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A strategical re-thinking on National Blood Donor Pool: Anti-HBc positivity related re-entry mechanisms

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ABSTRACT

Background and Objectives: Screening of blood donations for antibodies against hepatitis B core antigen (anti-HBc) is used to prevent transfusion transmitted hepatitis B virus (HBV) infection. In this study, we studied the magnitude of blood donor gain by using a re-entry mechanism in our Blood Bank of Gulhane Military Academy of Medicine.
Materials and Methods: Between January and May 2013, 5148 voluntary blood donors were screened by ELISA method for HBsAg, anti-HBc total and other screening markers, prospectively. Samples with repeated reactivity for the presence of anti-HBc were further tested with four supplemental assays.
Results: We detected 515 (10%) anti-HBc positive and 4612 (90%) anti-HBc negative cases in 5127 HBsAg negative serum samples. A total of 461 (89.5%) blood units were reactive for at least one additional serologic parameter and 54 were (10.5%) negative. Isolated anti-HBc positivity rate was 1.3% (69/5127). In the isolated anti-HBc positive samples, 54 were also anti-HBe and HBeAg negative. HBV DNA was not detected in any of the samples.
Conclusion: Applying the EDQM criteria would decrease our blood donor loss from 10% to 5.4%. As alternative re-entry mechanisms have already been presented in the literature, institution of a new policy is needed to enhance the limited blood donor pool in our system.

1. Introduction

Hepatitis B Virus (HBV) infection is a serious global health problem. It is estimated that more than two billion people have been infected with HBV at some time in their lives, and 350 million are thought to be chronically infected [1]. Microbiological screening tests for blood donation are performed in order to prevent infection transmitted by transfusion. Hepatitis B surface antigen (HBsAg) tests as a serological marker have been applied in blood donor screening procedures since 1972 [2]. As a screening test, HBsAg assays are not sensitive in pre-seroconversion window period, in the early convalescence period (core window) of acute hepatitis B infections and in chronic HBV infections, where HBsAg is often present at low levels. Moreover, HBV mutants with amino acid substitutions, within the common "a" determinant of viral gene region, cannot be detected by the currently available HBsAg screening assays [3,4]. Antibodies to hepatitis B core antigen (anti-HBc) and nucleic acid tests (NAT) were included in routine microbiological

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screening tests to decrease the risk of post-transfusion HBV infections [4]. The IgM form of anti-HBc occurs within the first 1–2 weeks after the appearance of HBsAg and persists for 4–6 months. During the core window phase, HbsAg is cleared and the long lived IgG form of anti-HBc develops and serves as a useful serological marker for Hepatitis B infection [5,6]. However, anti-HBc testing would not detect HBsAg-negative donors in the pre-seroconversion window period. Due to the slow ramp-up in viral load and the short diagnostic window period, NAT was expected to require a very high sensitivity to identify those donors [7].

Inclusion of anti-HBc test in the microbiological screening tests has been proposed by Food and Drug Administration (FDA) in 1991 and successively been implemented by France, Germany and Japan [6,8]. Implementation of this test in routine screening programs has also been recommended for countries with an anti-HBc seroprevalence of <10% [9]. However, anti-HBc test has a low specificity rate and this may result in significantly high false positivity rates. False positivity of anti-HBc tests may be due to non-specific EIA related reactions, Ig A and Ig M related substances derived from non-specific HBV-activated B lymphocytes or cross reactions caused by various serum-derived substances [10]. This situation inadvertently leads to the elimination of anti-HBc test positive donors unexposed to HBV from the already limited blood donor pool. Lack of anti-HBc confirmation tests led to the development of re-entry mechanisms to re-gain these test positive donors.

In this study, we aimed to stimulate the initiatives for national test algorithms to regain anti-HBc positive donors in to the donor pool, using the assessment of anti-HBs (quantitative), HBeAg, anti-HBe and HBV DNA (PCR) test results.

2. Materials and methods

2.1. Study group

After the approval of Gulhane Military Academy of Medicine (GMAM) Ethical Committee, serum specimens from 5148 blood donors, between January and May 2013, were included in the study. Of these, 4998 (97.1%) were female and 150 (2.9%) were male. Mean age of the blood donors was 27.54 ± 9.36. The donor population had not been previously screened for anti-HBc.

2.2. Serological study methods and test algorithm

Routine screening tests (HIV Ag/Ab combo, anti-HCV, syphilis TP, HBsAg and anti-HBc total) for 5148 blood donors were tested according to the manufacturer's instructions on an Architect i2000SR (Abbott Laboratories, Abbott Park, IL, USA) chemiluminescence immunoassay (CLIA). Donors with HBsAg positivity were excluded from the study. Samples that yielded repeated reactivity for the presence of anti-HBc was further tested with four supplemental assays: anti-HBs, HBeAg, anti-HBe and HBV DNA PCR.

Specimens with concentration values ≥10 mIU/mL are considered reactive by anti-HBs criteria. HBsAg, HBeAg and anti-HBe were interpreted using a ratio of the sample relative light unit (RLU) rate to cut off RLU(S/CO), where S/CO ≥ 1.00 was positive and S/CO ≤ 1.00 was anti-HBe positive.

An isolated positive test for anti-HBc is defined as the presence of anti-HBc positivity simultaneously with negative HBsAg and negative anti-HBs (without further testing for anti-HBe, HBeAg and HBV-DNA).

2.3. PCR study

Aliquots of 200 μL each of serum samples were tested by RTA HBV Real-Time PCR Kit version 2.0 on RTA VOLTRAN Viral Load Detection System, followed by amplification and detection using Bio-Rad CFX96 Real-Time PCR Detection System. Quantification of the amount of target in unknown samples is accomplished by measuring Ct and using the standard curve to determine starting copy number or IU/mL. RTA HBV Real-time PCR assay utilizes four external standards to gather quantitative results. It also includes an internal control, which controls for target isolation and amplification and detected in the HEX fluorescence channel. The target region is situated in S gene region of HBV genome and is 10^4-bases long. The kit has a linear range between 9.9 IU/mL and 1×10^9 IU/ mL; and it can detect HBV DNA at concentration of 10 IU/mL with a probability rate of 95% (confidence range is 7.9–14.9 IU/mL). RTA HBV Real Time PCR Kit can detect and quantify HBV genotypes A, B, C, D, E, F, G and H; and no cross-reactivity has been observed with other potential cross-reactive markers (RTA HBV Real-Time PCR Kit v2.0 Handbook, 2014). The assay has the Conformite Europeenne/European Conformity – In Vitro Diagnostic Medical Devices (CE-IVD) approval (Notified Body number: 0483).

2.4. Statistical analysis

Data analysis was performed with computer software (SPSS, Version 15.0, SPSS Inc., Chicago, IL). Frequencies and percentages were computed to present test positivity. Age of blood donors was presented as mean ± SD.

3. Results

Out of 5148 blood donors, 536 had anti-HBc positivity. HBsAg was detected in 21 of 536 donors and these donors were excluded from the study. The remaining anti-HBc positive 515 donors were investigated for the presence of HBV DNA by using the PCR method and the additional serologic markers (anti-HBs, HBeAg, anti-HBe).

Among 515 anti-HBc positive donors, 446 had anti-HBs titers ≥10 mIU/mL. In serum samples of 446 anti-HBs positive donors, 238 (46.2%) were highly positive (100 ≥ mIU/mL) and 208 (40.3%) were low positive (<100 mIU/mL) (Fig. 1). The isolated anti-HBc positivity rate was derived from the remaining 69 (1.3%) donors. Within the 515 anti-HBc total positive donors, 201 (39%) were anti-HBe positive. Of the 201 anti-HBe positive donors, 186 (93%) were tested positive for anti-HBs.

All of the HBeAg test results were negative. The exact serologic profile of 515 anti-HBc positive blood donors is shown in Table 1. None of the anti-HBc positive donors had HBV DNA positivity.