

Brief Original Article

Booster immunity – diagnosis of chronic hepatitis B viral infection

Radka T Komitova¹, Ani K Kevorkyan², Maria V Atanasova³, Aneta V Ivanova⁴, Elica Golkocheva-Markova⁵

¹ Department of Infectious diseases, Parasitology and Tropical medicine, Faculty of Medicine, Medical University, Plovdiv, Bulgaria

² Department of Epidemiology and Disaster Medicine, Faculty of Public Health, Medical University, Plovdiv, Bulgaria

³ Department of Microbiology and Immunology, Faculty of Pharmacy, Medical University, Plovdiv, Bulgaria

⁴ Laboratory of Porphyrias and Molecular Diagnostics of Liver Diseases. Clinic of Gastroenterology, Saint Ivan Rilski University Hospital, Sofia, Bulgaria

⁵ National Reference Laboratory “Hepatitis viruses”, National Center for Infectious and Parasitic Diseases, Sofia, Bulgaria

Abstract

Introduction: Diagnosis of chronic hepatitis B virus (HBV) infection particularly its occult form requires monitoring and repeat serological and molecular studies. The aim of the study was to investigate the possible relation between the case of a family outbreak of hepatitis A and the finding that a member of this family was diagnosed with chronic hepatitis B.

Methodology: A mother and her two sons, one previously diagnosed with chronic HBV infection, were hospitalized due to suspected acute hepatitis. Serological markers for hepatitis A, hepatitis B and hepatitis C were assessed. Additionally, HBV DNA was tested with a sensitive PCR. Hepatitis B vaccine was administered to the mother to differentiate resolved from occult HBV infection.

Results: A family outbreak of hepatitis A was confirmed, alongside a focus of chronic HBV infection. The serological profile for two brothers was HBsAg(+), anti-HBcIgM(-), anti-HBc(+), HBcAg(-)/anti-HBe(+). The mother was negative for all HBV markers except anti-HBc. HBV DNA was detected at a level of 461 IU/mL in the elder brother, 3647 IU/mL in the younger brother and was negative in the mother on two occasions. Her anti-HBc alone, having two sons with chronic HBV infection, and her lack of antibody response to hepatitis B vaccine despite being negative for HBV DNA, led to the diagnosis of probable occult HBV infection.

Conclusion: Our results confirmed that a vaccination approach could facilitate diagnosis of chronic HBV infection in the presence of isolated anti-HBc. If it were not for a family outbreak of hepatitis A, this unexpected family HBV focus would not have been revealed.

Key words: hepatitis B virus; occult HBV infection; anti-HBc alone; hepatitis B vaccine.

J Infect Dev Ctries 2019; 13(3):255-260. doi:10.3855/jidc.9929

(Received 10 November 2017 – Accepted 25 June 2018)

Copyright © 2019 Komitova *et al.* This is an open-access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Introduction

Chronic hepatitis B virus (HBV) infection is a potentially life-threatening liver condition with serious complications, such as liver cirrhosis and hepatocellular carcinoma. A panel of serological tests is used to differentiate the HBV status of the patients. Antibodies to hepatitis B core antigen (anti-HBc) are produced in all individuals exposed to HBV and usually remain detectable for life. For that reason, anti-HBc are generally accepted as the most reliable serological marker of HBV infection and serve for both diagnostic and epidemiological purposes [1,2]. Positive anti-HBc in the absence of other HBV serological markers are referred to as “isolated anti-HBc” [3]. The frequency of “isolated anti-HBc” has significant variability, ranging

between 1% and 20% depending on the prevalence of HBV infection in the population tested [4-6]. In areas non-endemic for HBV, some sub-populations such as persons with chronic hepatitis C virus infection, HIV infection, or users of injected drugs also have a relatively high frequency of “isolated anti-HBc” [7,8]. Based on the limited data in Bulgaria, the prevalence of “isolated anti-HBc” is low (2.08%) but this requires additional verification [9].

“Isolated anti-HBc” could be indicative of acute, resolved, overt, or occult chronic HBV infection [10]. Occult HBV infection (OBI) is a recently recognized entity and one of the most challenging and important issues in the field of viral hepatitis [11-15]. In 2008 it was defined as the detection of HBV DNA in the liver

(with or without HBV DNA in serum), in the absence of hepatitis B surface antigen (HBsAg) [16]. Distinguishing between these conditions is possible using additional serial serological and virological tests, as well as assessment of the degree of liver damage by biopsy, which is expensive and not always readily available [16]. It is possible that testing the level of protective antibodies to HBsAg (anti-HBs), following administration of hepatitis B vaccine could provide additional diagnostic information [17]. This vaccination approach could substitute molecular methods, especially in resource-limited countries.

Low viral replication in the liver in OBI generally indicates a favorable prognosis and low risk of chronic HBV consequences. Regarding the clinical significance of OBI, there are a few potential risks. First, there is a danger of HBV transmission during blood transfusion, albeit small, or through organ transplantation, especially liver transplantation from OBI positive donors. Second, there is a considerable risk of reactivation of HBV during immunosuppressive therapy, in particular with rituximab. Since this reactivation can manifest as fulminant hepatitis, all candidates for organ transplantation or immunosuppressive therapy should be screened for HBV markers and should receive prophylactic antiviral therapy to inhibit replication if necessary [18]. Finally, OBI might favor or accelerate the progression towards cirrhosis in patients with chronic hepatitis C infection and alcoholic liver disease [19,20].

The aim of the study was to investigate the possible relation between the case of a family outbreak of hepatitis A and the fact that one member of this family was diagnosed with chronic hepatitis B infection.

Methodology

Three patients from the same household were simultaneously admitted and treated for viral hepatitis at the Department of Infectious Diseases at St George University Hospital in Plovdiv, Bulgaria, in January 2016. They were two brothers (patient No.1, aged 33 years and patient No.2, aged 28 years) and their mother, aged 54 years (No.3) (Figure 1).

Clinical history

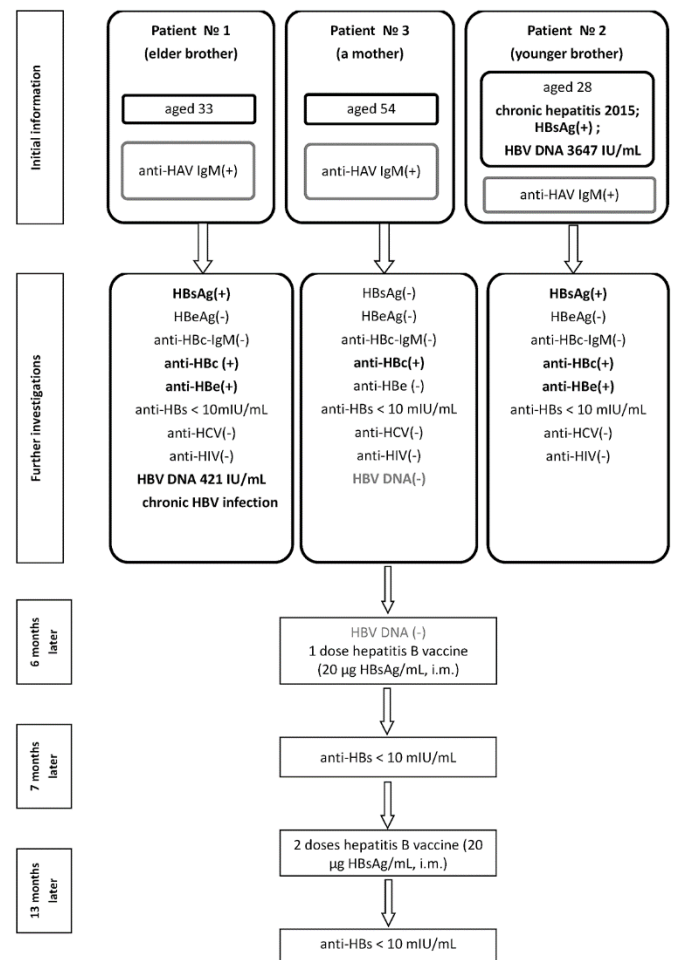
HBsAg was detected in the younger brother (patient No.2) on an attempt to donate blood in 2015. Later, he was diagnosed with chronic hepatitis B not eligible for antiviral therapy. The level of HBV DNA was 3647 IU/mL and liver biopsy showed chronic hepatitis with moderate activity (MetavirA2F1). At that time, no epidemiological study of the contacts was performed.

Their father died in 2015 (he had a history of liver and pancreatic disease, however, no direct medical evidence was available).

Serological tests

The patients were tested for anti-hepatitis A virus (HAV), HBV, comprising HBsAg, anti-HBs, hepatitis B e antigen (HBeAg) and antibodies to HBeAg (anti-HBe), anti-hepatitis C virus (HCV) as well as anti-human immunodeficiency virus (HIV1/HIV2), using enzyme-linked immunosorbent assay (ELISA). The following commercial kits were employed: anti-HAV-IgM (DiaPro, Milano, Italy); HBsAg (Biokit, Barcelona, Spain), HBeAg (DiaPro, Milano, Italy/Vidas), anti-HBc-IgM (DiaPro, Milano, Italy), anti-HBc-total (Biokit, city, Spain/Vidas), anti-HBe (DiaPro, Milano, Italy/Vidas), anti-HBs (Biokit, Barcelona, Spain/Vidas); anti-HCV (Biokit, Barcelona, Spain/Vidas); anti-HIV1/2 (Biokit, Barcelona, Spain). Samples with positive anti-HBc results were retested using Abbott Architect kit (Germany). In the absence of

Figure 1. Flowchart of the patients with HBV infection.



other HBV markers, those positive for anti-HBc-total by both kits were defined as “isolated anti-HBc”.

All testing was performed at the Virology Laboratory at St. George University Hospital, Plovdiv, and results were interpreted according to manufacturer’s instructions.

HBV DNA detection in plasma

Polymerase chain reaction (PCR) was used to test for HBV DNA employing two commercial kits:

1. Artus HBV DNA RG RT-PCR Kit, Time PCR, Rotor-Gene Q instrument (lower limit of detection, LLD 9 IU/mL). The test was performed at the Laboratory for Molecular Diagnosis of Liver Diseases at St. I. Rilski University Hospital, Sofia.

2. Abbott Real Time HBV PCR assay (Abbott Molecular, Wiesbaden, Germany) (LLD 9 IU/mL). The test was performed at the National Reference Laboratory for Hepatitis Viruses at the National Center for Infectious and Parasitic Diseases, Sofia.

All tests were performed and interpreted in accordance with manufacturer’s instructions.

Biochemical studies

Aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and gamma glutamyl-transferase were identified by the Central Clinical Laboratory of St George University Hospital, Plovdiv using common automated spectrophotometric methods. If necessary, tests were repeated at monthly intervals.

Hepatitis B vaccine immunization

Immunization with hepatitis B vaccine was used as an indirect approach to verify the diagnostic value of the “isolated anti-HBc” in the mother (patient No.3). A standard immunization schedule was used consisting of three doses (initial, at 1 month and 6 months). Recombinant hepatitis B vaccine (Engerix B, GSK, Belgium, 20 µg HbsAg, 1 mL) was employed and immunization started 6 months after the registration of the outbreak.

The immune response was evaluated based on the level of anti-HBs antibodies. Primary immune response and protective immunity were defined as having anti-HBs levels ≥ 10 mIU/mL one month after completion of the vaccination schedule [21]. Secondary (anamnestic) immune response was determined when anti-HBs level was ≥ 10 mIU/mL one month after a single (booster) dose. Lack of immune response was defined as levels of anti-HBs at <10 mIU/mL one month following the completion of the vaccine schedule [22].

All patients signed an informed consent for the testing; the immunized patient signed an additional consent.

Results

Two foci of infection were detected: acute hepatitis A and chronic HBV in two more patients: the elder brother (patient No.1) and the mother (patient No.3). The results of the serological and molecular tests of the patients from the family outbreak of hepatitis A are presented in Figure 1.

The laboratory profile of the elder brother (patient No.1) - HBsAg(+), anti-HBc-IgM(-), anti-HBc(+), anti-HBe(+), HBV DNA 461 IU/mL and persisting HBsAg during the six-month follow-up, supported the diagnosis of chronic HBV infection. After six months the patient was referred to a gastroenterologist for further management.

The mother (patient No.3) tested positive only for “isolated anti-HBc” on three separate occasions at two-month intervals, regardless of which of the two commercial kits was used. HBV DNA was tested repeatedly at 6 month intervals with sensitive PCR, but the result was always negative.

It was suspected that the mother had had previous exposure to HBV, with subsequent drop of anti-HBs below the level of detection. She received a booster vaccine dose, but after one month did not exhibit anti-HBs. The immunization was completed with two additional doses. One month and three months later, the level of anti-HBs remained undetectable. The mother was also referred to a gastroenterologist for further assessment for possible chronic HBV infection.

The elder brother was monitored for 6 months and the mother for up to a year. Over this period, they had no clinical complaints and their aminotransferase levels were normal. The younger brother (patient No.2) was also free of complaints.

Discussion

In the present report investigating a family outbreak of hepatitis A, an unexpected focus of chronic HBV infection was detected – two brothers and their mother, who was presumed to have been the likely source of infection for her sons.

Hepatitis A outbreak investigation has led to the diagnosis of chronic HBV infection in the elder brother. The presence of HBV DNA $<2,000$ IU/mL indicates a low replicative infection. This condition could have been identified one year earlier if he had been investigated as a contact of his younger brother who was then diagnosed with chronic HBV infection.

A key determinant of HBV chronicity is the age at which the infection is acquired. In infants up to one year of age, who have not received hepatitis B immunoglobulin and hepatitis B vaccine, the risk is 90%. It declines to 20-50% in infants 1 to 5 years of age [23]. The fact that both brothers had chronic HBV infection led us to consider that their mother was the likely source of infection for them both and that the transmission had occurred in the past.

The serological profile of the mother is particularly interesting – “isolated anti-HBc” as the only marker of HBV. “Isolated anti-HBc” might be a false positive, especially in countries with a low prevalence of the infection and in individuals without risk factors (e.g. blood donors). The mother’s positive anti-HBc antibodies in three separate investigations with two different commercial kits, however, make the latter explanation less likely. No viral replication was detected even when a sensitive PCR test was used.

To sum up, the mother’s “isolated anti-HBc” state, her having two sons with chronic HBV infection and her lack of antibody response to hepatitis B vaccine despite the negative HBV DNA result, taken together led to the diagnosis of probable OBI.

In a strict definition of OBI, the presence of HBV DNA in the hepatic tissue is a requirement, regardless of serum HBV DNA, with negative HBsAg [16]. This occurs frequently in combination with anti-HBc(+). HBV replication has been significantly, but not absolutely, suppressed by the host immune defenses. HBV has been preserved as covalently-closed-circular DNA (ccc DNA) in the nuclei of hepatocytes for a long period of time [16]. According to their history of HBV exposure, patients with OBI could be classified into three groups. The first group comprises the patients with definite past history of self-limiting acute HBV – they undergo a short period of negative HBsAg state with persistent low HBV DNA levels. The second consists of patients with known long-standing chronic HBV infection, who have achieved serum clearance of HBsAg (seroclearance). They have no complaints, their aminotransferase levels are normal and liver tissue histology shows normal or minimal damage. Most patients with OBI fall into this category, which is typical for regions with high HBV prevalence. And finally, a small group of patients without prior history have what is called ‘primary occult hepatitis’ – they are negative for all serum HBV markers in most cases [12,24].

The mainstay of diagnosis of OBI infection is the detection of HBV DNA in the liver tissue. Blood viremia is low, under 200 IU/mL, and commonly

fluctuates [16]. In 90% of the cases, the levels are under 20 IU/mL or even undetectable [25]. The low-level viremia and its undulating character makes detection of OBI difficult even when standardized and sensitive amplification assays are used. Consequently, continuous monitoring and sequential testing of patients for HBV DNA levels and liver enzymes are recommended [26]. The detection of HBV DNA in liver tissue is the most accurate method, albeit invasive and not always available [27]. In our case, liver biopsy was not performed because of ethical considerations. For logistic reasons, we were able to monitor and test the mother for DNA HBV only twice within one year, which is one limitation of the study. HBV genotype testing in the brothers was not performed, which represents another limitation.

When PCR is unavailable to confirm the diagnosis, “isolated anti-HBc” could be used as a diagnostic marker. These antibodies are considered a sentinel marker of HBV occult infection [16,27].

The response to hepatitis B vaccine could aid in the differentiation of immune patients from those with chronic infection. After the negative HBV DNA result in the mother, immunization with hepatitis B vaccine was used to detect whether anti-HBs antibodies were formed. Immune individuals with undetectable anti-HBs usually develop secondary (anamnestic) immune response following administration of a single dose. Conversely, individuals with chronic HBV infection do not respond to the vaccine [28,30], as in our case. The absence of protective immunity one month following the initial dose and after the completion of the immunization schedule in the mother is highly supportive of a diagnosis of OBI.

Conclusion

In conclusion, while investigating a family outbreak of hepatitis A, we diagnosed patients co-infected with chronic HBV infection. The result of our study has confirmed that hepatitis B vaccine might also be used as a diagnostic tool, especially in low-income settings where other tests are not available. The absence of immune response, together with the presence of “isolated anti-HBc” is highly supportive of the diagnosis of OBI in the mother. Ironically, it was the family outbreak of hepatitis A that resulted in revealing this HBV infection focus.

Acknowledgements

The authors would like to thank the patients for their cooperation and their patience, Prof. Anna Lok, University of

Michigan-USA, for her valuable and helpful comments and advice in the course of the investigation, and Prof. R. Argirova, Clinical Laboratory, Tokuda Hospital-Sofia for the additional sample testing for anti-HBc antibodies

References

- Laperche S, Guitton C, Smilovici W, Courouge AM (2001) Blood donors infected with the hepatitis B virus but persistently lacking antibodies to the hepatitis B core antigen. *Vox Sang* 80: 90–94.
- Busch MP (2004) Should HBV DNA NAT replace HBsAg and/or anti-HBc screening of blood donors? *Tranfus Clin Biol* 11: 26–32.
- Grob P, Jilg W, Bornhak H, Gerken G, Gerlich W, Günther S, Hess G, Hüdig H, Kitchen A, Margolis H, Michel G, Trepo C, Will H, Zanetti A, Mushahwar I (2000) Serological pattern “anti-HBc alone”: Report on a workshop *J Med Virol* 62: 450–455.
- Jilg W, Hottenträger B, Weinberger K, Schlottmann K, Frick E, Holstege A, Schölmerich J, Palitzsch KD (2001) Prevalence of markers of hepatitis B in the adult German population. *J Med Virol* 63: 96–102.
- Lok AS, Lai CL, Wu PC (1988) Prevalence of isolated antibody to hepatitis B core antigen in an area endemic for hepatitis B virus infection: implications in hepatitis B vaccination programs. *Hepatology* 8: 766–770.
- Gibney KB, Torresi J, Lemoh C, Biggs BA (2008) Isolated core antibody hepatitis B in sub-Saharan African immigrants. *J Med Virol* 80: 1565–1569.
- French AL, Operskalski E, Peters M, Strickler HD, Tien PC, Sharp GB, Glesby MJ, Young M, Augenbraun M, Seaberg E, Kovacs A (2007) Isolated hepatitis B core antibody is associated with HIV and ongoing but not resolved hepatitis C virus infection in a cohort of US women. *J Infect Dis* 195: 1437–1442.
- Cacciola I, Pollicino T, Squadrito G, Cerenzia G, Orlando ME, Raimondo G (1999) Occult hepatitis B virus infection in patients with chronic hepatitis C liver disease. *N Engl J Med* 341: 22–26.
- Atanasova MV (2016) Study of markers of hepatitis B and hepatitis C in the general population and risk groups. Doctorate thesis in Microbiology. Medical University of Plovdiv, Bulgaria. 232 p. [Thesis in Bulgarian].
- Wang Q, Klenerman N, Semmo (2017) Significance of anti-HBc alone serological status in clinical practice. *Lancet Gastroentero Hepatol* 2: 123–134.
- Ye X, Li T, Xu X, Du P, Zeng J, Zhu W, Yang B, Li C, Allain JP (2017) Characterisation and follow-up study of occult hepatitis B virus infection in anti-HBc-positive qualified blood donors in southern China. *Blood Transfus* 15: 6–12.
- Yuen MF (2017) Clinical implication of occult hepatitis B infection. *Ann Blood* 2: 1–10.
- Makvandi M (2016) Update on occult hepatitis B virus infection. *World J Gastroenterol* 22: 8720–8734.
- Raimondo, G, Caccamo G, Filomia R, Pollicino T (2013) Occult HBV infection. *Semin Immunopathol* 35: 39–52.
- Hollinger FB, Sood G (2010) Occult hepatitis B virus infection: a covert operation. *J Viral Hepat* 17: 1–15.
- Raimondo G, Allain JP, Brunetto MR, Buendia MA, Chen DS, Colombo M, Craxi A, Donato F, Ferrari C, Gaeta GB, Gerlich WH, Levrero M, Locarnini S, Michalak T, Mondelli MU, Pawlotsky JM, Pollicino T, Prati D, Puoti M, Samuel D, Shouval D, Smedile A, Squadrito G, Trépo C, Villa E, Will H, Zanetti AR, Zoulim F (2008) Statements from the Taormina expert meeting on occult hepatitis B virus infection. *J Hepatol* 49: 652–657.
- Yao J, Ren W, Chen Y, Jiang Z, Shen L, Shan H, Dai X, Li J, Liu Y, Qiu Y, Ren J (2016) Responses to hepatitis B vaccine in isolated anti-HBc positive adults. *Hum Vaccin Immunother* 12: 1847–1851.
- Raimondo G, Filomia R, Maimone S (2014) Therapy of occult hepatitis B virus infection and prevention of reactivation. *Intervirology* 57: 189–195.
- Covolo L, Pollicino T, Raimondo G, Donato F (2013) Occult hepatitis B virus and risk for chronic liver disease: A meta-analysis. *Dig Liv Dis* 45: 238–224.
- Squadrito G, Cacciola I, Alibrandi A, Pollicino T, Raimondo G (2013) Impact of occult hepatitis B virus infection on the outcome of chronic hepatitis C. *J Hepatol* 59: 696–700.
- McMahon BJ, Parkinson AJ, Helminiak C, Wainwright RB, Bulkow L, Kellerman-Douglas A, Schoenberg S, Ritter D (1992) Response to hepatitis B vaccine of persons positive for antibody to hepatitis B core antigen. *Gastroenterology* 103: 590–594.
- Silva AE, McMahon BJ, Parkinson AJ, Sjogren MH, Hoofnagle JH, Di Bisceglie AM (1998) Hepatitis B virus DNA with persons with isolated antibodies to hepatitis B core antigen who subsequently received hepatitis B vaccine. *Clin Infect Dis* 26: 85–87.
- World Health Organization (2015) Guidelines for the prevention, care and treatment of persons with chronic hepatitis B infection, first edition, France, 166 p. (ISBN 97892 4 154905 9).
- Lok A (2004) Occult hepatitis B infection: diagnosis, implications and management? *J Gastroent Hepatol* 19 Suppl 7: 114–117.
- Yuen MF, Lee CK, Wong DK, Fung J, Hung I, Hsu A, But DY, Cheung TK, Chan P, Yuen JC, Fung FK, Seto WK, Lin CK, Lai CL (2010) Prevalence of occult hepatitis B infection in a highly endemic area for chronic hepatitis B: a study of a large blood donor population. *Gut* 59: 1389–1393.
- Chemin I, Guillaud O, Queyron PC, Trépo C (2009) Close monitoring of serum HBV DNA levels and liver enzymes levels is most useful in the management of patients with occult HBV infection. *J Hepatol* 51: 824–825.
- Urbani S, Fagnoni F, Missale G, Franchini M (2010) The role of anti-core antibody response in the detection of occult hepatitis B virus infection. *Clin Chem Lab Med* 48: 23–29.
- Aoki SK, Finegold D, Kuramoto IK, Douville C, Richards C, Randell R, Fernando L, Holland PV, Zeldis JB (1993) Significance of antibody to hepatitis B core antigen in blood donors as determined by their serologic response to hepatitis B vaccine. *Transfusion*. 33:362–367.

29. Schiffman RB, Rivers SL, Sampliner RE, Krammes JE (1993) Significance of isolated hepatitis B core antibody in blood donors. *Arch Intern Med* 153: 2261-2266.

Corresponding author

Professor Radka Komitova, MD, PhD
Department of Infectious diseases, Parasitology and Tropical medicine, Faculty of Medicine, Medical University, Plovdiv, Bulgaria “Saint George” University Hospital 66 Peshtersko Shosse blvd 4006, Plovdiv, Bulgaria
Tel. +359 882 495 800
Email:radkakomitova@yahoo.com

Conflict of interests: No conflict of interests is declared.