Case report
Prikaz bolesnice

ISSN 0351-0093
Coden: MEJAD 54 (2024) 1

Detection of voluntary blood donor with previous hepatitis B infection – a case report

Detekcija dobrovoljnog darivatelja krvi s preboljelom infekcijom hepatitisa B- prikaz bolesnice

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Summary

Introduction: Hepatitis B is liver inflammation caused by the hepatitis B virus, with more than 250 million documented cases worldwide in the chronic form of infection. Markers of hepatitis B infection can be measured in the blood during or after infection and may be present in the form of specific hepatitis B surface antigen (HBsAg) or antibodies to hepatitis B surface (HBs), core (HBc), or envelope (HBe) antigens.

Case report: A 42-year-old female, who successfully donated blood for the first time, underwent mandatory serological and molecular testing along with additional testing for other hepatitis B markers. Standard screening showed the absence of HBsAg in the blood, but additional serological testing confirmed the presence of total antibody to hepatitis B core antigen (anti-HBc). Upon repeated reactive results, the sample was sent for confirmatory testing to a reference center. After obtaining all the results, it was determined that the voluntary blood donor had the presence of anti-HB cand HBs antibodies, indicating a prior contact with the hepatitis B virus. While serological tests suggested a resolved hepatitis B infection, the possibility of a persistent liver infection could not be ruled out, despite the absence of detectable hepatitis B virus DNA in the blood. Therefore, the individual has been permanently excluded from the list of potential blood donors.

Conclusion: Mandatory serological and molecular testing of blood donors for the hepatitis B virus successfully detects potentially infected individuals and carriers of hepatitis B markers. However, additional testing further enhances the safety of both recipients and blood donors. This case study highlights the importance of comprehensive screening for hepatitis B markers, as relying solely on HBsAg screening would not have identified the voluntary blood donor as a resolved hepatitis B case. Thus, comprehensive screening ensures a higher level of safety in blood transfusion and contributes to overall healthcare protection.

Keywords: anti-HBc antibodies, blood donor, case report, hepatitis B, hepatitis B surface antigen, serological testing

Sažetak

Uvod: Hepatitis B upala je jetre uzrokovana hepatitis B virusom s više od 250 milijuna zabilježenih slučajeva širom svijeta koji imaju kronični oblik infekcije. Biljezi hepatitis B infekcije mjerljivi su u krvi tijekom ili nakon infekcije, a mogu biti prisutni u obliku specifičnog hepatitis B površnog (engl. HB surface) antigena (HBsAg) ili antitijela na hepatitis B antigene HBs, HBc (engl. HBcore) ili HBe (engl. HBenvelope).

Prikaz bolesnice: Ženska osoba u dobi od 42 godine, koja je prvi put uspješno darovala krv, podvrgnuta

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Primljeno/*Received* 2023-07-03; Ispravljeno/*Revised* 2023-12-28; Prihvaćeno/*Accepted* 2024-02-05

je obveznom serološkom i molekularnom testiranju, kao i dodatnom testiranju na druge biljege hepatitisa B. Nadalje, standardnim probirom uočeno je odsustvo HBsAg u krvi, no dodatnim serološkim testiranjem utvrđeno je prisustvo ukupnih anti-HBc antitijela. Po ponovljenom reaktivnom rezultatu, uzorak je poslan na potvrdno testiranje u referentni centar. Nakon dobivanja svih rezultata, dobrovoljnoj darivateljici krvi utvrđeno je prisustvo ukupnih anti-HBc i HBs-antitijela, što je ukazalo na raniji kontakt s virusom hepatitisa B. Iako serološki testovi govore u prilog preboljeloj infekciji hepatitisa B, u ovom slučaju ne može se isključiti mogućnost postojanja infekcije u jetri, unatoč odsustvu detekcije DNA hepatitis B virusa u krvi. Stoga je osoba trajno isključena s popisa mogućih darivatelja krvi, organa, tkiva ili stanica.

Zaključak: Obvezno serološko i molekularno testiranje darivatelja krvi na hepatitis B virus uspješno otkriva možebitno zaražene osobe i nositelje markera hepatitisa B, no dodatno testiranje pridonosi većoj sigurnosti primatelja, ali i darivatelja krvi. U ovom prikazu bolesnice pokazano je kako probirom HBsAg dobrovoljna darivateljica krvi ne bi bila identificirana kao preboljeni slučaj hepatitisa B bez dodatnog testiranja hepatitis B biljega. Sveobuhvatniji probir na prisustvo hepatitisa B omogućuje višu razinu sigurnosti pri procesu transfuzije krvi, kao i bolju zdravstvenu zaštitu.

Ključne riječi: antitijela na hepatitis B antigene, dobrovoljni darivatelji krvi, hepatitis B, hepatitis B površinski antigen, prikaz bolesnice, serološko testiranje

Med Jad 2024;54(1):47-52

Introduction

The hepatitis B infection is liver inflammation caused by hepatitis B virus (HBV). It occurs in two forms: the primary form, also known as acute viral hepatitis B which usually ends in complete cure, and the chronic or persistent form, which occurs in 5-10% of patients with acute HBV infection.[1] More than 250 million people worldwide have a chronic form of the infection, and it is estimated that approximately one million people die annually from complications.[2] Markers of infection can be measured in the blood during or after the infection. They can manifest as antigens or antibodies indicating the presence of the virus.[3] To ensure their safety for use, blood products must meet national requirements. Blood is always considered potentially contagious. Sample testing results show the absence of causative agents of infectious diseases, for which mandatory testing is required by law. Therefore, the careful selection of donors and testing of blood products is of utmost importance.[4]

In voluntary blood donor hepatitis B testing, the presence of HBsAg is demonstrated through blood testing using enzyme immunochemical methods. The test must possess a high sensitivity and specificity (>99%).

The implementation of mandatory nucleic acid testing (NAT) has been a significant step in ensuring the safety of blood products. It has considerably reduced the window period (WP), which used to be measured in months and is now shortened to 5-15 days depending on the type of infection. NAT molecular testing involves multiplex testing for HBV, HCV, and HIV. In the case of a reactive result, the next step is a discriminatory test that determines which of these three types of infection is present. NAT has a sensitivity of 100%, and the discriminatory tests have a specificity of 100%. One of the great advantages of NAT is its ability to detect and amplify very small amounts of viruses that are not detectable by serological tests.[2]

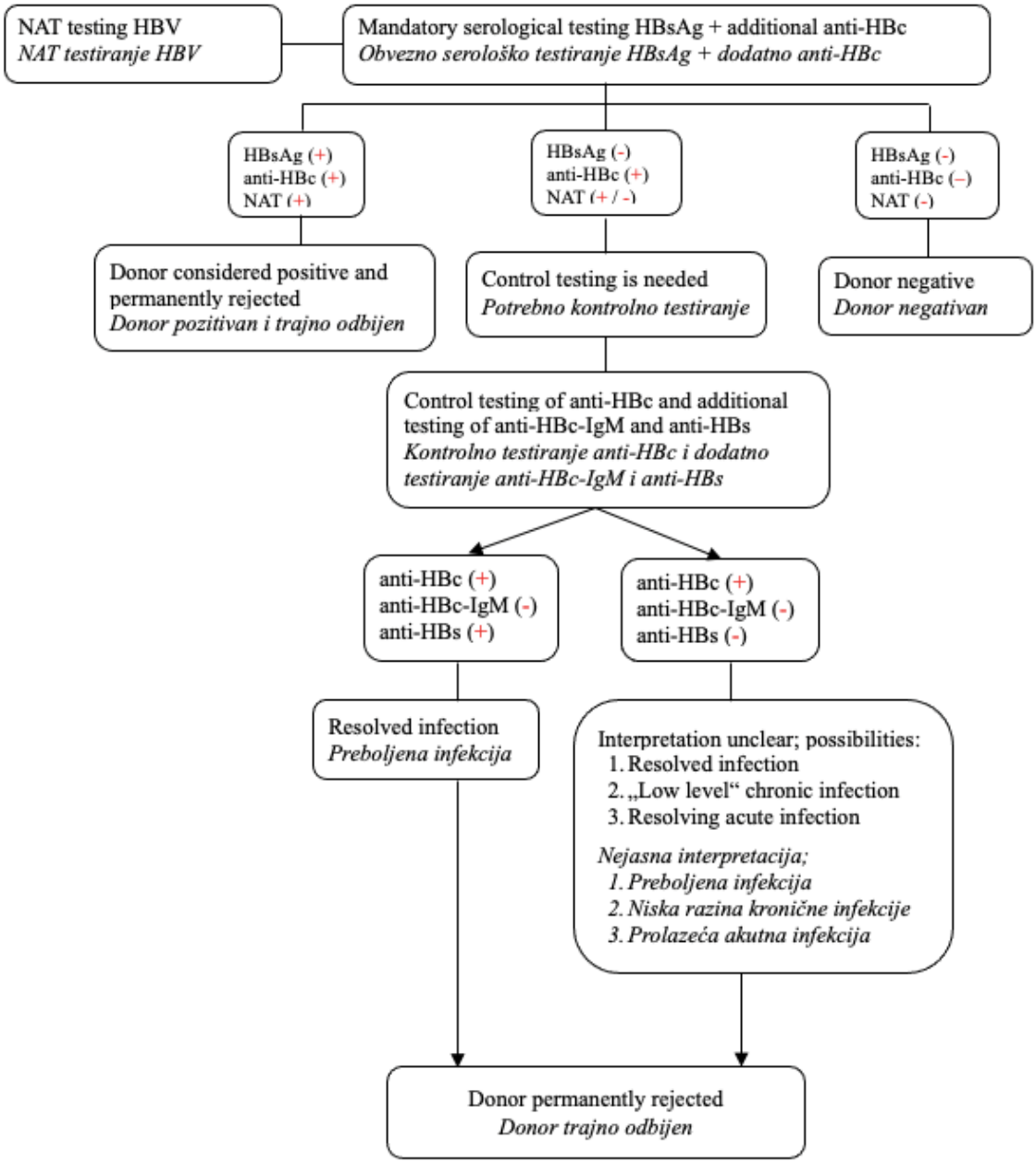
Due to the specificity of serological methods, NAT testing is also useful in resolving cases of false reactive donation results, which is crucial for the final evaluation of the donor.

This paper aims to present the testing of voluntary blood donors, the different tests performed to detect recovered hepatitis B infection, and discuss the testing methods used globally and the benefits of introducing additional testing. As testing guidelines, we will utilize the hepatitis B algorithm (Figure 1) employed in the Republic of Croatia. The algorithm provides detailed procedures for handling positive results and the criteria for rejecting donors based on molecular and serological testing. It is complemented by additional testing for total antibody to hepatitis B core antigen (anti-HBc), conducted by our Department for enhanced safety of blood products.

Case report

A 42-year-old female visited the Transfusion Department of Dubrovnik General Hospital to donate blood for the first time. She completed a pre-donation questionnaire that included relevant questions about her general health, potential diseases, and risks that could affect the safety of the donated blood. Additionally, she acknowledged and agreed that her blood would be tested for blood-borne diseases.

Figure 1 The testing algorithm for hepatitis B
Slika 1. Algoritam testiranja na hepatitis B



Legend: (+) – positive result; (-) – negative result;
Legenda: (+) - pozitivan rezultat; (-) - negativan rezultat;

After completing the questionnaire, her hemoglobin concentration was determined, which was satisfactory (>125 g/L), and she underwent an interview with a transfusion medicine specialist. During the interview, the potential donor stated that there was no history of hepatitis B in her family, no contact with hepatitis B, and no previous accidental needle stick injury. She also mentioned that she had not changed sexual partners and did not engage in risky sexual relationships. In addition to the donation of whole blood samples were collected for serological, immune hematological, and molecular testing. The NAT samples were sent to a reference center that conducts molecular testing for the entire country. The new blood donor underwent mandatory serological testing as required by the laws of Croatia. She also underwent additional testing for total antibody to hepatitis B core antigen (anti-HBc) using the Abbott Architect i2000sr analyzer (Abbott Diagnostics, Illinois, USA). The method employed for determination was chemiluminescent microparticle immunoassay (CMIA), and the unit of measurement used was S/CO (signal-to-cutoff).

The reactive result criterion was set at >1.00

S/CO. All test results are presented in Table 1.

Table 1 Serological and molecular test results
Tablica 1. Rezultati seroloških i molekularnih testova

Test ***Test***	**Test results** ***Rezultati testova***
HBsAg	Neg/*neg*
Syphilis/*Sifilis*	Neg/*neg*
Anti-HCV	Neg/*neg*
HIV	Neg/*neg*
Anti-HBc	Pos/*poz*
NAT	Neg/*neg*

The donor initially showed reactivity in the anti-HBc test, and in accordance with the manufacturer's recommendations, the test was repeated in duplicate. The manufacturer stated that the test must yield reactive results twice to be considered repeatedly reactive. Following another reactive result, additional markers of hepatitis B were examined, and a sample was sent to a reference center. The confirmatory testing involved several different methods on various analyzers to ensure good sensitivity and specificity of used tests. The NAT testing produced a nonreactive result, indicating no evidence of acute infection through molecular methods.

All the extended and repeated tests are presented in Table 2.

Table 2 Confirmatory testing
Tablica 2. Potvrdno testiranje

Test ***Test***	**Test results** ***Rezultati testova***
Anti-HBc	Pos/*poz*
Anti-HBc - IgM	Neg/*neg*
Anti-HBe	Pos/*poz*
Anti-HBs	Pos/*poz* (145 IU/L)

The test results confirmed that the donor had successfully recovered from hepatitis B infection in the past. If the result of a confirmatory test is positive, the donor is declared positive, and the finding must be confirmed by a control blood sample to affirm the donor's positivity or infection. The donor was contacted 21 days after the blood donation and scheduled for an interview with a transfusion medicine specialist. During the interview, an additional medical history was taken, and the findings were explained to the donor. A confirmatory sample was requested for further testing. The donor reiterated that there had been no risky behavior, affirmed that she had not received the hepatitis B vaccination, and provided evidence through a vaccination card. The control blood sample underwent analysis using the same tests and methods as the initial sample. It was also sent for confirmatory testing to a reference center, and all the results matched those of the first sample.

The donor was now considered positive and was personally informed of the finding through a letter. The letter stated that reactivity to markers of hepatitis B virus infection had been established.

The marker findings indicated a previous contact with the hepatitis B virus, although the presence of the virus in the blood was not confirmed. However, it could be present in the liver and potentially harm its function. Due to this finding, the donor was permanently disqualified from donating blood, organs, tissues, and cells.

The blood donor, along with a letter from a specialist in transfusion medicine, was referred for further assessment to an infectious disease specialist who would examine her current health condition and assess any potential damage that the hepatitis B virus might have caused. This evaluation will be important for her future health, and after the assessment is completed, she will receive recommendations for ongoing monitoring and visits to infectious disease specialists in the future.

Discussion

Here we have presented a case involving a 42-year-old female voluntary blood donor with a resolved hepatitis B infection. The donor successfully donated blood for the first time and underwent mandatory serological and molecular testing, including additional testing for other hepatitis B markers. The results of the mandatory HBV testing were negative. However, upon further serological testing, it was determined that the voluntary blood donor had a presence of anti-HBc and HBs antibodies, indicating prior contact with the hepatitis B virus. While the serological tests suggested a resolved hepatitis B infection, the possibility of a persistent liver infection could not be ruled out, despite the absence of detectable hepatitis B virus DNA in the blood. Therefore, the individual was permanently excluded from the list of potential blood donors.

The risk of transfusion-transmitted HBV infection is relatively low but still higher compared to the transmission risk associated with other viruses that are subject to mandatory testing. This difference in transmission risk may be attributed to the variability in the clinical presentation of HBV infection, host

immune responses, viral replication, and dynamics of serological markers. These factors pose challenges in both blood donor selection and testing procedures.[5]

While current HBsAg tests demonstrate high sensitivity, relying solely on HBsAg testing has its limitations and is more suitable for countries with high vaccination rates and low HBV prevalence. According to the Viral Hepatitis National Strategic Plan for the United States, research needs to be conducted to support changes in hepatitis B screening guidelines. This research should aim to demonstrate screening reliability, efficacy, safety, and cost-effectiveness. Moreover, there is a need to develop and implement quality measures for viral hepatitis testing, as well as an increase in hepatitis B testing in geographic regions where HBsAg prevalence is ≥2%.[6]

The window period (WP) for HBsAg testing is still relatively long, approximately 40 days, which limits its ability to detect infections during the early acute phase and early convalescence when HBsAg may no longer be present in the blood and viral replication is low. With this testing strategy, it is not possible to detect a donor with occult hepatitis B infection (OBI).[7]

OBI is characterized by the presence of viral DNA without detectable HBsAg in the blood outside WP. More than 80% of individuals with OBI have specific antibodies against HBV present in their blood.[8]

Detecting OBI in blood donors is crucial as they pose a risk of transmitting HBV infection to immunocompromised recipients. Furthermore, OBI is the most common cause of hepatitis B transmission through transfusion of blood products.[7]

The diagnosis of OBI is more frequent in individuals who test positive for anti-HBc compared to those who test negative for anti-HBc.[9]

In the absence of an HIV-specific test, anti-HBc has also been used in the USA as a marker of risky sexual behavior, demonstrating high efficacy in detecting established HIV infections.[8]

Therefore, implementing a strategy that combines HBsAg, NAT, and anti-HBc testing for HBV appears to be the best choice, particularly in countries with a high HBV prevalence.[6]

In countries with a low HBV prevalence and a substantial number of regular donors, testing for anti-HBc can be valuable in assessing the prevalence of resolved HBV infections in the population. However, the economic aspect of such testing poses a significant challenge, which is why NAT testing has not yet become a global standard, especially in low-income countries. In such cases, the use of anti-HBc testing provides a cost-effective alternative that contributes to the safety of blood products.

In Croatia, the anti-HBc test is employed as a supplemental test in the confirmation algorithm for initially reactive and repeatedly non-reactive samples in the NAT test when confirmation through discriminatory NAT or high-sensitivity quantitative HBV DNA testing is not feasible.[7]

At our department, we have decided to include anti-HBc testing in combination with HBsAg and NAT testing for all blood donors. This approach allows for the detection of HBV-infected blood donors who may be HBsAg negative and have low-level viremia that cannot be detected by even the most sensitive NAT tests available. By combining different tests, the overall effectiveness of detecting HBV-infected blood donors can be increased. In regions with low HBV endemicity, testing for anti-HBc has been utilized in blood transfusions to minimize the incidence of post-transfusion hepatitis when transfusing HBsAg-negative blood.[10]

In HBV non-endemic countries, the inclusion of anti-HBc testing in blood donor screening is not routine as it would result in the discard of many blood products, even though most of them would be safe for transfusion due to the presence of satisfactory levels of anti-HBs antibodies.[11]

Certain countries, such as Germany and the Netherlands, allow blood donations if the anti-HBs value is higher than 100 IU/L and 200 IU/L, respectively.[12,13]

In Croatia, individuals who test positive for anti-HBc are permanently excluded from being blood donors and are notified through a letter stating their rejection due to past contact with HBV. According to a study conducted in Croatia, deferring all anti-HBc positive donors would lead to the exclusion of 1.32% of blood donors.[7] Such a criterion may not be sustainable in the future, as demonstrated by the recent example of the SARS-CoV-2 pandemic, which has led to a decrease in the global blood supply and posed numerous challenges for transfusion services worldwide. Croatia has also experienced a decline in the number of blood donors due to restrictions on population movement. Additionally, the consumption of blood components in hospitals has been variable, further impact in the blood component supply in Croatia.[14]

Since HBV infections remain a global health concern, it is important to emphasize that in addition to testing for HBV, the blood donor population should also be screened for any risk-behavior factors through medical examinations and pre-donation blood donor questionnaires based on parenteral and sexual risk factors.[15]

It is crucial to strike a balance between ensuring the safety of blood products and maintaining a

continuous blood supply.

Mandatory serological and molecular testing of blood donors for hepatitis B successfully detects potentially infected individuals and carriers of hepatitis B markers. However, additional testing further enhances the safety of both recipients and blood donors. This case study highlights the importance of comprehensive screening for hepatitis B markers such as anti-HBc and anti-HBs, as relying solely on HbsAg screening would not have identified the voluntary blood donor as a resolved hepatitis B case. The absence of HBsAg in apparently healthy donors does not guarantee the absence of the virus in their blood or organs.

Without routine testing for anti-HBc and anti-HBs, individuals with low HBV viremia may go undetected. Moreover, in donors who deny vaccination, the presence of anti-HBs could serve as an indication of a previously resolved infection. Thus, comprehensive screening ensures a higher level of safety in blood transfusion and contributes to overall healthcare protection.

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