



Brazilian Journal of  
**OTORHINOLARYNGOLOGY**

[www.bjorl.org](http://www.bjorl.org)



ORIGINAL ARTICLE

**Osteitis and mucosal inflammation in a rabbit model of sinusitis** ☆,☆☆



Carlos Augusto Correia de Campos<sup>a,b,\*</sup>, Eduardo Landini Lutaif Dolci<sup>a,b</sup>,  
Leonardo da Silva<sup>a,b</sup>, José Eduardo Lutaif Dolci<sup>a,b</sup>,  
Carlos Alberto Herrerias de Campos<sup>b</sup>, Ricardo Landini Lutaif Dolci<sup>a,b</sup>

<sup>a</sup> Faculdade de Ciências Médicas, Santa Casa de São Paulo, São Paulo, SP, Brazil

<sup>b</sup> Department of Otorhinolaryngology, Santa Casa de São Paulo, São Paulo, SP, Brazil

Received 25 June 2014; accepted 5 August 2014

Available online 30 March 2015

**KEYWORDS**

Sinusitis;  
Osteitis;  
Animal models

**Abstract**

*Introduction:* Several experimental studies have shown osteitis after the onset of sinusitis, supporting the idea that bone involvement could participate in the dissemination and perpetuation of this inflammatory disease. However, procedures commonly performed for the induction of sinusitis, such as antrostomies, can trigger sinusitis by themselves.

*Objective:* To evaluate osteitis in an animal model of sinusitis that does not violate the sinus directly and verify whether this is limited to the induction side, or if it affects the contralateral side.

*Methods:* Experimental study in which sinusitis was produced by inserting an obstructing sponge into the nasal cavity of 20 rabbits. After defined intervals, the animals were euthanized and maxillary sinus samples were removed for semi-quantitative histological analysis of mucosa and bone.

*Results:* Signs of bone and mucosal inflammation were observed, affecting both the induction and contralateral sides. Statistical analysis showed correlation between the intensity of osteitis on both sides, but not between mucosal and bone inflammation on the same side, supporting the theory that inflammation can spread through bone structures, regardless of mucosal inflammation.

☆ Please cite this article as: de Campos CA, Dolci EL, da Silva L, Dolci JE, de Campos CA, Dolci RL. Osteitis and mucosal inflammation in a rabbit model of sinusitis. Braz J Otorhinolaryngol. 2015;81:312–20.

☆☆ Institution: Department of Otorhinolaryngology, Santa Casa de São Paulo, São Paulo, SP, Brazil.

\* Corresponding author.

E-mail: [guto-campos@uol.com.br](mailto:guto-campos@uol.com.br) (C.A.C. de Campos).

**PALAVRAS-CHAVE**

Sinusite;  
Osteíte;  
Modelos animais

**Conclusion:** This study demonstrated that in an animal model of sinusitis that does not disturb the sinus directly osteitis occurs in the affected sinus and that it also affects the contralateral side.

© 2015 Associação Brasileira de Otorrinolaringologia e Cirurgia Cérvico-Facial. Published by Elsevier Editora Ltda. All rights reserved.

**Osteíte e inflamação mucosa em um modelo experimental de rinossinusite****Resumo**

**Introdução:** Diversos estudos experimentais evidenciam osteíte após estabelecimento de sinusite, corroborando para a ideia de que o envolvimento ósseo poderia participar na disseminação e perpetuação do processo inflamatório. Porém procedimentos realizados para indução da doença nestes modelos, como antrostomias, podem, por si só, desencadear osteíte.

**Objetivo:** Avaliar osteíte em um modelo de rinossinusite em que não ocorre manipulação sinusal e verificar se esta é limitada ao lado de indução, ou se acomete o lado contralateral.

**Método:** Estudo experimental em que induziu-se rinossinusite em 20 coelhos, por meio de obliteração temporária com esponja de uma das cavidades nasais. Amostras de tecido sinusal foram submetidas à análise histológica semiquantitativa, após sacrifício dos animais em intervalos regulares.

**Resultados:** Foram observados sinais de inflamação óssea e mucosa mais intensa no lado de indução, mas também contralateral. Testes estatísticos evidenciaram correlação entre a osteíte de ambos os lados, porém não entre inflamação óssea e mucosa de um mesmo lado, apoiando a teoria de que a inflamação poderia se disseminar através do tecido ósseo, independente da inflamação mucosa.

**Conclusão:** O presente estudo evidenciou a existência de osteíte, tanto no lado de indução quanto no contralateral, em modelo experimental em que não ocorre manipulação sinusal.

© 2015 Associação Brasileira de Otorrinolaringologia e Cirurgia Cérvico-Facial. Publicado por Elsevier Editora Ltda. Todos os direitos reservados.

**Introduction**

Several factors may contribute to the onset and persistence of sinonasal inflammation, leading to chronic rhinosinusitis (CRS). These range from alterations related to the host, such as immunodeficiencies and mucociliary disease, to characteristics associated with etiological agents, such as the capacity to form biofilms and bacterial superantigens.<sup>1</sup>

Among these, the involvement of the paranasal bones in CRS development and maintenance has been investigated. The close contact between bone and mucosa in this region and radiological findings in patients with CRS suggest the involvement of this tissue.<sup>2-4</sup>

Several studies have disclosed the presence of sinus bone inflammation in patients with CRS, usually using tomographic assessment or histological analysis.<sup>2,5,6</sup> This incidence varies from 36% to 100%,<sup>4,7,8</sup> depending on the method chosen for patient inclusion and the form of assessment. Apparently, the incidence is greater when histological evaluation is performed, showing that, depending on the intensity of osteitis, there may not be evidence of inflammation on tomographic assessment.<sup>8,9</sup>

In this sense, Lee et al. prospectively evaluated 121 patients with CRS treated surgically. Based on tomography, they observed signs of osteitis affecting 36% of patients (82% ethmoid, 64% sphenoid, 45% maxillary, without evaluation of the frontal sinus) but observed histological signs

in 53%.<sup>4</sup> Other studies showed that tomographic signs of osteitis are associated with greater disease intensity in anatomopathological examinations<sup>7,9</sup> and worse outcome in surgical treatments.<sup>6</sup>

The most commonly reported signs indicative of osteitis in patients with CRS are periosteal thickening, osteoblast proliferation, bone resorption and new bone formation, and inflammatory cell infiltration.<sup>2,4,5,7-9</sup>

Although these studies have provided evidence of the existence of bone inflammation in cases of CRS and some clinical implications, others have pointed out that osteitis does not occur in all patients. Also, they have indicated that it greatly increases if the patient has been previously submitted to surgery (from 6.7% to 58%), which may suggest the importance of other factors for its onset, such as surgical trauma.<sup>4</sup> Another important fact is that we found no clinical studies that evaluated the presence of bone inflammation in acute episodes of rhinosinusitis. That is because acute rhinosinusitis (ARS) is usually treated non-surgically, making it difficult to collect samples for histological analysis. Determining whether osteitis is present at the early stages or if it arises only with the persistence of sinonasal inflammation would aid to further understand its role.

For this purpose, experimental animal models are used, usually rabbit models, in which bone and mucosal inflammation are assessed, and other factors commonly associated

with sinusitis can be ruled out, such as allergic and inflammatory disorders, drug use, previous surgery, and anatomical alterations. These studies show bone involvement as early as two weeks after the rhinosinusitis induction process has begun and persisting at varying intensities, for up to 13 weeks. The most commonly described findings of bone inflammation are similar to those reported in clinical trials: periosteal thickening, inflammatory infiltrate, increased osteoclastic and osteoblastic activity, new bone formation, and eventually fibrosis.<sup>3,10-13</sup>

The problem with these experimental models is the methods by which the sinusitis is induced. Normally, definitive obliteration of the maxillary ostium drainage with glue and sinus inoculation of an infectious agent through an external sinusotomy are performed. These procedures injure a certain area of the sinus wall and alter its physiology and, by themselves, trigger tissue inflammation regardless of the infection. Moreover, the definitive obliteration of the sinus containing a pathogen leads to an intense infectious process, that, if limited to the sinus cavity often does not correspond to the pathophysiology of this disease.<sup>14</sup> In ARS, infection usually has a nasal origin and maxillary ostium obstruction is reversible, as it is caused by mucosal edema.

There are experimental models where rhinosinusitis is induced by procedures in which there is less manipulation of the animals' nasal cavity. They are called rhinogenic models and are based on the introduction of a sponge into one of the nasal cavities, that remains in place for a set period. In this method, the sinuses are not violated, thus limiting inflammation caused by the procedure, and the ostial blockage is reversible. Therefore, these models better reflect the physiopathogeny of this disease in humans.<sup>14-16</sup>

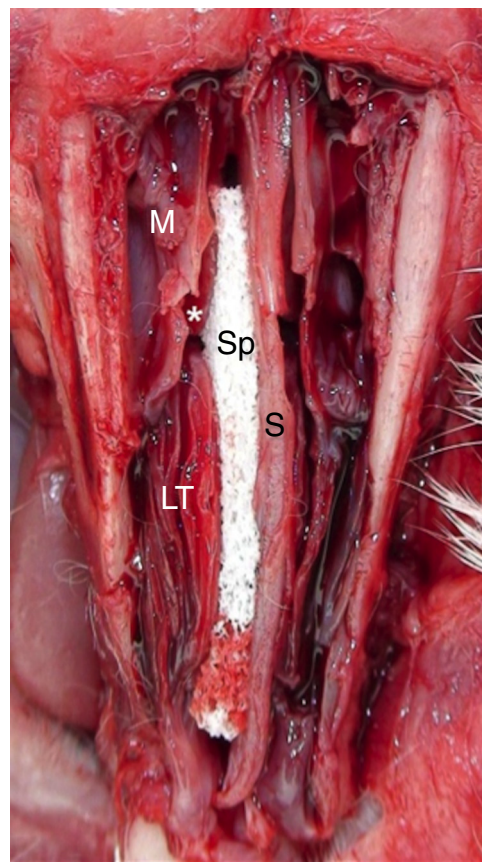
Therefore, the present study aimed to determine whether sinus bone inflammation occurs, to correlate it with mucosal inflammation in an experimental model of rhinosinusitis in which there is no manipulation of the paranasal sinus and to verify whether this inflammation is limited to the induction side, or if it also affects the contralateral side.

## Methods

A total of 22 adult, white, male and female New Zealand rabbits were used, weighing approximately 2500 g at the beginning of the experiment. Throughout the study, the animals were maintained in individual cages suitable for the breed and weight and had free access to food and water.

Bacterial rhinosinusitis was induced in 20 of 22 animals, by placing a small piece of porous polyvinyl sponge measuring 3.0 cm × 0.5 cm × 0.3 cm, previously sterilized with ethylene oxide, into the right nasal cavity of the animals (Fig. 1). The sponges were soaked in 1.0 mL solution containing streptococcal and staphylococcal toxoid. No procedure was performed in the left nasal cavity. Two animals used as controls were euthanized without undergoing any intervention.

After 10 days, the sponges were removed and six animals were randomly euthanized (10th day of the experiment). After another 7 days, during which the animals did not undergo any further intervention, seven additional animals were euthanized (17th day of the experiment). Finally, on the 30th day of the experiment, the last seven animals were euthanized.



**Figure 1** Anatomical specimen showing sponge (Sp) placed in the nasal cavity of rabbits. Observe the nasal septum (S), the maxillary sinus (M), the middle turbinate (\*), and the lower turbinate on the right (LT).

All procedures were performed under anesthesia with spontaneous breathing, according to standards established by the Brazilian Society of Laboratory Animal Science (Sociedade Brasileira de Ciência em Animais de Laboratório – SBCAL) and after approval by the ethics committee of the institution, under No. 2011-4.

After euthanization, opening of the outer wall of the nasal cavity and paranasal sinuses was performed. Next, the entire medial wall of the maxillary sinus on both the induced sinusitis side and the contralateral side was removed and samples containing bone and mucosal tissue were obtained. The material of each sample was fixed in buffered formalin, dehydrated at increasing concentrations of ethanol, cleared in xylene, and embedded in paraffin. It was then sliced with a microtome into 4-mm thick sections that were mounted on slides, and stained with hematoxylin and eosin (HE).

The slides were evaluated using optical microscopy by a pathologist blinded to each animal experiment protocol. The mucosal tissue and bone samples were graded according to inflammatory parameters, semi-quantitatively. The following was considered for mucosal inflammation: grade 0, absence of inflammation; grade 1, mild inflammation (slight inflammatory cell infiltrate in the mucosa); grade 2, moderate inflammation (diffuse inflammatory infiltrate); grade 3, intense inflammation (diffuse inflammatory infiltrate, epithelial cell injury, abnormal mucosal and submucosal

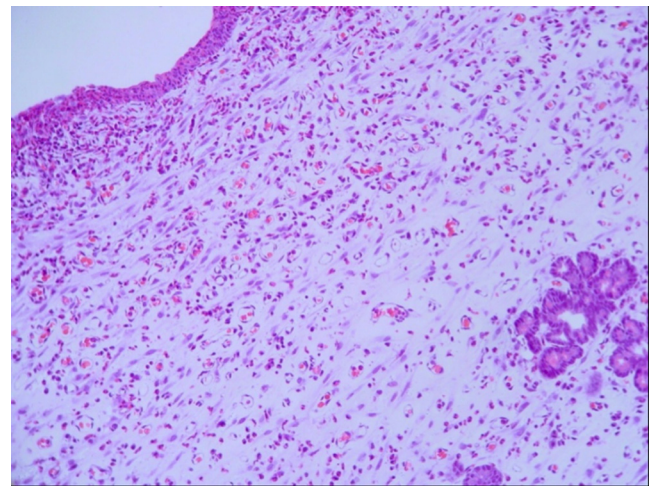
architecture). For bone inflammation classification, the following was considered: grade 0, absence of inflammation; grade 1, mild inflammation (mild periosteal thickening); grade 2, moderate inflammation (moderate periosteal thickening and osteoblastic rimming – osteoblast layer along the newly formed bone); grade 3, intense inflammation (pronounced periosteal thickening, presence of non-mineralized osteoid matrix and osteoblastic rimming).

Secretion from the maxillary sinus was also collected using swabs. These samples were plated on blood agar, Sabouraud agar, and chocolate agar culture media (Probac do Brasil). The blood and chocolate agar plates were incubated at  $35 \pm 2^\circ\text{C}$ , while Sabouraud agar plates were kept at room temperature. Daily readings of the plates were performed for up to 15 days.

Statistical analysis sought to correlate the degree of inflammation of the mucosal and bone tissue on the different sides, in order to verify how this inflammation behaved during follow-up and whether there was an association between the degree of inflammation and identified pathogens. For that purpose, the mucosal and bone histology data as well as culture test results were described according to the side of intervention and time of euthanization, using absolute and relative frequencies. These data were analyzed by paired Wilcoxon, Kruskal–Wallis, and likelihood ratio tests. Finally, Spearman's test was performed to correlate bone and mucosal inflammation on the different sides. All tests were performed with a significance level of 5%.

## Results

At the time of sponge removal, all animals had purulent rhinorrhea on the side where the sponge had been placed, whereas none of them had contralateral rhinorrhea. None of the animals died before the scheduled time for euthanization.



**Figure 2** Sample of right maxillary sinus mucosa, showing epithelial alterations, neovascularization, connective-fibrous proliferation, and inflammatory cell infiltrate – optical microscopy, hematoxylin and eosin staining, 100× magnification.

Histological evaluation of mucosal samples showed a range of outcomes, from animals with significant inflammatory process to animals with almost normal maxillary sinus mucosa. Alterations such as inflammatory cell infiltrate, neovascularization, subepithelial glandular hyperplasia and destruction, and epithelial alterations such as ulcerations and ciliary destruction were observed. Some signs that characterize chronicity, such as connective-fibrous proliferation and mucosal hyperplasia, were also identified in animals euthanized later in the experiment (Fig. 2). Of the 20 rabbits evaluated, three (15%) had no signs of mucosal inflammation on the induction side and nine (45%) did not show these signs on the contralateral side. Therefore, the right maxillary sinus mucosa showed a greater degree of inflammation than

**Table 1** Description of the sinus mucosa histology according to side and time of euthanization and results of the comparative tests.

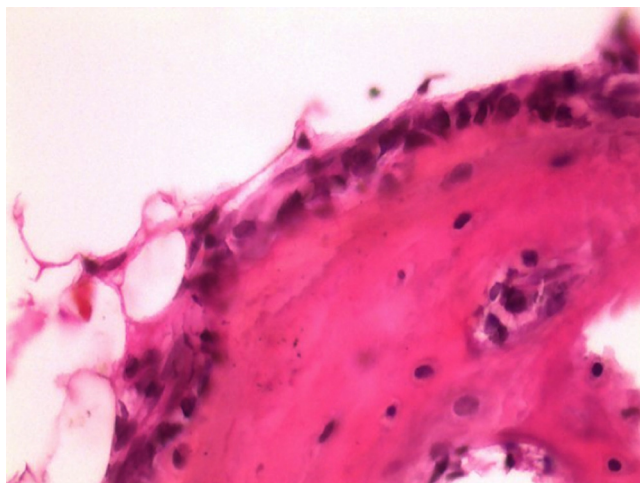
Mucosal inflammation	Day of euthanization						Total		$p^a$
	10		17		30		<i>n</i>	%	
	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%			
<i>Right maxillary sinus</i>									
Grade 0	0	0.0	1	14.3	2	28.6	3	15.0	0.009
Grade 1	1	16.7	3	42.9	5	71.4	9	45.0	
Grade 2	2	33.3	3	42.9	0	0.0	5	25.0	
Grade 3	3	50.0	0	0.0	0	0.0	3	15.0	
<i>Left maxillary sinus</i>									
Grade 0	1	16.7	4	57.1	4	57.1	9	45.0	0.267
Grade 1	5	83.3	3	42.9	3	42.9	11	55.0	
Grade 2	0	0.0	0	0.0	0	0.0	0	0.0	
Grade 3	0	0.0	0	0.0	0	0.0	0	0.0	
$p^b$	0.003								
Total	6	100	7	100	7	100	20	100	

*n*, number of animals; %, relative percentage of animals.

<sup>a</sup> Kruskal–Wallis test result.

<sup>b</sup> Paired Wilcoxon test result.



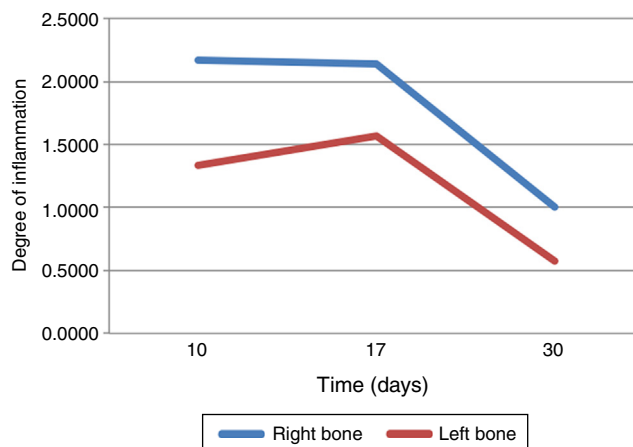


**Figure 3** Right maxillary sinus sample showing osteoblast layer over the bone matrix (osteoblastic rimming) and some osteocytes – optical microscopy, hematoxylin and eosin staining, 400× magnification.

that of the left maxillary sinus ( $p=0.003$ ) and this inflammation decreased over time in a statistically significant manner ( $p=0.009$ ), as shown in [Table 1](#).

The mucosal samples of the two control animals, which were euthanized before rhinosinusitis induction, showed no inflammatory sign. Therefore, they were classified as grade 0, both for the left and right maxillary sinuses.

Histological assessment of bone samples also showed several characteristics of inflammation, such as periosteal thickening, osteoblastic proliferation and border, osteoclast proliferation, altered bone architecture, presence of immature bone with disorganized collagen fibers, and non-mineralized osteoid matrix deposition ([Fig. 3](#)). On the right side, the animals showed a higher degree of inflammation than on the left ( $p=0.004$ ) and this inflammation



**Figure 4** Mean degrees of bone inflammation on both sides over time.

decreased over time on both sides, with statistical significance ( $p=0.046$  and  $p=0.037$ ). Two (10%) of the 20 assessed animals showed no signs of bone inflammation on the rhinosinusitis induction side and four (20%) did not show signs of inflammation on the contralateral side ([Table 2](#)). Histological signs of bone inflammation were also not observed in samples taken from control animals.

A direct correlation was observed between the intensity of inflammation observed in the right maxillary bone samples and that of the left maxillary bone collected over time ( $p<0.001$ ). That is, as bone inflammation decreased on the induction side, this also occurred on the contralateral side ([Table 3](#) and [Fig. 4](#)). This direct association of inflammation evolution over time was not observed among the mucosal samples obtained from both sides or between the mucosal and bone samples collected from the same side.

**Table 2** Description of sinus bone histology according to side and time of euthanization and results of the comparative tests.

Bone inflammation	Day of euthanization						Total		$p^a$
	10		17		30		n	%	
	n	%	n	%	n	%			
<b>Right maxillary sinus</b>									
Grade 0	0	0.0	0	0.0	2	28.6	2	10.0	0.046
Grade 1	2	33.3	1	14.3	3	42.9	6	30.0	
Grade 2	1	16.7	4	57.1	2	28.6	7	35.0	
Grade 3	3	50.0	2	28.6	0	0.0	5	25.0	
<b>Left maxillary sinus</b>									
Grade 0	0	0.0	0	0.0	4	57.1	4	20.0	0.037
Grade 1	3	50.0	3	42.9	2	28.6	8	40.0	
Grade 2	2	33.3	4	57.1	1	14.3	7	35.0	
Grade 3	1	16.7	0	0.0	0	0.0	1	5.0	
$p^b$	0.004								
<b>Total</b>	6	100	7	100	7	100	20	100	

n, number of animals; %, relative percentage of animals.

<sup>a</sup> Kruskal–Wallis test result.

<sup>b</sup> Paired Wilcoxon test result.

**Table 3** Correlation between the degree of inflammation observed in samples of bone tissue and mucosal tissue.

Correlation between tissue inflammation <sup>a</sup>	Right mucosal	Left mucosal	Right bone
<i>Left mucosal</i>			
<i>r</i>	0.176		
<i>p</i>	0.459		
<i>Right bone</i>			
<i>r</i>	0.375	0.118	
<i>p</i>	0.103	0.619	
<i>Left bone</i>			
<i>r</i>	0.410	0.139	0.781
<i>p</i>	0.073	0.560	<0.001

*r*, correlation value (ranging from 1 to -1; when closer to 1, the correlation is direct and when closer to -1, the correlation is indirect).

<sup>a</sup> Spearman's correlation test.

Culture test results showed higher positivity on the side where rhinosinusitis was induced, compared to the contralateral side, but without statistical significance. Culture tests of the two control animals were negative.

*Staphylococcus aureus* and *Streptococcus pneumoniae*, the agents used in rhinosinusitis induction, were found only in the right maxillary sinus of the animals euthanized earlier. Other microorganisms, mostly Gram-negative, such as *Escherichia coli*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*, were found on both the induction and contralateral sides and at the different times of follow-up. No statistically significant differences were observed for the microorganisms found on both sides. Additionally, no association was observed between the etiological agents and side of induction or time of euthanization, or between the etiological agents and inflammation intensity (Table 4).

## Discussion

Experimental models allow assessments ranging from aspects related to the pathogenesis of rhinosinusitis to the effectiveness of different forms of treatment for this disease.<sup>17-22</sup> Among several methods described for rhinosinusitis induction, this study utilized the introduction of a sponge containing a toxoid agent into one of the nasal cavities of the animals. In addition to being a technically simple and efficient procedure to produce sinusitis in 100% of animals in several studies,<sup>22,23</sup> it causes little injury to the nasal mucosa and no sinus injury.

Perhaps this is the main characteristic that differentiates the present investigation from others described in the literature, in that it assesses bone inflammation in experimental models.<sup>3,10-13</sup> Procedures performed in these studies, such as maxillary ostium obliteration with glue and sinus inoculation of an infectious agent performed through sinusotomy, rupture the osteomucosal wall, alter the mucociliary flow pattern, damage the vascular network and cause, by themselves, an inflammatory process. These procedures also strip the mucosa from small areas of the sinus bone in the sinusotomy sites; this bone exposure could facilitate the involvement of this tissue by pathogenic agents and their toxins. Similar reasons might explain, at least in part, the increased incidence of osteitis in patients with CRS submitted to previous nasal surgery, compared with those

that were never submitted to surgical procedures (from 6.7% to 58%).<sup>4</sup>

More intense signs of inflammation were observed affecting the mucosal and bone tissue in the early-euthanized groups and on the induction side. These findings suggest that bone inflammation, which had already been observed by other authors<sup>3,11-13</sup> even in experimental models in which maxillary ostium obstruction was temporary; is probably due to sinus infection rather than the trauma related to the induction procedures. Still, this inflammation significantly affects the mucosa and bone, not only on the induction side, but also on the contralateral side.

The present study used the histological classification proposed by Antunes et al., as this method was employed in an experimental model of rhinosinusitis that assessed both bone and mucosal tissue.<sup>24</sup> This semi-quantitative assessment demonstrated a direct correlation between the intensity of bone inflammation that occurred on the right side and on the left side. That is, although less severe on the left side, both similarly decreased over time. This association was not observed between the mucosal inflammation on both sides or between bone inflammation and mucosal tissue on the same side.

It is possible that the inflammation often observed in the maxillary sinus on the opposite side of the induction could be caused by an opportunistic bacterial infection, which would spread through the nasal and paranasal sinuses to both sides, first affecting the mucosal tissue and later the underlying bone tissue. This hypothesis would be consistent with the positive results in culture tests from both sides. However, in this case one would expect to see, a direct correlation between the intensity of inflammation observed in bone tissue and that observed in the mucosal tissue on the same side, but not between the bone tissue on one side and the contralateral bone tissue.

Perloff et al. observed signs of bone and mucosal inflammation on the contralateral side, distant from the site of induction in animals euthanized between seven and 13 weeks after the start of the experiment. They also described signs of chronicity, and in the bone histology, they observed increased vascularization and expansion of the Haversian canals containing inflammatory cells. Fibrosis was found in these canals in some animals euthanized at a later date.<sup>3</sup> Khalid et al. reported the same alterations in

**Table 4** Description of the bacteria found in the cultures, according to side and time of euthanization.

Bacteria found in the cultures	Day of euthanization						Total		<i>p</i> <sup>a</sup>
	10		17		30		<i>n</i>	%	
	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%			
<i>Staphylococcus aureus</i>									
Right									0.051
Negative	3	50.0	6	85.7	7	100.0	16	80.0	
Positive	3	50.0	1	14.3	0	0.0	4	20.0	
Left									>0.999
Negative	6	100.0	7	100.0	7	100.0	20	100.0	
Positive	0	16.7	0	0.0	0	0.0	0	0.0	
<i>p</i> <sup>b</sup>							0.106		
<i>Streptococcus pneumoniae</i>									
Right									0.068
Negative	4	66.7	7	100.0	7	100.0	18	90.0	
Positive	2	33.3	0	0.0	0	0.0	2	10.0	
Left									>0.999
Negative	6	100.0	7	100.0	7	100.0	20	100.0	
Positive	0	0.0	0	0.0	0	0.0	0	0.0	
<i>p</i> <sup>b</sup>							0.487		
<i>Other Gram-positive cocci</i>									
Right									0.333
Negative	6	100.0	7	100.0	6	85.7	19	95.0	
Positive	0	0.0	0	0.0	1	14.3	1	5.0	
Left									0.282
Negative	5	83.3	7	100.0	7	100.0	19	95.0	
Positive	1	16.7	0	0.0	0	0.0	1	5.0	
<i>p</i> <sup>b</sup>							>0.999		
<i>Gram-negative bacilli</i>									
Right									0.958
Negative	3	50.0	4	57.1	4	57.1	11	55.0	
Positive	3	50.0	3	42.9	3	42.9	9	45.0	
Left									0.341
Negative	2	33.3	3	42.9	5	71.4	10	50.0	
Positive	4	66.7	4	57.1	2	28.6	10	50.0	
<i>p</i> <sup>b</sup>							0.752		
<i>Gram-positive bacilli</i>									
Right									0.282
Negative	4	66.7	2	28.6	2	28.6	8	40.0	
Positive	2	33.3	5	71.4	5	71.4	12	60.0	
Left									0.510
Negative	4	66.7	3	42.9	5	71.4	12	60.0	
Positive	2	33.3	4	57.1	2	28.6	8	40.0	
<i>p</i> <sup>b</sup>							0.206		

*n*, number of animals; %, relative percentage of animals.

<sup>a</sup> Likelihood ratio test result.

<sup>b</sup> McNemar test result.

the Haversian canal system, implying that this could be a pathway for dissemination of inflammation to distant sites. Signs of contralateral osteitis were observed in animals euthanized between seven and nine weeks after infection. They even suggested the following sequence of events to explain the dissemination: maxillary sinus mucosal disease on one side, entry of infectious and inflammatory agents into adjacent bone, activation of bone remodeling process, access

to the vascular network, dissemination through bone to the contralateral side, and secondary inflammation of contralateral mucosa.<sup>13</sup>

In the present study, inflammatory cell infiltrate, osteoblast proliferation, and other characteristics of bone remodeling were observed. However, no alterations were observed in the Haversian system canals, perhaps because the animals were euthanized earlier than in other

studies and because the maxillary sinus clearance limited the inflammatory process. But even without histological alterations, this canal system may allow inflammatory mediators to spread to non-adjacent bone structures. This would explain the fact that signs of inflammation were found in the left maxillary sinus in this study and the finding that the left osteitis intensity was correlated to the right osteitis intensity rather than the underlying left mucosal inflammation. The dissemination of inflammation to distant sites through bone tissue implies that these sites only improve after improvement of the site of the original inflammation.

A higher percentage of *S. aureus* and *S. pneumoniae*, the agents inoculated into the rabbits, was found on the induction side and in the animals that were euthanized earlier. Regarding other pathogens, the most frequently isolated was *E. coli*. Several other bacilli were also observed, both Gram positive and Gram negative. Many of these micro-organisms are opportunistic pathogens of the respiratory and digestive system of rabbits, which after the prolonged course of rhinosinusitis and the resulting alterations in the upper respiratory tract, acquire the means to multiply and often replace the original infection-causing agent, as described by other authors.<sup>23,25</sup>

Perloff et al. and Khalid et al. isolated the agents used for induction in all animals euthanized at the end of the experiment. This is perhaps due to the fact that these authors used more pathogenic agents (*P. aeruginosa* and *S. aureus*), associated with definitive sinus obliteration.<sup>3,13</sup> Westrin et al. used *S. pneumoniae* and *Bacteroides fragilis* for the induction of experimental bacterial rhinosinusitis and analyzed the subsequent bacteriologic alterations. On average, they observed the substitution of pneumococcus after 5 days of culture. However, they identified *B. fragilis* on the day of euthanization of all animals that were inoculated.<sup>10</sup>

The present study found no correlation between the tests identified in bacterial culture and the degree of inflammation in the mucosal or bone tissue on both sides. This is explained by the diverse flora found at those sites, mostly consisting of opportunistic agents that are capable of proliferating in the inflamed sinus, perhaps related to the induction method.

The findings of this study show that the sinus bone inflammation occurs early after rhinosinusitis induction. They also demonstrate that prolonged maintenance of infection or surgical trauma is not necessary for the underlying bone to be affected and for this involvement to extend to distant sites. It can be observed that, despite this initial involvement, bone inflammation at the induction site tends to improve with sinus clearing and early ventilation, and that this improvement is accompanied by improvement in bone inflammation at distant sites. Finally this inflammation does not occur only in the presence of a specific etiological agent, but also in the presence of diverse flora.

The involvement of bone in the pathogenesis of rhinosinusitis, already addressed in previous clinical and experimental research, needs to be better understood. This capacity to transmit inflammation to distant sites, as suggested by the results of this study, could explain the characteristics observed in the clinical picture of this disease, such disease dissemination from a frontal or sphenoid sinus to the other, through the inter-sinus septum, or from the

ethmoid sinus to the middle turbinate. It could also explain the reason for the persistence of symptoms in some patients, even with medical treatment, and the need to remove not only the mucosa but also the underlying bone, in specific cases, in order to obtain clinical improvement.

However, care must be taken in extrapolating the findings of experimental studies into daily clinical practice. CRS is not only an infectious disease, but a multifactorial process, with environmental, individual, and host genetic predisposing factors. Even the inflammatory-infectious findings of this study, compatible with the ARS picture, need to be tested in other models, in different periods, and evaluating other agents. However, it is evident that the sinonasal inflammation in this process is not limited to the mucosa, but also exists in the underlying bone tissue. And its role needs to be better understood, so that treatments that re-establish normality in both tissues can be formulated.

## Conclusion

In an experimental rhinosinusitis model in which there was no manipulation of the paranasal sinus, this study demonstrated the presence of inflammatory signs in the sinus bone tissue, which affected both the induction and the contralateral side.

We documented a correlation between bone inflammation on both sides, but not between bone and mucosal inflammation on the same side.

## Conflicts of interest

The authors declare no conflicts of interest.

## References

1. Fokkens W, Lund V, Mullol J, Bachert C, Alobid I, Baroody F, et al. European position paper on rhinosinusitis and nasal polyps 2012. *Rhinology*. 2012;50:1–298.
2. Kennedy DW, Senior BA, Gannon FH, Montone KT, Hwang P, Lanza DC. Histology and histomorphometry of ethmoid bone in chronic rhinosinusitis. *Laryngoscope*. 1998;108:502–7.
3. Perloff JR, Gannon FH, Bolger WE, Montone KT, Orlandi R, Kennedy DW. Bone involvement in sinusitis: an apparent pathway for the spread of disease. *Laryngoscope*. 2000;110:2095–9.
4. Lee JT, Kennedy DW, Palmer JN, Feldman M, Chiu AG. The incidence of concurrent osteitis in patients with chronic rhinosinusitis: a clinicopathological study. *Am J Rhinol*. 2006;20:278–82.
5. Tovi F, Benharroch D, Gatot A, Hertzanu Y. Osteoblastic osteitis of the maxillary sinus. *Laryngoscope*. 1992;102:426–30.
6. Kim HY, Dhong HJ, Lee HJ, Chung YJ, Yin YJ, Oh JW, et al. Hyperostosis may affect prognosis after primary endoscopic sinus surgery for chronic rhinosinusitis. *Otolaryngol Head Neck Surg*. 2006;135:94–9.
7. Giacchi RJ, Lebowitz RA, Yee HT, Light JP, Jacobs JB. Histopathologic evaluation of the ethmoid bone in chronic sinusitis. *Am J Rhinol*. 2001;15:193–7.
8. Biedlingmaier JF, Whelan P, Zoarski G, Rothman M. Histopathology and CT analysis of partially resected middle turbinates. *Laryngoscope*. 1996;106:102–4.



9. Cho SH, Min HJ, Han HX, Paik SS, Kim KR. CT analysis and histopathology of bone remodeling in patients with chronic rhinosinusitis. *Otolaryngol Head Neck Surg.* 2006;135:404–8.
10. Westrin KM, Norlander T, Stierna P, Carlsoo B, Nord CE. Experimental maxillary sinusitis induced by *Bacteroides fragilis*. A bacteriological and histological study in rabbits. *Acta Otolaryngol.* 1992;112:107–14.
11. Norlander T, Forsgren K, Kumlien J, Stierna P, Carlsoo B. Cellular regeneration and recovery of the maxillary sinus mucosa. An experimental study in rabbits. *Acta Otolaryngol Suppl.* 1992;492:33–7.
12. Bolger WE, Leonard D, Dick EJ Jr, Stierna P. Gram negative sinusitis: a bacteriologic and histologic study in rabbits. *Am J Rhinol.* 1997;11:15–25.
13. Khalid AN, Hunt J, Perloff JR, Kennedy DW. The role of bone in chronic rhinosinusitis. *Laryngoscope.* 2002;112:1951–7.
14. Marks SC. Acute sinusitis in the rabbit: a new rhinogenic model. *Laryngoscope.* 1997;107:1579–85.
15. Marks SC. Acute sinusitis in the rabbit model: histologic analysis. *Laryngoscope.* 1998;108:320–5.
16. Kara CO, Cetin CB, Demirkan N, Sengul M, Topuz B, Pinar HS, et al. Experimental sinusitis in a rhinogenic model. *Laryngoscope.* 2004;114:273–8.
17. Min YG, Kim YK, Choi YS, Shin JS, Juhn SK. Mucociliary activity and histopathology of sinus mucosa in experimental maxillary sinusitis: a comparison of systemic administration of antibiotic and antibiotic delivery by polylactic acid polymer. *Laryngoscope.* 1995;105:835–42.
18. Bende M, Fukami M, Arfors KE, Mark J, Stierna P, Intaglietta M. Effect of oxymetazoline nose drops on acute sinusitis in the rabbit. *Ann Otol Rhinol Laryngol.* 1996;105:222–5.
19. Maeyama T. A study of experimental sinusitis in rabbits. *Auris Nasus Larynx.* 1981;8:87–98.
20. Cable BB, Wassmuth Z, Mann EA, Hommer D, Connelly G, Klem C, et al. The effect of corticosteroids in the treatment of experimental sinusitis. *Am J Rhinol.* 2000;14:217–22.
21. Sutbeyaz Y, Aktan B, Yoruk O, Ozdemir H, Gundogdu C. Treatment of sinusitis with corticosteroids in combination with antibiotics in experimentally induced rhinosinusitis. *Ann Otol Rhinol Laryngol.* 2008;117:389–94.
22. Cheng Y, Wei H, Li Z, Xue F, Jiang M, Chen W, et al. Effects of intranasal corticosteroids in the treatment of experimental acute bacterial maxillary sinusitis in rabbits. *ORL J Otorhinolaryngol Relat Spec.* 2009;71:57–65.
23. Costa HO, Luchi GER, Augusto AG, Castro M, Souza FC. Estudo comparativo entre diversas técnicas de confecção de modelo experimental de sinusite inflamatória em coelhos. *Braz J Otorhinolaryngol.* 2007;73:627–31.
24. Antunes MB, Feldman MD, Cohen NA, Chiu AG. Dose-dependent effects of topical tobramycin in an animal model of *Pseudomonas sinusitis*. *Am J Rhinol.* 2007;21:423–7.
25. Jyonouchi H, Sun S, Kennedy CA, Roche AK, Kajander KC, Miller JR, et al. Localized sinus inflammation in a rabbit sinusitis model induced by *Bacteroides fragilis* is accompanied by rigorous immune responses. *Otolaryngol Head Neck Surg.* 1999;120:869–75.