ORIGINAL ARTICLE

QUALITY OF ORTHOPEDIC REHABILITATION OF PATIENTS WITH POST-TRAUMATIC DEFECTS OF THE UPPER JAW BY CHARACTERISTICS OF BIOCENOSIS OF THE ORAL CAVITY

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ABSTRACT

The aim of the research was to study the dynamics of the microbiota's features of oral mucosal membrane during orthopedic rehabilitation of patients with removable dentures which has an obturating part with two-layer bases.

Materials and methods: To achieve this goal, our bacteriological examination of oral cavity mucosa was performed for 25 patients with partial adentia of the upper jaw and defect of hard palate and alveolar process.

Results and conclusions: Of the conducted studies indicate significant shifts in the qualitative and quantitative composition of microbiocenosis in the oral cavity in patients with partial adenia of the upper jaw and a defect of hard palate and alveolar process due to representatives of moraksel, enterobacteria (representatives of the kinds *Klebsiella* and *E. coli*). The comparing of frequency of extraction and the density of microbial colonization showed us the persistence in biotope of representatives near 13 kinds of bacteria and yeast-like fungi of the kind Candida in averages from lg (2,5 ± 0,19) to lg (5,4 ± 0,17) CFU/g.

For patients who have been made a two-layered basis, materials of which are based on carboxymethylcellulose and polyvinialacetate in the period of adaptation to removable dentures, showed us that the detection of 5 component associations at 30 days was reduced by 2 times ($\chi 2 = 5{,}991$; $v = 2$; $p < 0{,}05$) . The frequency of removal and density of microbial colonization of the experimental group did not differ statistically. Among patients in the control group, the microbial colonization density increased for *Enterococcus spp*, for *Klebsiella spp* and for *Candida spp*. *Yeast-like fungi*. A significant decrease in the microbial density of the resident microflora was 1.4 times for *Neisseria spp*, 1.6 times for *Lactobacillus spp* ($p < 0{,}05$).

KEY WORDS: microecology, two-layer basis, removable dentures, obturating part, mucous membrane of oral cavity

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INTRODUCTION

About 700-1000 species of various microorganisms have been identified among representatives of oral microbiocenosis, their identification and quantification are quite a challenge [1]. The oral cavity is a complex ecological system in which external factors interact with the internal and are in dynamic equilibrium.

However, the variability of microflora with age, because the oral cavity differs favorable conditions for its reproduction [2, 3]. Bacterial colonization is facilitated by optimal temperature and humidity, the presence of a slightly alkaline environment, different in structure of tissues and food residues.

Under the influence of various endogenous and exogenous factors, qualitative and quantitative changes in the microflora can occur, which contribute to the formation of dysbiosis. Dysbiotic condition of the oral cavity can leads to exacerbation or chronic course of stomatitis, ulcerative gingivitis, periodontitis and other dental diseases [4, 5].

Removable plastic dentures instigate violation of the microecology of the oral cavity [6, 7]. The fact of the direct dependence of the rate of formation of microbial plaque on the prosthesis material was also established [8, 9]. Especially colonization of biotopes increases with the use of acrylic materials having a certain degree of porosity [10].

High level of microbial colonization is established both on the mucous membrane of the prosthetic area and on the surface of the prosthesis, analyzing the results of the study. The need to improve the quality of prosthetics by using more inert base materials is proved [11]. The diversity of the spectrum of microorganisms and the aggressiveness of the inflammatory changes provoked by local and general character confirm the special importance of studies of the microbial «scenery» of the mouth [12, 13, 14].

The development and implementation of effective methods of prevention and treatment of oral microenvironment's disorders, especially in dental prosthetics, are extremely important and necessary for practical health care in modern conditions [15, 17]. Therefore, the role of oral biocenosis in the formation of pathological processes in the orthopedic treatment of patients with upper jaw's post-traumatic defects requires further study and can be used as an additional criterion for determination the effectiveness of corrective therapy.

The use of different liners between the prosthesis base and the mucous membrane should be considered the

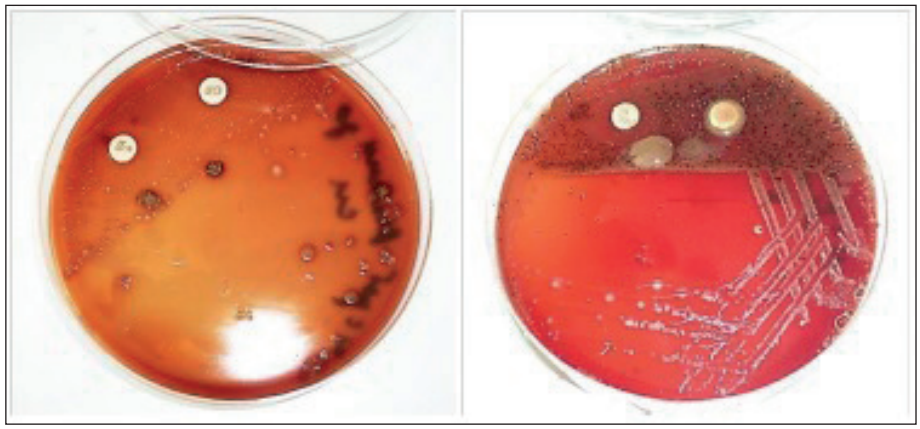

Fig. 1. Identification of extracted microbial cultures

most promising which improve fixation and eliminate incidental effects – irritation, hypersensitivity. At the same time, the term of adaptation to plate prostheses is significantly reduced.

THE AIM

The purpose of the research is to study the dynamics of the representatives of the oral mucosa's microbiota during prosthetic rehabilitation of patients with removable dentures which has an obturating part with two-layer bases.

MATERIALS AND METHODS

The study was conducted at the Department of Orthopedic Dentistry, University Dental Center, Kharkiv National Medical University.

Deontological aspects are resolved within the framework of the legislation in force in Ukraine, the Law of Ukraine "On Medicines", 1996, Art. 7, 8, 12, principles of ICH GCP (2008), order of the Ministry of Health of Ukraine No. 690 of 23.09.2009 "On approval of the Rules for clinical trials and expertise of materials of clinical trials and model regulations on the ethics commission", as amended; World Health Association Declaration of Helsinki. The study was performed with minimal psychological loss for patients. Patients were fully informed about the purpose and methods of the study, the potential gains and risks, and the possible discomfort with the diagnosis and treatment. All ethical requirements for maintaining the confidentiality of the information received during the study are fulfilled. The work was reviewed and approved by the Bioethics Commission of the KhNMU of the Ministry of Health of Ukraine.

To achieve this goal, a bacteriological study of the oral mucosa was performed on 25 patients. Clinical patient groups were formed by the following criteria: the main group consisted of 13 patients with partial adentia of the upper jaw and defect of the hard palate and alveolar process (groups 1A and 1B by V.Y. Kurlandsky) which made two-layer removable prostheses with a obturating part using "PM-SN" JSC "Stoma". The control group consisted of 12 patients with partial adentia of the upper jaw and defect of the hard palate and alveolar process (groups 1A and 1B according to V.Y.Kurlandsky), which made obturating removable prostheses by the usual method.

Material collection, transportation and bacteriological examination were carried out in accordance with current regulatory documents by conventional methods [16]. Removable prosthesis and oral cavity were thoroughly rinsed with physiological solution for remove food's residue. The material was collected 20 minutes after mouthwash with physiological solution: before the prosthesis, after a week and after a month of prosthesis.

Material from the oral mucosa was removed with a cotton swab, which is in a tube with a Stuart transport medium.

The crops were made on 5% blood agar, Endo medium, enterococar, yolk-salt agar to extract aerobic and optional anaerobic bacteria. Saburo medium was used for yeast and molds. The crops were incubated at 37 °C from 24 till 120 hours under aerobic conditions, depending on the group of microorganisms tested (Fig. 1).

The identification of the extracted bacterial cultures was carried out on the basis of morphological, cultural, biochemical characteristics according to the «The determinant of the bacteria is Berdy», 1997; identification of fungal strains – according to the « The determinant of pathogenic and conditionally pathogenic fungi», 2001 by standard methods.

The quantity of microorganisms was determined by counting colony forming units in 1g of material and expressed in decimal logs (lg CFU/g).

Formation of the database on the results of the research was carried out in Microsoft Excel, 2007. Statistical processing of the research results was carried out using the software package Statistica v. 8.0. The arithmetic mean of the quantitative indicators presented in the text ($M \pm m$) was calculated, where "M" is the sample mean and "m" is the error of the mean. The results of the description of qualitative indicators (frequency of withdrawal) were expressed in percentage. In all statistical analysis procedures, the achieved significance level (p) was calculated, with the critical significance level in this study assumed to be 0.05. The hypothesis of the equality of the general averages in the two groups compared was tested using the nonparametric Wilcoxon-Mann-Whitney criterion for independent samples, and the percentages using the χ-square criterion [18, 19].

Table 1. Quantitative characterization of microbial associations isolated from the alveolar ridge in the examined patients of the experimental and control group depending on the time of adaptation to the removable prosthesis

Groups of patients examined	Subgroups of patients examined	The frequency of removal of microbial associations, %			
		2-components	3-components	4- components	5- components
Patients with a two-layer basis n=13	before putting the prosthesis	26,1	34,8	21,7	17,4
	7 days	30,4	26,1	30,4	13,1
	30 days	34,8	30,4	26,1	8,7
Control group, n=12	before putting the prosthesis	25,0	33,3	25,0	16,7
	7 days	16,7	25,0	41,6	16,7
	30 days	8,3	16,7	50,0	25,0

Table 2. Characterization of oral microbiocenosis in the adaptation period to removable prosthesis.

Removal frequency (%)	Representatives of aerobic and optional anaerobic microflora	Experimental group, n=13			Control group, n=12		
		before putting the prosthesis	7 days	30 days	before putting the prosthesis	7 days	30 days
		The number of strains removed (%)					
>50,0%	*Streptococcus spp with á- hemolytic properties*	73,9	69,6	69,6	66,7	58,3	66,7
30,1-50,0 %	*Corynebacterium spp*	39,1	39,1	39,1	50,0	41,6	50,0
	Neisseria spp	43,5	7,8	43,5	41,6	33,3	41,6
20,1-30,0 %	*Lactobacillus spp*	26,1	26,1	26,1	33,3	33,3	25,0
	S. pyogenes	21,7	21,7	21,7	25,0	33,3	33,3
	Micrococcus sp	21,7	26,1	21,7	25,0	25,0	25,0
	Moraxella spp	21,7	21,7	21,7	33,3	33,3	33,3
	E. coli	21,7	21,7	21,7	25,0	25,0	25,0
	M. morganii	21,7	26,1	26,1	33,3	33,3	33,3
10,0-20,0 %	*Haemophillus spp*	17,4	17,4	13,1	16,7	16,7	16,7
	Enterococcus spp	13,1	17,4	13,1	8,3	8,3	16,7
	Klebsiella spp	13,1	13,1	13,1	8,3	8,3	8,3
	Candida spp	17,4	17,4	13,1	16,7	16,7	16,7
	Staphylococcus spp	13,1	13,1	13,1	8,3	8,3	8,3

Note: * the difference is significant between the indicators ($p<0,05$).

RESULTS AND DISCUSSION

Microbiological studies included the determination of qualitative and quantitative composition of biocenosis. The microflora of patients with partial adentation of the upper jaw and defect of the hard palate and alveolar process were found to consist of associations of yeast fungi with 2-5 representatives of the microbial world. (Table 1).

No significant differences were found between the persistence of microbial associations of the oral mucosa of the test and control groups of individuals prior to use of the removable prosthesis during the study..

The deletion of 3-component microbial associations in patients of the experimental group was found to decrease 1.3 times on the 7th day of the study, and the deletion of 4-component associations among the experimental group on the 7th day was 1.4 times more frequent. For patients with a two-layer basis, the frequency of detection of 2-component microbial associative on the 30th day of the study was more than 1.3 times more frequent. Detection of 5 component associations on the 30th day decreased by 2-fold ($\chi2 = 5,991$; $\nu = 2$; $p < 0.05$). Instead, the dynamics of the distribution of 4-component microbial associations in the oral cavity after a week of adaptation to the prosthesis was 1.6 times more frequent in patients of the control group, 5-component associations remained at the initial level. However, after 30 days the distribution of 4 component microbial associations among the patients of the control group was 2 times more frequent, than the initial indicators, the percentage of 5 component associations was 1.5 times more frequent ($\chi2 = 5,991$; $\nu = 2$; $p < 0.05$).

The structure of microbiocenoses of the oral mucosa of the examined patients is represented by 13 bacterial species and yeast-like fungi of the genus *Candida* in average quantities from lg (2.5 ± 0.19) to lg (5.4 ± 0.17) CFU/g (Tables 2, 3).

Table 3. The density of microbial colonization of the oral cavity in the adaptation period to the removable prosthesis

p / n	Representatives of aerobic and optional anaerobic microflora	Experimental group, n=13 (lg CFU/g)			Control group, n=12 (lg CFU/g)		
		before putting the prosthesis	7 days	30 days	before putting the prosthesis	7 days	30 days
1	*Streptococcus spp with á- hemolytic properties*	4,6±0,15	4,5±0,19	4,8±0,21	4,3±0,18	3,8±0,15	4,2±0,2
2	*Corynebacterium spp*	4,2±0,26	4,1±0,2	3,9±0,18	4,3±0,1	3,8±0,16	3,6±0,11
3	*Neisseria spp*	5,2±0,22	5,0±0,12	4,9±0,1	5,1±0,14	4,6±0,1	3,6±0,18*
4	*Lactobacillus spp*	3,4±0,17	3,2±0,1	3,2±0,25	3,6±0,11	2,7±0,12	2,3±0,18*
5	*S. pyogenes*	3,2±0,29	3,6±0,2	3,4±0,18	3,3±0,2	3,8±0,12	4,0±0,18
6	*Micrococcus sp*	3,2±0,2	3,5±0,1	3,8±0,09	3,3±0,24	3,8±0,19	4,1±0,23
7	*Moraxella spp*	4,2±0,21	4,6±0,25	4,8±0,11	4,3±0,2	4,8±0,27	5,4±0,17
8	*E. coli*	3,4±0,1	3,2±0,2	3,4±0,09	3,3±0,22	3,9±0,1	4,1±0,09
9	*M. morganii*	3,0±0,13	3,0±0,1	2,7±0,2	3,1±0,17	3,2±0,1	3,7±0,14
10	*Haemophillus spp*	3,6±0,19	3,8±0,1	3,8±0,22	3,3±0,1	3,8±0,14	3,7±0,1
11	*Enterococcus spp*	2,5±0,19	2,7±0,1	3,0±0,21	2,6±0,1	3,1±0,12	4,0±0,1*
12	*Klebsiella spp*	2,8±0,15	3,0±0,11	3,2±0,13	2,5±0,1	3,0±0,13	3,6±0,18*
13	*Staphylococcus spp*	4,1±0,23	4,0±0,2	3,8±0,11	4,2±0,09	3,7±0,09	3,5±0,16
14	*Candida spp*	3,1±0,09	3,3±0,1	3,6±0,2	3,0±0,1	3,7±0,19	4,7±0,1*

Note: * the difference is significant between the indicators ($p<0,05$).

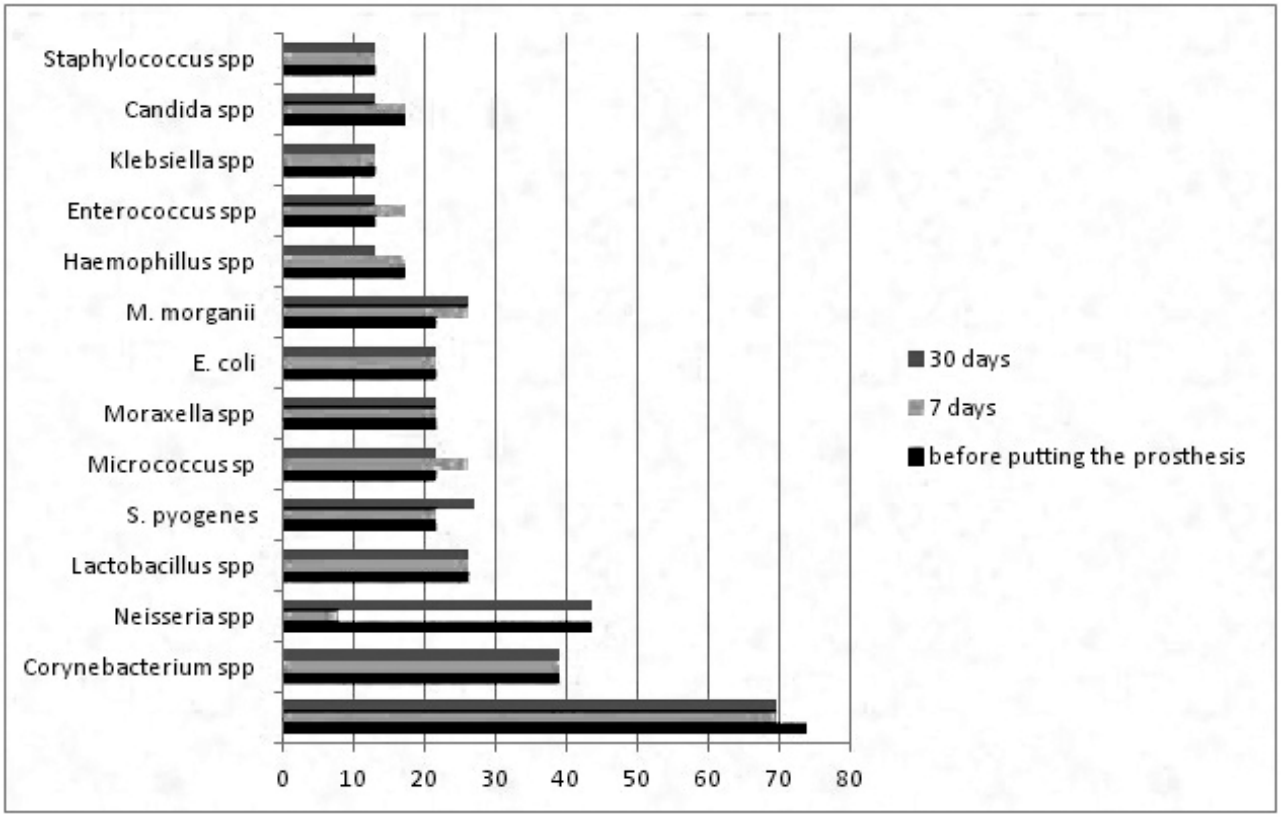

Fig. 2. Characterization of oral microbiocenosis in the adaptation period to the removable prosthesis.

In addition, an expansion of the species composition of microbiocenosis of the oral mucosa was established, due to representatives of moroccelles, enterobacteria (representatives of *Klebsiella* and *E. coli* species), as well as fungi Candida spp. Against this background, a decrease in the frequency of extraction of representatives of resident microflora (*neisseria, corynebacteria, lactobacilli*) inherent in this biotope is normal (Figs. 2, 3).

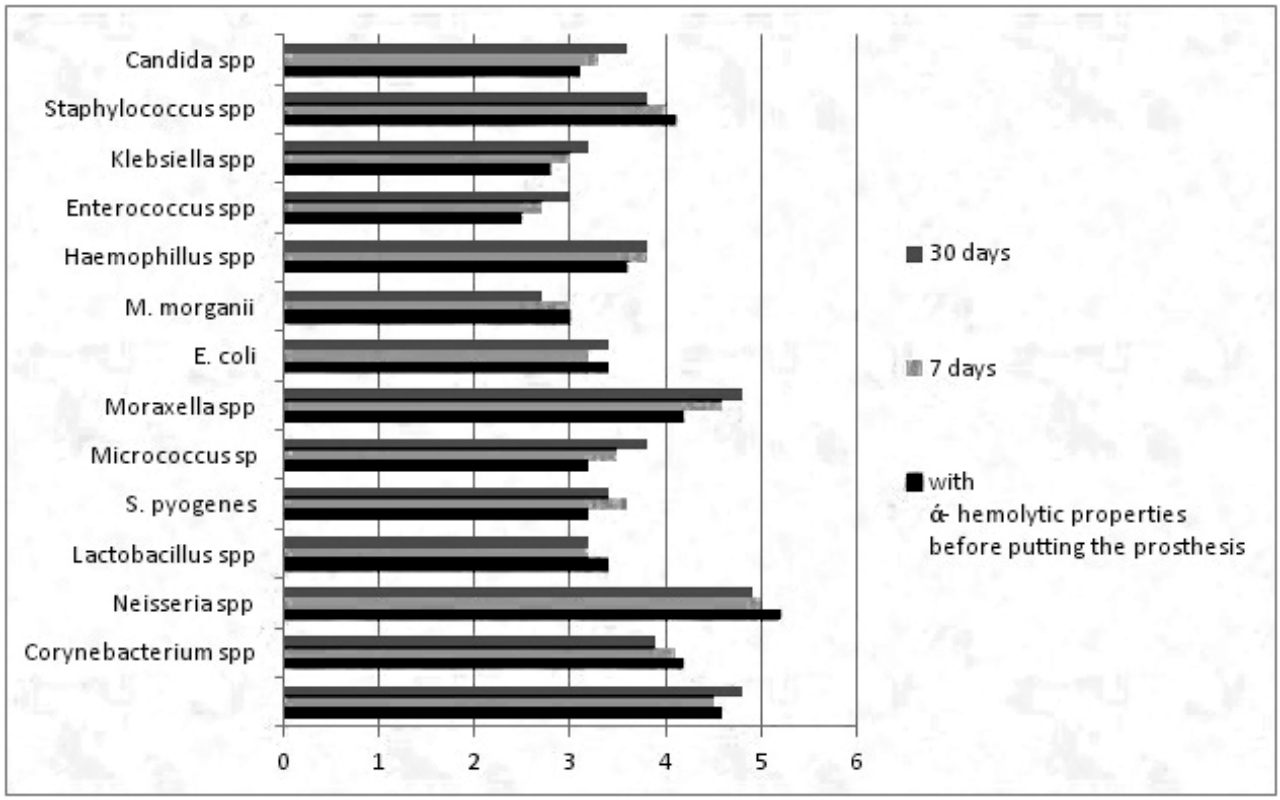

Fig. 3. The density of microbial colonization of the oral cavity in the adaptation period to the removable prosthesis

A primary examination of the alveolar crest microflora in patients in both groups showed a high level of microbial contamination.

When using a two-layer basis, the frequency of extraction and density of microbial colonization was not statistically different.

Instead, the microbial population density in the control group increased 1.5-fold for *Enterococcus spp*, 1.4-fold for *Klebsiella spp*, and 1.6-fold for the yeast-like *fungus Candida spp*. A significant decrease in the microbial density of representatives of resident microflora was found 1.4 times for *Neisseria spp*, 1.6 times for *Lactobacillus spp* ($p < 0.05$).

CONCLUSIONS

The results of the studies indicate significant shifts in the qualitative and quantitative composition of oral microbiocenosis in patients with partial adentia of the upper jaw and defect of the hard palate and alveolar process due to representatives of moroxel, enterobacteriaceae (representatives of the genera *Klebsiella* and *E. coli*). Comparison of the frequency of extraction and the density of microbial colonization showed the persistence in the specified biotope of representatives of 13 kings of bacteria and yeast-like fungi of the *king Candida* in average quantities from lg (2.5 ± 0.19) to lg (5.4 ± 0.17) CFU/g.

For patients treated with a two-layer base based on carboxymethylcellulose and polyvinyl acetate, during the adaptation to the removable prosthesis, there was a 2-fold decrease in the detection of 5 component associations on the 30th day ($\chi 2 = 5{,}991$; $\nu = 2$; $p < 0{,}05$). The frequency of extraction and the density of microbial colonization of the experimental group was not statistically different.

The microbial colonization density for *Enterococcus spp*, for *Klebsiella spp* and for yeast *fungi Candida spp* increased in the control group. A significant decrease in the microbial density of representatives of resident microflora was found 1.4 times for *Neisseria spp*, 1.6 times for *Lactobacillus spp* ($p < 0.05$).

The revealed microbiological features in patients with adentia dictate the need to include a scheme for the correction of oral microbiocenosis of patients with partial adentia of the upper jaw and defect of the hard palate and alveolar process of the means with directed anti-inflammatory action and ensure the restoration and storage of normal biocenosis of the specified biotope.

Prospects for further research. The study of the dynamics of the representatives of the microbiota of the oral mucosa allows to assess the level of influence of changes in microecology on the tissues of the prosthetic area, and therefore further studies will be directed to the search for improving the chewing efficiency of patients with partial adentia of the upper jaw and defect of the hard palate and alveolar process for improving their quality of life.

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Conflict of interest:
The Authors declare no conflict of interest.

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