



## Clinical and microbiological effects of photodynamic therapy applied in non-surgical treatment of periodontitis

Klinički i mikrobiološki efekti primene fotodinamske terapije u nehirurškom lečenju parodontopatije

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### Abstract

**Background/Aim.** Treatment of periodontitis undergoes several treatment phases. Non-surgical periodontal treatment (NSPT) represents the basic treatment stage, and it is applied to all the patients undergoing periodontal treatment. Adjunctive antimicrobial photodynamic therapy (aPDT) is one of several contemporary and relatively new possibilities with a role to inactivate microorganisms responsible for the occurrence and progression of the disease. The aim of this study was to comparatively analyze the clinical and microbiological effects of the NSPT alone, as well as combined with aPDT. **Methods.** A split-mouth method design was used in a prospective randomized controlled trial. The following clinical parameters were registered and monitored: plaque index (PI), bleeding on probing, probing depth (PD), and clinical attachment level (CAL). The presence of microorganisms *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Treponema denticola* was detected by the polymerase chain reaction (PCR) method. Samples were tested before the therapy, as well as three months after the therapy. Therapeutic modalities of NSPT and NSPT with adjunct aPDT were examined. **Results.** All of the analyzed clinical parameters proved

statistically significant improvement after the application of both treatment modalities ( $p < 0.001$ ). Microbiological analyses showed that the total number of microorganisms was statistically significantly lower after the application of both methods ( $p < 0.001$ ). Following the treatment, there was a statistically significantly reduced number of microorganisms *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Treponema denticola* ( $p < 0.001$ ). NSPT combined with aPDT led to a statistically significant improvement of both clinical parameters and microbiological status compared to NSPT applied on its own. **Conclusion.** The study showed improvement of all clinical indicators after the application of both treatment modalities. The total number of microorganisms was reduced as well as the number of specific microorganisms. Combining aPDT with NSPT led to a statistically significantly higher reduction in the number of microorganisms compared to NSPT alone.

**Key words:**  
aggregatibacter actinomycetemcomitans; periodontitis; photochemotherapy; polymerase chain reaction; porphyromonas gingivalis; treponema denticola; treatment outcome.

### Apstrakt

**Uvod/Cilj.** Lečenje parodontopatije prolazi kroz nekoliko faza. Kauzalna (inicijalna, bazična) faza lečenja parodontopatije predstavlja osnovnu terapijsku fazu kroz koju prolaze svi pacijenti. Antimikrobna fotodinamska terapija (aPDT) predstavlja jednu od savremenih i relativno novih mogućnosti, čija je uloga inaktivacija mikroorganizama odgovornih za nastanak i progresiju oboljenja. Cilj istraživanja bio je uporedna analiza kliničkih i mikrobioloških efekata primene samo kauzalne terapije i njene kombinacije sa aPDT. **Metode.** U prospektivnoj randomizovanoj studiji bila je primenjena

metoda podeljenih usta. U istraživanju su bili praćeni sledeći klinički parametri: plak indeks (PI), krvarenje na provokaciju, dubina sondiranja (DS) i nivo pripojnog epitela (NPE). Metodom lančane reakcije polimeraze (PCR) praćeno je prisustvo mikroorganizama *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis* i *Treponema denticola*. Testirani su uzorci pre, kao i tri meseca nakon terapije. Ispitivani su terapijski modaliteti kauzalne terapije i njene kombinacije sa aPDT. **Rezultati.** Analizirani klinički parametri: PI, krvarenje gingive, DS i NPE pokazali su statistički značajno poboljšanje nakon primene oba modaliteta lečenja ( $p < 0,001$ ). Mikrobiološke analize pokazale su da je ukupni broj mikroorganizama

bio statistički značajno manji primenom obe metode lečenja ( $p < 0,001$ ). Mikroorganizmi *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis* i *Treponema denticola* nalazili su se u statistički značajno manjem broju nakon primenjenih terapija ( $p < 0,001$ ). Kauzalna terapija u kombinaciji sa aPTD je dovela do statistički značajnog poboljšanja kako kliničkih parametara, tako i mikrobiološkog statusa u odnosu na kauzalnu terapiju primenjenu samostalno. **Zaključak.** Istraživanje je pokazalo da je došlo do poboljšanja svih kliničkih pokazatelja nakon primene oba modaliteta lečenja. Ukupni broj mikroor-

ganizama, kao i broj specifičnih mikroorganizama je bio smanjen. Kombinacija aPDT sa kauzalnom terapijom dovela je do statistički značajno većeg smanjenja broja mikroorganizama u poređenju sa samom kauzalnom terapijom.

#### **Ključne reči:**

**aggregatibacter actinomycetemcomitans; periodontitis; fotohemioterapija; polimeraza, reakcija stvaranja lanaca; porphyromonas gingivalis; treponema denticola; lečenje, ishod.**

## **Introduction**

Periodontitis is a chronic inflammatory disease with bacterial infection playing one of the key roles in the etiology of the disease<sup>1</sup>. Socransky et al.<sup>2</sup> and Socransky and Haffajee<sup>3</sup> have classified periodontal pathogens into six complexes based on pathogenesis and subgingival biofilm colonization in adults.

Red complex bacteria are dominant in subgingival dental plaque among patients who suffer from chronic periodontitis with deep periodontal pockets. It is known that *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* are able to invade certain periodontal tissues. Their persistence over a long period of time and after the treatment leads to the reactivation of the disease<sup>4,5</sup>.

Periodontal treatment consists of several phases. Non-surgical treatment of periodontitis (NSPT) presents a basic and inevitable treatment phase. In certain cases, further chemical plaque control and/or antibiotic therapy are performed<sup>6,7</sup>.

The removal of dental plaque and calculus from the root surface, elimination of necrotic cementum, and root planning are achieved by mechanical scaling and root planning (SRP). The above-mentioned procedures result in the activation of reparatory and regenerative processes in periodontium<sup>8</sup>. However, SRP alone cannot completely remove subgingival periodontal pathogens in inaccessible or hardly accessible areas. Incomplete elimination of periodontal pathogens leads to a relapse of the infection. Germ removal and infection elimination, present in dental apparatus, stand as crucial factors that create conditions for possible regeneration of lost tissues. This is the aim of contemporary periodontal therapy<sup>9-11</sup>.

The use of systemic antibiotics has shown positive effects on the periodontium when treating periodontitis<sup>12</sup>. However, additional research is needed in order to evaluate real effects on the periodontium due to increased microbial resistance on antibiotics<sup>13</sup>. Local or systematic antibiotic therapy is commonly used as additional treatment in initial active periodontal treatment (APT), aimed at bacterial removal for a longer period of time<sup>14</sup>. Mentioned pharmacological therapy has many side effects, therefore, it is necessary to look for alternative methods. There is an ongoing search for ideal alternative methods for eliminating microorganisms, thus improving the effects of periodontal treatment<sup>15</sup>.

Antimicrobial photodynamic therapy (aPDT) represents one of the several contemporary and relatively new possibilities for the inactivation of microorganisms responsible for the pathological occurrences in the periodontium. Antimicrobial photodynamic therapy is defined as an oxygen-based photochemical reaction that involves photosensitizer (PS), light source, and oxidative molecules. Owing to its high antibacterial potential, aPDT is suggested as a potential adjunctive treatment for periodontitis, peri-implantitis<sup>16</sup>, as well as in endodontic therapy<sup>17-19</sup>. The advantage of aPDT over the use of antibiotics is in the fact that microorganisms do not develop resistance, therefore, the procedure can be repeated as many times as needed without any negative effects on the patient's organism and general health<sup>20</sup>.

The aim of this research was to conduct a comparative analysis of clinical and microbiological effects of NSPT alone and NSPT supplemented by adjunctive use of aPDT.

## **Methods**

This was a prospective, randomized study with a split-mouth method. This method means that one half of both jaws represents a test group, while the other half of both jaws served as a control group. The test group was treated by NSPT combined with aPDT, while the control group was treated by NSPT applied alone. The research was approved by the Ethics Committee of the Faculty of Medical Sciences in Kosovska Mitrovica (number 05-1691, from September 13, 2016). Patients were included in the study from October 2016 until December 2018. The research was conducted at the Periodontology and Oral Medicine Clinic, Faculty of Medical Sciences, University of Priština/Kosovska Mitrovica. The research included 25 patients. Patients who participated in this study were 30 to 70 years old; they suffered from chronic periodontitis and had not used local or systemic antibiotic therapy at least three months prior to the inclusion in the study. The participants had to be healthy non-smokers and with at least three teeth in each quadrant except for the third molar, as well as with at least two teeth with probing depth of 5 and more millimeters in each quadrant. Criteria for exclusion were the presence of chronic diseases, smoking, periodontal therapy in the last six months, pregnancy, and lactation. Determining which side of the mouth will be tested and which control was done by means of randomization envelopes.

*First stage – clinical parameters followed in the study*

After the anamnesis, clinical examination, and analysis of additional diagnostic tools (orthopantomographic image – OPG), the following parameters were registered: plaque index (PI) according to Silness L ou, bleeding on probing (BOP) according to M uhlemann, probing depth (DP), and clinical attachment level (CAL). The amount of dental plaque in one-third of the gingival dental crown was examined, with a periodontal probe, on four tooth surfaces (distobuccal, buccal, bucco-mesial, and oral surface) and graded from 0 to 3<sup>21</sup>. Determining BOP was performed by interdental papilla bleeding provocation with Williams periodontal probe (Hu-Friedy, USA). Probing was performed from the papilla base to its highest point and graded from 0 to 4<sup>22</sup>. PD is the measured distance from the gingival margin to the bottom of the periodontal pocket. CAL is the measured distance from the cemento-enamel junction (CEJ) to the coronal part of the junctional epithelium. DP and CAL measurements were performed using a periodontal probe according to Williams (Hu-Friedy, USA) in six points at each tooth (three points on the vestibular side and three points on the oral side). Values were expressed in millimeters, and numbers were rounded towards a higher value. Periodontal condition assessment was done before the therapy as well as three months after the therapy.

*Examiner calibration*

One examiner (ZA) recorded all the clinical parameters. A standard calibrated probe was used for measuring. Periodontal pocket depth, CAL, and BOP were tested on three patients and measured in two separate sessions with an interval of seven days. Calibration was accepted if the percentage between the measuring at the beginning and the measuring after seven days was higher than 90%.

*Second stage – Microbiological procedure*

The samples of subgingival dental plaque were collected from all patients after the second visit and three months after the therapy. The sampling of subgingival dental plaque for microbiological analysis was performed 24 hours after the clinical parameters' registration (in order to avoid blood contamination). DP value determined the choice of periodontal pocket used for sampling, meaning that sampling was performed in the region with the highest value. Sterile paper points (four in total, one for each quadrant) from PET-diagnostic set (MIP Pharma GmbH, Germany) were placed until the first mild resistance and held in place for twenty seconds. They were placed in separate plastic test tubes (Ependorf) and kept at room temperature until microbiological analysis.

The presence of the following microorganisms: *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Treponema denticola* was analyzed by means of polymerase chain reaction (PCR). Samples were tested prior to the treatment and three months after it.

*Third stage – non-surgical periodontal treatment (NSPT)*

Samples for microbiological analysis were taken during the second visit, after which NSPT had been applied. This study utilized Full Mouth Disinfection protocol (FMD), meaning that NSPT was performed within 24 hours. Quirynen et al.<sup>23</sup> introduced a one-session approach in treating diseased periodontium, with the main goal being elimination or reduction of all periodontopathogens from the oropharynx's region (periodontal pocket, all of the oral mucous membrane, spit, and tonsils) within 24 hours. Removal of supragingival and subgingival layers while treating periodontal pockets was conducted under local infiltration anaesthesia (2% lidocaine with adrenaline in scale 1:100,000). Heavy dental deposits were removed with an ultrasonic device (Mini Piezon, EMS, Nyon, Switzerland). NSPT was applied in all quadrants at once due to possible contamination of other periodontal pockets with residual microorganisms, which might diminish the effects of the therapy using Gracey's curettes (Gracey curettes, Hu-Friedy, Chicago, IL, USA). Pockets were rinsed deeply with sterile physiological solution. Removal of soft deposits and final polishing of the teeth surfaces was performed by using abrasive paste (Proxyl RDA 36), middle-sized particles (Liechtenstein) without fluoride, and a rotating brush.

*Fourth stage – application of antimicrobial photodynamic therapy*

After NSPT (0–24 hours in minor and 24–48 hours in greater gingiva bleeding), aPDT was applied. Procedure for aPDT application included creating a relatively dry workspace, using aspiration tools and cotton rolls, followed by photosensitizer placement in the periodontal pocket. The photosensitizer used was phenothiazine chloride (HELBO® Blue photosensitizer, Bredent Medical GmbH & Co KG) which was thoroughly rinsed with the physiological solution for periodontal pockets up to 5 millimeters after one minute and for pockets over six millimeters after three minutes according to the manufacturer manual. Photosensitizer was activated with a 660 nm diode laser powered by 100 mW (HELBO® TheraLite Laser, HELBO® Zenden, Germany). Fibers 450 µm (3D Pocket Probe; Bredent Zenden Germany) with activated laser light were applied on periodontal pockets for 1 minute (10 seconds at the time on 3 points on vestibular and on 3 points on oral side).

*Statistical analysis*

In order to analyze primary data, descriptive statistical methods were used, methods for testing statistical hypotheses, and methods for analyzing outcomes and potential predictor relations. Depending on variable type and type of distribution, data description was shown as n (%), mean ± standard deviation (SD), or median (minimum-maximum). When testing the statistical hypothesis, we used the follow-

ing methods: the *t*-test, Wilcoxon's test,  $\chi^2$  test, and variance analyses of repeated measurements. The statistical hypothesis was tested at the statistically significant level (alpha level) of 0.05. All the data were processed in IBM SPSS Statistics 22 (SPSS Inc., Chicago, IL, USA) software package or R programming environment (R Core Team, 2018).

## Results

All participants completed the study successfully, and there were no side effects after the treatment or during the research period. The research was conducted on 25 subjects. Their average age was 35 years (30–64 years). There was a total of 13 male (52%) and 12 female (48%) participants.

One hundred samples were gathered from 25 subjects (four samples each) using the split-mouth method. Fifty samples were analyzed for the test group and 50 samples for the control group.

There was no statistically significant difference in clinical values such as PI, BOP, and DP for both treatment modalities before the treatment. However, there was a statistically significant difference in the values of CAL before the treatment.

Three months after the therapy, all clinical parameters monitored (PI, BOP, PD, and CAL) were statistically signifi-

cantly reduced in comparison to their initial values. The results showed continual clinical improvement three months after the therapy, especially in clinical parameter values where additional aPDT was applied. Overall, statistically significant changes in PI, PD, and CAL for both treatment modalities were observed ( $p < 0.001$ ). For the given parameters concerning therapy models, a statistically significant difference in values was obtained after the treatment ( $p < 0.001$ ) (Table 1).

There was no statistically significant difference in the values of total bacteria number in relation to therapy modalities before the treatment ( $p = 0.836$ ), while after the treatment, that difference was statistically significant ( $p < 0.001$ ) (Table 2).

Statistically significant differences in the total number and prevalence of microorganisms *Aggregatibacter actinomycetemcomitans* and *Treponema denticola* were not shown before the treatment, while for *Porphyromonas gingivalis* a statistically significant difference was shown ( $p < 0.001$ ). By observing bacterial microorganisms individually after the treatment, it was demonstrated that there was a statistically significant difference in the values for *Aggregatibacter actinomycetemcomitans* ( $p = 0.035$ ), *Porphyromonas gingivalis* ( $p < 0.001$ ), and *Treponema denticola* ( $p < 0.001$ ) in relation to therapy modalities (Table 3).

The clinical status of periodontium before and after applied treatments is shown in Figure 1.

**Table 1**

Clinical parameters' values before and after the therapy					
Parameter	Treatment	Values (mean $\pm$ SD)		Overall <i>p</i> -value	
		before	after	in time	between treatment
Plaque index	C	1.53 $\pm$ 0.44	0.83 $\pm$ 0.29	< 0.001	0.685
	E	1.54 $\pm$ 0.42	0.74 $\pm$ 0.28		
	<i>p</i> (C vs. E)	0.621	< 0.001		
Gingiva bleeding index	C	2.03 $\pm$ 0.98	1.17 $\pm$ 0.60	< 0.001	0.861
	E	2.04 $\pm$ 0.97	1.08 $\pm$ 0.59		
	<i>p</i> (C vs. E)	0.496	< 0.001		
Depth sounding	C	3.78 $\pm$ 0.75	2.92 $\pm$ 0.61	< 0.001	0.652
	E	3.75 $\pm$ 0.70	2.79 $\pm$ 0.61		
	<i>p</i> (C vs. E)	0.536	< 0.001		
Junctional epithelium level	C	2.88 $\pm$ 0.51	2.41 $\pm$ 0.49	< 0.001	0.714
	E	2.86 $\pm$ 0.50	2.33 $\pm$ 0.57		
	<i>p</i> (C vs. E)	0.040	0.058		

C – control group treated with causal therapy alone; E – test group treated with causal therapy combined with antimicrobial photodynamic therapy; SD – standard deviation.

**Table 2**

Total bacteria number before and after the treatment		
Treatment	Total bacteria number, median (minimum– maximum)	
	before	after
C	1.7 $\times 10^8$	2.3 $\times 10^6$
	(4.5 $\times 10^6$ –8.3 $\times 10^9$ )	(5.3 $\times 10^4$ –9.2 $\times 10^7$ )
E	1.1 $\times 10^8$	5.6 $\times 10^4$
	(3.8 $\times 10^6$ –7.7 $\times 10^9$ )	(3.7 $\times 10^3$ –1.9 $\times 10^7$ )
<i>p</i> (C vs. E)	0.836	< 0.001

C – control group treated with causal therapy alone; E – test group treated with causal therapy combined with antimicrobial photodynamic therapy.

Table 3

Prevalence of microorganisms before and after the treatment					
Bacterium	Treatment	Prevalence, n (%)		Overall <i>p</i> -value	
		before	after	in time	between treatment
<i>Aggregatibacter actinomycetemcomitans</i>	C	18 (36.0)	9 (18.0)	< 0.001	0.998
	E	17 (34.0)	2 (4.0)		
	<i>p</i> (C vs. E)	0.655	0.035		
<i>Porphyromonas gingivalis</i>	C	28 (56.0)	20 (40.0)	< 0.001	< 0.001
	E	22 (44.0)	4 (8.0)		
	<i>p</i> (C vs. E)	0.025	< 0.001		
<i>Treponema denticola</i>	C	49 (98.0)	33 (67.3)	< 0.001	0.001
	E	47 (94.0)	12 (24.0)		
	<i>p</i> (C vs. E)	0.317	< 0.001		

**C** – control group treated with causal therapy alone; **E** – test group treated with causal therapy combined with antimicrobial photodynamic therapy.



**Fig. 1– A) Clinical status before therapy; B) Non-surgical periodontal treatment (NSPT); C) HELBO® Blue photosensitizer application; D) Diode laser activation; E) Clinical status after therapy.**

## Discussion

Present clinical data unambiguously proved that mechanical treatment of the root surface provides satisfying results with improvements of clinical parameters, as shown in

other studies<sup>24–27</sup>. However, complete elimination of the present subgingival periodontogens is not possible<sup>26, 27</sup>. The present study showed that the application of both treatment modalities significantly improved clinical parameters after the therapy. The results showed continuous clinical im-

improvements three months after the therapy, especially in the group where additional aPDT was applied. Results gathered by Husejnagic et al.<sup>28</sup> also correlate with our research (PI was  $58.83 \pm 25.31$  at baseline and was reduced to  $31.32 \pm 15.12$  at the point of reevaluation).

This study followed up several clinical parameters, but it also analyzed microbiological parameters through the application of the PCR technique, quantitative-qualitative analysis of subgingival periodontal pathogens, showing reduction of certain examined pathogens. Our research, using the quantification method (PCR), showed more accurate results in examining pathogens in relation to a study conducted by Petrovic et al.<sup>29</sup>. The use of the PCR method showed that the following bacteria predominated in our samples: *Porphyromonas gingivalis* and *Treponema denticola*, which is a characteristic finding in patients affected by chronic periodontitis. Kumawat et al.<sup>30</sup> established that the prevalence of these two microorganisms correlates with the progression of pathological processes and that their presence is connected with the level of periodontal tissue damage. By following each of the three microorganisms, our study proved that the total number of *Aggregatibacter actinomycetemcomitans* was reduced after the treatment by both therapy modalities. However, antimicrobial photodynamic therapy gave better results. Corrêa et al.<sup>31</sup> have followed this reduction of *Aggregatibacter actinomycetemcomitans* during a period of 3, 7, 14, and 90 days, and proved that antimicrobial photodynamic therapy significantly affects the reduction of microorganisms in a shorter period of time, while improving clinical parameters in longer time periods.

Examining microbiological status during the assessment of the same pathogens as in this study, Husejnagic et al.<sup>28</sup> have also found out that the number of *Porphyromonas gingivalis* and *Treponema denticola* significantly declined, while the number of *Aggregatibacter actinomycetemcomitans*, after two applications of aPDT during the therapy, also declined, but not significantly. Unlike our study, the above-mentioned study also included smokers. In relation to microbiological status, our achieved results showed superior effects with only one application of aPDT. This could be explained by the fact that smokers were not included in our study. Smoking plays an important role in periodontitis etiology, as well as in its further development. Smoking can change the microbial status of oral flora, leading to increased growth of pathological periopathogens *Porphyromonas gingivalis* and *Treponema denticola* in the subgingival region and consequently to the destruction of the periodontal tissue over time<sup>32, 33</sup>.

NSPT alone cannot achieve long-term results in improving clinical parameters and reducing subgingival periodontopathogens in smokers<sup>34</sup>. Xue et al.<sup>35</sup> have examined the clinical efficiency of aPDT as a supplement to SRP in treating chronic periodontitis and have not achieved significant improvements in the values of periodontal probing depth decrease nor in the clinical attachment level increase in groups of patients which included smokers. Multiple applications of aPDT using the “split-mouth” method did not

achieve clinical, microbiological, or immunological improvements during the treatment<sup>36</sup>. There are few studies able to prove that aPDT application results in bacterial species' reduction in smokers affected by chronic periodontitis.

The number of laser applications, as well as the time of exposure of aPDT, is not crucial in achieving clinical results during the treatment. Applying aPDT in 3 sessions (0, 7, and 14 days) and with two-minute exposure time has not achieved better results compared to our study<sup>37</sup>. Some studies also imply that there is no long-term method that may be able to completely remove microorganisms from periodontal pockets. The results of Lulic et al.<sup>38</sup> have shown that recolonization of deep periodontal pockets happens even though the numbers of microorganisms are reduced in a short period of time. Comparative analysis of therapy modalities after the therapy, conducted in our study, proved that initial periodontal therapy combined with aPDT led to a significant reduction in microorganism number compared to NSPT alone. Moreira et al.<sup>39</sup> conducted a study with an immunological analysis and proved that the application of aPDT combined with NSPT therapy gives promising results in the long-term prognosis of treating patients with aggressive forms of periodontitis. Similar results have also been reported by other authors<sup>38, 40</sup>.

Given the above, it may be concluded that aPDT produces a positive effect only in combination with previously applied mechanical treatment of the root canal. However, many studies are dealing with these questions and there is no consensus concerning the approach and treatment of patients affected by periodontitis. This is primarily related to the antibiotic application in patients who smoke and to the question of which antibiotic is more efficient in treating non-smokers and *vice versa*<sup>41</sup>. There is also the question of the number of aPDT applications and the choice of photosensitizers. Over the last decade, several clinical studies have concluded that repeated aPDT application gives better results in treating periodontitis<sup>31, 36, 42</sup>. On the other hand, some other authors reduce the number of applications or apply only one aPDT treatment<sup>43</sup>.

## Conclusion

This research showed that all clinical parameters significantly improved after the application of both treatment modalities. The total number of microorganisms was significantly reduced, as well as the number of specific microorganisms: *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Treponema denticola*. The combination of antimicrobial photodynamic therapy with non-surgical periodontal therapy showed improvement of all clinical parameters and the reduction of the number of microorganisms compared to NSPT therapy alone.

## Conflict of interest

There is no conflict of interest.

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