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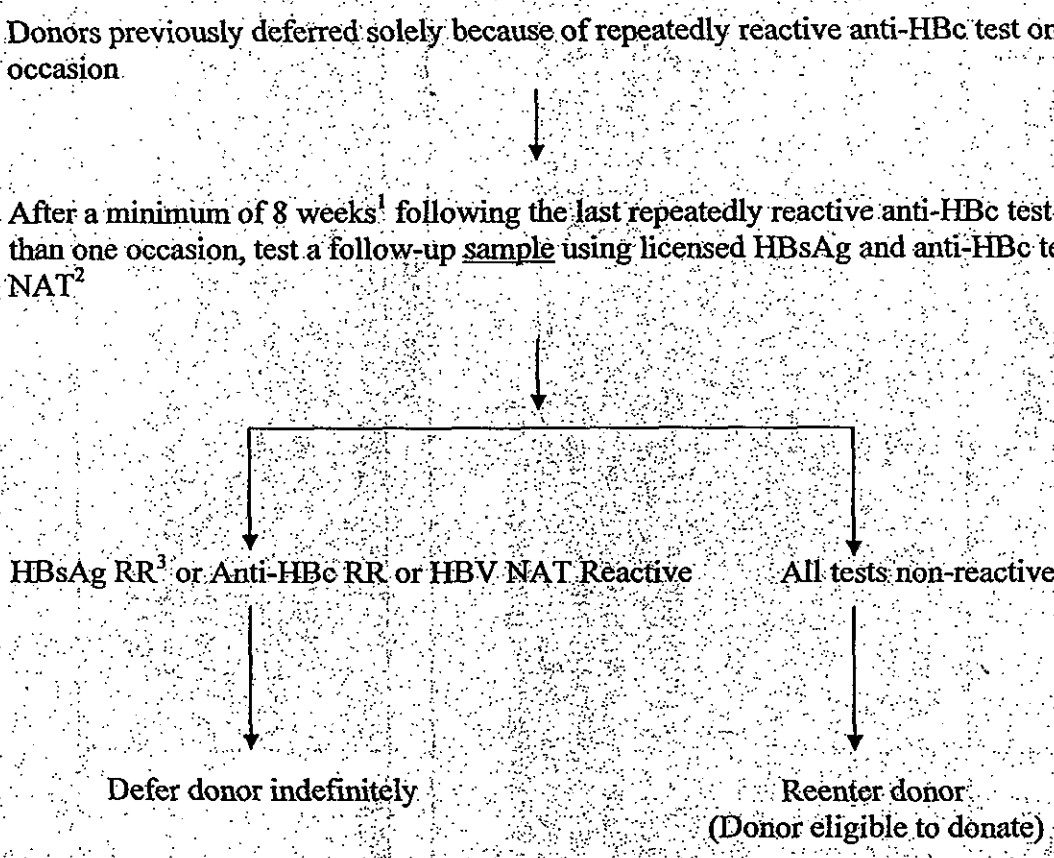
APPENDIX

REENTRY FOR DONORS DEFERRED BECAUSE OF REPEATEDLY REACTIVE TEST RESULTS FOR ANTI-HBc



[1] If the donor sample is tested before 8 weeks following the last repeatedly reactive anti-HBc test results on more than one occasion, a) if the sample tests HBsAg RR or anti-HBc RR or HBV NAT reactive, the donor is indefinitely deferred, and b) if the sample tests negative on all three of these tests, the donor should be retested after a minimum of 8 weeks following the last repeatedly reactive anti-HBc test result on more than one occasion using licensed HBsAg and anti-HBc tests, and HBV NAT.

[2] The sensitivity of the HBV NAT used should be ≤ 10 copies/mL, at 95% detection rate.

[3] Regardless of the neutralization test result.

別紙様式第２－１

医薬品　研究報告・調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
一般的名称	新鮮凍結血漿、濃厚赤血球		研究報告の公表状況	Journal of hepatology (England) Jun2008, 48 (6) p1022-5.	公表国 英国	
販売名（企業名）	－					

研究報告の概要

潜在性 B 型肝炎ウイルス感染者 (OBI) の血液は抗 HBs 抗体が陽性であれば感染性がないと考えられているが、スロヴェニアにおいて、冠動脈バイパス術で濃厚赤血球と新鮮凍結血漿 (HBs 抗原陰性で抗 HBc 抗体陽性、抗 HBs 抗体低力価陽性、HBV DNA 陽性) が輸血された 59 歳の患者が、その 4 ヵ月後に急性 B 型肝炎を発症した。
また、もう一例、先の例の感染源と同じ供血血液から得られた濃厚赤血球 (RCC) の輸血を受けた 71 歳の患者が、受血の 7 ヵ月後に HBV 感染を認めた (HBV に感染した 2 例はドナーと同じ配列を有するジェノタイプ D 型が感染していた)。
原因となった供血血液は、抗 HBc 抗体及び抗 HBs 抗体 (12IU/L) が陽性であったが、HBV DNA も陽性であり、この供血者のこれまで及びそれ以後のサンプルには低量のウイルスと抗 HBs 抗体が含まれていたが、過去 2 回分の供血血液では HBV 感染は起きていなかった。
今回の 2 例の受血者は手術の外傷に加え、加齢により免疫が低下していたことがウイルスに対する感受性を増大させたとも考えられる。
OBI は感染性を持つが HBV DNA スクリーニングで検出可能であるので、抗 HBc 抗体も HBV NAT も実施されていない国の保健当局は慎重に考慮すべきである。

使用上の注意記載状況・その他参考事項等

重要な基本的注意

(1) 本剤の原材料となる (献血者の) 血液については、HBs 抗原、抗 HCV 抗体、・・・・陰性で、かつ ALT (GPT) 値でスクリーニングを実施している。さらに、プールした試験血漿については、HIV-1、HBV 及び HCV について核酸増幅検査 (NAT) を実施し、適合した血漿を本剤の製造に使用しているが、当該 NAT の検出限界以下のウイルスが混入している可能性が常に存在する。

報告企業の意見	今後の対応
HBs 抗原陰性で抗 HBs 抗体低力価陽性、HBV DNA 陽性の血液による B 型肝炎感染の報告である。 血漿分画製剤の原料血漿はミニプール血漿における NAT 検査で HBV DNA 陰性を確認しており、最終製品においても HBV DNA 陰性を確認している。	今後ともに潜在性 B 型肝炎ウイルス感染に関する安全性情報に留意していく。

③

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Case Report

Anti-HBs positive occult hepatitis B virus carrier blood infectious in two transfusion recipients☆

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***Background/Aims*: Occult hepatitis B infection (OBI) in blood donations is not considered infectious when anti-HBs is present.**

***Methods*: Four months after transfusion of eight blood components during coronary arterial bypass surgery, a 59-year-old patient developed acute hepatitis B. A second 71-year-old patient transfused with a red cell concentrate (RCC) from one of these donations had early HBV infection 7 months post-transfusion. Samples were tested for HBV serological markers and HBV DNA was quantified and sequenced.**

***Results*: One implicated donation contained anti-HBc, anti-HBs (12 IU/L) and 180 IU/ml of HBV DNA. Previous and subsequent samples contained 3–10 times lower viral load and slightly variable anti-HBs. Two previous donations did not cause HBV infection. Recipients of the FFP and RCC from the index donation were both HBV infected and carried genotype D strains with sequences identical to the donor strain.**

***Conclusions*: Despite anti-HBs, an OBI carrier transmitted HBV to two immunocompetent transfusion recipients.**

***Keywords*: HBV; Occult HBV; Infectivity; Blood transfusion**

1. Introduction

In Slovenia, approximately 100,000 donations per year are collected. However, in 2005–2007, six cases of HBV transmission by transfusion were reported. Incidence was probably underestimated due to a high frequency of subclinical infection. Since HBsAg serological screening with a sensitive assay is systematically performed, transfusion transmission of HBV can originate from either recent infections in the pre-HBsAg seroconversion window period or occult HBV infection (OBI). OBI is defined as an atypical carrier state characterized by the presence of HBV DNA in plasma without detectable hepatitis B surface antigen (HBsAg) with or without antibodies to hepatitis B core antigen (anti-HBc) and hepatitis B surface antigen (anti-HBs) [1].

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Abbreviations: OBI, occult HBV infection; HBV, hepatitis B virus; RCC, red cell concentrate; FFP, fresh frozen plasma; anti-HBc, antibody to hepatitis B virus core antigen; anti-HBs, antibody to hepatitis B virus surface antigen; QPCR, real-time PCR; BCP/PC, basic core promoter/pre-core.

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It is generally accepted that HBV DNA in blood may carry the risk of transmission, particularly in the pre-HBsAg window phase [2]. However, the transmission risk of OBIs is not well defined, although some cases of OBIs with anti-HBc only infectious by transfusion were described [2,3].

HBV transmission by blood components from a single anti-HBs positive OBI donation to two recipients is presented.

2. Case report

A patient who had been transfused 4 months previously with five units of fresh frozen plasma (FFP) and three units of RCC was suspected of acute hepatitis B. Stored samples from each implicated donation were tested for HBV markers. Seven samples were HBV marker negative. One sample was anti-HBc reactive and contained HBV DNA. The implicated donor was identified and stored samples from eight previous donations and one donation subsequent to the index donation as well as three follow-up samples were tested for HBV markers.

The first recipient of an FFP unit from the index donation was a 59-year-old male who was screened negative for HBV markers 3 days prior to cardiac arterial bypass. He was transfused on 23rd June, 2005. Four months later, clinical and laboratory evidence of acute Hepatitis B was obtained. ALT level was 1821 IU/L, HBsAg and anti-HBc IgM became reactive. No sample was available for HBV DNA testing. In a sample collected 4 months later, HBsAg was undetectable, IgM anti-HBc remained present and HBV DNA was at low level (Table 1).

The second recipient of the index donation was a 71-year-old female who received two units of RCC following orthopedic surgery. No pre-surgical HBV screening was performed and no post-surgical evidence of HBV infection was noted. A blood sample obtained 7 months after transfusion was anti-HBc negative but HBsAg positive and contained a high level of HBV DNA (Table 1). Nine months post-transfusion, ALT level was 566 IU/L. At 14 months post-transfusion the patient had recovered.

2.1. Methods

Routine blood donation screening for HBsAg was performed using Abbott PRISM (Abbott laboratories, Delkenheim, Germany). HBsAg repeat testing, anti-HBc and anti-HBs assays were performed with Abbott AxSYM. Cobas Amplicor HBV Monitor (Roche, Basel, Switzerland) and in-house real-time PCR (QPCR) as previously described were used to detect and quantify HBV DNA [4]. Basic core promoter/pre-core region (BCP/PC), Pre-S/S regions and full HBV genome were amplified, sequenced and phylogenetically analyzed as described [5].

3. Results and discussion

The index donation met the criteria defining 'occult' hepatitis B virus carriage since the plasma contained no detectable HBsAg but HBV DNA, anti-HBc and low titer of anti-HBs. This pattern was consistent 7 and 16 months after the index donation. Seven prior donations carried anti-HBc and anti-HBs although HBV DNA ranged between 7 and 63 IU/ml when tested

Table 1
Hepatitis B virus markers in the OBI donor and two HBV infected recipients

	Time from Index donation (m)	HBsAg	Anti-HBc	Anti-HBc IgM	Anti-HBs (IU/L)	HBV DNA (IU/ml)		HBV genotype
						Amplicor	QPCR	
Donor	−45	−	+	ND	29	Neg	63	
	−37	−	+	ND	15	Neg	Neg	
	−31	−	+	ND	16	Neg	35	
	−23	−	+	ND	16	Neg	45	
	−19	−	+	ND	19	Neg	7	
	−13	−	+	ND	15	Neg	ND	
	−6	−	+	ND	15	Neg	ND	
	Index	−	+	ND	12	<60	180	D
	+3	−	+	ND	53	Neg	ND	
	+7	−	+	ND	31	Neg	16	
	+12	−	ND	ND	ND	ND	Neg	
	+16	−	+	ND	25	Neg	40	
Recipient 1	−3 days	−	−	ND	−	ND	ND	
	+4	+	+	+	−	ND	ND	
	+8	−	+	+	−	12	185	D
Recipient 2	+7	+	−	−	−	1.1×10^6	1.7×10^6	D
	+14	−	+	+	−	Neg	ND	

−, non-reactive; ND, not done; Neg, negative.

with a sensitive in-house assay but was consistently undetectable by a commercial assay except in the Index sample. This pattern indicates recovery from >5 years past HBV infection (Table 1). Despite being tested with the high sensitivity assay, two of the nine donor samples tested remained HBV DNA negative, suggesting fluctuations of viremia. Prior to the index donation, anti-HBs levels were essentially stable (15–29 IU/L) but increased from 12 to 53 IU/L 3 months later suggesting minimal immune response. There was no clinical evidence that 14 previous donations and one subsequent donation were infectious to recipients. Pre- and post-transfusion samples from recipients of −71 and −13 month-donations showed no serological evidence of HBV infection. The −71 recipient was negative for HBsAg, anti-HBc and anti-HBs pre-transfusion, and 4 months post-transfusion, HBsAg was negative but anti-HBc was not tested. The −13 month recipient did not carry HBsAg, anti-HBc or anti-HBs 42 months after transfusion.

In contrast, there is strong evidence that both recipients of the index donation were HBV infected since acute hepatitis B occurred in recipient 1, 4 months after transfusion. In recipient 2, the 7-month post-transfusion sample containing HBsAg and high HBV DNA load without anti-HBc strongly suggested recent acute HBV infection and was followed by serological evidence of recovery (Table 1). A high ALT level 9 months post-transfusion that normalized after 14 months further supported this conclusion. The 4-month and probably 7-month long incubation time observed in recipients 1 and 2, respectively, could be explained by a relatively low infectious dose further decreased by partial anti-HBs neutralization (calculated on the basis of 180 IU/ml of HBV DNA and 200 ml of FFP for recipient 1 at 200,000 copies and 20,000 copies in 20 ml of RCC plasma for recipient 2). Published data indicated that lower infectious dose prolonged HBV incubation time and milder symptoms [6]. Transfusion transmission was further demonstrated by the Pre-S/S sequence identity between the index donation, recipient 1 and recipient 2 strains from follow-up samples. The whole genome sequences of recipient 2 and index donation were identical. Strains were of genotype D. Of note, the deduced amino acid sequence of the S protein was wild-type when compared to the genotype D consensus sequence except for A117T and S133Y, neither of these substitutions being recognized as escape mutants. An escape mutant mechanism explaining the infectivity of the index donation but not of the other donations from the donor was thus excluded. Similar cases of breakthrough HBV infection with wild-type strains have been described [7]. Although suppression of the HBV replication and gene expression is a reported cause of occult HBV [8], no mutation in the parts of the genome implicated in replication was found. Imperfect containment of viral replication by the donor immune system is the most likely cause of low levels of HBV DNA.

The stability of HBV DNA load and anti-HBs in multiple samples preceding the index donation and tested simultaneously contained 6–10 times less viral DNA than the index donation (Table 1). It is therefore speculated that the main factor singling out the index donation was a temporarily higher viral load sufficient to overcome the relatively weak neutralizing capacity of a low anti-HBs level (Table 1). This interpretation is supported by the subsequent increase in anti-HBs level suggesting a weak immune response.

Published data reporting the infectivity of OBIs by transfusion are rare. One case of transmission by a donation carrying anti-HBc without anti-HBs was reported in Japan [2]. Another study reported five donors (4 genotype D, one genotype A2) with OBI also carrying only anti-HBc transmitting to recipients. Of 51 traced recipients, 28 (54.9%) either developed fulminant, fatal, hepatitis B (3 cases) or carried anti-HBc post-transfusion although no pre-transfusion testing was performed [3]. In the Japanese study, 16 donations contained both anti-HBc and anti-HBs and no evidence of HBV transmission was found [2] confirming previous results [9]. The two cases reported here appear to be the first related to an OBI donor with anti-HBs. Data collected in Poland indicated that approximately 50% of OBIs in asymptomatic, apparently healthy, blood donors carry anti-HBs [10] and that levels of DNA and anti-HBs are variable as reported here.

Considering that the recipients at age 59 and 71, respectively, might have presented a mild, age-related, immunodeficiency added to the trauma of major surgery might have played a role in increasing susceptibility to viral infection [11]. The fact that approximately 50% of recipients of blood components in Western Europe present some degree of immunodeficiency related to age, chemotherapy or therapeutic immunosuppression suggests an increased susceptibility to HBV infection [12]. Limited but convincing evidence that OBIs can be infectious and can be detected by HBV DNA screening should be carefully considered by the health authorities of countries where neither anti-HBc nor HBV NAT are implemented.

Despite their apparent uniqueness, our two cases of HBV transmission need to be factored in discussions regarding HBV blood safety policy. They clearly illustrate that the neutralizing capacity of low-level anti-HBs is limited and reinforce the validity of considering anti-HBs below 100 IU/L to be poorly protective from infectivity when HBV DNA is present. However, even in the presence of higher levels of anti-HBs, in a severely immunodeficient recipient, HBV DNA-containing blood might be infectious and the clinical expression severe.

Acknowledgements

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医薬品
医薬部外品　研究報告　調査報告書
化粧品

識別番号・報告回数			報告日	第一報入手日 2008 年 3 月 13 日	新医薬品等の区分	厚生労働省処理欄

一般的名称	①献血アルブミン-Wf ②献血アルブミン(5%)-Wf ③ノイアート ④ノイアート静注用 1500 単位 ⑤ハプトグロビン注-ヨシトミ ⑥コンコエイト-HT	研究報告の公表状況	Veterinary Science in China 2007; 37(11): 921-925	公表国 中国
販売名 （企業名）	①②人血清アルブミン ③④乾燥濃縮人アンチトロンビンⅢ ⑤人ハプトグロビン ⑥乾燥濃縮人血液凝固第Ⅷ因子			

研究報告の概要

文献中のヒト HBV の S 遺伝子の配列に従って、ブタ HBV の S 遺伝子のための 2 つのプライマーを設計し合成した。ブタの肝臓と血清のサンプルを中国の畜殺場から集めた。次いで、RT-PCR を使って S 遺伝子を増幅し配列決定を行った。その結果、ブタとヒトの HBV の S 遺伝子の配列は 98-100%の相同性を示した。HBV 陽性血漿の発光透過型電子顕微鏡による測定の結果、ウイルス粒子は直径 20 および 40nm であることが分かった。それら粒子は、ヒトの HBV 粒子と直径と形状が類似していた。陽性血清は、ELISA 法による HBV の表面抗原の存在によって確認した。ORF2/ORF3 のオーバーラップ領域から設計された 1 対の degenerated primers に対する nested RT-PCR アッセイから、HEV の遺伝子配列はブタの肝臓には存在するが、血清には存在しないことが示唆された。

報告企業の意見	今後の対応
中国の畜殺場から集めたブタの肝臓および血清からブタ B 型肝炎ウイルス、ブタ肝臓から E 型肝炎ウイルスを検出したとの報告である。 万一、ヘパリンの原料であるブタ小腸粘膜にHBVまたはHEVが混入したとしてもそれぞれPRVおよびPPVをモデルウイルスとしたウイルスバリデーション試験成績から、ヘパリンの製造工程において十分に不活化・除去されると考えている。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

使用上の注意記載状況・その他参考事項等

代表としてノイアート（献血）の記載を示す。
2. 重要な基本的注意
(1) 本剤の原材料となる献血者の血液については、HBs 抗原、抗 HCV 抗体、抗 HIV-1 抗体、抗 HIV-2 抗体、抗 HTLV-I 抗体陰性で、かつ ALT (GPT) 値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV 及び HCV について核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該 NAT の検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した血漿を原料として、Cohn の低温エタノール分画で得た画分から人アンチトロンビン III を濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において 60℃、10 時間の液状加熱処理及びウイルス除去膜によるろ過処理を施しているが、投与に際しては、次の点に十分注意すること。