# **ORIGINAL PAPER**



# Efficacy of finisher files in the removal of calcium hydroxide paste from the root canal system – preliminary results

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## ABSTRACT

**Introduction.** Successful endodontic treatment is affected by a number of factors associated with the disinfection and filling of the root canal. The chemical-mechanical root canal preparation consists in a thorough removal of any content from the pulp space, including inflamed pulp, bacteria, as well as canal filling materials. **Aim.** The aim of the study was to analyse the efficacy of the XP-endo Finisher and the Brush-Finisher on the removal of a calcium hydroxide dressing.

**Material and Methods.** The study was conducted using extracted single-rooted human teeth prepared according to sample standardization. Calcium hydroxide with iodoform was inserted into the canals. After two weeks, canal cleaning was performed with the use of 2% sodium hypochlorite solution and both finisher files. A conventional endodontic needle and syringe (SNI) were used in the control group. Following rinsing activation, two projection radiographs were performed and uploaded to software developed specifically for the study. The graphic files were evaluated in terms of the remaining amount of dressing. In order to analyse whether the percentage of the canal area that remained untreated was statistically significant, the Kruskal-Wallis ANOVA test with Dunn's post-hoc test were employed.

**Results.** The intracanal dressing was most effectively removed in the XP-endo Finisher group (in both projections 96.32% and 91.35%), and its removal was considerably better than that in the control group (p<0.0001), although not significantly different from the Brush-Finisher group (89.68% and 81.85%).

**Conclusions.** Supplementary irrigant activation with either the XP-endo Finisher or the Brush-Finisher improved the removal of calcium hydroxide from the root canal walls.

# Introduction

Prior to a root canal obturation, the intracanal dressing has to be completely removed from the

canals in order to minimize its negative impact on the treatment prognosis [1]. A variety of different techniques have been used for the removal of material including stainless steel hand files, sonic

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instruments and ultrasounds, rotary instruments, as well as lasers. Sonic and ultrasonic devices are reported to be more effective; however, until now there has been no universal consensus regarding the most effective method of removing intracanal calcium hydroxide (Ca(OH)<sub>2</sub>) [2]. In endodontics, Ca(OH)<sub>2</sub> paste is the most commonly used intracanal medication, due to its antibacterial efficacy against the majority of endodontic pathogens and to its biocompatibility [3,4]. Nevertheless, residual parts the dressing which remain on canal walls, interact with some sealers, thus changing their physical properties, as well as reducing flow and setting time. Furthermore, the aforementioned residues also prevent the penetration of a sealer into the dentinal tubules, and may increase apical leakage of gutta-percha root fillings [1,5]. In fact, some clinicians consider abandoning the use of calcium hydroxide, since it cannot be completely removed from the entire canal system. However, the treatment of the infected canals, e.g. cases with the open apex and perforation or apexification procedures, still may require the use of this material. Therefore, the objective of the present study was to compare the effectiveness XP-endo Finisher and the Brush-Finisher files in removing calcium hydroxide paste from wide root canals.

# Material and Methods

The Bioethics Committee of the Medical University of Lodz approved this experimental study (approval no. RNN/3988/19/KE of 12th December 2019). One hundred and fifty extracted human single-rooted teeth obtained from the Department of Dental Surgery at the Medical University of Lodz (Poland) and a private orthodontic dental clinic were radiographed (in two dimensions) in order to select those with single canals, curved up to 10 degrees (according to the Schneider' methods), without any canal fillings, calcifications or resorption. Thirty teeth (samples) were selected for the preliminary examinations. They were subsequently stored in 10% buffered formalin solution until use, and randomly divided into three groups: Group I (XP-endo Finisher, FKG Dentaire; La Chaux-de- Fonds, Switzerland), Group II (Brush-Finisher, MedicNRG, MedicNRG, Kibbutz Afikim, Israel) and Group III (conventional syringe and endodontic needle irrigation, SNI) consisting of 10 samples each as a preliminary study. The crowns were removed using a diamond disk to provide roots measuring 15 mm ± 1 mm in length. On the basis of the radiographs taken in two projections and the measurement of the apical foramen performed by means of hand files, wide root canals groups were established. If it was possible to wedge a hand initial file, larger than number 30 ( $\geq$ 0.3 mm), at the apical foramen, the canal was classified into a large root canal group (LC group, provided the middle and coronal part of the were loose (Figure 1). The ProTaper Next file number X3 (size 0.30 and taper: 7.5%; Dentsply Maillefer, Ballaigues, Switzerland), was introduced loosely (without a shaping procedure) up to the apical foramen to remove residual parts of the pulp and confirm the right classification of the canal width.

Following a thorough irrigation, using 2% NaOCI (5 ml) and 17% EDTA (5 ml), all canals were dried with paper points and filled with calcium hydroxide paste with iodoform (Calcipast



**Figure 1.** Classification of the canal as a wide root canal. If hand initial file was used first, larger than number  $30 (\ge 0.3 \text{ mm})$ , wedged at the apical foramen, the canal was classified as belonging to the large root canal group (LC group).



Figure 2. A buccolingual and mesiodistal radiograph of the canal filled with calcium hydroxide with iodoform paste

J, Cerkamed, Poland) (placed at 1 mm short of the apical foramen and withdrawn during dressing application) using an applicator. Additionally, the cavities were temporarily closed with materials based on zinc oxide and zinc sulphate. Next, each sample was placed into a silicone model in order to take two radiographs in the buccolingual and mesiodistal projection (GEN-DEX expertDC, 65kV, 7mA, 0.250 s, KaVo, Brea, USA) (Figure 2). If there was any void observed in the canal filling, the procedure was repeated until the sealing was acceptable. Subsequently, all samples were stored in an incubator at 37°C and 100% humidity for 14 days. Finally, the intracanal dressing was removed using three methods described below.

## Group I

In the first group, the XP-endo Finisher files were used. The task was performed by one operator according to the manufacturer's instructions. The speed was 1000 rpm and the torque amounted to 1 Ncm. The working length was set using a plastic tube (a manufacturer's ruler). During the rotations, the file was worked in vertical movements in order to reach the full working length, and new portions of the solution were delivered continuously. The irrigant was heated up to 40°C and subsequently activated for approximately 15 seconds inside the canal. The procedure was repeated three times using 5 ml of the solution (working time: 1 minute ± 5 seconds). Decrease in the temperature of the solution (below 36°C) was minimized by a cyclic procedure of 15-second agitations and proper environmental conditions. The ambient temperature was approximately 20-26°C. The XP-endo Finisher files were cleaned from the residual parts of the dressing between individual work stages using abundant water irrigation and gauze, and their status was inspected. Finally, the "last drop" of the solution was aspirated - the canals were not dried using paper points, but allowed to dry for 3 minutes and subsequently radiographed.

## **Group II**

In the second group, the Brush-Finisher was applied, which is an additional instrument to the Gentlefile system. The task, as previously, was conducted by one operator according to the manufacturer's recommendations. The needle was inserted into the canal orifice, and the rinsing solution (5 ml) was introduced continuously (as in the previous group). Then, it was agitated by pecking motions up to the working length for approximately 1 minute  $\pm$  5 seconds. A short break provided every 15 seconds after activation in order to clean the Brush-Finisher and check the condition of the instrument. The remaining solution was aspirated and after a few minutes two radiographs of the canal were taken.

#### Group III

In the control group (SNI), endodontic syringes (with Luer-Lock, 5 ml) and needles (with a lateral

opening, measuring 0.3 mm x 25 mm, EndoTop, Cerkamed, Poland) were used in a longitudinal and swivel movements. The rinsing was performed for approximately 1 minute by one operator. Finally, the remaining solution was aspirated and the radiographs were taken after 3 minutes.

In all the groups, one irrigating solution of the same volume, i.e. 5 ml of 2% NaOCl was applied. Each tooth specimen was coated entirely with silicone material and placed in a special holder, so as to protect it from the apical extrusion of the dressing, as well as from the operator's impact on the irrigation procedure. In group I, a special model bath was created to ensure a required body temperature. Following the dressing removal, two projection radiographs were repeated using a designed platform which ensured the same radiological position. Some samples with wide canals remained in excellent condition, and were further distributed to both groups where



Figure 3. From left: a – The outlined and excited apical part of the canal space; b – The outlined and excited middle part of the canal space; c – The outlined and excited coronal part of the canal space



**Figure 4.** Analysis using custom-made software showing 95.5% of  $Ca(OH)_2$  filling in the middle part of the canal. The first image (on the left) shows the middle part of the canal in the radiograph, the second image (on the right) presents pixels (PX) corresponding to the dressing residues

two different instruments were applied. All those canals were cleaned with an air abrasive technology, controlled radiographically and re-filled with a dressing. Subsequently, the images were uploaded to the graphic software (GIMP 2.8.22) and the root canals were divided into three parts: apical, middle and coronal. In order to make the image more legible for the analysis, graphic tools were applied to mark the canal space (Figure 3a, 3b, 3c). Next, new, custom-made software was applied to calculate pixel quantity (PX), corresponding to the dressing residues, and to convert the obtained values to a percentage of the canal surface area which remained untreated (Figures 4, 5, Table 1). Evaluations were performed twice, by one operator who was blinded to the study group.

# Statistical analysis:

All the results were presented as a mean with a standard deviation and medians with interquartile range (IQR). The Kruskal-Wallis ANOVA test was used with the Dunn's post-hoc test in order to compare differences between >2 independent variables. A *p* value below 0.05 was considered statistically significant. All calculations were performed using Statistica 13 software.

## Results

In terms of the entire canal length, the Kruskal-Wallis ANOVA test indicated that the SNI group presented poorer scores than the other groups



**Figure 5.** Analysis using custom-made software showing 3.54% of the remaining material in the middle part of the canal following the dressing removal. The first image (on the left) demonstrates the middle part of the canal in the radiograph, the second image (on the right) presents pixels (PX) corresponding to the dressing residues

 Table 1. Table presents an excel sheet clipping of representative scores showing the process of calculation the percentage (2.22%) of unremoved calcium hydroxide dressing from the medial part of the canal after calibration procedure

Tooth code	Void	PX without voids	PX with voids	[%] canal	[%] apical	[%] middle	[%] coronal	[%] canal†	[%] apical†	PX middle*	PX middle after calibration**	PX middle remained***	[%] medial†	[%] coronal†
	Before Ca(OH) <sub>2</sub> removal						Afte	r Ca(OH) <sub>2</sub> ren	noval					
4.3C	Yes	56609	51621	91.18	78.22	95.33	92.68	7.27	4.97	17939	17101.24	380	2.22	8.08

[%] canal/apical/medial/coronal, actual amount of  $Ca(OH)_2$  filling expressed as a percentage; PX medial\*, pixels of the image when the medial part was filled completely; PX medial after calibration\*\*, pixels of the image taking into account that the medial part of the canal was filled incompletely (with voids); PX medial remained\*\*\*, pixels of the image of the remaining  $Ca(OH)_2$  in the medial part of the canal; [%] medial†, actual amount of the remaining  $Ca(OH)_2$  in the medial part of the canal; [%] medial†, actual amount of the remaining  $Ca(OH)_2$  in the medial part of the canal; [%] medial†, actual amount of the remaining  $Ca(OH)_2$  in the medial part of the canal; [%] medial†, actual amount of the remaining  $Ca(OH)_2$  in the medial part of the canal expressed as a percentage.

(P< 0.0001) (Table 2), both with regard to the buccolingual, as well as the mesiodistal projection. In the mesiodistal projection, the median residue dressing amounted to 77.29% in the SNI group, 22.68% in the Finisher-brush group and 11.75% in the XP-endo Finisher group (P=0.0001). None of the investigated groups achieved 100% cleaning efficacy in removing calcium hydroxide from the root canal walls. The XP-endo Finisher removed the largest amount of the canal dressing and its results were superior in the apical and middle portions of the canal (in the BL=buccolingual projection 96.32% and 96.92%;

and in the MD=mesiodistal projection 91.35% and 92.79%), as compared to the coronal portion (in the BL=buccolingual dimension 91.3%, and in the MD=mesiodistal projection 87.63%) (Table 3). In the Finisher-brush group, the observed scores were close to those of the XP-endo Finisher files, without a significant difference (in the BL projection 89.68% and 90.01%; and in MD projection 81.85% and 79.46%) (Table 3). In all the groups, the largest amount of the remaining intracanal dressing was found in the coronal thirds (Fig. 6a, Fig. 6b). In both Finisher groups, the difference was statistically insignificant (Table 4).

**Table 2.** Remaining  $Ca(OH)_2$  [%] in the whole canal length concerning the control group, the Brush-Finisher and XP-endo Finisher group in buccolingual and mesiodistal projection

Canal %	SNI		Brush-	Finisher	XP-endo Finisher		P-value	
	B-L	M-D	B-L	M-D	B-L	M-D	-	
Median	63.81	77.29	12.46	22.68	5.05	11.75	<0.0001	
1 <sup>st</sup> quartile	55.36	64.45	9.44	14.00	1.83	6.00		
3 <sup>rd</sup> quartile	66.05	81.68	19.01	32.69	15.83	26.02		
Min.	32.89	37.78	5.13	12.30	0.30	0.40		
Max.	76.90	91.10	36.34	61.87	22.66	39.75		

Data was presented as medians (squares) with interquartile range (boxes) and min-max values (whiskers). The Kruskal-Wallis ANOVA test was used.

**Table 3.** Residual  $Ca(OH)_2[\%]$  in the SNI, XP-endo Finisher and the Brush-Finisher group in the buccolingual and mesiodistal projection in the apical, middle and coronal portion of the canal

<b>Canal portion</b>	SNI	X-Pendo Finisher	Brush-Finisher	p-value
apical %	32.99	3.68	10.32	0.0061
middle %	56.39	3.08	9.99	0.0001
coronal %	78.22	8.70	16.15	<0.0001
apical %	54.26	8.65	18.15	0.0058
middle %	74.57	7.21	20.54	0.0001
coronal %	71.56	12.37	23.18	0.0001
	Canal portion apical % middle % coronal % apical % middle % coronal %	Canal portion         SNI           apical %         32.99           middle %         56.39           coronal %         78.22           apical %         54.26           middle %         74.57           coronal %         71.56	Canal portion         SNI         X-Pendo Finisher           apical %         32.99         3.68           middle %         56.39         3.08           coronal %         78.22         8.70           apical %         54.26         8.65           middle %         74.57         7.21           coronal %         71.56         12.37	Canal portionSNIX-Pendo FinisherBrush-Finisherapical %32.993.6810.32middle %56.393.089.99coronal %78.228.7016.15apical %54.268.6518.15middle %74.577.2120.54coronal %71.5612.3723.18

Data were presented as medians. The Kruskal-Wallis ANOVA test was used.



**Figure 6a.** The chart presents results in the apical third of the canal in all groups (buccolingual projection). The significant difference is marked between the tested groups. The Kruskal-Wallis ANOVA test was applied with the Dunn's post-hoc test



**Figure 6b.** The chart presents the results in the coronal third of the canal in all groups (buccolingual projection). The significant difference is marked between the tested groups. The Kruskal-Wallis ANOVA test was applied with the Dunn's posthoc test

Table 4. A comparison of the significant differences between the SNI, XP-endo Finisher	and the Brush-
Finisher group in the buccolingual and mesiodistal projection in the apical, middle and co	onal portion of
the canal	

Buccolingual d	limension	X-	Pendo Finisl	ner	Brush Finisher		
		apical %	middle %	coronal %	apical %	middle %	coronal %
SNI	apical %	0.0049	-	-	ns	-	-
	middle %	-	0.0001	-	-	0.0144	-
	coronal %	-	-	0.0000	-	-	0.0029
X-Pendo Finisher	apical %				ns	-	-
	middle %				-	ns	-
	coronal %				-	-	ns
Mesiodistal d	imension	Х-	Pendo Finis	her	B	Brush Finishe	er
Mesiodistal d	imension	X- apical %	Pendo Finis middle %	her coronal %	B apical %	rush Finishe middle %	er coronal %
Mesiodistal d	imension apical %	<b>X-</b> <b>apical %</b> 0.0041	Pendo Finis middle % -	her coronal % -	B apical % ns	Brush Finishe middle % -	er coronal % -
Mesiodistal d	imension apical % middle %	X- apical % 0.0041 -	Pendo Finis middle % - 0.0001	her coronal % -	B apical % ns -	Frush Finishe middle % - 0.0123	er coronal % - -
Mesiodistal d	apical % middle % coronal %	X- apical % 0.0041 - -	Pendo Finisl middle % - 0.0001 -	her coronal % - 0.0001	B apical % ns - -	rush Finishe middle % - 0.0123 -	er coronal % - - 0.0029
Mesiodistal d SNI X-Pendo Finisher	imension apical % middle % coronal % apical %	X- apical % 0.0041 - -	Pendo Finis middle % - 0.0001 -	her coronal % - 0.0001	B apical % ns - - ns	Brush Finishe middle % - 0.0123 - -	er coronal % - - 0.0029 -
Mesiodistal d SNI X-Pendo Finisher	imension apical % middle % coronal % apical % middle %	X- apical % 0.0041 - -	Pendo Finis middle % - 0.0001 -	her coronal % - 0.0001	B apical % ns - - ns -	rush Finishe middle % - 0.0123 - - ns	er coronal % - - 0.0029 - -

The Dunn's post hoc test was used.

## Discussion

The aim of this study was to compare the impact of the XP-endo Finisher, the Brush-Finisher, and the use of a syringe and an endodontic needle on the removal of an intracanal dressing. The null hypothesis that the irrigation groups do not differ from each other in removing calcium hydroxide from wide canals was rejected. The use of XPendo Finisher and the Brush-Finisher demonstrated a better efficacy in removing the dressing, with 94.95% and 87.54% of the calcium hydroxide removed, respectively. These results were applicable to the buccolingual dimension and did not differ significantly from each other. In contrast, they were considerably better than the results achieved in the control group (36.19%, P<0.0001). Furthermore, in the mesiodistal projection, the XP-endo Finisher and the Brush-Finisher also removed a large portion of the canal dressing (88.25% and 77.32%). Additionally, their results were significantly better than in the control group (22.71%, P=0.0001). These findings are in accordance with the observations made by Uygun et al. [6], who claim that the TRUShape 3D Conforming File and the XP-endo Finisher can be beneficial in removing calcium hydroxide from root-canal walls via continuous irrigation. In the present study, the XP-endo Finisher was compared to the Brush-Finisher.

In the literature, we found only two studies assessing Brush-Finisher's efficacy. Firstly, Neelakantan et al. [7] conclude that the use of the Finisher GF Brush (i.e. Brush-Finisher) improved the debridement of canals. Therefore, in our research, we confront files which both work at high speed (XP-endo Finisher: 800-1000 rpm and Brush-Finisher: 6500 rpm) and rotate in one direction. Moreover, their application in the course of the cleaning procedure is similar. According to the manufacturer, the files go in up-and-down movements with an amplitude of 7-8 mm for approximately 1 minute. Both instruments are made of different material (the XP-endo Finisher is made of Ni-Ti alloy, whereas the Brush-Finisher is made of stainless steel) and have a different design, thus, we may compare their impact on the effectiveness of Ca(OH)<sub>2</sub> removal. Irrigation protocols were highly standardized, performed with an exact positioning of tips, identical flow rates and activation times, executed by one person (an endodontist) and the analysis was performed twice with a double blinded test at a three-week interval. Interestingly, as shown in Table 3, the activation with the XP-endo Finisher and the Brush-Finisher resulted in a similar pattern of the intracanal dressing removal, as described by De Deus et al. [8]. The coronal portion of canals was cleaned less effectively than the other areas in both groups, but the difference was not statistically significant (P>0.01).

The material used in the study included extracted, single-rooted (with a single canal) human teeth, verified with radiographs and measurements of the apical foramen. Cases where a hand file larger than 30 (≥0.3 mm) was possible to be wedged at the apical foramen, were included into the wide root canal group, if both the middle and coronal portion of the file were loose. Apart from the advantages of a biological material and a great emphasis on the standardization of samples, this material possibly presents certain drawbacks, e.g. differences in the extent of the canals (e.g. taper, 3D dimensions). This is a common disadvantage of using extracted teeth; however, it does not exclude the suitability of the material. To address this, we created several groups (e.g. the XP-endo Finisher, the Brush-Finisher, and the control group), each of them consisting of a representative number of samples resulting from the preliminary research (thus, forming the separate wide and narrow canal groups). In the literature, there are numerous studies which involve the artificial groove model [9], whereby such a pre-drilled groove is assumed to imitate the canal isthmus and irregularities. According to the model described by Lee et al. [10], it is 4 mm long, 0.5 mm deep and 0.2 mm wide. Nevertheless, this model has not been chosen for our study, since it does not represent the complexity of the physiological root canal anatomy [9,11] and does not correspond to the anatomical conditions present in wide canals.

In addition, apart from the high magnification image analysis, the artificial groove model allows to evaluate the presence of a dressing still in two dimensions (length and width). In other studies, cone-beam computed tomography is used for the calculation of remaining Ca(OH)<sub>2</sub>. This technique enables volumetric analysis, however, it may also be based on particular scans of the root canal sections and uniform grayscale threshold to visualize and quantify the volume of the residual Ca(OH)<sub>2</sub> material [12]. Nowadays, MicroCT is one of the most effective methods, although remains still quite unavailable [13]. In this study, we provided the same protocol conditions taking the canal radiographs in two projections following the dressing insertion and after its removal. Image acquisition was standardized by means of repetitive sample positioning (with the use of a custom-made platform) and a uniform radiological setting. Moreover, new, custom-made software was used to calculate pixels quantity (PX), corresponding to the dressing residues, and finally to convert them to the percentage of the canal surface area which had not been cleaned. In fact, similar software was used in another study assessing biofilm-mimicking hydrogel removal [14].

Since numerous articles indicate serious difficulties in removing intracanal dressing from the root canal system, in our study we decided to use calcium hydroxide with iodoform as endodontic medication [9,15]. Firstly, it is vital to bear in mind that a thorough debridement of the apical part of the canal constitutes an exceptional impediment [16]. Another issue is obtaining optimal cleanliness of oval or very wide canals, as the use of mechanical instrumentation might be limited due to the necessity of preserving root tissue [17]. Nevertheless, it is crucial that the canal system be hermetically filled with an intracanal dressing. Thus, we inserted calcium hydroxide paste with an applicator, beginning the insertion from the apical to the coronal part of the canal, and its homogeneity was confirmed radiologically. Any smallest void visible in the canal path was accounted for before and after pixel calculation. This resulted in the objective outcome variable, which constitutes the percentage of the remaining calcium hydroxide paste. A similar technique was employed in other studies, e.g. assessing cleaning efficacy during root canal retreatment [18,19].

The most commonly used method of removing  $Ca(OH)_2$  is a combination of abundant rinsing and the preparation with a master apical file (MAF) of the entire working length [20]. According to the literature, one of the most effective methods of removing dressing from the apical third of the canal is ultrasonic rinsing activation [21]. On the other hand, Wigler et al. [22] compared the ultrasonic technique with the XP-endo Finisher activation lasting 1 minute and achieved comparable results. Moreover, there was also no statistically significant difference between the XP-endo Finisher and ultrasonic activation in the study by Leoni[23]. In our study, the best efficacy of removing the dressing from the apical part of the canal was obtained in the XP-endo Finisher group in both dimensions (96.32% and 91.35%). Additionally, similar results were achieved in the Brush-Finisher group (89.68% and 81.85%) without any significant difference. In fact, the XP-endo Finisher is very flexible and it can expand during rotation. Additionaly, the design of the Brush-Finisher's may affect the cleaning efficacy due to splitting its filaments in the course of application and reaching the canal surface in the apical portions. This, in turn, is in accordance with the results of Hristov and Gateva [24], who compared six irrigation protocols eliminating bacteria in root canals of immature permanent teeth. They reported that the reduction in the number of colony-forming units was significantly superior in the XP-endo Finisher (and EDTA) group and the Gentlefile brush (and citric acid, i.e. Brush-Finisher) group, which may support the findings in the present study.

Since the 1-min operation time was not sufficient for the effective removal of Ca(OH)<sub>2</sub> from the canals in this study, longer activation periods should be verified before formulating final, probably more comprehensive, conclusions. We present preliminary results with regard to the cleaning efficacy of large canals, thus, it is necessary to perform comparable research concerning narrow canals in the immediate future.

# Perspectives

Concluding, none of the methods used in the study was able to entirely remove Ca(OH)<sub>2</sub> from wide root canals. However, considering the limitations of this study, it can be concluded that the XP-endo Finisher and the Finisher-brush are beneficial in removing calcium hydroxide from the root canal walls.

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### Contribution

AK: concept design, definition of intellectual content, literature search, clinical studies, experimental studies, data acquisition, data analysis, statistical analysis, manuscript preparation, manuscript editing and manuscript review; PK: Contributed to: data acquisition, data analysis, statistical analysis, manuscript editing

### **Conflict of interest statement**

The authors declare no conflict of interest.

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