Optical Coherence Tomography Angiography in Penetrating Keratoplasty

Angiografia por Tomografia de Coerência Ótica na Queratoplastia Penetrante

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ABSTRACT

INTRODUCTION: Penetrating keratoplasty (PK) is one of the most common surgical procedure in corneal transplantation worldwide. Graft failure and rejection risk progressively increases with the increasing number of quadrants with corneal neovascularization (CNV). Optical coherence tomography angiography (OCTA) is a noninvasive imaging technique that has been widely used to visualize vascular abnormalities in the retina and some studies have shown its potential use in the anterior segment (AS) of the eye. The purpose of this study was to investigate the potential of OCTA technology to image and describe quantitatively CNV in eyes submitted to PK.

MATERIAL AND METHODS: A cross-sectional study was performed, including 20 eyes from 18 patients submitted to PK at least 5 years before and with no history of graft rejection. All eyes underwent anterior segment slit-lamp photography (SLP) and OCTA with en face, b-scans and c-scans imaging. The vessel density (VD) was analyzed in the inferior, nasal and temporal corneal margin in all patients. The measurements were calculated after binarization with *ImageJ* software, using OCTA scans with 6 × 6 mm in a depth of 800 µm.

RESULTS: The mean age was 59 years-old and most patients were submitted to PK due to corneal leucoma, followed by keratoconus, and a few had Fuchs endothelial corneal dystrophy and bullous keratopathy. The mean total VD was 50.16% and it was higher in the temporal quadrant and lower in the inferior one. However, there were no statistically significant differences between the 3 analyzed areas (p=0.801) or between each area and the other two and there was no correlation between the areas. OCTA was able to identify abnormal vessels when SLP apparently showed no abnormal vessels; OCTA was able to distinguish between larger and smaller vessels; OCTA scans allowed the investigation of several corneal planes.

CONCLUSION: OCTA can become a new method for monitoring corneal diseases. It may allow the qualitative and quantitative follow-up of patients submitted to PK over time and to detect the appearance of CNV earlier than through SLP. OCTA applied to the anterior segment has promising and valuable features.

KEYWORDS: Corneal Neovascularization; Fluorescein Angiography; Keratoplasty, Penetrating; Tomography, Optical Coherence.

RESUMO

INTRODUÇÃO: A queratoplastia penetrante (QP) é um dos procedimentos cirúrgicos mais realizados na transplantação corneana mundialmente. A falência do enxerto e o risco de rejeição aumentam progressivamente com o aumento do número de quadrantes com neovascularização corneana (NVC). A angiografia por tomografia de coerência ótica (OCTA) é um exame não invasivo que tem sido largamente utilizado na visualização de anomalias vasculares da retina e alguns estudos demonstraram a sua potencial utilização no segmento anterior do olho. O objetivo deste estudo foi investigar o potencial da tecnologia da OCTA na aquisição de imagens e descrição quantitativa da NVC em olhos submetidos a QP.

MATERIAL E MÉTODOS: Estudo transversal, com 20 olhos de 18 doentes submetidos a QP pelo menos 5 anos antes e sem história de rejeição do transplante. A todos os olhos foi realizada uma fotografia do segmento anterior na lâmpada de fenda (LF) e uma aquisição de imagens *en face*, *b-scans* e *c-scans* da OCTA. A densidade vascular (DV) foi analisada na margem corneana inferior, nasal e temporal em todos os doentes. A medição da DV foi obtida após criação de imagens binárias com o *software ImageJ*, usando imagens da OCTA com 6 × 6 mm numa profundidade de 800 μm.

RESULTADOS: A idade média dos doentes foi de 59 anos e a maioria foi submetido a QP por leucoma corneano, seguido de queratocone. A DV total média foi de 50,16% e foi mais alta no quadrante temporal e mais baixa no quadrante inferior. No entanto, não se verificou uma diferença estatisticamente significativa entre as 3 áreas analisadas (p=0,801) ou entre cada área e as duas restantes e não se verificou uma correlação entre as áreas. A OCTA foi capaz de identificar vasos anormais quando as fotografias na LF não os identificavam; a OCTA foi capaz de distinguir entre vasos de grande e pequeno calibre; a OCTA permitiu a investigação em diferentes planos corneanos.

CONCLUSÃO: A OCTA poderá tornar-se um novo método para a monitorização de doenças corneanas e permitir o seguimento qualitativo e quantitativo de pacientes submetidos a QP, com a deteção precoce de NVC.

PALAVRAS-CHAVE: Angiofluoresceinografia; Neovascularização da Córnea; Queratoplastia Penetrante; Tomografia de Coerência Óptica.

INTRODUCTION

Corneal disease is the fifth leading cause of blindness in the world affecting primarily the younger population and thus having a disability-adjusted life years much greater than other eye diseases that affect mostly the elderly such as cataracts or macular degeneration.[1] Corneal transplantation remains the main method for visual rehabilitation once the cornea has lost its transparency.[2] Even though the greatest burden of corneal blindness is in the developing countries, most corneal transplants are performed in developed ones. The main indication for transplantation in Europe is keratoconus, followed by keratitis.[1] Penetrating keratoplasty (PK), which involves the transplantation of the five corneal layers, is now the second most common surgical procedure in corneal transplantation worldwide, once it was over past by endothelial keratoplasty since 2011.[1]

PK survival depends on various factors which affect donor endothelial cell survival and which may lead to their loss and subsequent transplant failure.[2] PK in nonvascularized corneas has a 5-year survival rate up to 81%, even without histocompatibility matching or use of systemic prophylactic immunosuppressive therapies, being one of the most successful solid tissue transplantation procedures, which is attributed to the immunological privilege of the cornea. However, this rate is markedly reduced when the surgery is performed in "high-risk" corneas which are the ones that have corneal neovascularization (CNV) in 2 or more quadrants or an inflamed recipient bed (pre-existing inflammatory processes or a previously rejected graft).[3-5] Graft failure and rejection risk progressively increases with the increasing number of quadrants affected. Furthermore, postgraft neovascularization may also be a risk factor for transplant rejection in low and high-risk corneas.[3]

To evaluate CNV, the use of slit lamp photography is limited and does not allow the performance of quantitative measurements.[6] On the other hand, the use of angiography techniques, which utilizes intravenous injections of fluorescein and indocyanine green as contrast dyes, provides better details but is time-consuming and is associated with risks of serious adverse events.[7,8] Optical coherence tomography angiography (OCTA) is a noninvasive imaging tech-

nique that has been widely used to visualize vascular abnormalities in the retina and some studies have shown its potential use in the anterior segment (AS) of the eye.[8-11] This imaging technique also provides accurate depth information on the flow signal in its corresponding cross-sectional image. Thus, OCTA may supply an additional method for the diagnosis and assessment of grafts neovascularization after PK.[8] The purpose of this study was to investigate the potential of OCTA technology to image and describe quantitatively CNV in eyes with PK.

MATERIAL AND METHODS

We conducted a cross-sectional study in order to analyze vessel density (VD) measurements in patients submitted to PK, followed by the Corneal Department of our hospital. An approval of the Ethic Committee of our hospital to the realization of this investigation was obtained. The research adhered to the tenets of the Declaration of Helsinki. All patients completed and signed informed consent forms.

Twenty eyes from 18 patients were included in the investigation, all submitted to surgery at least 5 years before and with no history of graft rejection. Demographic and clinical data were evaluated: age, sex, previous diseases of the transplanted eyes, best-corrected visual acuity (BCVA), intraocular pressure (IOP), slit-lamp biomicroscopy of the anterior and posterior segment and specular microscopy. First, an AS slit-lamp photography (SLP) was taken from each patient through a digital slit-lamp camera system (SL-D Digital Slit-Lamp; Topcon, Tokyo, Japan) with a standard diffuse illumination (x10 magnification, 45º angle). After that, OCTA images of the inferior, nasal and temporal corneoscleral margin were obtained. VD measurements were performed through binary vessel maps that were generated from OCTA slabs analysed using *ImageJ* software.

OCT ANGIOGRAPHY

In OCTA, blood flow is detected through the decorrelation signal generated from the red blood cell motion through consecutive A-scans of the same location, generating a volumetric blood flow analysis. In our investigation, all OCTA scans were conducted by one expert professional operator. The OCTA system used to take the OCT angiograms of the AS was the spectral domain OCTA system Avanti XR AngioVue (Optovue, Inc., Fremont, CA, USA). The Angio Retina mode (split-spectrum amplitude-decorrelation angiography) was adapted for AS imaging by coupling the long cornea adaptive module lens (CAM-L; Optovue, Inc). In each image acquisition, the patient was initially correctly positioned in the device and the observation room was put on dim illumination. A manual adjustment of the cornea adaptor lens was performed until the corneal surface appeared in the OCT window (approximately 2 to 3 cm). Patients were instructed to look in different positions, placing a fixation point on the right, left and on top of the patient in order to expose the different parts of the conjunctiva (nasal, temporal and inferior). Due to the lack of a standard protocol for imaging of the AS OCTA, 6 × 6 mm scans (250 × 250 pixels) to a depth of 800 µm were performed in inferior, nasal and temporal quadrants along the limbus. A square of 2.5 × 2.5 mm (150 × 150 pixels) tangent to the corneoscleral margin, which comprises the most part of corneoscleral margin vascular networks, at 3 o'clock, 6 o'clock and 9 o'clock bilaterally was defined as the representative region of interest (ROI) (Fig. 1). The ROI for VD measurement were validated by two different researchers.[9,10]

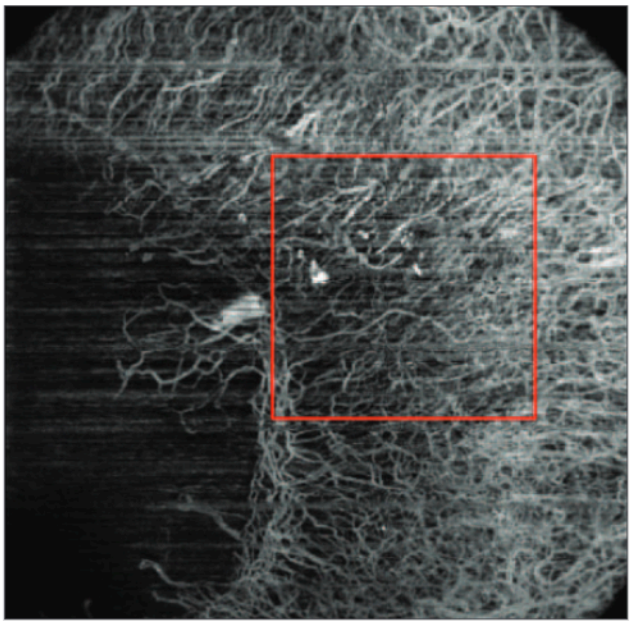

Figure 1. Representative ROI in the OCTA slab. The red square shows the 2.5 x 2.5 mm (150 x 150 pixels) ROI used to assess vascular density.

IMAGE PROCESSING AND VESSEL DENSITY MEASUREMENTS

The OCTA images were exported from the equipment in Portable Network Graphics (PNG) image file into Fiji Software, a distribution of the open-source Java-written software *ImageJ*, (V.1.49p, National Institutes of Health, Bethesda, Maryland, USA)[12] focusing on biological-image analysis. VD was defined as the percentage area occupied by the large vessels and microvasculature in the analyzed region. To determine the VD, the OCTA images first were

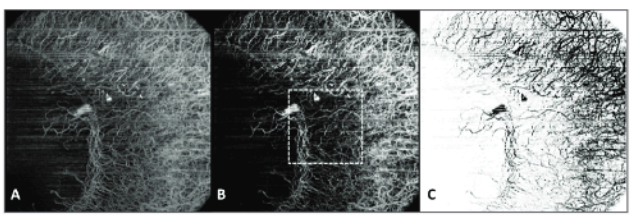

Figure 2. Illustration of OCTA image analysis. A. OCTA images were imported as 8-bit color images in the ImageJ software; B. The contrast was enhanced to reduce the surrounding noise in the ROI (contrast limited adaptative histogram equalization filter); C. The images were converted to binary using locally calculated thresholds.

binarized and then converted to an 8-bit image for processing (Fig. 2). To improve image quality, the program adjusts threshold through the Shanbhag method which highlights blood vessels and reduces the surrounding noise and adjusts the contrast using the Equalize Histogram of this algorithm. The percentage of vessels was calculated by taking the ratio of the total vessel area to the total area of the analyzed region (area with the highest number of whites: vessel pixels = 1; background = 0). Only clear images were studied, and images with poor quality (quality index < 3), caused by motion artifacts such as poor fixation and blinking artifacts, were excluded.[9,10]

RESULTS

The study included a total of 20 eyes from 18 patients, 9 female and 9 male. The mean age was 59 years-old. Patients had a mean BCVA of 0.4 (logMAR) and a normal mean IOP. The eyes had been submitted to surgery 11 years ago (mean time), with a minimum of 5 and a maximum of 20. Regarding the specular microscopy, the mean cell density (CD) was 1400 cells/mm^2 and the mean central corneal thickness (CCT) was 576 µm. Most patients were submitted to PK due to corneal leucoma, followed by keratoconus, and a few had Fuchs endothelial corneal dystrophy (FECD) and bullous keratopathy. This data is presented in Table 1.

In what concerns OCTA imaging evaluation, the results and statistical analysis are presented in Table 2. The mean total VD was 50,16% and it was higher in the temporal quadrant and lower in the inferior one. However, there were no statistically significant differences between the 3 analyzed areas (p=0.801) or between each area and the other two (Table 3). Also, there was no correlation between the areas (Table 4).

The photographs taken with the slit-lamp were compared qualitatively with the OCTA images to demonstrate possible advantages of this technique. The first case (Fig. 3) corresponds to a 40-year-old patient who was submitted

Table 1. Demographic and clinical characterization of the sample.

		n (valid)	Mean ± SD	Minimum	Maximum
Age (years)		18	59 ± 19	28	83
BCVA (logMAR)		20	0.4 ± 0.55	+1.3	0.0
IOP (mmHg)		20	16 ± 2	12	20
Graft time surgery (years)		20	11 ± 5	5	20
Specular microscopy	CD (cells/mm^2)	20	1400 ± 340	714	1932
	CCT (µm)	20	576 ± 62	474	675
Previous diseases	Leucoma (n)	9	-	-	-
	Keratoconus (n)	8	-	-	-
	FECD (n)	2	-	-	-
	Bullous Keratopathy (n)	1	-	-	-

Table 2. VD in the three quadrants evaluated and in total.

AREA	Mean VD (%) ± SD	Minimum	Maximum	Shapiro-Wilk	p-value of Shapiro-Wilk
Inferior	49.99 ± 2.47	46.16	56.36	0.928	0.138
Nasal	50.02 ± 2.75	44.55	55.03	0.970	0.758
Temporal	50.47 ± 2.44	43.73	54.45	0.906	0.053
Total	50.16 ± 2.52	43.73	56.36	-	-

Table 3. Comparison between the areas VD.

TEST		Quadrant	Mean difference	SD	p-value	95% confidence interval for difference	
						Lower bound	Upper bound
One-Way ANOVA test	**Between groups**		-	-	0.801	-	-
Bonferroni	Inferior	Nasal	-0.034	0.808	1.000	-2.026	1.959
		Temporal	-0.483	0.808	1.000	-2.476	1.510
	Nasal	Inferior	0.034	0.808	1.000	-1.959	2.026
		Temporal	-0.449	0.808	1.000	-2.442	1.543
	Temporal	Inferior	0.483	0.808	1.000	-1.510	2.476
		Nasal	0.449	0.808	1.000	-1.543	2.442

a. Adjustment for multiple comparisons: Bonferroni.

Table 4. Correlation between the areas VD.

	Temporal	Nasal	Inferior
Pearson's r temporal	-		
p-value	-		
Pearson's r nasal	0.25	-	
p-value	0.39	-	
Pearson's r inferior	0.34	0.33	-
p-value	0.15	0.16	-

to a PK on his right eye due to a leucoma. In the SLP (Fig. 3A) it is possible to observe a clear graft without abnormal vessels; en face OCTA image (Fig. 3B) is able to localize vessels in the edge and central area of the graft not visible

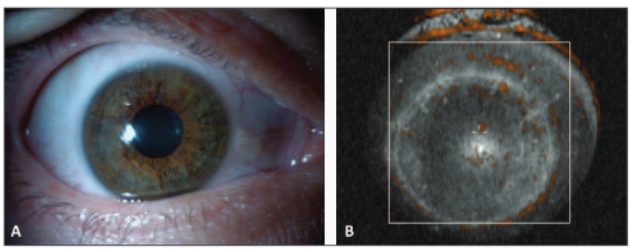

Figure 3. Clinical case 1 – A. Right eye SLP showing a clear graft; B. vessels at the edge and central area of the graft.

in the SLP. The second case (Fig. 4) also corresponds to a 67-year-old patient with a previous leucoma on her left eye. SLP also shows a clear graft in an innocent limbus (Fig. 4A); OCTA allows the distinction between smaller (Fig. 4B) and larger (Fig. 4C) corneal and limbal vasculature. The third

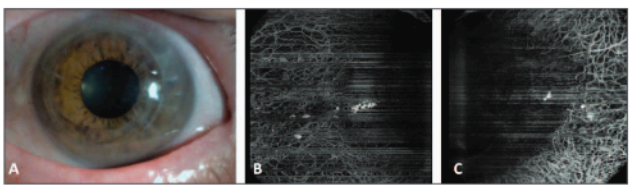

Figure 4. Clinical case 2 – A. Left eye SLP showing a clear graft in an innocent limbus; B. smaller nasal corneal and limbal vessels; C. larger temporal corneal and limbal vessels.

case (Fig. 5) is another example of a patient with a previous leucoma, 75 years old, but with a hyperemic limbus at the SLP (Fig. 5A). The graft is clear despite the visible vessels at the limbus and corneal receptor bed. OCTA demonstrates

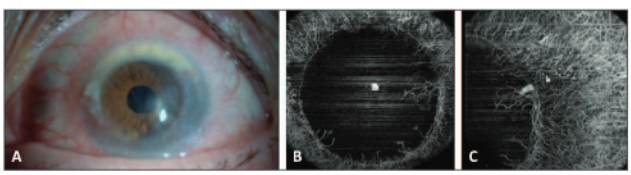

Figure 5. Clinical case 3 – A. Left eye SLP showing a hyperemic limbus; B. dense and smooth vascularization within the graft; C. dense and smooth vascularization at the graft's temporal edge.

a dense as well as smooth vascularization within the graft and at the graft's edge (Figs. 5B and 5C). The fourth case is one of a 28-year-old patient with a keratoconus (Fig. 6) submitted to a PK, with a clear graft in the SLP (Fig. 6A). It is possible to observe several corneal planes acquired through the OCTA. Using the defined retinal protocol, the C-scans are determined by two parallel lines automatically delineated, with the default thickness of 50 µm. A posterior manual modification is possible in order to know the extent of corneal pathology and abnormal vasculature. Analysis were made in the nasal and superficial vascularization (50-150 µm) (Fig. 6B), nasal and anterior vascularization (150-200 µm) (Fig. 6C) and nasal and deep vascularization (250-300 µm) (Fig. 6D).

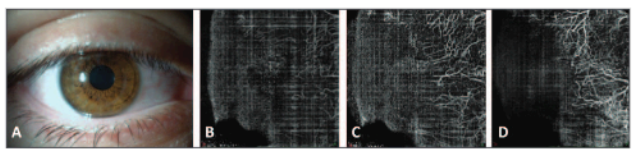

Figure 6. Clinical case 4 – A. Left eye SLP showing a clear graft at an innocent limbus; Corneal nasal planes acquired with OCTA: B. 50-150 µm; C. 150-200 µm; D. 200-300 µm.

DISCUSSION AND CONCLUSION

This investigation describes the use of OCTA imaging in the evaluation of CNV in patients submitted to PK. Inappropriate and pathologic blood vessels formation and infiltration of the clear matrix of the cornea, besides leading to edema, exudation of lipids and corneal scarring, increases the risk of graft rejection after PK. Since traditional methods for evaluating the CNV are imprecise such as SLP or invasive and time-consuming such as angiography, noninvasive imaging modalities to objectively assess these patients are warrant.[13] OCTA detects erythrocyte movement via motion contrast within the vessels by comparing sequential OCT cross-sectional scans.[13,14] It can be used in an en face orientation to provide a projection map of CNV or in a volumetric way to evaluate CNV in three dimensions in order to quantify vessel depth with an in vivo assessment.[13]

Immune rejection remains the main cause of graft failure following PK and CNV is its principal risk factor. This occurs because PK promotes the growth of blood and lymphatic vessels into the avascular recipient bed leading to the loss of the immune-privileged status of the normal

cornea, since it provides a passage for the transportation of alloantigens from the ocular surface to the peripheral lymphoid organs and of the immune cells back into the graft.[4,15] It is thought that the sutures of the PK induce local release of proinflammatory cytokines and mobilization of macrophages which express high levels of lymphangiogenic factors. These macrophages may also derive from the iris-ciliary body and can also promote the initiation of the immune cascade (especially for endothelial immune rejection).[15] Abnormal CNV may arise from the corneal marginal arcade or the limbal palisades vessels, therefore objective imaging is important to understand the etiology and potential treatment of corneal pathological features.[14]

Patients in our study had a wide range of ages, varying from 28 to 83 years old. This variation, along with other elements such as gender, previous diseases or other ocular factors, may influence vessel size and branching of similar corneal quadrants between subjects[14]; however, the aim of our investigation was to demonstrate the possible useful application of OCTA in PK rather than a detailed description of the corneal vasculature of each patient.

We observed no statistically significant differences between the vascularization in the quadrants evaluated or any statistical correlation between them. Nevertheless, with the clinical cases presented, we have seen that OCTA allows us to detect CNV in patients that apparently had a clear graft without blood vessels invasion; distinguish between smaller and larger vessels; monitor vessels in patients with SLP alterations; and study corneal vascular planes. These four examples demonstrate the possible utility in our daily practice of this imaging technique, with a system that is rapid and non-invasive. OCTA may be a promising tool to monitor changes in corneal vascular areas and treatment response.[16]

One of the main advantages of OCTA is the fact that it acquires images in a rapid way, with no contact, even though the current systems are not designed for the AS and they need to be adapted to it. Still, this technique has great potential for clinical application in the AS.[17-19] At the moment, diseases are monitored through serial SLP and this can become a new method for their monitoring, not only to observe the response of diseases to treatments but also to detect the appearance of CNV or its recurrence. OCTA may allow the qualitative and quantitative follow-up of patients submitted to PK over time.[20] In these patients, vessels localization may allow us to create possible patterns of graft neovascularization concerning the type of transplantation; and the precise location of CNV may be useful for guiding treatments, for instance the use of fine needle for deep stromal injections of bevacizumab.[9] Therefore, this tool can become useful to allow an earlier detection of patients at greater risk.

Despite the possible advantages of OCTA applied to the AS, some limitations must be noted. The lack of an eye-tracking system makes the occurrence of image distortion due to patient movement more frequent and may lead to artifacts. Since the equipment is optimized for the retina rather than the AS, image resolution may not be the best to distinguish between conjunctival and scleral vessels and between normal and abnormal vessels. In our investigation, being a descriptive study, the lack of a comparison group was a limitation. When comparing with another similar study performed with glaucoma patients, we observed that VD of patients with healthy eyes was 32.13% on the nasal side and 29.67% on the temporal side, a lower value than that observed for the patients in our investigation (50.05% and 50.47%, respectively).[21] Moreover, the delineation of the major extent of CNV was performed manually and did not include the superior quadrant due to technical difficulties (presence of lashes and more time of image acquisition). In cases of PK, it is known that lympangiogenesis also plays an important role in CNV, mainly in immune rejections[15]; however, lymphatic fluid is clear and does not have blood cells to provide a backscattered OCT signal using current techniques, therefore it was not assessed in this study. Our study was performed with a small sample of eyes, but it may be the basis for future investigations.

This study demonstrated that OCTA may be a useful tool in the assessment and monitoring of patients submitted to PK. It may be able to detect early CNV in the graft and allow early intervention and a closer follow-up. This ancillary test has the potential to be included into the flow of the clinical work along with the evaluation through the slit lamp, allowing a follow up of the corneal changes in eyes submitted to PK in multiple subsequent office visits, comparing the current exam to previous ones. OCTA applied to the anterior segment has promising and valuable features that can be routinely used in our daily clinical practice.

CONTRIBUTORSHIP STATEMENT / DECLARAÇÃO DE CONTRIBUIÇÃO:

CPA, JJ e IA: Contribuição intelectual substancial, direta, no desenho e elaboração do artigo; análise e interpretação dos dados; redação do manuscrito, revisão de versões e revisão crítica do conteúdo; aprovação da versão final.

LD, SG, MJM, VM, JA e JCP: Contribuição intelectual substancial, direta, no desenho e elaboração do artigo; -análise e interpretação dos dados e aprovação da versão final.

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Proteção de Pessoas e Animais: Os autores declaram

que os procedimentos seguidos estavam de acordo com os regulamentos estabelecidos pela Comissão de Ética responsável e de acordo com a Declaração de Helsínquia revista em 2013 e da Associação Médica Mundial.

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Protection of Human and Animal Subjects: The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki as revised in 2013).

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