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10.2478/AMB-2022-0039

ORIGINAL ARTICLE

PROINFLAMMATORY CYTOKINE POLYMORPHISMS AND SEVERITY OF PERIODONTITIS IN A COHORT OF BULGARIAN PATIENTS

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Abstract. *In this manuscript we are discussing our research of the presence of single nucleotide polymorphisms (SNPs) of two proinflammatory cytokines – interleukin 17F (IF-17F) and interleukin 23 (IL-23) in a cohort of Bulgarian patients.* ***The aim*** *of the study was to investigate the presence of SNPs of IL-17F and IL-23 and to explore a relation between IL-23 and IL-17F and severity of periodontitis. In the study 83 individuals were included – 62 patients with severe periodontitis and 21 healthy participants. Detailed periodontal status was taken in order to estimate the diagnosis of each participant. A Real-Time PCR for determination of SNP of IL-17 and IL-23 was performed. The data was summarised and processed by the means of statistical analysis.* ***The results*** *showed that when testing individuals for SNP of IL-17F, three genotypes were detected – TT, CT, CC, with prevalence of the TT genotype. In regards to SNP of IL-23, the widespread distribution of GG genotype represents the major share among all individuals with only three representatives with GA genotype. Important tendencies about the severity of the periodontal disease with regards to the SNPs and their simultaneous presence were found.* ***Conclusion:*** *In the present study two proinflammatory cytokine gene polymorphisms were explored and studied in relation to the severity of the periodontal disease. Their distribution among the tested individuals was established and relation to major parameters of the periodontal disease was evaluated.*

Key words: *periodontitis, single nucleotide polymorphism, interleukin-17F, interleukin-23*

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Received: *23 June 2022 –* ***Revised:*** *22 August 2022 –* ***Accepted:*** *20 September 2022*

INTRODUCTION

Periodontitis is an inflammatory destructive disease that is triggered by the elevated levels of pathogenic bacteria, but is modulated by the host response to inflammation and by some environmental factors. The scientific data confirm that individual genetic and systemic factors along with environmental factors can modify the course of some complex diseases such as periodontitis, obesity, diabetes, osteoporosis, etc. The pathogenic mechanisms involved in the bacterial virulence are relatively well explored and it is proved that they can be controlled by non-surgical periodontal therapy. The literature data shows that the presence of genetic determinants is a real modifier in terms of the periodontal disease. Some genes and intergenetic connections are involved in the remodelling of the clinical course, the severity and the prognosis of the periodontal disease.

The role of the genetic factors involved in the individual host response to the bacterial challenge and especially the single nucleotide polymorphisms (SNPs) is well explored. Based on many clinical studies the concept that some individuals might be at high risk of development of specific disease was introduced [1]. The results from a large number of studies show that the genes involved in periodontitis are related to the individual's immune response, thus influencing both development and progression of the disease. The variations in SNP could have non-significant or moderate role in some diseases, but in other cases they could lead to serious interactions with other genetic variations or environmental factors.

Multiple genetic investigations have revealed the polygenic nature of the periodontal disease [2]. It is suggested that the disease modifying genes for a same clinical condition are not equal in terms of severity of the disease or in different populations [1]. Some SNPs are specific for a given population [3].

In the recent years, the cytokine family polymorphisms have been well explored. Many studies suggest that the tissue destruction and progression of the periodontal lesion is related to the cytokine dysregulation [4, 5]. The research among different populations shows variable results in regard to SNPs of some Interleukins. In research among juvenile individuals between 14 and 17 years old, Gonçalves et al. have established 15.1 times higher risk of development of localised form of aggressive periodontitis and 24.6 times higher possibility of development of the generalised form of the disease [6].

IL-17F is a proinflammatory cytokine, secreted by activated T-cells. The IL-17 family includes six members: IL-17A, IL-17B, IL-17C, IL-17D, IL-17E (or IL-25) and IL-17F and five receptors: IL-17RA-RD and SEF. IL-17 is related to Th17 cells which are associated with variety of autoimmune diseases and are related to the development of severe inflammatory conditions. The cytokines related to the Th17 cells, such as IL-17 and IL-22, have a major impact on the host defence against the pathogenic microorganism's action [7, 8, 9]. IL-23 is a member of the IL-12 family, but it has a different effect on the cell-mediated immunity [10]. IL-23 is produced by activated macrophages and dendritic cells after they locate bacteria and triggers the Th17 cell to produce IL-17 [11]. IL-23 is associated with significant expansion of the Th17 cells, which are involved in many destructive diseases including periodontitis. The relation between IL-17 and IL-23 is substantial. The dysregulation of the genetic expression of IL-17 increases the susceptibility not only to periodontitis development, but also to Porphyromonas gingivalis (animal study) [12]. IL-17F +7383A/G and IL-17F-7488 C/T SNPs have been studied in relation to periodontitis and periimplantitis [13]. Interleukin 23 is a recently identified pro-inflammatory cytokine, belonging to the IL-12 heterodimeric family [14,15]. IL-23 stimulates the Th17 cells to produce IL-17. The simultaneous presence of IL-17 and IL-23 is important because of its role in the development of chronic inflammation and influence in the host response against bacterial infection [16]. The working hypothesis of this study is formed on the results from literature data and is based on the suggestion that the presence of SNP of IL-23R (rs11209026) could be involved in the susceptibility to development of severe periodontitis.

The significance of tobacco smoking, associated with periodontal disease is widely discussed. Smoking is considered a major modifying factor when describing the grade of the periodontitis [17-19].

AIM

The aim of the study was to study the presence of IL-23 in a cohort of Bulgarian patients and to explore the relation between SNPs of IL-23 simultaneous with IL-17F and severity of periodontitis.

MATERIALS AND METHODS

In the recent study 83 individuals were recruited. Of them, 62 patients were diagnosed with severe periodontitis, while the other 21 were healthy controls. For estimation of the correct periodontal diagnosis

the following methods were applied: hygiene status and gingival status estimated by Full Mouth Plaque Score/Full Mouth Bleeding Score – FMPS/FMBS; Probing Pocket Depth (PPD) evaluated in six sites around each tooth; Clinical Attachment Level (CAL); Bleeding on Probing (BoP); Furcation involvement (F), tooth mobility (M) and gingival recession (R).

The clinical measurements were performed by UNC 15 (Hu Friedy) periodontal probe.

Radiographic examination was performed to all patients, diagnosed with periodontitis. A radiographic method, aiming to confirm periodontitis or periodontal health was used. The following diagnostic x-rays were made: orthopantomography and intraoral retro alveolar x-ray. Bl/Age ratio was calculated for each patient with periodontitis.

Periodontitis was defined as interdental clinical attachment loss in at least two non-adjacent teeth ≥ 2 mm or buccal or oral clinical attachment loss ≥ 3 mm with pocketing > 3 mm detectable at ≥ 2 teeth.

All participants with periodontitis met the including criteria: systemically healthy individuals with generalized stage III-IV periodontitis; presence of periodontal pockets more than 5 mm at least at 10 teeth; presence of periodontal pockets of more than 7 mm at least at 4 teeth; radiographically detected bone loss; presence of interdental CAL of 3-4 mm and ≥ 5 mm in the most advanced tissue destruction. Criteria for exclusion from the study: patients with systemic diseases; patients with immunosuppressive therapy, pregnant and breastfeeding women.

Statistical analysis

The data processing was implemented by SPSS – IBM SPSS Statistics Version 22. The following methods were applied:

1. Descriptive analysis – the frequencies distribution of the tested parameters is presented in tables, presented by subgroups.
2. Variation analysis – measures of central tendency and variability.
3. Student T-test – for hypothesis testing for two independent sample means. The basic level of significance in the hypothesis testing is 0.05.

Correlation coefficients were evaluated for each pair of the following parameters: Age, FMPS (%), FMBS (%), PD 1-3 mm, PD 3-5 mm, PD 5-7 mm, PD > 7 mm, CAL 1-2 mm, CAL-3-4 mm, CAL ≥ 5 mm, BoP (%), Bl/Age.

Laboratory methods for determination of SNPs of IL-17(-7488C/T rs763780) and IL-23 (rs11209026) were performed by means of Real-Time PCR. DNA from epithelial cells collected from buccal mucosa was extracted. The Termo Fisher Scientific (USA) TaqMan SNP genotyping kit was used. Each tampon with epithelial cells sample was incubated in buffer solution with Proteinase K at 55 degrees Celsius for 30 minutes with constant mixing. After removal of the tampon 400 microliters buffer solution optimized for DNA connection were added to each sample. The sample was centrifuged through column which was rinsed twice. DNA was eluted in 50 microliters buffer solution and was stored at -20 degree Celsius until the PCR analysis was performed. The PCR analysis was performed in 5 microliters genomic DNA with optimised sets of primers and two probes for genotype sampling. The real-time PCR was performed with 20 microliters total volume, 1 x 95C/10 min; 40 x 95C/15 sec and 60C/1 min. For SNP of IL17:rs763780C/T the target sequence was GTGGATATGCACCTCTT ACTGCACA[C/T] GGTGGATGACAGGGGTGACGCAGGT. For SNP of IL23R: rs11209026A/G the target sequence of ATTGGGATATTTAACAGATCATTCC[A/G] AACTGGGTAGGTTTTTGCAGAATTT was used.

RESULTS

Out of 83 tested individuals, 62 (74.7%) were confirmed as patients with periodontitis and 21 (25.3%) were defined as healthy individuals. A mild prevalence of the non-smokers was observed (55.6% non-smokers vs. 43.4% smokers). The genetic analysis of the two pro-inflammatory cytokine polymorphisms showed a variable distribution among the studied individuals. In regard to IL-17F, three genetic variations were found with significant prevalence of the TT genotype, while the SNP of IL-23 showed only two genotypes. The three genotypes in regards to SNP of IL-17F were distributed as follows: 77.1% TT or 64 participants (48 patients with periodontitis and 16 healthy individuals), 19.3% CT (12 patients with periodontitis and 4 healthy individuals) and 3.6% CC genotype (two patients with periodontitis and only 1 healthy participant). The genotype variations of SNP of IL-23 showed no presence of AA genotype, but general distribution of GG genotype – 96.4% (60 patients with periodontitis and 20 healthy controls), and a modest presence of GA genotype – 3.6% (two patients with periodontitis and just one healthy individual).

When exploring the SNP IL-23 G-allele homozygotes and the heterozygote cases, basic periodontal parameters about the patients were described and represented in Table 1 and Table 2. A rare GA genotype was located in 3 participants – two periodontitis patients and one healthy person. Their clinical results were similar to the homozygotes subgroup and showed no statistical significance.

Table 1. Descriptive Statistics for Patients with SNP IL-23 GG Genotype and Periodontitis

Characteristics	N	Average	Median	Standard deviation	Range	Minimal value	Maximum value
Age	60	48.77	47.00	11.42	56.00	29.00	85.00
FMPS (%)	60	86.03	92.00	15.88	54.00	46.00	100.00
FMBS (%)	60	72.66	74.00	25.11	92.20	7.80	100.00
PD 1-3 (%)	60	42.21	40.30	18.66	90.00	0.00	90.00
PD 3-5 (%)	60	34.02	34.95	14.93	61.70	5.50	67.20
PD 5-7 (%)	60	16.97	16.45	9.36	39.10	1.90	41.00
PD >7 (%)	60	6.03	4.25	6.96	43.60	0.00	43.60
CAL 1-2 (%)	60	32.20	32.70	18.57	72.00	0.00	72.00
CAL 3-4 (%)	60	40.44	39.60	15.17	71.80	7.30	79.10
CAL ≥ 5 (%)	60	25.01	21.90	15.74	69.30	0.90	70.20
BoP (%)	60	79.48	85.50	20.68	83.00	17.00	100.00
BI/Age	60	1.07	1.03	0.44	2.52	0.34	2.86

Table 2. Descriptive Statistics for Patients with SNP IL-23 GA Genotype and Periodontitis

Characteristics	N	Average	Median	Standard deviation	Range	Minimal value	Maximum value
Age	2	44.00	44.00	7.07	10.00	39.00	49.00
FMPS (%)	2	100.00	100.00	0.00	0.00	100.00	100.00
FMBS (%)	2	81.50	81.50	26.16	37.00	63.00	100.00
PD 1-3 (%)	2	30.90	30.90	15.70	22.20	19.80	42.00
PD 3-5 (%)	2	35.25	35.25	3.89	5.50	32.50	38.00
PD 5-7 (%)	2	29.15	29.15	18.46	26.10	16.10	42.20
PD >7 (%)	2	4.70	4.70	6.65	9.40	0.00	9.40
CAL 1-2 (%)	2	0.00	0.00	0.00	0.00	0.00	0.00
CAL 3-4 (%)	2	36.85	36.85	31.04	43.90	14.90	58.80
CAL ≥ 5 (%)	2	63.15	63.15	31.04	43.90	41.20	85.10
BoP (%)	2	78.00	78.00	31.11	44.00	56.00	100.00
BI/Age	2	1.10	1.10	0.04	0.05	1.07	1.12

When comparing the deepest periodontal sites (PD > 7 mm) in males and females we observed a similar distribution with a higher level of the median in male patients. There was a significant prevalence in the CAL ≥ 5 mm in male patients. Almost 50 males represented severe attachment loss compared to the female subgroup. The BoP had equal distribution among both genders. There was a single male individual with some extremely low level of BoP. From the gender/BI/Age ratio, we have established one very high and one low value of the BI/Age ratio among the male patients. In general, this parameter was comparable between males and females.

In regard to the SNP of IL-17F, three genotypes were established – TT, CT and CC. The main share among the patients was in TT genotype, while only two patients with CC genotype were identified. The periodontal parameters of the patients with periodontitis are summarised in Tables 3 and 4.

The strength of the relationship, measured by Pearson's correlation coefficients between some of the periodontal parameters related to the severity, the rate of progression of the periodontal disease and the patient's inflammatory status were explored. The values of PD > 7 mm, CAL ≥ 5 mm, BoP and BI/Age were higher in patients with CT genotype. When comparing the FMPS and the tested periodontal parameters it was established that the correlation was greater in the CT genotype patients.

The hypothesis testing showed no statistically significant differences in the periodontal parameters between the two SNP IL-17F genotypes (TT and CT).

The distribution of the PD > 7 mm and the BP had prevalence in the TT genotype patients, but the CAL ≥ 5 mm was greater in the CT genotype patients.

Regarding the interrelation between the two pro-inflammatory cytokines, we have noted some solid tendencies about periodontal parameters like PD > 7 mm and CAL ≥ 5 mm and BI/Age – clinical parameters characterising the periodontal disease as severe, and BI/Age – as a primary criterion for the grading, respectively the rate of progression of the periodontitis. We found that the presence of different genotypes especially in SNP of IL-17F were influencing the clinical expression of the abovementioned parameters. The results are summarized in Figures 1-2.

Table 3. Descriptive Statistics for Patients with SNP IL-17F TT Genotype

Characteristics	N	Average	Median	Standard deviation	Range	Minimal value	Maximum value
Age	64	43.94	44.00	12.83	53.00	22.00	75.00
FMPS (%)	64	75.44	84.00	28.07	92.00	8.00	100.00
FMBS (%)	64	62.85	64.50	31.35	97.00	3.00	100.00
PD 1-3 (%)	64	55.93	50.70	29.26	100.00	0.00	100.00
PD 3-5 (%)	64	26.27	29.55	19.45	67.20	0.00	67.20
PD 5-7 (%)	64	12.53	12.35	10.55	42.20	0.00	42.20
PD > 7 (%)	64	4.90	1.95	7.09	43.60	0.00	43.60
CAL 1-2 (%)	64	24.60	25.05	22.45	72.00	0.00	72.00
CAL 3-4 (%)	64	31.29	33.25	22.75	79.10	0.00	79.10
CAL ≥ 5 (%)	64	18.04	14.70	17.97	85.10	0.00	85.10
BoP (%)	64	60.31	73.10	39.68	100.00	0.00	100.00
BI/Age	64	0.80	0.92	0.60	2.86	0.00	2.86

Table 4. Descriptive Statistics for Patients with SNP IL-17F CT Genotype

Characteristics	N	Average	Median	Standard deviation	Range	Minimal value	Maximum value
Age	16	40.88	42.50	14.43	43.00	22.00	65.00
FMPS (%)	16	75.92	88.75	34.97	94.00	6.00	100.00
FMBS (%)	16	63.71	79.25	36.91	100.00	0.00	100.00
PD 1-3 (%)	16	55.38	45.35	33.06	100.00	0.00	100.00
PD 3-5 (%)	16	24.48	27.05	19.73	55.20	0.00	55.20
PD 5-7 (%)	16	16.13	16.25	14.40	41.00	0.00	41.00
PD > 7 (%)	16	2.63	0.75	3.51	10.10	0.00	10.10
CAL 1-2 (%)	16	18.41	14.80	17.65	50.00	0.00	50.00
CAL 3-4 (%)	16	26.31	31.30	20.03	54.00	0.00	54.00
CAL ≥ 5 (%)	16	25.13	23.50	21.78	58.30	0.00	58.30
BoP (%)	16	59.12	76.80	38.60	100.00	0.00	100.00
BI/Age	16	0.86	0.97	0.63	1.74	0.00	1.74

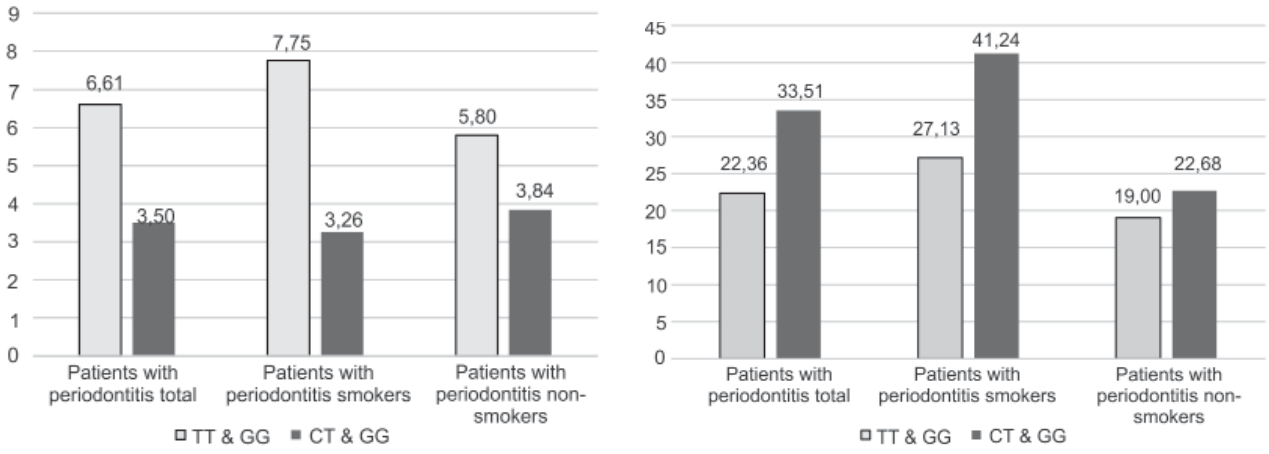


Fig. 1. PD > 7 mm and CAL ≥ 5 mm – simultaneous presence of different genotypes regarding IL-17F and IL-23

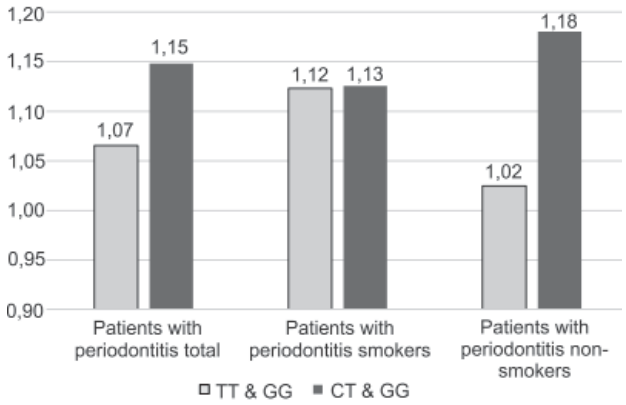


Fig. 2. BI/Age – simultaneous presence of different genotypes regarding IL-17F and IL-23

DISCUSSION

The suggestion that certain genetic polymorphisms are involved in the pathogenesis of variety of chronic diseases is not novel. The IL-23/IL-17 pathway could be considered possible determinant in the periodontal disease [20]. Despite the suggested role in the pathogenesis of periodontitis the distribution of their genetic polymorphisms is different in a given population and shows a variety of genotype distributions.

The recent study, conducted among Bulgarian patients with periodontitis and healthy controls, have provided information about the presence of SNPs of IL-17F and IL-23. We have hypothesised that the presence of specific genotype and even the common presence of different genotypes of two proinflammatory cytokines is related to severity of periodontitis. Without aiming to identify quantitative expression of these cytokines, but only their presence, we have established some particular conclusions. The SNP of IL-17F is demonstrated by significant prevalence of TT genotype. The careers of this genotype are represented by patients with periodontitis (75%) and healthy controls (25%). The CT genotype is isolated in 16 participants (75% in patients and 25% in controls as a common share). Only three participants were found to carry the CC genotype – 66% of this sample had periodontitis (only two participants) and 33% or just one person with this genotype was healthy (see Table 2). Based on some literature data, showing the results from the recent study, we have established some similarities in regard to the dominant TT genotype. Research aiming to identify the role of SNP of IL-17F in the periodontal disease was conducted in Brazil. Zacharias et al. did not detect CC genotype in the control group and identified only one person among 140 periodontitis patients with this obviously rare genotype. They have established a higher frequency of TT genotype than CT (91.3% TT / 8.7% CT) and concluded that the genetic polymorphism was not related to a higher risk of periodontitis development [9]. In similar research from Saraiva et al., an extremely high presence of TT genotype among patients with periodontitis was found (96.3% TT/ 6.4% CT) and no detection of CC genotype was found [21]. Erdemir et al. conducted a study aiming to identify a relation between SNP of IL-17F and periodontitis in Turkish population and the collected data is similar to the one in the recent study. Their results support our findings for a prevalence of TT genotype (90.4%), followed by CT (8.4%) and CC (1.2%) [22]. The genetic polymorphism of IL-17 in relation to periodontitis was studied by Mazurek-Mochol et al. in Polish individuals. The results showed presence of two genotypes – TT genotype presents 95% of the patients with periodontitis, CT genotype only 5%, while the distribution of those genotypes among the control group is 92.50% for TT genotype and 7.25% for CT genotype. In the studied population CC genotype is not detected [22].

Our search for specific genotype about polymorphism of IL-23 shows a remarkable prevalence of GG genotype with only three individuals, representing the GA genotype. No A-allele homozygotes were detected. The dominant genotype is equally distributed among periodontitis patients and healthy controls (Table 2). In contrast to our study, Erdemir at al. found three genotypes with specific distribution in a Turkish population with highest prevalence of GG genotype, followed by GA genotype and very discrete distribution of the obviously rare AA genotype [20]. The rare AA genotype was encountered with a very low frequency in a group of periodontal patients in Brazilian population, but not in the healthy controls group. Both studies found no significant relation between the IL-23 SNP and the severity of periodontitis [21].

Regarding the clinical presentation of the given genotypes, we have found solid tendencies about the prevalence of the deepest periodontal sites (PPD ≥ 7 mm) in patients with concomitant presence of TT genotype (IL-17F) and GG genotype (IL-23), irrespective of the smoking habits. The distribution of periodontal sites with CAL ≥ 5 mm is higher in the patient's subgroup with CT genotype (IL-17F) and GG genotype (IL-23) in the smoker's subgroup. The ratio BI/Age is directly related to the grade of the periodontal disease. Our research has detected that the patients with simultaneous presence of CT genotype (IL-17F) and GG genotype (IL-23) have greater value of the particular index only in the non-smoker's subgroup. The TT genotype of SNP of IL-17f could be considered a potential risk factor regarding the deepest periodontal pockets despite the

presence of the tobacco smoking as a major periodontitis modifying risk factor.

CONCLUSIONS

Within the limitations of the recent study, we identified two SNPs among Bulgarian individuals. With regards to IL-17F polymorphism, we found three genotypes (TT, CT, CC) with higher prevalence of TT genotype. The results about IL-23 polymorphism showed a significant prevalence of GG genotype and very limited distribution of GA genotype. No statistical significance between the IL-17F and IL-23 and periodontitis was found. Some tendencies about the distribution of the severely affected periodontal sites in patients with specific genetic profile combining different genotypes in both IL-17F and IL-23 were detected. A larger study among a wider group is needed in order to provide a solid data about the significance of these genetic polymorphisms in the pathogenesis of the periodontal disease.

Acknowledgements: *The recent study is supported by Grant project No. 4321/06.07.2020. with contract No. D-47/08.03.2021. Part of the results from the project were presented as an oral presentation in the 20th BZS congress (16-18.06.2022).*

Disclosure Summary: *The authors have nothing to disclose.*

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