Advs Exp. Medicine, Biology - Neuroscience and Respiration (2017) 29: 29–37
DOI 10.1007/5584_2016_164

Published online: 14 October 2016

Prevalence of *Neisseria meningitidis* Carriage with Identification of Serogroups and Genogroups in Professional Soldiers

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Abstract

The article presents the prevalence of *Neisseria meningitidis* carriage with the identification of sero- and genogroups in professional soldiers serving in the Polish Armed Forces. A total of 1246 soldiers from the 10th Armored Cavalry Brigade in Świętoszów, Poland were examined in the period January-February 2016. Microbiological tests were performed using standard methods (culture, incubation, microscopy, biochemical, and automated identification with VITEK cards). *Neisseria meningitidis* isolates from carriers were subjected to a slide agglutination test for the identification of serogroups, next bacterial DNA was isolated and genogroups were identified based on the results of PCR. Of the 1246 soldiers tested, 65 were found to be carriers of *N. meningitidis*. Serogroups of 36 isolates and genogroups of 56 meningococcal isolates were determined. The genogrouping identified the isolates as belonging to group B (n = 34; 52.3 %), E29 (n = 8; 12.3 %), C (n = 6; 9.2 %), Y (n = 6; 9.2 %), and W (n = 2; 3.1 %). The primers which were used did not make it possible to determine the genogroup of nine isolates. In conclusion, the overall carrier rate of *N. meningitidis* amounted to 5.2 %, with the serogroup B being predominant, which is similar to that reported in the general population in Poland and Central Europe.

Keywords

Neisseria meningitidis • Serogrouping • Genogrouping • Carriage • Prevalence • Soldiers

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1 Introduction

Neisseria meningitidis (*N. meningitidis*) is a major etiological factor associated with infections of the central nervous system (CNS). CNS infections commonly take the form of meningitis or sepsis and are referred to as invasive meningococcal disease (Rosenstein et al. 2001). The bacteria colonize the nasopharyngeal mucosa asymptomatically and *N. meningitidis* carriers are the major source of infection (Soriano-Gabarro et al. 2011). Estimated prevalence of *N. meningitidis* in the general population ranges from 5 to 10 % (Skoczyńska and Hryniewicz 2012), whereas in closed environments such as prisons, boarding schools, or military camps, meningococcal carriage rate may reach 40–80 % (Tyski et al. 2000). There are 12 serogroups of meningococcal strains described as A, B, C, E29, H, I, K, L, W135, X, Y, and Z; the serogroups are determined by the biochemical composition of a polysaccharide capsule of *N. meningitidis*. Serogroups A, B, C, Y, and W135 are most commonly isolated from carriers or in invasive infections. In 30–45 % of cases, it is impossible to identify the bacterial serogroup (Caugant et al. 2007; Bennett and Cafferkey 2006). Serogroups B and C are a major causes of meningococcal infections in North and South America as well as in Europe, while serogroups A and C account for a majority of infections in Asia and Africa. In recent years, especially in the United States, the United Kingdom, Sweden and Finland, the incidence of meningococcal infections caused by serogroup Y has increased (Skoczyńska and Hryniewicz 2012; Rosenstein et al. 2001). In general, serogroup prevalence varies between countries and changes over time.

Invasive meningococcal disease (IMD) remains one of the most serious infectious illnesses in the world, despite the use of antibiotics at an early stage and the development of intensive care facilities. IMD is an acute illness which may lead to death in less than 24 h. Mortality from IMD reaches 10–13 %, and in case of a septic shock – as much as 70 % (Skoczyńska and Hryniewicz 2012; Caugant et al. 1994). Two hundred and eighteen cases of the diseases have been reported in Poland in 2015 (National Institute of Public Health 2015), and there were 103 deaths from IMD between 2010 and 2014 (KOROUN 2015). The IMD outbreaks are rare in the Polish Armed Forces, yet they may pose a significant health hazard. In 2006, four microbiologically confirmed IMD cases were reported in a military unit in Skwierzyna; two of the infected soldiers died (Grecki and Bienias 2006). In 2007, another 15 cases of IMD were reported from a military airbase in Warsaw; two of the patients died (Kadłubowski et al. 2007). Between 2006 and 2008, there were reports of microbiologically confirmed IMD cases from military units in the cities of Wrocław, Gliwice, Gołdap, Warszawa-Wesoła, Toruń, Koszalin, and Przemyśl. In December 2011, a Polish soldier died from IMD while serving in Afghanistan. Although there have been a number of confirmed cases of IMD among soldiers, some of which were fatal, there have been few studies into the prevalence of *N. meningitidis* carriage in the military environment. The studies by Tyski et al. (2001) conducted in 1998 and 1999 have reported that out of the 151 and 168 soldiers tested, 36 % and 61 %, respectively, were carriers of *N. meningitidis*. These studies, however, involved conscripts and Poland suspended conscription in 2009. The only study on *N. meningitidis* carriage in professional soldiers serving in the Polish Armed Forces was conducted in 2013 by research staff from the Department of Epidemiology and Tropical Medicine of the Military Institute of Medicine as part of the department's statutory activity. The study, involving 559 soldiers from the 25th Brigade stationed in the city of Tomaszów Mazowiecki, the unit in which the soldier who died from IMD in Afghanistan in 2011 had served, has found that 5.7 % of the soldiers tested were carriers of *N. meningitidis*. In non-vaccinated soldiers ($n = 302$), the carriage rate was 9.6 %, whereas among the vaccinated individuals ($n = 257$) it was only 1.2 %. The carriage rate was thus eight-fold lower in the vaccinated soldiers

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than that in the non-vaccinated ones, which suggests that vaccination is an effective method of inducing herd immunity (Korzeniewski et al. 2015). To investigate whether it is necessary to vaccinate all the Polish military personnel with a quadrivalent conjugate vaccine against *N. meningitidis* serogroups A, C, Y, and W135 and with a newly introduced vaccine against serogroup B, we were obliged to conduct a large-scale population study to investigate the prevalence of *N. meningitidis* carriage in the military environment. The obligation was in compliance with a regulation of the Minister of Defense of February 2014 on a vaccination schedule for professional soldiers. Therefore, the aim of the present study was to assess the prevalence of *N. meningitidis* carriage and to identify the sero- and genogroups in professional soldiers serving in the Polish Armed Forces.

2 Methods

The research task was approved by the Ethics Committee of the Military Institute of Medicine in Warsaw, Poland (permit 24/WIM/2014 of 18 Aug 2014).

2.1 Study Population

We tested 1246 professional soldiers from the 10th Armored Cavalry Brigade stationed in the city of Świętoszów after they had provided informed consent and completed a questionnaire concerning personal information such as military rank, age, gender, place of residence, cigarette smoking, symptoms of a respiratory tract infection, medications used on a regular basis, and vaccinations against meningococcal infections. The inclusion criteria were as follows: age of 20–55 years and a good general health, with a possible respiratory tract infection but no pathological lesions in the nasopharynx that would make it impossible to take a swab sample. The biological material (nasopharyngeal swabs) was taken during the winter season (January-February) of 2016 on the premises of the military unit.

2.2 Laboratory Workup

Identification of Isolates The cultures were inoculated, using a streaking procedure, onto the Columbia Agar medium with 5 % sheep blood and PoliVitex VCA3 and incubated under elevated CO_2 concentration at 37 °C for 48 h. They were then transported to a microbiological laboratory of the Military Institute of Medicine in Warsaw where the colonies grown were macroscopically evaluated. The colonies morphologically similar to *N. meningitidis* strains were isolated onto Columbia Agar with 5 % sheep blood plates, and were then incubated under elevated CO_2 concentration at 37 °C for another 24–48 h. Catalase and cytochrome oxidase tests were performed. Gram-stained preparations were examined with light microscopy. All catalase and cytochrome oxidase-positive strains, as well as strains morphologically similar to Gram-negative cocci (microscopic evaluation) were then identified with biochemical tests. The identification was carried out by means of API NH biochemical sets and an automated system for identification of microorganisms using Vitek 2 NH cards (bioMerieux; Marcy l'Etoile, France). The strains identified as *N. meningitidis* were stored at −20 °C and further transported to the National Reference Center for the Diagnostics of Bacterial Infections of Central Nervous System (KOROUN) in Warsaw, Poland for re-identification, serogrouping, DNA isolation, and genogrouping.

Serogrouping The strains delivered to KOROUN were revived by placing them onto the Columbia Agar medium; they were incubated in elevated CO_2 atmosphere at 37 °C for 24 h. Serogroups of the isolates were identified with a slide agglutination test using a set of primers according to the manufacturer's recommendations. The serogroup-specific reagents included the serogroups: A, B, C, Y, and W (Thermo

Fisher Scientific, Remel Products; Lenexa, KS), E29 (Bio-Rad Laboratories LTD; Hemel Hempstead, UK), and X and Z (Becton Dickinson; Franklin Lakes, NJ).

DNA Isolation Chromosomal DNA was isolated from meningococcal isolates with Genomic DNA Prep Plus (A&A Biotechnology; Gdynia, Poland), following the manufacturer's recommendations.

Genogrouping Genogroups were identified with PCR assays using the genogroup-specific oligonucleotide primers *orf-2*(A), *siaD*(C), *siaD* (W135), and *siaD*(Y) according to the description of Taha (2000) and *siaD*(B) according to Guiver et al. (2000).

2.3 Statistical Elaboration

Quantitative variables were characterized by the arithmetic mean ± SD or median with max/min range and 95 % confidence interval. Qualitative variables were presented as count and percentage. To check if a quantitative variable derives from a population of normal distribution the Shapiro-Wilk test was used. The Leven test was used to demonstrate the homogeneity of variances. Statistical significance of differences between two groups was processed with a *t*-test or Mann-Whitney *U* test. A logistic model was used to examine dependency of socio-demographic variables and the non-carrier/carrier groups. A p-value under 0.05 was considered statistically significant. The analyses were performed using a commercial STATISTICA package of StatSoft Inc. ver. 12.0.

3 Results

Out of the 1246 professional soldiers tested, 65 (5.2 %) were found to be carriers of *N. meningitidis*. The serogroups were determined for 36 *N. meningitidis* isolates. Five serogroups were identified: B (n = 25, 38.5 %), Y (n = 4, 6.15 %), E29 (n = 2, 3.1 %), C (n = 2, 3.1 %), W (n = 2, 3.1 %), and A (n = 1, 1.5 %). A large number of isolates polyagglutinated (n = 23, 35.4 %), other isolates autoagglutinated (n = 5, 7.7 %), and one isolate (1.5 %) did not agglutinate. The genogroups were determined for 56 isolates: B (n = 34, 52.3 %), E29 (n = 8, 12.3 %), C (n = 6, 9.2 %), Y (n = 6, 9.2 %), and W (n = 2, 3.1 %). It was impossible to determine the genogroup of nine isolates (NG, 13.8 %) with the primers used (Fig. 1). The results of serogrouping and genogrouping are presented in Table 1.

The mean age of *N. meningitidis* carriers was 30.7 ± 5.0 years (range 21–45 years) and that of non-carriers was 31.7 ± 5.4 years (range 20–59 years); the difference between the two groups was insignificant. There was one woman in the carrier group and 116 women (9.8 %) in the non-carrier group; the proportion of women among the non-carriers was significantly higher (p = 0.044). As for the carrier group, the proportion of subjects living in rural areas was 38.5 %, which was grossly akin to that in the non-carrier group of 40.9 %. There were no significant

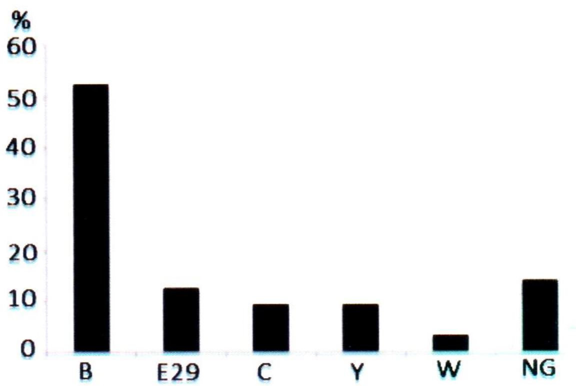

Fig. 1 Percentage distribution of carriers of the *N. meningitidis* genogroups B, E29, C, Y, and W in the soldiers examined (n = 65) (*NG* isolates that could not be allocated to any specific genogroup with the primers used)

Table 1 Serogrouping (monoclonal serum), presented in rows, and genogrouping (PCR), presented in columns, of *N. meningitidis* isolates (n = 65)

Sero/Genogrouping	A	B	C	E29	W	Y	NG	Total
A	–	–	–	–	–	–	1	1
Autoagglutination	–	2	–	1	–	–	2	5
B	–	25	–	–	–	–	–	25
C	–	–	2	–	–	–	–	2
E29	–	–	–	2	–	–	–	2
Polyagglutination	–	7	4	5	–	2	5	23
NG	–	–	–	–	–	–	1	1
W	–	–	–	–	2	–	–	2
Y	–	–	–	–	–	4	–	4
Total	0	34	6	8	2	6	9	65

NG isolates that could not be allocated to any specific genogroup with the primers used

Table 2 Socio-demographic variables of non-carriers and carriers of *N. meningitidis*

Socio-demographics	Non-carriers (n = 1181)	Carriers (n = 65)	p-value
Age (years)			
Mean (SD)	31.7 (5.4)	30.7 (5.0)	0.188
Range	20.0–59.0	21.0–45.0	
Median	31.0	31.0	
95%CI	31.4–32.0	29.5–32.0	
Gender			
Women	116 (9.8 %)	1 (1.5 %)	0.044
Men	1065 (90.2 %)	64 (98.5 %)	
Place of residence			
Rural area	483 (40.9 %)	25 (38.5 %)	0.693
Urban area	697 (59.1 %)	40 (61.5 %)	
Cigarette smoking			
Yes	396 (33.5 %)	30 (46.2 %)	0.037
No	785 (66.5 %)	35 (53.8 %)	
Respiratory tract infection			
Yes	86 (7.3 %)	2 (3.1 %)	0.198
No	1095 (92.7 %)	63 (96.9 %)	
Military rank			
Private	742 (62.8 %)	47 (72.3 %)	0.087
Non-commissioned officer	371 (31.4 %)	18 (27.7 %)	
Commissioned officer	68 (5.8 %)	0 (0 %)	
Vaccinated			
Yes	178 (15.1 %)	6 (9.2 %)	0.196
No	1003 (84.9 %)	59 (90.8 %)	

correlations between the place of residence and the study group (p = 0.693). The proportion of smokers in the carrier group was 46.2 %, which was significantly greater than the 33.5 % in the non-carrier group (p = 0.037). The proportion of privates in the carrier group was 72.3 % compared with 62.8 % in the non-carrier group; the proportion of non-commissioned officers (NCO) was 27.7 % and 31.4 % and the proportion of commissioned officers (CO) was 0.0 % and 5.8 % in the respective groups. There were no significant differences in the distribution of military ranks between the two study groups (p = 0.087). The proportion of vaccinated soldiers in the *N. meningitidis* carrier group was 9.2 % and in the non-carrier group it was 15.1 %, with no significant difference between the two groups (p = 0.196) (Table 2).

In the *N. meningitidis* non-carrier group, the mean age of the vaccinated soldiers was 33.5 ± 4.8 years (range 23–49 years) and of the non-vaccinated ones it was 31.4 ± 5.4 years (range 20–59 years); the latter were significantly younger (p = 0.0001). The proportion of women among the vaccinated soldiers was 2.7 % and among the non-vaccinated ones it was 10.5 %; the difference was significant, p = 0.0008. The proportion of privates among the vaccinated soldiers was 45.1 %, and among the non-vaccinated ones it was 66.5 % (the difference was significant, p = 0.0001) (Table 3).

In the *N. meningitidis* carrier group, the mean age of the vaccinated soldiers was 34.5 ± 2.6 years (range 32–38 years) and of the non-vaccinated ones it was 30.3 ± 5.0 years (range 21–45 years); the latter were significantly younger (p = 0.025). There were no women in the group of vaccinated soldiers; the proportion of women in the group of non-vaccinated soldiers was 1.7 %. No significant differences were found between gender and vaccination (p = 0.156). The proportion of privates among the vaccinated soldiers was 0.0 %, and among the non-vaccinated soldiers it was 79.7 % (the difference was significant, p = 0.0002) (Table 4).

The logistic regression analysis (univariate and multivariate) identified two factors significantly associated with increased risk of *N. meningitidis* carriage, i.e., smoking cigarettes and low military rank (Table 5).

4 Discussion

The only natural habitat of *N. meningitidis* in humans is the nasopharyngeal mucosa. The bacteria colonize the nasopharynx and spread through inhalation of droplets of respiratory secretions or through a direct contact with a carrier (e.g., sharing a drink or a cigarette with an infected person). In most cases, meningococcal carriage does not lead to invasive disease. Yet it may cause an invasive meningococcal disease (IMD) under certain conditions such as concomitant respiratory tract infections, dental diseases,

Table 3 Socio-demographic variables among soldiers vaccinated with quadrivalent vaccine A, C, W135, and Y, and non-vaccinated soldiers in the group of *N. meningitidis* non-carriers

Socio-demographics	Vaccinated (n = 184)	Non-vaccinated (n = 1062)	p-value
Age (years)			
Mean (SD)	33.5 (4.7)	31.3 (5.4)	0.0001
Range	23.0–49.0	20.0–59.0	
Median	34.0	31.0	
95%CI	32.8–34.2	31.0–31.6	
Gender			
Women	5 (2.7 %)	112 (10.5 %)	0.001
Men	179 (97.3 %)	950 (89.5 %)	
Place of residence			
Rural area	68 (37.0 %)	440 (41.5 %)	0.250
Urban area	116 (63.0 %)	621 (58.5 %)	
Cigarette smoking			
Yes	79 (42.9 %)	347 (32.7 %)	0.007
No	105 (57.1 %)	715 (67.3 %)	
Respiratory tract infection			
Yes	16 (8.7 %)	72 (6.8 %)	0.349
No	168 (91.3 %)	990 (93.2 %)	
Military rank			
Private	83 (45.1 %)	706 (66.5 %)	0.0001
Non-commissioned officer	89 (48.4 %)	300 (28.2 %)	
Commissioned Officer	12 (6.5 %)	56 (5.3 %)	

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Table 4 Socio-demographic variables among soldiers vaccinated with quadrivalent vaccine A, C, W135, and Y, and non-vaccinated soldiers in the group of *N. meningitidis* carriers

Socio-demographics	Vaccinated (n = 6)	Non-vaccinated (n = 59)	p-value
Age (years)			
Mean (SD)	34.5 (2.6)	30.3 (5.0)	0.025
Range	32.0–38.0	21.0–45.0	
Median	34.0	30.0	
95%CI	31.8–37.2	29.0–31.6	
Gender			
Women	0 (0.0 %)	1 (1.7 %)	0.156
Men	6 (100.0 %)	58 (98.3 %)	
Place of residence			
Rural area	1 (16.7 %)	24 (40.7 %)	0.477
Urban area	5 (83.3 %)	35 (59.3 %)	
Cigarette smoking			
Yes	2 (33.3 %)	28 (47.5 %)	0.817
No	4 (66.7 %)	31 (52.5 %)	
Respiratory tract infection			
Yes	0 (0 %)	2 (3.4 %)	0.434
No	6 (100 %)	57 (96.6 %)	
Military rank			
Private	0 (0 %)	47 (79.7 %)	0.0002
Non-commissioned officer	6 (100 %)	12 (20.3 %)	
Commissioned officer	0 (0 %)	0 (0 %)	

Table 5 Factors associated with increased risk for *N. meningitidis* carriage according to the logistic regression analysis (univariate and multivariate)

Socio-demographics	Logistic regression – univariate			Logistic regression – multivariate		
	Assessment	Odds ratio	p-value	Assessment	Odds ratio	p-value
Age	−0.1	1.0	0.163	−0.1	1.0	0.341
Gender						
Men	1.0	2.6	0.055	1.0	2.7	0.050
Women	−1.0	0.4	0.055	−1.0	0.3	0.050
Place of residence						
Rural area	−0.1	1.0	0.693	−0.1	0.9	0.578
Urban area	0.1	1.1	0.693	0.1	0.1	0.578
Cigarette smoking						
Yes	0.3	1.3	0.039	0.3	1.3	0.042
No	−0.3	0.8	0.039	−0.3	0,8	0.042
Respiratory tract infection						
Yes	−0.5	0.6	0.213	−0.5	0.6	0.255
No	0.5	1.6	0.213	0.5	1.6	0.207
Military rank						
Private	5.8	340.6	0.0010	5.7	290.4	0.0001
Non-commissioned officer	4.9	133.9	0.0001	4.9	137.5	0.0001
Commissioned officer	−10.1	0.01	-	−9.9	0.1	-
Vaccinated						
Yes	−0.3	0.8	0.202	−0.3	0.7	0.050
No	0.3	1.3	0.202	0.3	1.3	0.187

and the like. Studies concerning the prevalence of carriage conducted among military recruits have shown that despite a high asymptomatic infection rate, hypervirulent *N. meningitidis* strains rarely colonize the nasopharyngeal mucosa (Tzanakaki et al. 1993; Caugant et al. 1988). In fact, epidemic strains are found only in 1.4–1.6 % of healthy individuals (Cartwright et al. 1987). In an outbreak of IMD, however, the prevalence of hypervirulent strains in carriers can be significantly higher (Edwards et al. 1977). *N. meningitidis* carrier rates among European recruits are high, regardless of the size of a given study group or country of origin. Andersen et al. (1998) have found that the carriage rate among 1069 Danish recruits was 39–47 %. The surveillance study conducted among 1179 German recruits has found that 32.6 % of the soldiers are carriers of *N. meningitidis* (Claus et al. 2005). In Norway, a meningococcal carriage study in 126 military recruits has demonstrated the prevalence of *N. meningitidis* carriage at 61.9 % (Caugant et al. 2007). By contrast, our previous investigation on meningococcal carriage, conducted in 559 professional Polish soldiers, has found that only 5.7 % of them were *N. meningitidis* carriers, which is similar to the overall meningococcal carriage rates in the general population. That study has also revealed that serogroup B was the most prevalent one among the carriers (28 %) (Korzeniewski et al. 2015). In fact, serogroup B is still the most common of all *N. meningitidis* strains isolated from soldiers in Europe. This group is the most frequently identified serogroup among recruits studied in France (46 %) (Chapalain et al. 1992), Poland (32 %) (Tyski et al. 2001), and Germany (42 %) (Claus et al. 2005). It is important that meningococcal carriage studies focus not only on the identification of serogroups but also on the risk factors shaping the *N. meningitidis* carriage rate. Smoking, both active and passive, is a major contributing factor for the nasopharyngeal carriage of *N. meningitidis* (Stuart et al. 1989). This was confirmed in the present study as the logistic regression analysis found that smoking was associated with increased risk for meningococcal carriage.

Studies into the prevalence of meningococcal carriage in the military environment are widely available. However, the majority are limited to one type of community only, i.e., young, newly drafted recruits. Some European countries, including Poland, have suspended compulsory conscription and transformed their national armies into fully professional organizations. As a result of those changes, the mean age of soldiers has increased; 19–20 years old recruits are replaced by 25–30 years old professional privates. The past young recruits used to serve on a 24/7 basis, were accommodated in barracks, and had meals in military dining facilities; the conditions that are conducive to *N. meningitidis* carriage. The present time older professional soldiers, on the other hand, typically work eight hours a day and are not accommodated on the premises of a military unit, with the exception of rather infrequent 24-h duties, military exercises, or military operations. In fact, professional military service has changed from 24/7 service to a regular job, with risk factors similar to those found in the civilian environment. That is reflected by a relatively higher age of thirty odd years of soldiers in the present study; the age at which the prevalence of meningococcal carriage seems reduced.

5 Conclusions

The overall carrier rate of *N. meningitidis* in the study group was 5.2 %, with serogroup B being predominant (52.3 %), which is similar to the rate reported in the general population in Poland and Central Europe. Considering the fact that meningococcal carrier state may be chronic and last for several months, or it may be irregular and intermittent as shown by variability in colonization of the nasopharyngeal mucosa, it is necessary to conduct further research into the prevalence of *N. meningitidis* carriage among soldiers, especially in the context of the validity of introducing immunoprophylaxis against meningococcal infections for the entire military personnel of the Polish Armed Forces.

Acknowledgments We are grateful to the Commander of the 10th Armored Cavalry Brigade in Świętoszów for permission to carry out this study and to the soldiers of this military unit for their participation in examination. The paper was supported by the Polish Ministry of Science and High Education, grant no. 326/2015.

Conflicts of Interest The authors declare no conflicts of interest in relation to this article.

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