Rotavirus gastroenteritis in children less than five years of age in primary care settings in Bulgaria: an observational study

Mayda Tiholova[1], Kusuma Gopala[2], Magda Berberova[3], Margarita Strokova-Stoilova[4], Monica Tafalla[5,]*

Abstract

Background **Rotavirus (RV) causes a high proportion of acute gastroenteritis (AGE) cases, especially among children under the age of five years old. This surveillance study was undertaken to study the incidence and severity of rotavirus gastroenteritis (RVGE) in primary care settings in Bulgaria over a one-year period.**

Methods **In this prospective, observational study of AGE cases in children under five years of age presenting in the primary care setting over a one year period, stool samples were collected and tested for RV using a rapid visual immunochromatographic test kit. After the first visit, parents monitored their child for about two weeks and reported the symptoms experienced by the child during the follow-up period to the physician in a follow-up phone call. The percentage of RVGE cases among AGE was calculated and the severity of AGE (according to the 20-point Vesikari scale) was assessed by the physician based on the symptoms reported by the parents. The seasonality of RVGE was also studied.**

Results **The proportion of RVGE among the 624 AGE cases examined was 25.5%. Severe AGE was experienced by 81.8% RV-positive and 54.6% RV-negative children (p-value <0.001) and a third of all severe AGE cases occurred in RV-positive patients. A multivariate logistic regression analysis of the determinants of hospitalization indicated that severity of disease and RV-positivity were the statistically significant variables explaining hospitalization of AGE cases; even controlling for severity, RV-positive patients were more often hospitalized than RV-negative ones. RVGE cases occurred throughout the year, with peaks during August and September.**

Conclusion **Our study emphasizes that RV is an important cause of AGE in children under five presenting in primary care settings in Bulgaria and a disproportionately high proportion of severe AGE cases may be attributed to RV infections.**

Trial registration number **NCT01733849**

Keywords **Rotavirus, acute gastroenteritis, RV disease burden, primary care settings**

Background

Rotavirus (RV) is the most common cause of diarrhea in the first two years of life[1] and contributes to approximately 40% (range 34%-45%) of diarrhea-related hospitalizations among children under the age of five years.[2] RV is also globally the most common cause of acute gastroenteritis (AGE)[3] and worldwide accounted for around 453,000 deaths (420,000–494,000)[4] and over two million hospitalizations among children under five in 2008.[3] Most deaths due to RV occur in developing countries where there is underlying malnutrition and the access to healthcare facilities for life-saving rehydration may be difficult and delayed.[5]

Interventions such as improved sanitation and anti-microbial treatments, which have shown promise against some diarrhea-associated bacterial and parasitic diseases, are less effective

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[1]MD, Department of Infectious Diseases, Medical University Sofia, Bulgaria; [2]MSc, GSK Pharmaceuticals Ltd., Bangalore, India; [3]MD, GSK Bulgaria, Sofia, Bulgaria; [4]MD, GSK Bulgaria, Sofia, Bulgaria; [5]MD, MPH, GSK Vaccines, Wavre, Belgium.

*Corresponding author: Monica Tafalla, MD, MPH, Senior Manager, Epidemiology Europe, GSK Vaccines, Avenue Fleming 20, 1300 Wavre, Belgium. monica.x.tafalla@gsk.com

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against RV. However, vaccination against RV is effective[6] and since 2009 the World Health Organization (WHO) has recommended the inclusion of RV vaccination in all National Immunization Programs (NIP).[7-9]

Two safe and effective oral vaccines are available since 2006 but their uptake has been limited in many countries and relatively few countries have included RV vaccination in their NIP although many more have issued recommendations for parents to have their infants vaccinated. One reason for the decision of many countries not to include RV vaccination in their NIP is that the cost-effectiveness of such vaccinations is controversial.[10] Part of the reason for the controversy about the cost-effectiveness of RV vaccination is that the available information about the epidemiology and actual burden of RV disease is rather limited in many countries.

One such country is Bulgaria, where reporting of rotavirus gastroenteritis (RVGE) cases has been mandatory since 2011 and RV vaccination is recommended but not included in the NIP. A steep increase was seen from 2011 to 2014 in the number of cases reported but the 1861 cases reported in 2014 is still very low compared to a rough estimate of the actual number of cases. Thus, the number of children less than five years old was approximately 340,000 and European studies assess the yearly incidence of AGE in this child population to range from 4 to 16 per 100 children,[5] or an expected 13,600 to 54,400 AGE cases per year in Bulgarian children under five. If the proportion of RVGE cases is assumed to be a relatively low 25%,[11-14] the expected yearly number of RVGE cases in this child population would range from 3,400 to 13,600.

The existing incidence data for Bulgaria are based on hospitalized cases[15-17] but they include only the more severe cases and do not give an accurate impression of the epidemiology and actual burden of RV disease. By one estimate, up to one in five AGE cases will see a physician and no more than two percent will be admitted to a hospital or present to an emergency room while the remaining cases will be cared for in their home by the parents or other caregivers.[18]

The present study aimed to add to the available information about the epidemiology and burden of disease of RVGE in Bulgaria by investigating children less than five years old presenting in primary care settings with AGE. The primary objectives were to estimate the proportion of RVGE cases among all AGE, to characterize them in terms of severity of symptoms and treatment and also to study the seasonality of RVGE over a one-year period. The results will be useful for short term decisions about RV vaccinations and as baseline data for potential future studies of the impact of vaccinations, if RV vaccination is included in the NIP or if the vaccine uptake increases based just on parents' autonomous decisions.

Methods

Study design

This was a prospective, observational study conducted from December 2012 to January 2014 in a convenience sample of 30 primary care centers throughout Bulgaria, equally representing urban and rural areas (NCT01733849). The centers were selected based on the estimated number of children each general practitioner could include in the study.

The study was conducted in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki and was approved by the local NCIPD ethics committee (N°00006384 approved 22 November 2012). Written informed consent was collected from the parents of each child before enrolment; no data that could identify the patient were collected.

Study population and case definition

Children younger than five years old, whose parents consulted their primary care doctor for their child's AGE, were eligible for enrolment in the study. A case of AGE was defined according to the WHO definition: "three or more loose stools and/or two or more vomiting episodes within a 24-hour period, with the onset of symptoms ≤14 days before the primary care consultation".[2] Children were only excluded from enrollment if they did not meet the case definition of AGE.

Data collection

During the initial visit at the physician's consultation or in the child's home, the parents

completed a questionnaire regarding their child's age, gender, medical history, previous RV vaccination and AGE symptoms. The parents were also asked to monitor and record the child's condition (episodes of vomiting, diarrhea) and temperature for about two weeks following the initial visit and diagnosis. At the end of this follow-up period, the investigator made a follow-up phone call to ask about the child's current condition and to record the information noted by the parents about the duration of diarrhea and vomiting and the maximum number of diarrhea and vomiting episodes experienced over a 24-hour period. The severity of each AGE case was assessed based on the information recorded by the investigator during the follow-up phone call using the 20-point Vesikari scale (Vesikari score ≤6: mild; 7-10: moderate; ≥11: severe).[19]

Diagnosis of RV

Stool samples were collected from each child at the initial visit or up to four days after. The samples were tested for the presence of RV at the study site using a rapid visual immunochromatographic test kit (*IMMUNOQUICK-RV*, Biosynex, Strasbourg, France). The stool samples were not tested for other intestinal pathogens.

Statistical analysis

Assuming an RVGE/AGE proportion of 35% based on several European incidence studies[11-14] and an acceptable width of the two-sided 95% confidence interval (CI) of 10%-points, we considered that an evaluable sample of 700 AGE patients would allow us to estimate the proportion of RVGE among all AGE cases with acceptable precision. Assuming the percentage of non-evaluable children to be 10%, the target enrollment was approximately 780 children with AGE to achieve an evaluable number of 700. The analysis was performed on all children who met the eligibility criteria, complied with the protocol-defined procedures and had available data. Demographic characteristics, symptoms, severity, medical history including previous RV vaccination were reported with appropriate descriptive statistics, including 95% CIs. Statistical comparisons of categorical variables between RV-positive and RV-negative cases were made by Chi-square tests or Fisher's exact p-values. Multivariate logistic regression analysis models with Wald test for statistical significance were used to examine the determinants of hospitalization of these AGE patients first presenting in primary care. All statistical analyses were carried out using SAS Version 9.2 or Excel 2007.

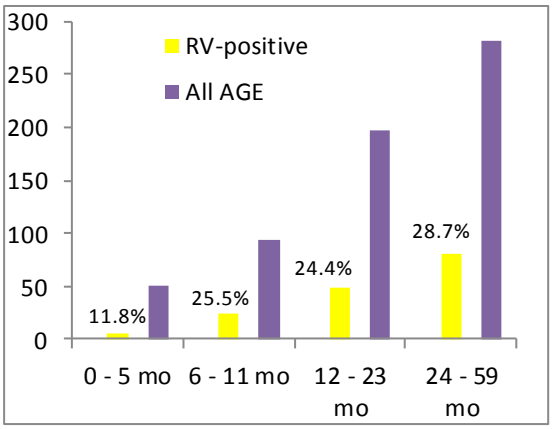
Results

Demographic characteristics

Of 629 enrolled children, 624 were analyzed and five patients were excluded because of protocol violation or non-compliance with the study procedures. Ten children were enrolled twice for two separate episodes of AGE. The mean age of the children was 24.4 months (standard deviation 15.1 months) and 50.3% were male. More than 96% of the initial visits took place in the physician's consultation, the rest in the child's home. Thirty-eight patients had been vaccinated against RV prior to the AGE episode, three of them were RV-positive.

Proportion of RVGE cases

The proportion of RV-positive among all the AGE cases was 25.5% (95% CI: 22.1%-29.1%). Figure 1 shows the number and proportion of RVGE cases in each age group.



AGE – acute gastroenteritis; **RVGE** – rotavirus gastroenteritis.

Figure 1. Number of RVGE and AGE cases and RVGE as a proportion of all AGE cases, by age group (months, mo)

The severity of AGE by age group and RV status is described in Table 1. Severe AGE was experienced by 81.8% (130/159) of the RV-positive and 54.6% (254/465) of the RV-negative children (p<0.001). Of the children under the age of one year with severe AGE, 26/77 (33.8%) were RV-positive, and among children between one and five years old with severe disease 104/307 (33.9%) were RV-positive. Among the severe AGE cases, the proportion of RV-positive patients ranged from 5/22 (22.7%) of the children up to five months old to 21/55 (38.2%) of those from six to 11 months.

particular, dehydration, with 127/159 (79.9%) of the RV-positive and 255/465 (54.8%) of the RV-negative (p<0.001) patients presenting mild/moderate to severe dehydration.

Table 2 shows the distribution of the patients with regard to the severity of AGE symptoms (the items making up the Vesikari score) over the entire episode according to RV status.

Regardless of the symptom, the proportion of patients experiencing worse symptoms in terms of maximum number of episodes per day or the duration of the symptom was obviously higher among those with RVGE than among patients

Table 1. Severity of AGE by RV-status and age group

		RV-positive N = 159		RV-negative N = 465		
Age (months)	**Severity**	**n**	%	**n**	%	**p-values**
0-5	Mild (1-6)	0	0.0	9	20.0	0.160
	Moderate (7-10)	1	16.7	19	42.2	
	Severe (≥11)	5	83.3	17	37.8	
6-11	Mild (1-6)	1	4.2	10	14.3	0.003
	Moderate (7-10)	2	8.3	26	37.1	
	Severe (≥11)	21	87.5	34	48.6	
12-23	Mild (1-6)	1	2.1	18	12.1	0.017
	Moderate (7-10)	9	18.8	45	30.2	
	Severe (≥11)	38	79.2	86	57.7	
24-59	Mild (1-6)	5	6.2	23	11.4	0.001
	Moderate (7-10)	10	12.3	61	30.3	
	Severe (≥11)	66	81.5	117	58.2	
0-11	Mild (1-6)	1	3.3	19	16.5	< 0.001
	Moderate (7-10)	3	10.0	45	39.1	
	Severe (≥11)	26	86.7	51	4.3	
12-59	Mild (1-6)	6	4.7	41	11.7	< 0.001
	Moderate (7-10)	19	14.7	106	30.3	
	Severe (≥11)	104	80.6	203	58.0	
Overall	Mild (1-6)	7	4.4	60	12.9	< 0.001
	Moderate (7-10)	22	13.8	151	32.5	
	Severe (≥11)	130	81.8	254	54.6	

AGE – acute gastroenteritis; **RV** – rotavirus; N = number of AGE patients; n = number of subjects in a given category; P-value = Fisher's exact test to test the significant difference between the RV status with respect to severity.
Note: age computed at GP/pediatrician visit date

At first presentation at the initial visit, about 90% of the children had experienced diarrhea, regardless of their RV-status, but they differed with regard to vomiting (132/159 [83.0%] of the RV-positive had been vomiting and 306/465 [65.8%] of the RV-negative, p=0.003), and, in

with AGE due to other causes. Of note, despite almost half the RV-negative patients presenting with no signs of dehydration at first visit, almost 72% of them received preventive rehydration in a polyclinic during the follow-up.

Table 2. AGE symptoms and severity by RV-status

Symptom	Categories	Levels	RV-positive N = 159		RV-negative N = 465	
			n	%	n	%
Diarrhea	Max. number of stools per day	1-3	5	3.1	25	5.4
		4-5	48	30.2	211	45.4
		≥6	106	66.7	229	49.2
	Days of diarrhea	1-4	46	28.9	253	54.4
		5	43	27.0	112	24.1
		≥6	70	44.0	100	21.5
Vomiting	Max. number of vomiting episodes per day	1	4	3.1	40	12.4
		2-4	83	63.8	229	70.9
		5-7	33	25.4	44	13.6
		≥8	10	7.7	10	3.1
		Missing	29	-	142	-
	Days of vomiting	1	17	13.1	94	29.1
		2-4	102	78.5	212	65.6
		≥5	11	8.5	17	5.3
		Missing	29	-	142	-
Fever	Max. temperature	37.1-38.4°C	13	8.7	78	18.8
		38.5-38.9°C	35	23.3	125	30.2
		≥39.0°C	102	68.0	211	51.0
		Missing	9	-	51	-
Dehydration	Degree of dehydration	No dehydration	32	20.1	210	45.2
		Mild/moderate (1-5%)	113	71.1	246	52.9
		Severe (≥6%)	14	8.8	9	1.9
Treatment	Type of treatment for dehydration	None	33	20.8	132	28.4
		Polyclinical rehydration	126	79.2	333	71.6

AGE – acute gastroenteritis; **RV** – rotavirus;
N = number of AGE patients; n = number of patients in a given category

Table 3. Number and proportion of hospitalized AGE patients by RV-status and by age group

	RV-positive N = 159				RV-negative N = 465			
Age (months)	N	n	%	95% CI	N	n	%	95% CI
0-5	6	1	16.7	0.4-64.1	45	2	4.4	0.5-15.1
6-11	24	7	29.2	12.6-51.1	70	1	1.1	0.0-7.7
12-23	48	11	22.9	12.0-37.3	149	1	0.7	0.0-3.7
24-59	81	13	16.0	8.8-25.9	201	3	1.5	0.3-4.3
All	159	32	20.1	14.2-27.2	465	7	1.5	0.6-3.1

AGE – acute gastroenteritis; **RV** – rotavirus; N = Number of AGE cases in a given category; n = Number of hospitalized AGE cases; 95%CI = exact confidence interval
Note: age computed at GP/pediatrician visit date

Proportion of patients hospitalized for AGE

Altogether 39 children were hospitalized, 32/159 (20.1%; CI: 14.2%-27.2%) of the RV-positive and 7/465 (1.5%; CI: 0.6%-3.1%) of the RV-negative ($p<0.001$). The proportions of children hospitalized in each age-interval and RV-status group are shown in Table 3. Regardless of RV-status, most (over 70%) of the hospital admissions lasted for five days or more (data not shown). A multivariate, stepwise logistic regression analysis of the determinants of hospitalization of the AGE cases resulted in a

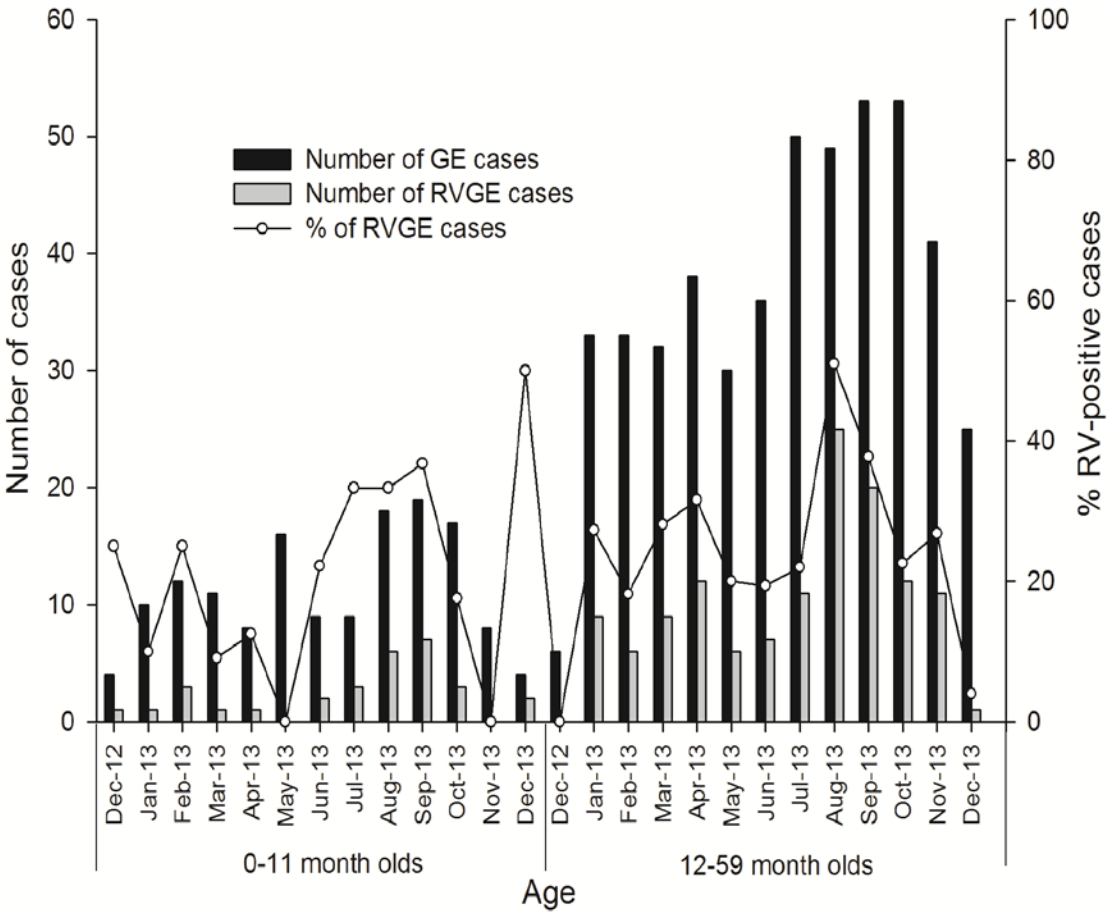


Figure 2. Monthly incidence of AGE and RVGE cases

final model with two statistically significant determinants with the following odds ratios (OR) and p-values: severe disease (Vesikari score ≥11): OR=6.8, p=0.010) and RV-positive (OR=12.4, p<0.001). Even when controlled for the severity of AGE, hospital admission was thus significantly more frequent for RV-positive than for RV-negative patients.

Seasonal distribution of RVGE

Both AGE and RVGE occurred throughout the year. The highest monthly number of AGE cases (72) was observed in September 2013, of which 37.5% were attributed to RV. During the winter months from November to May the proportion of AGE cases attributed to RV ranged from 10% to 28%. The highest proportion of AGE cases attributed to RV was seen during August 2013 (31/67, 46.3%). This pattern of RVGE was observed in both infants (0-11 months) and toddlers and young children (12-59 months) (Figure 2).

Discussion

This primary care surveillance study is the first one carried out in Bulgaria and provides recent epidemiological and burden of disease information on RVGE in children less than five years old. We followed 624 children with a diagnosis of AGE between December 2012 and January 2014. A quarter of all AGE cases (25.5%) and 33.9% of those with severe disease were found to be RV-positive. Among the RV-positive patients, the incidence of severe disease was highest (87.5%) in the age interval from six to 11 months, consistent with the findings of other studies.[2]

Our study complements previous hospital-based studies in Bulgaria and provides a more complete view of the epidemiology and burden

of RVGE in the country. These hospital-based studies performed between 2005 and 2008 reported that 28%-43% of the incidence of AGE and at least a third of all the hospitalizations of children less than five years old could be attributed to RV.[15,16]

Overall, only 6.3% of the children in the study were hospitalized, 20.1% of the RV-positive and 1.5% of the RV-negative.

Remarkably, the multivariate regression analysis of the determinants of hospitalization indicated that when controlling for the severity of disease, RV-positive patients were more likely to be hospitalized than RV-negative ones. Our data do not allow an elucidation of the reasons for this finding but it might suggest that primary care physicians are particularly cautious when a child with AGE is tested RV-positive.

The rate of hospital referrals of RVGE patients was comparable to that found in many other European studies of the incidence and burden of RVGE in primary care but there are wide variations, perhaps indicating differences in physician attitudes and treatment patterns. Thus, the REVEAL study carried out in six western European countries reported proportions of hospitalization of RVGE patients varying from 13% to 57%.[13]

The proportion of patients with severe disease was remarkably high both among RV-positive and RV-negative AGE cases, 81.8% and 54.6%, respectively. The manual for the Vesikari scale notes that if the scale is correctly applied one would expect about 50% of RVGE cases to be rated as severe.[19] In our study, the scoring was done by the investigator during the follow-up call with the parents about two weeks after the initial visit and based on the parents' recall of the number, severity and duration of symptom episodes. The parents were instructed during the initial visit that they would be asked about this at follow-up but they were not provided with diary cards to record the episodes each day. It is not possible to assess the extent of the potential recollection bias and how this may have affected the parents' reporting of symptoms but there is no obvious reason to expect that any recollection bias should be systematic and one-sided.

The relatively low rate of hospitalizations for RVGE underlines that hospital based data collection only provides a limited impression of the epidemiology and real burden of RV disease. However, it must also be kept in mind that no point of observation can give the complete picture as many or most of the milder AGE cases will be cared for in their home without any involvement of primary care physicians or other personnel. Unsurprisingly, there are wide variations in the estimates of the proportion of AGE cases leading to medical attention, ranging between 20% and 50%.[18]

During this one-year observational study we observed that RVGE cases occurred all year round but peaked both in infants and in toddlers and young children during August and September. In contrast, a previous study in Bulgaria reported also year-round circulating RV but with peaks between January and March.[17] This year-round incidence of RVGE was considered as unusual for developed countries with a temperate climate but this conventional notion has been challenged by recent studies of the seasonality of RV disease.[20-22] A comprehensive review of studies of the seasonality of RVGE concluded that it is unlikely that a single, unifying explanation of RVGE seasonality variations may be found, given that distinct patterns of RVGE seasonality have been observed in countries with similar geographical location, climate and level of economic development.[22] Factors like different birth rates and transmission rates and interaction between other local factors and the host may also play a role and country-specific data showed that some countries had a predictable annual pattern while in other countries the timing of peak incidence of RVGE is more sporadic.[23] The contrast between our findings and the pattern reported previously[17] may suggest that Bulgaria belongs to the countries where the timing of peak incidence is more variable.

Our results must be interpreted with caution as the study was limited by a number of factors. The 30 study centers were constituted as a convenience sample located throughout Bulgaria with about half in the regions surrounding the

largest cities where the RVGE incidence based on hospital data has been estimated to exceed the national average somewhat. Apart from these data, we are not able to assess the representativeness of the primary care units selected and, as a consequence, the generalizability of the findings to the whole country is uncertain. Further, the results may have been affected by differences in clinical practice, in parents' healthcare seeking behavior and, perhaps, in local rules for reimbursement by the Bulgarian social health insurance system.

Conclusion

This study emphasizes that RV is an important cause of AGE cases presenting in primary care. RV causes the most severe forms of the disease and is the most important cause of AGE-related hospitalizations. Vaccination is an effective public health intervention against RV and observational data from real-world settings after introduction and widespread uptake of RV vaccination have documented substantial reductions of the disease burden and also some indication of herd effects for unvaccinated older children and adults.[2,23] The results of this study may be used as baseline data for possible future studies of the impact of RV vaccination in Bulgaria if it is decided to include RV vaccination in the NIP or if the uptake of RV vaccinations increases substantially for other reasons.

Authors' contributions statement: All named authors have contributed in the design/acquisition of data or analysis and interpretation of data. They have provided substantial intellectual and scientific input in the development of this manuscript. All authors were involved in critically reviewing the content and revising the manuscript and they all approved the final version of the manuscript.

Conflicts of interest: Prof. Tiholova received grants from the GSK group of companies, and personal fees from GSK group of companies, Sanofi-Pasteur, MSD, and Biocodex, outside the submitted work. Kusuma Gopala, Magda Berberova, Margarita Strokova-Stoilova and Monica Tafalla are employed by the GSK group of companies. Monica Tafalla holds stock options and restricted shares from the GSK group of companies.

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