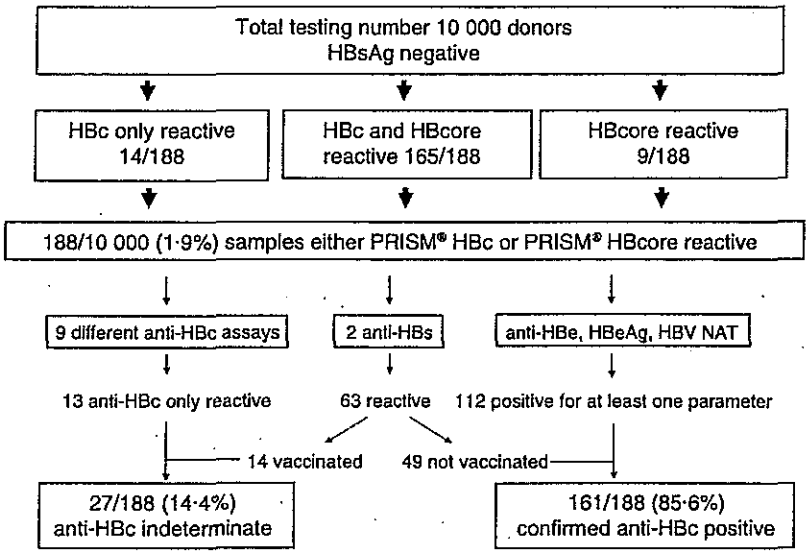

Fig. 1 Testing procedure. A total of 10 000 hepatitis B surface antigen (HBsAg)-negative donors were screened with PRISM® HBc and PRISM® HBcore. Reactive samples were analysed by nine different tests for antibody to hepatitis B core antigen (anti-HBc) and tested for antibody to hepatitis B surface antigen (anti-HBs), antibody to hepatitis B envelope antigen (anti-HBe) and hepatitis B virus by nucleic acid amplification technology (HBV NAT). Samples that were also positive for anti-HBe, hepatitis B e antigen (HBeAg), HBV NAT or anti-HBs without vaccination were interpreted as confirmed anti-HBc positive. Samples that were anti-HBc-only reactive or anti-HBs with vaccination were interpreted as anti-HBc indeterminate.

HBV NAT

Samples diagnosed as anti-HBc reactive by PRISM® HBc or PRISM® HBcore were tested using an in-house real-time HBV DNA polymerase chain reaction (PCR) assay with primers targeted to the surface (S) gene (nucleotides 338–430) [15]. To increase sensitivity of the in-house NAT, we performed enrichment of viruses from 9·6 ml of single-donor plasma by centrifugation at 58 000 *g* for 1 h before extraction (single-sample enrichment PCR). After centrifugation, supernatants were decanted and the pellets were subjected to nucleic acid extraction using the QIAamp DNA blood mini kit (Qiagen, Hilden, Germany). Nucleic acids were eluted from the Qiagen columns in a final volume of 50 µl. Aliquots of 20 µl were subjected to amplification by HBV PCR in duplicate. Single-sample enrichment PCR was independently repeated at least four times. The analytical sensitivity (95% detection limit) of the single-sample enrichment PCR was 1·86 IU/ml, based on the World Health Organization (WHO) International Standard for HBV DNA (NIBSC Code 97/746). Furthermore, all anti-HBc-reactive specimens were tested in two commercially available HBV DNA screening NAT assays by ID-NAT, HBV Cobas AmpliScreen (Roche Molecular Systems, Pleasanton, CA, USA) and TMA Ultrio (Chiron, Emeryville, CA, USA), according to the manufacturers' instructions. The test systems HBV Cobas AmpliScreen and TMA Ultrio yield a sensitivity of 6·7 IU/ml [16] and 11 IU/ml [17], respectively.

Serological testing

A total of 10 000 blood donors were screened with PRISM® HBc, as well as with PRISM® HBcore. Samples that were positive in at least one of the assays were additionally tested in the following seven anti-HBc assays: AxSym Core™ (Abbott, Wiesbaden, Germany); Immulite® 2000 Anti-HBc (DPC, Bad Naunheim, Germany); Enzygnost® Anti-HBc Monoclonal (Dade Behring, Marburg, Germany); Ortho™ HBc ELISA (Ortho Clinical Diagnostic, Neckargemuend, Germany); Cobas® Anti-HBc (Roche Diagnostics, Mannheim, Germany); Murex® Anti-HBc (Abbott/Murex Biotech Ltd, Dartford, Great Britain); and ADVIA Centaur HBc® (Bayer Health Care, Tarrytown, NY, USA). To determine HBsAg, hepatitis B envelope antigen (HBeAg), anti-HBs and anti-HBe, the following tests were used: PRISM HBsAg®, AxSYM HBe 2·0®, AxSYM AUSAB®, and AxSYM anti-HBe 2·0®, respectively (all Abbott); ADVIA Centaur Anti-HBs (Bayer Health Care); and Architect Anti-HBe (Abbott). All serological tests were conducted strictly in accordance with the manufacturers' instructions. All anti-HBc assays were competitive tests, with the exception of ADVIA Centaur HBc® and Ortho™ HBc ELISA, which were non-competitive anti-HBc assays.

Statistical analysis

The standard deviation (SD) and coefficient of variation (CV) of the antibody assays were calculated with Excel 2000. The Student's unpaired *t*-test was performed with the data from sample cut-off (S/Co) values. Fisher's test was performed for sensitivity, specificity, positive and negative predictive value between PRISM® HBc and PRISM® HBcore. Statistical significance was considered if the *P*-value was < 0·05. Results were highly significant if the *P*-value was < 0·01.

Results

Prevalence of anti-HBc in the German Red Cross donor population

A total of 10 000 HBsAg-negative donors from our blood donation service were screened, in parallel, with PRISM® HBc

Table 1 Characterization of 188 antibody to hepatitis B core antigen (anti-HBc)-reactive samples by nine different anti-HBc assays

		Anti-HBc reactivity in nine different assays		
		Class A (9 assays)	Class B (5–8 assays)	Class C (1–4 assays)
Group 1				
Anti-HBc only	13	0	8	5
Group 2[a]				
Anti-HBc + anti-HBs	63	50	11	2
Anti-HBc + anti-HBe	7	7	0	0
Group 3[b]				
Anti-HBc + anti-HBs + anti-HBe	105	105	0	0
Total	188 [168 (89·4%) anti-HBs positive]	162 (86·2%)	19 (10·1%)	7 (3·7%)

[a]One second marker positive.

[b]Both second markers positive.

All 188 samples reactive by PRISM® HBc and/or by PRISM® HBcore were re-analysed by seven additional assays for anti-HBc. Samples were categorized into three groups [1 = anti-HBc-only reactives; 2 = one second marker positive for antibody to hepatitis B surface antigen or antibody to hepatitis B envelope antigen (anti-HBs or anti-HBe); 3 = samples reactive for anti-HBc and anti-HBe + anti-HBs). The samples were further classified into three classes (class A = reactive in all nine anti-HBc assays; class B = reactive in five to eight anti-HBc tests; class C = reactive in four or fewer anti-HBc tests).

and PRISM® HBcore (Fig. 1). One-hundred and eighty eight of 10 000 (1·88%) samples were either PRISM® HBc or PRISM® HBcore reactive. The majority of these samples were additionally positive for anti-HBs (168/188, 89·4%) or for anti-HBe (112/188 59·6%) (Table 1). All of these anti HBc-reactive samples were HBeAg negative. Only one sample, which was anti-HBc reactive in all nine assays, negative for anti-HBs and positive for anti-HBe, was HBV DNA positive by single-sample enrichment NAT. Quantification yielded a virus load of 2·5 IU/l. However, both commercial HBV NAT systems designed for NAT blood screening (Cobas Ampliscreen and TMA Ultrio) gave negative results after triplicate testing of the individual plasma.

Sensitivity and specificity of PRISM® HBc and PRISM® HBcore assays

One-hundred and sixty five of 188 samples were reactive in both anti-HBc screening assays, whereas 14 and nine samples were only PRISM® HBc and PRISM® HBcore reactive, respectively. Ten of 14 PRISM® HBc-only reactive samples were not confirmed by other HBV parameters. The remaining four samples were positive for anti-HBs. Three of nine PRISM® HBcore-reactive samples were not confirmed by other HBV parameters. The remaining six samples were positive for anti-HBs.

Diagnostic sensitivity is defined as the ratio of positive tested samples divided by all positive samples, whereas diagnostic specificity is defined as the ratio of negative tested samples divided by all negative samples. Based on 'anti-HBc-positive' samples (samples that were also positive for an additional HBV parameter after exclusion of vaccination), diagnostic sensitivity was equal for both screening assays (96·9% and 99·4% for PRISM® HBc and PRISM® HBcore, respectively; not significant $P = 0·5$; 159/161 samples reactive for PRISM® HBc and 160/161 samples reactive for PRISM® HBcore). In contrast, diagnostic specificity was significantly higher for PRISM® HBcore (9812/9822 samples negative for PRISM® HBc and 9812/9815 samples negative for PRISM® HBcore; $P = 0·046$) and the positive predictive value was also significantly increased for PRISM® HBcore (159/169 samples positive for PRISM® HBc and 160/163 samples positive for PRISM® HBcore; $P = 0·049$).

Detailed investigation of 188 anti-HBc reactive samples

Samples that were either PRISM® HBc or PRISM® HBcore reactive were tested in parallel with seven additional anti-HBc assays, two anti-HBs tests and three NAT assays. Arbitrary confirmation of anti-HBc reactivity was either decided on the basis of additional detection of anti-HBs, anti-HBe or HBV-DNA, or in conjunction with the frequency of reactivity in the total of nine different anti-HBc assays. As shown in Table 1, the samples were categorized into three groups in regard to the presence of additional serological HBV markers. The first group represented anti-HBc-only reactive samples (no additional serological HBV marker), the second group consisted of samples that had exclusively anti-HBs or anti-HBe as a second HBV marker, and the third group was made up of anti-HBe- and additional anti-HBs-reactive samples.

To address the theoretical possibility that the reactive anti-HBc screening result might be non-specific (false-positive) and the anti-HBs reactivity caused by an HBV vaccination with recombinant HBsAg vaccine, we interviewed 63 of the

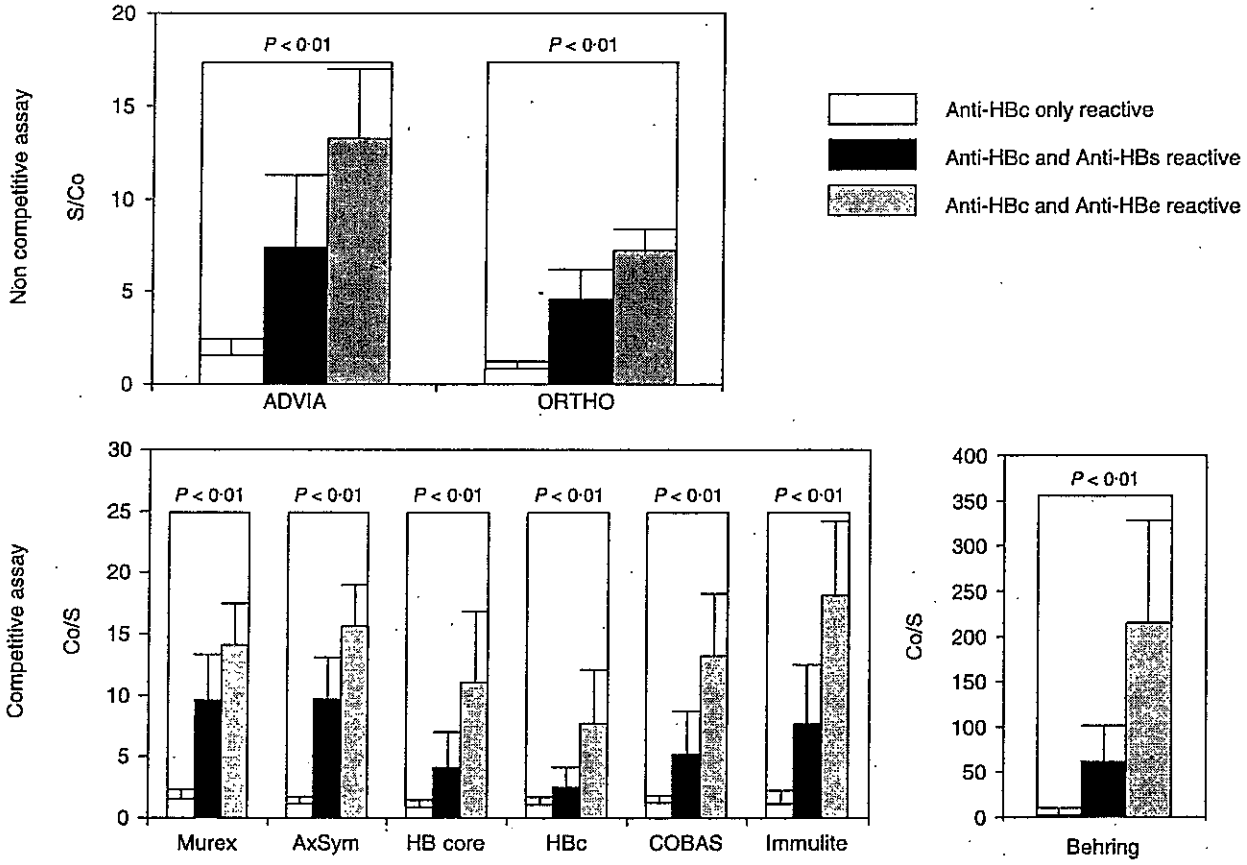

Fig. 2 Analysis of 188 antibody to hepatitis B core antigen (anti-HBc)-reactive samples: comparison between nine anti-HBc assays. Anti-HBc assays were divided into competitive (Murex, AxSYM, PRISM® HBcore, PRISM® HBc, COBAS Immulite and Behring) and non-competitive (ADVIA and Ortho) assays. Sample cut-off values (S/Co) differed significantly between anti-HBc-only reactive samples and anti-HBc + antibody to hepatitis B envelope antigen (anti-HBe)-reactive samples.

anti-HBc/anti-HBs dual-reactive donors about their vaccination history: 14 (22·2%) reported having had an HBV vaccination in the past.

All 112 anti-HBe-reactive samples were also concordantly reactive in the nine anti-HBc assays, providing strong evidence for anti-HBe as the most specific marker for a past HBV infection. Figure 2 shows S/Co values for each group according to the different anti-HBc assays. One might also consider the S/Co ratio of anti-HBc results as an indication of distinguished true and false-positive anti-HBc results: significantly lower anti-HBc signals were obtained with the anti-HBs- and/or anti-HBe-negative samples compared with anti-HBs- and/or anti-HBe-reactive samples (Fig. 2, $P < 0·01$). The student's unpaired *t*-test was performed for the S/Co values between these groups of samples and differed significantly ($P < 0·05$) in all nine anti-HBc tests.

Discussion

Blood donors chronically infected with HBV, but without detectable HBsAg, contribute to the residual risk of transfusion-transmitted HBV infections, which is higher for HBV than for HIV or HCV [6]. Blood donor screening with HBsAg assays and HBV MP or single-donation NAT may fail to detect chronic HBV-infected persons, because the low virus burden may remain undetected by these assays. However, a significant proportion of these HBV infections might be detected by testing for anti-HBc.

In this study we first compared two anti-HBc assays (PRISM® HBc and PRISM® HBcore) with each other. One-hundred and sixty one of 188 anti-HBc-reactive samples were confirmed by other HBV markers. This corresponds to a prevalence of 1·61% of confirmed anti-HBc-positive donors in our population. The diagnostic sensitivity was comparable between both assays, whereas the diagnostic specificity was significantly enhanced for the PRISM® HBcore. Based on these data, we suggest the use of the PRISM® HBcore for blood donor screening in order to enhance the specificity of the anti-HBc assay without compromising sensitivity.

Screening for anti-HBc is considered a potential measure to improve blood safety further. However, because of presumed non-specificity of assays, the implementation of anti-HBc as

a screening marker for blood donors is the subject of controversial discussion. A golden rule for the confirmation of anti-HBc reactivity as an indicator of a past HBV infection has not yet been established. It is likely that anti-HBc reactivity, in conjunction with both anti-HBs and anti-HBe, indicate a past HBV infection. In our study, 105/188 (55·9%) of the donors showed this. Anti-HBe as the only second marker also suggests a past HBV infection. This conclusion is supported by the fact that all anti-HBe-reactive samples, with or without anti-HBs coreactivity, were clearly anti-HBc reactive in all nine anti-HBc assays used in this study.

Under this premise, 112/188 (59·6%) anti-HBc-reactive samples were truly anti-HBc positive. At first glance, one might also consider anti-HBs as the only second marker as a confirmation for anti-HBc positivity (group 2). However, both the reactivity of some of these specimens in only a few anti-HBc assays, and the low anti-HBc S/Co values combined with very high anti-HBs titres, raised some doubts. Indeed, in interviews, 14/63 donors confirmed that past HBV vaccinations with recombinant HBsAg were presumably responsible for the high anti-HBs titres. Therefore, we cannot retrospectively differentiate between vaccinated donors who comprise non-specific anti-HBc reactivity and vaccinated donors with anti-HBc as a marker of past HBV infection. Both constellations seemed to be represented in our population. After exclusion of all anti-HBs-positive donors with a vaccination history (including most of the weak anti-HBc reactives), probably 49/63, both anti-HBs and truly anti-HBc-positive donors remain. Altogether, 27/188 (14·4%) of anti-HBc-reactive samples remain as probably non-specific anti-HBc reactive (false-positive), and 161/188 (85·6%) of anti-HBc-reactive samples appear to be truly positive.

A total of 160/161 and 159/161 of these samples were reactive with PRISM® HBcore and PRISM® HBc, respectively. This low percentage of unconfirmed anti-HBc results contrasts previous reports [12,14] and may be explained, in part, by the use of modern, more specific, assays for our blood donor screening.

In an attempt to classify the anti-HBc status by the S/Co values, the samples were classified into three groups according to the level of the S/Co values. The highest S/Co values were found for samples that were at least anti-HBc and anti-HBe reactive, followed by samples that were anti-HBc and anti-HBs reactive (Fig. 2). S/Co values for anti-HBc-only reactive samples were higher for those detected by five to nine assays than those which were reactive in only one to four tests. Therefore, the analysis of the S/Co values enabled us to define confidence intervals for each anti-HBc test in order to classify unknown samples to be potentially confirmed, indeterminate, or potentially negative. There were, however, two samples that could not be classified in this manner because they were both anti-HBc weak and anti-HBs highly reactive. The weak anti-HBc result could be a non-specific finding in donors recently vaccinated for HBV. These preliminary conclusions, however, should be challenged by studies that use a larger number of samples.

In this study, only one anti-HBc-positive donation was found to be positive for HBV DNA in one of the three NAT tests, and again only in three of four respective test runs. Clearly, HBV DNA in this plasma would have hardly been detected with state-of-the-art NATs, even when performed on a single-donation basis. The donor was a repeat donor who had previously donated 18 times, with a normal alanine aminotransferase (ALT) value obtained at each time point. HBV infection has not occurred in the recipients (six of 18 were retested by antibody screening and ultrasensitive NAT; 12 were already dead at the time of the look-back). In a previous study, Roth *et al.* [5] reported on seven HBV-positive samples among 729 HBsAg-negative, anti-HBc-positive donors. Six of these seven donors were also positive for anti-HBs. Kleinman and colleagues [13] found four HBV DNA-positive samples by testing 395 anti-HBc-positive samples with anti-HBs titres of < 100 IU/l. The risk associated with transfusion of those low-level viraemic donations is difficult to assess. Look-back examinations could eventually offer some clarification of this issue.

Based on the data presented, all anti-HBc assays tested were suitable for blood donor screening. However, we prefer a highly sensitive and specific screening test, such as PRISM® HBcore. Confirmation with a second anti-HBc test might be one strategy to identify non-specific reactivity, but we must bear in mind that many assays use the same antigens for testing. Therefore, a sample that could be confirmed with a second assay might well be a result of the non-specificity of both tests. In this study, confidence intervals for S/Co values were defined for each assay, allowing differentiation between truly anti-HBc-positive samples and anti-HBc-indeterminate samples. In very rare cases, however, anti-HBc-only reactive samples with a very weak S/Co value may represent a past HBV infection where anti-HBs and anti-HBe disappeared and anti-HBc waned to low levels. These cases might be outside the confidence intervals for the S/Co values defined in this study. Currently, there were no data, based on look-back examinations, that these donors were infectious.

In summary, ≈ 1·88% of our blood donors were initially reactive for anti-HBc, as determined by screening with PRISM® HBc and PRISM® HBcore, and, in 161/188 donors, anti-HBc reactivity was confirmed by additional HBV parameters. The diagnostic specificity and positive predictive value of PRISM® were significantly enhanced for PRISM HBcore® when compared with the PRISM® HBc. The approach to define confidence intervals for anti-HBc S/Co values might be useful for classifying an unknown sample as anti-HBc positive or anti-HBc indeterminate. The infection risk resulting from anti-HBc-positive donors is under examination in a separate study by the German Red Cross Research Foundation in which ≈ 1300 look-back analyses are being conducted. Results of

these look-back examinations are eagerly awaited and may allow us to assess the impact of donors, chronically infected with HBV, on blood safety more accurately.

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別紙様式第 2-1
番号 5

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 2006 年 9 月 4 日	新医薬品等の区分 該当なし	厚生労働省処理欄

一般的名称	人ハプトグロビン	研究報告の公表状況	肝臓 2006;47(8):384-391	公表国 日本
販売名 (企業名)	ハプトグロビン注－ヨシトミ(ベネシス)			

研究報告の概要

わが国の E 型肝炎の実態を明らかにする目的で、全国から総数 254 例の E 型肝炎ウイルス感染例を集め、これを解析した。
その結果、以下の知見を得た。
1)HEV は全国に浸透している。
2)感染者の多くは中高年（平均年齢約 50 歳）で、男性に多い(男女比約 3.5 対 1)。
3)我国に土着の HEV の遺伝型は 3 型と 4 型であり、後者は北海道に多い。
4)年齢と肝炎重症度との間に相関がある。
5)遺伝型 3 型に比べて、4 型は顕在化率も重症化率も高い。
6)発症時期は無季節性である。
7)感染経路は、動物由来食感染が約 30%、輸入感染が 8%、輸血感染が 2%、不明が約 60%であった。

使用上の注意記載状況・その他参考事項等

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ハプトグロビンを濃縮・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濾過膜処理(ナノフィルトレーション)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。

報告企業の意見	今後の対応
日本におけるE型肝炎ウイルス感染の統計学的･疫学的･ウイルス学的特徴を求めた調査の解析結果報告である。 万一原料血漿にHEVが混入したとしても、EMC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、本剤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。

別紙様式第2-1

No. 9

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	機構処理欄
			2006. 10. 23	該当なし	

一般的名称	人赤血球濃厚液	研究報告の公表状況	Hladik W, Dollard SC, Mermin J, Fowlkes AL, Downing R, Amin MM, Banage F, Nzaro E, Kataaha P, Dondero TJ, Pellett PE, Lackritz EM. N Engl J Med. 2006 Sep 28;355(13):1331-8.	公表国
販売名(企業名)	赤血球M・A・P「日赤」(日本赤十字社) 照射赤血球M・A・P「日赤」(日本赤十字社) 赤血球濃厚液-LR「日赤」(日本赤十字社) 照射赤血球濃厚液-LR「日赤」(日本赤十字社)			ウガンダ

研究報告の概要

○ヒトヘルペスウイルス8型の輸血伝播

背景:ヒトヘルペスウイルス8型(HHV-8)が輸血によって伝播するかどうかについては明らかにされていない。HHV-8の流行地域であるウガンダにおいてHHV-8の輸血伝播のリスクを検討した。

方法: ウガンダのKampalaで、2000年12月～2001年10月に輸血を受けた患者を登録した。HHV-8抗体について輸血前の血液検体と複数の輸血後検体(6ヶ月の期間中で最高9回行われた来院調査時に採取)を検査した。HHV-8血清反応陰性血液の受血者と比較した、HHV-8血清反応陽性血液受血者におけるセロコンバージョンの超過リスクを経時的に算出した。

結果:登録した受血者1811名のうち、輸血前のHHV-8血清反応が陰性で、必要な追跡調査を完了した患者は991名であった。991名のうち、43%はHHV-8血清反応陽性の血液の輸血を、57%はHHV-8血清反応陰性の血液の輸血を受けた。HHV-8セロコンバージョンは991名中41名に発現した。HHV-8血清反応陽性血液受血者の方が、HHV-8血清反応陰性血液受血者よりも、セロコンバージョンのリスクが有意に高く(超過リスク2.8%; P<0.05)、また、リスクの増加は、主に輸血3～10週後にセロコンバージョンを起こした患者に認められ(超過リスク2.7%; P=0.005)、このウイルスの輸血による感染に合致する結果であった。保存期間が4日を越える血液製剤と比較して、保存期間が4日以内の血液製剤では、セロコンバージョンの頻度が高かった(超過リスク4.2%; P<0.05)。

結論:本試験は、HHV-8が輸血によって伝播する強力なエビデンスを示す。伝染リスクは、血液の保存期間が長くなるほど低下する可能性がある。

報告企業の意見	今後の対応
ヒトヘルペスウイルス8型が輸血によって伝播する疫学的証拠が示されたとの報告である。	今後も引き続き情報の収集に努める。

使用上の注意記載状況・その他参考事項等

赤血球M・A・P「日赤」
照射赤血球M・A・P「日赤」
赤血球濃厚液-LR「日赤」
照射赤血球濃厚液-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

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ORIGINAL ARTICLE

Transmission of Human Herpesvirus 8 by Blood Transfusion

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ABSTRACT

BACKGROUND

Whether human herpesvirus 8 (HHV-8) is transmissible by blood transfusion remains undetermined. We evaluated the risk of HHV-8 transmission by blood transfusion in Uganda, where HHV-8 is endemic.

METHODS

We enrolled patients in Kampala, Uganda, who had received blood transfusions between December 2000 and October 2001. Pretransfusion and multiple post-transfusion blood specimens from up to nine visits over a 6-month period were tested for HHV-8 antibody. We calculated the excess risk of seroconversion over time among recipients of HHV-8–seropositive blood as compared with recipients of seronegative blood.

RESULTS

Of the 1811 transfusion recipients enrolled, 991 were HHV-8–seronegative before transfusion and completed the requisite follow-up, 43% of whom received HHV-8–seropositive blood and 57% of whom received seronegative blood. HHV-8 seroconversion occurred in 41 of the 991 recipients. The risk of seroconversion was significantly higher among recipients of HHV-8–seropositive blood than among recipients of seronegative blood (excess risk, 2.8%; $P<0.05$), and the increase in risk was seen mainly among patients in whom seroconversion occurred 3 to 10 weeks after transfusion (excess risk, 2.7%; $P=0.005$), a result consistent with the transmission of the virus by transfusion. Blood units stored for up to 4 days were more often associated with seroconversion than those stored for more than 4 days (excess risk, 4.2%; $P<0.05$).

CONCLUSIONS

This study provides strong evidence that HHV-8 is transmitted by blood transfusion. The risk may be diminished as the period of blood storage increases.

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KAPOSI'S SARCOMA IS THE MOST COMmon cancer associated with the acquired immunodeficiency syndrome (AIDS) worldwide, and human herpesvirus 8 (HHV-8), also known as Kaposi's sarcoma–associated herpesvirus, was identified a decade ago as the causative agent of Kaposi's sarcoma.[1] The burden of Kaposi's sarcoma in Africa is high; in Uganda, Kaposi's sarcoma accounts for half of all reported cancers.[2] In industrialized countries, the seroprevalence of HHV-8 is relatively low (2 to 8%),[3] whereas in sub-Saharan Africa, the seroprevalence of HHV-8 can exceed 50%. The modes of transmission of HHV-8 in Africa remain poorly understood. Studies indicate that the seroprevalence increases throughout childhood and reaches a plateau by adolescence, suggesting that transmission occurs mainly in the community, probably through saliva or other nonsexual routes.[3]

Whether HHV-8 is transmitted by blood transfusion remains controversial.[4] Transmissibility of the virus by this route may be limited by the cell-associated nature of the virus and the low frequency of circulating virus in asymptomatic seropositive persons.[5] Previous studies that did not find evidence of transfusion-transmitted infection enrolled small numbers of patients, most of whom received leukocyte-reduced or acellular blood components.[6-8]

The potential for blood-borne transmission of HHV-8 has been supported by the results of a number of studies. The transmission of HHV-8 has been associated with the use of injection drugs[9,10] and transplantation of infected organs.[11,12] HHV-8 infection has been seen among U.S. patients undergoing cardiac surgery who received multiple units of non–leukocyte-reduced blood.[13] Several case reports of Kaposi's sarcoma have described an association with blood transfusions.[14-17] Infectious HHV-8 has been recovered from a U.S. blood donor,[18] and viral DNA has been detected in blood donors in Africa.[19] The seroprevalence of HHV-8 has increased with increasing numbers of blood transfusions among patients with sickle cell anemia in Uganda.[20]

To evaluate the risk of the transmission of HHV-8 by blood transfusion, we conducted a prospective observational cohort study of transfusion recipients in Uganda, where the seroprevalence among blood donors was 40%,[21] leukocyte reduction was not used, and blood storage time was usually short. If transmission of HHV-8 by transfusion occurs, it is likely to be detected in such a setting.

METHODS

BLOOD DONATIONS

All volunteers who donated blood to the national blood-transfusion service in central Uganda between November 2000 and September 2001 were invited to participate in the study. A sample of blood from each consenting donor was stored for HHV-8 serologic testing. The samples were screened at the Nakasero Blood Bank in Kampala, Uganda, for human immunodeficiency virus (HIV), hepatitis B surface antigen, and *Treponema pallidum* and stored at 4° to 8°C according to routine procedures. Blood units were transfused as whole blood or separated into packed red cells and plasma. Some units were split into pediatric blood packs for use in young children. Leukocyte-reduction filters were not used; the buffy coat was partially removed in packed-cell units.

TRANSFUSION RECIPIENTS

Enrollment and follow-up of transfusion recipients took place between December 2000 and October 2001 at Mulago Hospital, Kampala. Transfusion recipients were eligible for enrollment if their pretransfusion specimen (left over from blood typing and cross-matching) was available and their transfusion could be linked to an identified blood unit. Patients who had received transfusions within the previous 6 months were not eligible. Follow-up visits were scheduled 1, 2, and 4 weeks after transfusion and monthly thereafter for up to 6 months; unscheduled visits also occurred. At enrollment and at each follow-up visit, blood was drawn, demographic data were recorded, and information was obtained about any repeated transfusions.

Transfusion recipients were included in the analysis if their pretransfusion specimen was seronegative for HHV-8 and they completed at least 2 months of follow-up. Patients who received more than one transfusion during the first 7 days of enrollment remained in the analysis, and their transfusion date was considered to be the midpoint between the first and last transfusions. The follow-up period for analysis began on day 10 after transfusion to exclude the earliest seroconversions, which were probably the result of community-acquired infections. Follow-up ended at the