age of 48.84 years, and there were 21 women (63.6%) and 12 men. Seventeen patients (51.5%) had asthma and aspirin intolerance. At the end of the study, 22 patients (66.7%) reported good symptom control and chose not to undergo surgical treatment. These patients remained under clinical follow-up at the Rhinology Outpatient Clinic, São Geraldo Hospital (Hospital São Geraldo). Eleven patients (33.3%) chose to undergo the surgical procedure because they did not feel fully satisfied with the results after the treatment. The sample diagram if shown in Fig. 1.

Staging

None of the patients exhibited worsening of the staging after the treatment compared with before the treatment. On average, the staging decreased by 3.4 units, and 23 patients (69.7%) exhibited improved staging after the treatment. One patient exhibited a nasal cavity free from polyps (even with nasofibroscopy) after the treatment.

Quality-of-life Questionnaire (SNOT-22)

Only two patients exhibited a worse SNOT-22 evaluation (increased from 1 to 4 points, values considered not significant according to the literature[9]). The SNOT-22 index (difference between values from before and after the treatment) of the patients decreased, on average, 20.3 points. Nineteen patients (57.6%) exhibited a decrease of >14 points, which was considered significant[9] (Table 2).

Histologic Evaluation

In the analysis of the percentage of eosinophils, the values obtained from each patient before and after the treatment were compared ($N = 31$). Regarding the absolute number of eosinophils, the mean of the five fields was calculated, and the results were compared ($N = 20$). The percentage of eosinophils in the patients decreased, on average, by 5.9%. Eighteen patients (58.1%) exhibited a decrease in the percentage of eosinophils. The mean absolute number of eosinophils per field in the patients increased by 8.7. Ten patients (50.0%) exhibited a decrease in the absolute number of eosinophils (Table 2).

There were no significant changes in the intensity, distribution, or pattern of the inflammatory infiltrate

Table 3 **Analysis for subgroups with and without asthma or intolerance to aspirin and with and without advanced initial staging**

Criterion	Asthma and/or Intolerance to Aspirin, no. (%)			Initial Staging of >14, no. (%)		
	Yes ($n = 17$)	No ($n = 16$)	p Value	Yes ($n = 14$)	No ($n = 19$)	p Value
Reduced SNOT-22 > 14	8 (47.1)	11 (68.8)	0.364	7 (50.0)	12 (63.1)	0.689
Reduction staging	11 (64.7)	12 (75.0)	0.792	6 (42.8)	17 (89.5)	0.012*

SNOT-22 = 22-Item Sino-Nasal Outcome Test.
**Significant ($p < 0.05$).*

($p > 0.5$). Seven patients (22.6%) exhibited improved intensity (from intense to moderate or from moderate to mild), and 11 patients (35.5%) exhibited a worsened intensity (from mild to moderate or from moderate to intense). Two patients (6.4%) exhibited an improved distribution (from diffuse to multifocal), and one patient (3.2%) exhibited a worsened distribution (from multifocal to diffuse). Eleven patients (35.5%) exhibited an improved pattern (inflammatory infiltrate trend of being mononuclear), and six patients (19.3%) exhibited a worsened pattern (inflammatory infiltrate trend of being polymorphonuclear).

Subgroups

We evaluated whether the response to the treatment was different in the subgroups with or without asthma or aspirin intolerance and with or without advanced staging. Seventeen patients (51.5%) had asthma and aspirin intolerance. There was no significant difference between the decrease in SNOT-22 or decreased staging between the subgroups with or without asthma and/or aspirin intolerance (Table 3). Fourteen patients (42.4%) exhibited advanced initial staging (staging degree of >14), and, in this subgroup, the decreased staging was significantly lower than in the subgroup with nonadvanced initial staging. The results of the subgroup analyses are listed in Table 3.

Adverse Effects

In general, the medication was well tolerated by the patients. Only one patient reported having adverse effects (heartburn and/or burning sensation) during the use of the medication. However, it was not necessary to interrupt the treatment. Even during targeted questioning, the remaining patients did not report having adverse effects. When considering that ENP is predominantly found in men and that the present sample had a higher percentage of women (63.6%), the result of each variable was evaluated separately in the group of men and in the group of women. There was no difference between the groups ($p > 0.05$).

Follow-up

After 12 months of study, the 22 patients who chose not to undergo surgery, 17 still had good clinical control of the disease (77.3%). Five patients had to undergo surgery because of worsening symptoms.

DISCUSSION

The search for other therapeutic options has spurred research on medications that can act by controlling the inflammatory process, minimizing the undesirable adverse effects that result from the chronic use of corticosteroids, and maintaining a prolonged therapeutic response.[4] Thus, macrolides have become important in this context. The variables analyzed in this study were supported by the literature. In the studied patients, we observed a clinical improvement in staging and in the quality of life (evaluated by the SNOT-22) after the treatment with azithromycin for 2 months (8 weeks). These findings corroborate previous studies,[17,22–24] but, to our knowledge, this was the first time that these data were obtained from an investigation conducted in a specific and well-determined group. In the clinical variables analyzed, no significant differences were observed regarding the response to the treatment between groups with or without asthma and/or aspirin intolerance.

The present study was a pioneer study in using three-dimensional staging as a clinical parameter to evaluate treatment with macrolides.[11] We observed that the advanced staging (>14) in the patients studied led to a less significant response to the treatment regarding subjective improvement and reduction of staging, which differed from the results found by Suzuki *et al.*[25] The present findings also differed from those of Videler *et al.*,[16] in which the absence of a response found by the investigators after azithromycin could be justified by the advanced staging of the studied patients.

In the present study, tissue eosinophilia was evaluated according to the literature[18,20,21] by a double-blinded pathologist (D.C.R.). However, at the end of

the study, the investigators evaluated the samples and observed a noticeable difference in the tissues before and after treatment. The difference was not in eosinophil count or in the inflammatory process classification used, but, in the biopsy specimens of these polyps of the same patient before and after treatment had different histologic characteristics. These differences should be investigated in the next study.

The treatment interval chosen in the present study was based on the literature,[15,17] furthermore noted that the interval between the first appointment and the surgery (usually 2 months). Thus, we tried not to cause any additional inconveniences to the patients associated with traveling to the hospital. Conversely, the study did not delay any previously recommended surgical treatment in case no improvement in symptoms was obtained with the proposed treatment. However, in some studies, the longer the treatment duration was, the greater the improvement or benefits resulting from it.[26–29] Hashiba and Baba[27] showed that treatments that lasted for 2, 4, 6, or 12 weeks had an improvement rate of 4.7, 47.7, 62.8, and 70.6%, respectively.

It is speculated that mucosal restoration is slow and requires >12 weeks.[26] Thus, the treatment time used in the present study may have been short, and, in theory, we might have obtained even better results if the study were prolonged for additional weeks. In chronic inflammatory diseases, azithromycin in the dosage used has already been maintained for longer periods without adding significant adverse effects.[12–14,28,29] The absence of a placebo control group was the main weakness of the study. A new study is already being carried out that uses a control group with placebo; in a near future, it may be possible to make more consistent statements about our findings.

Because ENP is a multifactorial disease, it is possible that a combination of treatments is required to obtain adequate symptom control. Based on the results of the present study, further investigations should evaluate a combination of clinical treatments, such as topical corticosteroids and azithromycin. In patients with advanced initial staging and who exhibit a less significant response, the use of azithromycin during the postoperative period may be evaluated with the aim to decrease the likelihood of disease recurrence.

CONCLUSION

In the studied population, treatment with azithromycin (500 mg three times a week for 8 weeks) caused a clinical improvement based on the polyposis staging and the quality-of-life questionnaire (SNOT-22). However, the absence of a control group makes this a finding with a low level of evidence. No significant changes were observed in the characteristics evaluated in the study of the inflammatory infiltrate between samples obtained before and after the treatment with azithromycin. Based on these results, azithromycin can be considered an additional therapeutic option for ENP. However, further studies are necessary to define the real mechanism of action involved and confirm the efficacy of the treatment.

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