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Histological evaluation of the effects of corticotomy on induced orthodontic movement in rats

Avaliação histológica dos efeitos da corticotomia no movimento ortodôntico induzido em ratos

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Resumo

Introdução: A corticotomia alveolar é um procedimento cirúrgico utilizado para aumentar a velocidade do movimento dentário. **Objetivo:** Identificar evidências histológicas do efeito da corticotomia no movimento ortodôntico no rato. **Material e método:** Quarenta e cinco ratos Wistar (Rattusnorvegicus Albinus) foram igualmente divididos em três grupos: Grupo de Controle (GC) - sem movimento dentário ou corticotomia; Grupo de movimento (GM) – apenas movimento ortodôntico do dente; e Corticotomia e Movimento Grupo (GCM) - movimento ortodôntico dentário cirurgicamente assistido por corticotomia. Os procedimentos cirúrgicos de GCM consistiram de uma incisão no palato, da mesial a distal do primeiro molar superior direito. O movimento do dente no GM e GCM foi aplicado com uma força da mola helicoidal de 40 gF do primeiro molar superior direito para o incisivo superior direito. Os ratos foram sacrificados no 1º, 3º e 7º dia e, após este período, foram realizadas seções histológicas para avaliar a contagem de osteoblastos e osteoclastos nas áreas de tensão e pressão. **Resultado:** A análise histológica mostrou que o GCM apresentou melhor resposta celular na neoformação óssea quando comparado aos outros grupos. Em áreas de pressão, no 3º dia, houve uma maior proliferação de osteoclastos, resultando em maior reabsorção. Em áreas de tensão, no 1º dia, houve uma maior proliferação de osteoblastos, indicando aumento da formação óssea. **Conclusão:** A diferença entre os grupos tratados ocorreu apenas no período inicial do movimento. Portanto, as alterações causadas pela corticotomia não são significativas no movimento ortodôntico para justificar o procedimento invasivo.

Descritores: Técnicas de movimentação dentária; osteoclastos; osteoblastos; ratos; ortodontia.

Abstract

Introduction: Alveolar corticotomy is a surgical procedure used to increase the velocity of tooth movement. **Objective:** Identify histological evidence of the effect of corticotomy on orthodontic movement in rats. **Material and method:** Forty-five Wistar rats (Rattusnorvegicus Albinus) were equally divided into three groups: Control Group (CG) - no tooth movement or corticotomy; Movement Group (MG) - tooth orthodontic movement only; and Corticotomy and Movement Group (CMG) - tooth orthodontic movement surgically assisted by corticotomy. In the CMG, surgical procedures consisted in an incision in the palatal, reaching from the mesial to the distal regions of the maxillary right first molar. Tooth movement in the MG and CMG was applied with coil spring force of 40 gF from the maxillary right first molar to the maxillary right incisor. The rats were sacrificed at days 1, 3, and 7, and histological sections were performed to evaluate the counting of osteoblasts and osteoclasts throughout the areas of tension and pressure. **Result:** Histological analysis showed that the CMG presented better cell response to bone neoformation compared with that of the other groups. Greater proliferation of osteoclasts was observed in areas of pressure on day 3, resulting in increased reabsorption, whereas greater proliferation of osteoblasts was observed in areas of tension on day 1, indicating increased bone formation. **Conclusion:** Differences between the treated groups occurred only in the initial period of tooth movement. Therefore, the changes caused by corticotomy are not significant in orthodontic movement to justify this invasive procedure.

Descriptors: Tooth movement techniques; osteoclasts; osteoblasts; rats; orthodontics.

INTRODUCTION

Patient concerns regarding duration of orthodontic treatment are part of the daily clinical routine of orthodontists who are frequently faced with the traditional question "How long will I use braces for?" The anxiety of patients for having a short-term treatment has led many orthodontists to search for alternatives that would enhance histological response during tooth movement and, consequently,

accelerate orthodontic treatment as a whole[1,2]. In this context, there has been an increase in number of studies addressing the matter of Alveolar Corticotomy to increase the velocity of tooth movement This procedure consists in an intentional surgical intervention limited to the cortical portion of the alveolar bone, with no damage to the bone marrow, associated with orthodontic treatment aiming to accelerate dental movement[2-5].

Despite its revolutionary aspect, this treatment approach was not very well accepted at the time, and still is not, due to its invasive nature[3,5]. Since 2001, however, because of modifications associated with this technique, subapical osteotomies were replaced by selective cuts limited to the cortical portion of the alveolar bone, described as the first attempt to surgical intervention aimed to enhance conventional orthodontic treatment in areas where movement was desired[6].

Studies investigating the biological mechanism of corticotomy have reported cortical bone turnover, suggesting Regional Acceleratory Phenomenon (RAP), which is a transitory biological stage of bone regeneration[4,6-8]. The main role of the RAP is the formation of younger bone, that is, a faster-than-usual increase in the number of osteoclasts and osteoblasts occurs in the region, consequently increasing bone remodeling[4-9]. The hypothesis raised by many orthodontists, which revolves around the realization of this study, is that this mechanism accelerates tooth movement; however, most studies available in the specific scientific literature associating corticotomy with orthodontic treatment present limited inference, because they are based solely on clinical case reports, which are considered weak scientific evidence to support the mechanism and results of this technique.

In order to contribute to increase scientific information on this theme and, consequently, subsidize the decision-making of professionals that wish to consider the use of this procedure in their clinical activities, this study aims to identify and evaluate the histological effect of corticotomy on the alveolar bone, on the areas of tension and pressure, after orthodontic movement of molars in rats.

MATERIAL AND METHOD

Forty-five male Wistar rats (Rattusnorvegicus, Albinus), aged nine weeks and weighing 300-350 g, were selected for the present experiment. The animals were kept in polyethylene housing boxes (16×40×30) in groups of three. They were provided with ground dry feed to avoid damage to the orthodontic device[10] and water *ad libitum*. They were kept under light/dark 12-hour cycle with controlled temperature and humidity at 23 °C and ± 40%, respectively. After a four-week adaptation period, they were placed in their new environment and had their body weight recorded throughout the experimental period. This study was previously approved by the Ethics Committee on Animal Use (CEUA) of the School of Dentistry (Araraquara) at Sao Paulo State University - UNESP, under protocol no. 19/2012). All the animals were originated from the vivarium of this institution.

Initially, the animals were randomly divided into three groups according to the treatment protocol as follows: (1) Control Group (CG), no tooth movement or corticotomy (n=15); (2) Movement Group (MG), tooth orthodontic movement only (n=15); and (3) Corticotomy and Movement Group (CMG), tooth orthodontic movement surgically assisted by corticotomy (n=15).

At time 0 (T0), for the installation of the orthodontic device and surgical procedures of alveolar corticotomy, all the animals from the MG and CMG underwent general anesthesia with intramuscular injection of a combination of Ketamine Chloride (Ketamina Agener 10%, Agener – Nacional Pharmaceutical Chemical Union S/A, São Paulo, São Paulo, Brazil) and Xylazine Chloride (Xilazin 2%, Syntec Ltda, Cotia, São Paulo, Brazil) with proportion of 8mL:3mL, respectively, and a dosage of 1.2mL/kg of body weight. Animals in the CG group were maintained untreated in their housing boxes throughout the time of experiment.

Alveolar Corticotomy Procedure in the CMG

Surgical procedures of alveolar corticotomy were performed in all animals of the CMG prior to installation of the orthodontic devices. Corticotomy involved an incision in the palatal, reaching from the mesial to the distal regions of the maxillary right first molar. With the aid of a low-speed, 1/4-mm, spherical carbide bur 1 (KG Sorensen Ltda, Cotia, São Paulo, Brazil), three concise perforations (0.25 mm wide; 0.25 mm deep) were performed in the cortical bone of the mesial and distal regions of the maxillary right first molar (Figure 1). The perforations were made under irrigation with saline solution. The tissues were subsequently sutured with absorbable thread (Vicryl-Ethicon 5-0, Johnson & Johnson, São José dos Campos, São Paulo, Brazil).

Installation of the Orthodontic Device in the MG and CMG

The anesthetized animals were placed on a stand table for distancing of peribuccal tissues. After distancing of the tissues, the orthodontic device - composed of a 9 mm-long, nickel-titanium, closed-spring coil (Orthometric, Marília, São Paulo, Brazil) - was placed from the maxillary right first molar to the maxillary right incisor, promoting a mesial molar movement (Figure 1). To ensure greater spring stability, the dental crowns of these teeth were previously preconditioned with phosphoric acid at 37% (Alpha Acid, DFL Indústria e Comércio S.A, Rio de Janeiro, Brazil) for 30 seconds. Next, these dental elements were cleaned with running water and dried with abundant blowing air. Then a thin layer of adhesive (Adper™ Single Bond 2, 3M ESPE Dental Products, St. Paul, Minnesota, USA) was applied and polymerized according to the manufacturer's instructions. After that, to promote displacement of the mesial molar, the springs were tied using a 0.010-inch, stainless steel ligature wire (Morelli, São Paulo, São Paulo, Brazil) and stretched until a force of 40 gF was delivered, verified by dynamometer (Correx Tension Gauge, Haag-Sreit International, Koeniz, Switzerland), and then bonded together using composite resin (Transbond™ XT, 3M Unitek, Monrovia, California, USA) from one tooth to another in both groups. In the CMG, the orthodontic device was installed on the animals soon after bleeding containment. For both the MG and CMG, the springs were activated only once during the experiment.

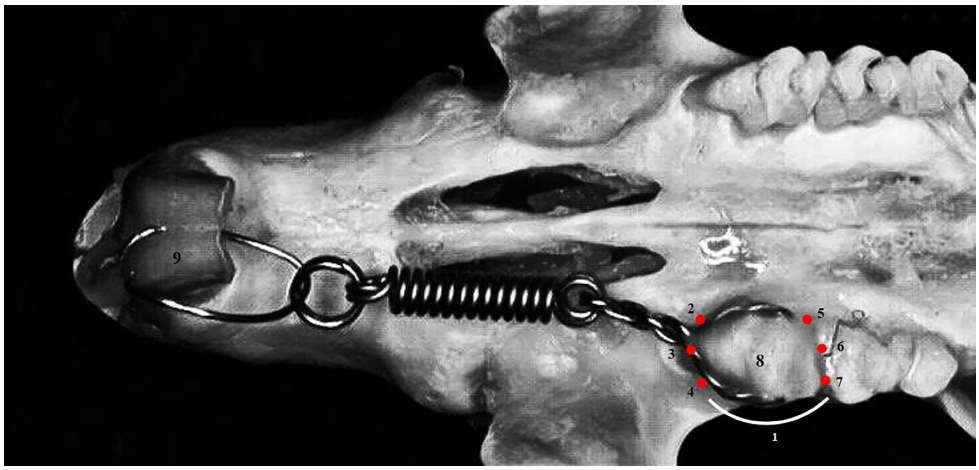

Figure 1. Illustration demonstrating the location of the incision (1), the three concise points of application of alveolar corticotomy: mesial (2, 3, and 4) and distal (5, 6 and 7) regions; and closed-spring coil installed from the maxillary right first molar (8) to the maxillary right incisor (9).

Tooth Movement Rate

Application of orthodontic force and the surgical procedure were performed on day 0. In all groups, the maxillary right first molar movements were evaluated at different periods according to the time the orthodontic force of the spring was maintained: day 1 after installation of the orthodontic device, day 3, and day 7. Each of these time periods represented a subgroup and comprised five rats. All rats were monitored and weighed daily during the time of experiment. A slight decrease in body mass was observed after 48 h, which was recovered during the following days.

The animals were sacrificed with the application of intraperitoneal injections of sodium pentobarbital (100 mg/kg) (Thiopentax, Cristal Pharma Ltd, Contagem, Minas Gerais, Brazil)[10] after completion the time periods of days 1, 3 and 7 of tooth movement in each subgroup.

Histological Analysis

After euthanasia, the usual laboratory procedures were conducted. The right hemi-maxilla were removed and fixed in 10% formaldehyde for 48 hours. The specimens of each group were then decalcified in ethylene-diamine-tetra-acetic acid (EDTA, Sigma-Aldrich Corp., St. Louis, Missouri, USA) for approximately five weeks (with six EDTA changes). Immediately after that, the specimens were washed, dehydrated, cleaned, and embedded in paraffin. The specimens were then cut into 6-µm sections and stained with Harris hematoxylin and alcoholic eosin to enable counting of the osteoclastic and osteoblastic cells.

Histometry was performed by capturing and digitizing the images (Leica DM 2500 Microscope, Wetzlar GmbH, Alemanha) and measuring them in mm using ImageLab 2000 software (Bio Diracon Informatics Ltda., Vargem Grande do Sul, São Paulo, Brazil). To this end, it was standardized that, in all groups, the defined areas of tension (T) and pressure (P) around the mesial and distal root of the maxillary right first molar would be evaluated (Figure 2). After that, the recorded images showing the presence of osteoblastic and osteoclastic cells (Figure 2) in contact with the root were counted by the same operator. The number of cells on each slide was tabulated in a Microsoft® Excel® spreadsheet.

Statistical Analysis

Data were analyzed using IBM SPSS 16.0 software (Statistical Package for the Social Sciences, SPSS Inc., Chicago, IL, USA). Normality of the variables was confirmed by the Kolmogorov-Smirnov test. To check the reliability of the measurement procedure, 50% of the histological slides of the randomly selected sample had cell count repeated by a same operator with a two-week interval. The Intraclass Correlation Coefficient (ICC) was used as a measure of reliability. The ICC values, estimated by means of confidence intervals, are >0.91 for the osteoblastic cell count and >0.90 for the osteoclast cell count, indicating excellent reliability of the measurement technique. ANOVA was used to compare the means between the three groups. As difference was observed between them, the Tukey test was applied as complementary analysis to identify which groups were different. A maximum significance level of 5% ($p<0.05$) was adopted for all statistical analyses.

RESULT

In total, 45 Wistar rats were included in the study. All rats remained healthy throughout the experimental period. The histological evaluation enabled identification of the areas of tension and pressure distributed along the molar alveolus (Figure 2); therefore, it was didactic to present the results in the following order: areas of pressure (Table 1) and areas of tension (Table 2).

At the pressure areas, no statistically significant differences were observed between the groups on days 1 and 3 for osteoblastic cell count, whereas statistically significant difference ($p<0.005$) was found between CG and CMG on day 7 (Table 1). Regarding the presence of osteoclastic cells, no statistically significant differences were observed between CG-MG and CG-CMG on days 1 and 7; however, significant differences ($p<0.005$) between all the groups were found on day 3 (Table 1), indicating that the amount of bone resorption/osteoclasts was greater in the CMG.

On day 1, statistically significant differences were observed between all the groups with respect to the number of osteoblasts in the tension area, whereas on day 3, differences were found only between CG-MG and CG-CMG, and on day 7 only between

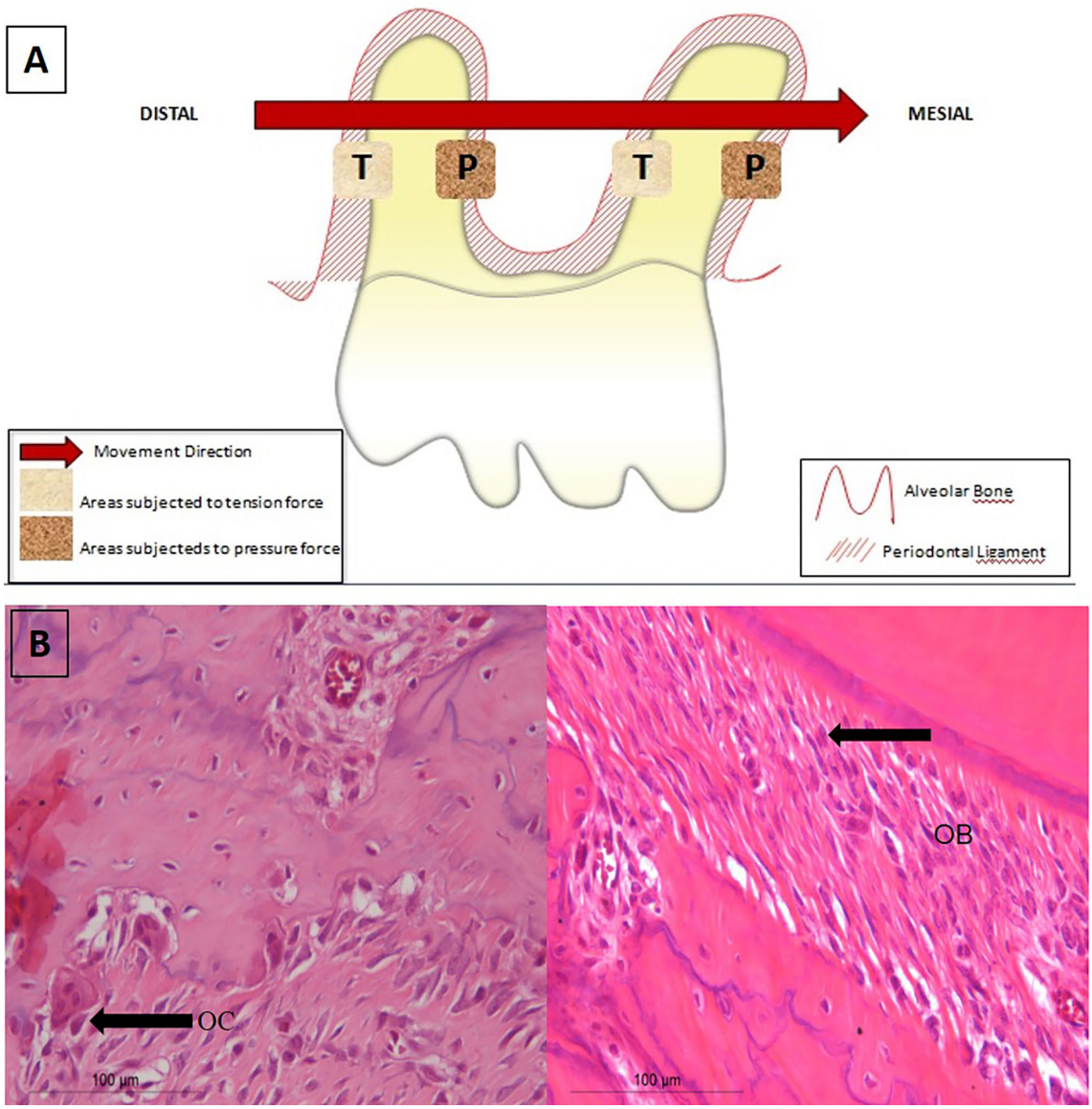

Figure 2. Areas of tension (T) and pressure (P) of the distal and mesial root of the maxillary right first molar (A) delimited for histological analysis and photomicrographs (B) of the region around the maxillary right first molar root, indicating presence of osteoclasts (OC) and osteoblasts (OB).

Table 1. Mean and standard deviation values assigned to osteoclast and osteoblast counts on the areas of pressure

Variable	Day 1				Day 3				Day 7			
	CG	MG	CMG	*p*	CG	MG	CMG	*p*	CG	MG	CMG	*p*
Osteoblasts	8.8(2.5)	7.0(5.1)	6.8(3.5)	0.681	2.0(1.8)	2.8(2.2)	5.0(1.4)	0.068	1.2(1.3)	3.2(2.0)	5.0(2.3)	0.030*C
Osteoclasts	0.8 (0.8)	5.6(2.7)	8.0(3.0)	0.002*AC	1.0(1.0)	5.0(2.2)	12.2(2.5)	0.000*ABC	2.0(2.0)	6.8(1.9)	8.6(3.3)	0.004*AC

*significant values for $p<0.005$; A significant CG-MG; B significant MG-CMG; C significant CG-CMG

Table 2. Mean and standard deviation values assigned to osteoclast and osteoblast counts on the areas of tension

Variable	Day 1				Day 3				Day 7			
	CG	MG	CMG	*P*	CG	MG	CMG	P	CG	MG	CMG	*P*
Osteoblasts	28.2(4.3)	58.2(6.8)	84.4(18.6)	0.000*ABC	28.8(6.8)	69.8(13.1)	71.8(24.0)	0.002*AC	47.8(4.6)	52.0(17.1)	71.4(12.0)	0.024*C
Osteoclasts	0.2(0.4)	1.4(0.5)	2.2(1.7)	0.044*C	1.4(1.1)	0.6(0.5)	1.6(1.1)	0.273	1.4(1.1)	2.2(1.1)	2.2(1.1)	0.446

*significant values for $p<0.005$; A significant CG-MG; B significant MG-CMG ; C significant CG-CMG

CG-CMG (p<0.005) (Table 2). Osteoclastic cell count in the tension area showed statistically significant difference between CG-CMG only on day 1, and no differences were found on days 3 and 7 (p<0.005) (Table 2).

DISCUSSION

Several factors support the choice of rats as experimental animals for the study of tooth movement. The anabolic and catabolic phases of bone remodeling in rats, as well as the periodontal region in the area of the molars of these animals are structurally and functionally similar to those of humans[11,12]. Although it is clinically speculated that the rate of bone turnover and the dynamics of orthodontic movement of a rat is not identical to that of a human being[13], rodent models enables the study of biological phenomena that, for ethical reasons, could not be reproduced and executed in a human model[9]. For these reasons, a choice was made of an animal model in this study.

For a better understanding of the results of this laboratory study, it is worth noting the plastic property of the bone, which is characterized by adaptation to functional forces[14]. When a pressing force is applied on this tissue, the local ligament compression stimulates the fusion of monocytes that are transformed into giant cells known as osteoclasts, which are responsible for bone reabsorption in this region. The opposite is observed in bone areas where tensile force is applied. The distension of the ligaments occurred in this region will cause the transformation of undifferentiated mesenchymal stem cells into osteoblasts, which will trigger the bone apposition process. Thus, the occurrence of resorption and affixing processes enables orthodontic movement.

In this study, histological analysis of the area subjected to pressure revealed a larger difference in osteoclast cell count on day 3; a fact previously observed in the studies by Wang et al.[15] and Peron et al.[10]. In this period, the group which received implementation of corticotomy with application of orthodontic mechanical device (CMG), produced a statistically larger number of osteoclastic cells, suggesting that this was the most intense response time for movement in rats[10,15]. However, after 7 days, no statistical difference was observed in osteoclast cell count between the MG and CMG. It is known that these Niti springs are particularly super-elastic, releasing lighter and constant strength for a long period of time, considering that there is a minor variation of force per millimeter of activation[16]. The importance of this fact lies on the constant maintenance of drive. Thus, on day 7, it would not be enough to cause deactivation systems, suggesting that association with cellular corticotomy was not capable of causing differences with regards to acceleration of orthodontic movement, a fact also reported in the studies by Murphy et al.[14] and Peron et al.[10]. This suggests the possibility that the tissue recovery process started and the cellular effect caused by corticotomy came to an end, thus lowering the expected orthodontic movement, as also observed by Baloul et al.[17] and Gandini et al.[18]

Histological analysis of the areas of tension in this study, as elucidated by the results obtained, showed greater difference in osteoblastic cell count between the MG and CMG. The significant difference in osteoblastic cell neogenesis indicates the occurrence of greater bone tissue neoformation in rats, as previously observed by Gandini et al.[18]

These findings are in agreement with those of other studies[17,19-21], suggesting that the acceleration tooth movement caused by this procedure occurs only in the early stages of treatment, apparently declining after the second month of observation and returning to normal rate after the healing phase. Therefore, the changes caused by corticotomy are not significant in orthodontic movement to justify the exposure of patients to this procedure.

Based on the results of this study and on a literature review, we conclude that the biological response obtained with alveolar corticotomy should be considered when the objective is to offer a differentiated treatment to patients. However, orthodontists should be alert to the clinical procedures involved in implementing this technique, as well as to the biological effects that result in bone tissue neoformation and the time period at which this procedure presents positive effects on acceleration of tooth movement, so that the use of this combination therapy could be justified in certain cases. However, further testing in humans is still required to confirm the advantages of this technique and evaluate its long-term effects.

CONCLUSION

The results obtained in this study demonstrated that alveolar corticotomy in rats caused greater proliferation of osteoclasts by day 3 on the pressure side and of osteoblasts by day 1 on the tension side. Differences between the treatment groups occurred only in the initial period of tooth movement. Therefore, the changes caused by corticotomy are not significant in orthodontic movement to justify the exposure of patients to this invasive procedure.

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CONFLICTS OF INTERESTS

The authors declare no conflicts of interest.

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