HBV NAT in Japan, and other countries reduce the risk of transmission by using assays with increased sensitivity for the detection of HBV surface antigen (HBsAg) [2–8]. These approaches have reduced the window period in the early stage of infection. The problem of occult HBV infection, recently defined as individuals who are HBsAg-negative and HBV NAT-positive regardless of the presence or absence of antibody to hepatitis B core antigen (anti-HBc) and antibody to hepatitis B surface antigen (anti-HBs), however, remains to be solved. Anti-HBc screening of blood donations has reduced the risk of occult HBV infection [9–13]. However, in HBV endemic areas such as Asia, anti-HBc screening is not generally utilized, because the rate of positivity is so high that many blood products would be discarded. One possible solution to this problem is to modify the cut-off value of the anti-HBc test and also to take into account the titre of anti-HBs. Using this approach, the Japanese Red Cross (JRC) has succeeded in reducing the frequency of post-transfusion HBV infections, particularly post-transfusion fulminant HBV infection [14,15]. However, the problem of occult HBV infection has not been completely removed and each year a number of cases of transfusion-associated HBV continue to be reported [16,17]. In an attempt to address this, the cut-off value of anti-HBc has been decreased and the sensitivity of HBV NAT testing increased by reducing the pool size from 50 to 20 and also increasing the input volume for the NAT assay from 0·2 ml to 0·85 ml [15]. However, there are limitations for the strategy from the view point of cost-effectiveness.

We have developed a new method of concentrating HBsAg and HBV, which could improve the detection of occult HBV infection. The principle of virus concentration is to induce the agglutination of viruses and poly-L-lysine in the presence of a bivalent metal. Poly-L-lysine-coated magnetic beads are used to shorten each step in the concentration procedure.

Materials and methods

Samples

Hepatitis B virus surface antigen-positive and/or anti-HBc-positive donations that did not meet standard JRC requirements were collected with the cooperation of blood centres in the eastern part of Japan from March 2003 to June 2006. None of these donations were used for transfusion purposes. Two hundred and fifty-nine donations were available. These were subdivided into 2·5-ml tubes and stored at –20°C. The remaining plasma from the donation was also stored at –20°C. Of the 259 donations, 182 were HBsAg-positive by enzyme immunoassay (EIA) (AxSYM®; Abbott Laboratories, North Chicago, IL, USA) and 77 were anti-HBc-positive ($\geq 2^5$ by haemagglutination inhibition assay (HI), JRC in-house], HBsAg-negative (EIA; AxSYM®) and anti-HBs-negative [$< 2^4$ (less than 200 mIU/ml)] by passive haemagglutination assay (JRC in-house). An anti-HBc titre $\geq 2^5$ by HI is equal to $\geq 2^7$–2^8-fold diluted sample that is positive ($\geq$ 50% inhibition) by anti-HBc EIA (AxSYM®).

The 77 anti-HBc-positive donations were used to study the efficacy of the HBV DNA and HBsAg concentration techniques.

Preparation of poly-L-lysine-coated magnetic beads

COOH magnetic beads (125 mg/2·5 ml) (IMMUTEX-MAG™; Japanese Synthetic Rubber, Tokyo, Japan) were added to 0·1 M 2-morphorinoethansulphate (MES) (Wako Pure Chemical, Tokyo, Japan) solution (final volume, 5·0 ml; pH 5·0) and were incubated for 10 min. Activated magnetic beads (25 mg/ml) were suspended in a coupling buffer [5 ml of 100 mM MES (pH 5·0), 50 µl of 100 mg/ml poly-L-lysine (Wako) and 1·2 ml of distilled water] and mixed by continuous inversion at room temperature for 15 min. Then 1·25 ml of 1-ethyl-3-(3-dymethyl-aminopropyl)-carbodiimido (Wako) solution was added to the mixture and mixed by continuous inversion at 10°C for 20 h. Then the solution was replaced with 1 M ethanolamine (Wako) to block reactions at 4°C overnight. Poly-L-lysine-coated magnetic beads were washed five times with phosphate-buffered saline (PBS) and stored at 4°C at a concentration of 50 mg/ml.

It takes 3 days to prepare the poly-L-lysine-coated magnetic beads. Initially, the poly-L-lysine-coated magnetic beads were manufactured in house as described above. Subsequently they have been purchased from JSR.

Concentration of HBsAg and HBV DNA

Poly-L-lysine-coated magnetic beads were added to 2 ml of plasma at a final concentration of 1 mg/ml. Then, 30 µl of 1·1 M $Zn(COOH)_2$ was added to the sample. The resulting mixture was mixed and left to stand for 5 min. The agglutinated HBsAg/HBV DNA and magnetic beads were trapped in a magnetic field (MagicalTrapper®, Toyobo, Tokyo, Japan) and washed twice with PBS to remove impurities. The concentrated HBsAg was eluted with 0·25 ml of 0·4 M ethylenediaminetetraacetic acid (EDTA) solution. The whole volume of the sample was eluted for EIA testing (AxSYM®, Abbott) (effective eightfold concentration). HBV DNA was eluted with 100 µl of 0·4 M EDTA solution and 50 µl or 100 µl was used for individual NAT (10- or 20-fold concentration, respectively). The concentration and elution process takes 30 min.

HBV DNA extraction and quantification

Hepatitis B virus DNA was extracted using an Ex-R&D kit® (Sumitomo Chemical, Tokyo, Japan). HBV DNA was detected quantitatively as described previously [3]. Briefly, to quantify

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the HBV DNA, nucleic acid extracts were amplified and titrated by using a sequence-detection system (TaqMan, ABI Prism 7700 Sequence Detector; PE Applied Biosystems, Foster, CA, USA). Quantification of the HBV DNA was calculated from the working curve (10^7, 10^6, 10^5, 10^4, 10^3 and 10^2 copies/ml) produced by domestic standard samples that were prepared based on the international standard (NIBSC: National Institute for Biological Standards and Control). Calculation was carried out using Sequence Detector version 1·7 (PE Applied Biosystems). The qualitative detection limit was assumed to be 60 copies/ml (95% confidence interval) and quantitative detection limit was assumed to be 100 copies/ml (95% confidence interval).

The AxSYM® HBsAg assay was used for detection of HBsAg. Tests were carried out in accordance with the manufacture's instructions. A positive result is defined as a signal/noise (s/n) ratio ≥ 2. Samples with different concentrations of HBsAg were used to assess the effectiveness of HBsAg concentration. High-titre HBsAg samples (AxSYM®; s/n ratio 266) were sequentially diluted 10-fold up to a final dilution of 10 000-fold using normal plasma. Lower low-titre HBsAg samples (AxSYM®; s/n ratio 12) were diluted up to a final dilution of 1000-fold. Samples known to have HBsAg below the level of detection in the AxSYM assay (s/n ratio 1·7) were diluted to a final dilution of 100-fold. The respective diluted samples were then concentrated eightfold as described above.

The parallel translation of linear line of dilution curves caused by HBsAg dilution and concentration was studied, plotting the s/n ratio of the EIA on the vertical axis to the dilution fold of the samples on the horizontal axis in both logarithm scales.

The effect of anti-HBs on HBV DNA concentration was studied by adding anti-HBs obtained from immunized horse serum. The titre of purified anti-HBs was 51 200 IU/l. The volumes of anti-HBs added to the samples were 0 µl, 20 µl (1024 mIU/l) and 35 µl (1792 mIU/l).

The effects of other viruses on HBsAg and HBV DNA concentrations were studied in the presence of parvovirus B19 (non-enveloped DNA virus) or HCV (enveloped RNA virus).

Data shown in the tables represent the average of the results of two or three experiments.

Results

Hepatitis B virus was concentrated quantitatively by our new method in a broad range of HBV DNA loads. However, the efficacy of concentration varied from sample to sample. The efficacy of concentration (measured value/expected value: original × concentration times) is shown in Table 1. The efficacy of the concentration process decreased from 0·76 to 0·49 as the HBV DNA load increased from 10^3 to 10^6 copies/ml (Table 1).

Table 1 Effect of the concentration method on concentration of HBV DNA samples

Sample no.	Original (copies/ml)	10-fold concentration (copies/ml)	Efficacy of concentration[a]
1	1·6 E + 06	7·8 E + 06	0·49
2	4·2 E + 05	2·1 E + 06	0·50
3	9·0 E + 04	5·7 E + 05	0·63
4	2·2 E + 04	1·6 E + 05	0·73
5	4·6 E + 03	3·5 E + 04	0·76

[a]Efficacy = 10-fold concentration (copies/ml)/original × 10 (copies/ml).

Table 2 Effect of hepatitis B surface antibody (HBsAb) on concentration of HBV DNA

Original sample	10-fold concentration		
HBV DNA (copies/ml)	HBsAb (mIU)	HBV DNA (copies/ml)	Efficacy of concentration
	0	860	0·72
120	1024	1400	1·17
	1792	1300	1·08

The efficacy of HBsAg concentration is shown in Fig. 1. For the high-titre HBsAg samples (s/n ratio 266·03), 100-fold dilution samples were more than limit for detection (s/n ratio 4·88) and 1000-fold dilution samples were less than the limit for detection (s/n ratio 1·16). Following eightfold concentration of HBsAg, the 1000-fold dilution sample was found positive (s/n ratio 3·24). Similarly, in the low-titre sample the undiluted sample was above the detection limit (s/n ratio 11·91). The 10 times dilution sample (s/n ratio 1·69) was negative but became positive following eightfold concentration (s/ratio 4·36). The negative samples (s/n ratio 1·66) became positive by eightfold concentration (s/n ratio 3·49). Based on the parallel translation of linear line shown in Fig. 1, the relative efficacy of concentration was about 0·64(5·1/8) in high-titre samples and 0·56(4·5/8) in low-titre samples.

The effects of anti-HBs and other viruses on HBsAg/HBV DNA concentration were determined. The effect of anti-HBs on HBV DNA concentration is shown in Table 2. The efficacy of HBV DNA concentration in the presence of anti-HBs was superior to that in the absence of anti-HBs. However, in the presence of anti-HBs (antigen–antibody coexistence samples), anti-HBs prevented the detection of HBsAg.

The effect of the coexistence of HCV or parvovirus B19 on the efficiency of HBsAg/HBV DNA concentration is shown in Table 3. HCV (10^6 copies/ml) and parvovirus B19 (2^{11} by RHA: receptor-mediated haemagglutination assay) had no

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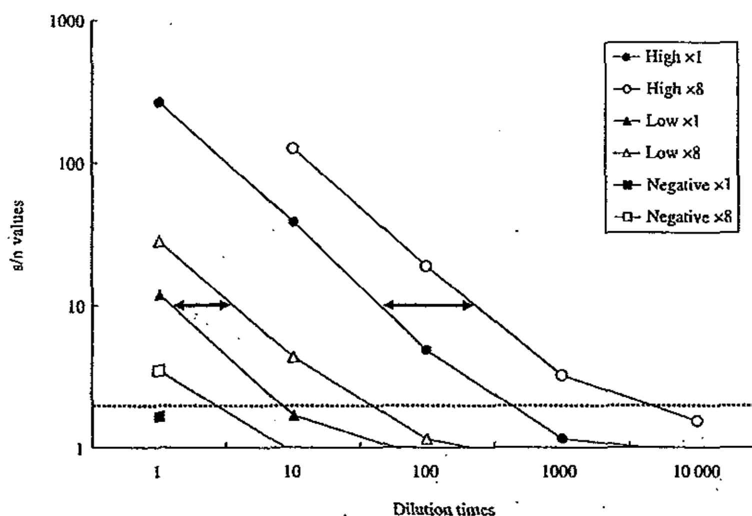

Data for Fig.1

		HBsAg: EIA(AxSYM: s/n[a])				
		dilution with normal plasma				
		1	10	100	1000	10 000
High	×1	266·03	38·81	4·88	1·16	0·91
	×8		126·77	18·95	3·24	1·54
Low	×1	11·91	1·69	0·86	0·77	
	×8	28·28	4·36	1·15	0·76	
Negative	×1	1·66				
	×8	3·49	0·93	0·8		

Fig. 1 Parallel translation of linear line caused by hepatitis B surface antigen (HBsAg) concentration. Vertical axis shows signal/noise (s/n) values of enzyme immunoassay (EIA) indicated by logarithm, and horizontal axis shows dilution fold of samples indicated by logarithm. The linearity was observed more than two (s/n value). Closed circle, high titre of HBsAg (×1: non-concentration); open circle, eightfold concentration of high titre of HBsAg (×8: concentration); closed triangle, low titre of HBsAg (×1:non-concentration); open triangle, eightfold concentration of low titre of HBsAg (×8: concentration); closed square, negative (s/n: < 2) titre of HBsAg (×1: non-concentration); open square, eightfold concentration of negative titre of HBsAg (×8: concentration). The dotted line shows two s/n values (cut-off values). Arrows show the distance of parallel translation by HBsAg concentration.

Table 3 Effect of coexistence of HCV or parvovirus B19 on efficiency of hepatitis B surface antigen (HBsAg) concentration

	AxSYM (s/n[b])	
Plasma for dilution	HBsAg dilution with various kinds of plasma[a]	10-fold concentration of diluted HBsAg plasma
Normal plasma	1·39	3·80
HCV-positive plasma[c]	1·18	3·47
Parvovirus B19-positive plasma[d]	1·31	3·77

[a]The original HBsAg-positive plasma titre is 6·19: EIA (AxSYM; s/n).
[b]More than 2 means positive.
[c]The titre of anti-HCV was > 2^{12} and the load of HCV RNA was 10^6 copies/ml.
[d]The titre of B19 antigen was 2^{11} by receptor–mediated-haemagglutination assay.

effects on the concentration of HBsAg/HBV DNA. Although the parvovirus B19 could not be concentrated by this method because of its lack of envelope, HCV RNA could be concentrated quantitatively (data not shown).

Seventy-seven anti-HBc positive (≥ 2^5 by HI assay by JRC criteria) and HBsAg-negative (EIA, AxSYM®) donations were selected to study the efficacy of HBsAg and HBV DNA concentrations. Of the 77 samples, 35 were positive by individual NAT and a further five became NAT positive following concentration (Table 4). Of 35 samples (Table 4; lanes d, e), 16 (Table 4; lane e) had HBV DNA loads of 120–1500 copies/ml and the other 19 samples (Table 4; lane d) had HBV DNA loads less than the quantitative detection limit (< 100 copies/ml). However, the HBV DNA loads of all these samples exceeded 100 copies/ml following concentration (Table 4; lanes d, e). Five samples (Table 4; lanes b, c) that were negative by individual NAT became positive (less than 100–510 copies/ml) following concentration.

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Table 4 Detection of occult HBV by concentration of HBV DNA and hepatitis B surface antigen (HBsAg)

				HBV DNA (copies/ml)				
				a	b	c	d	e
			Original	Negative	Negative	Negative	< 100	≥ 100
			Concentration (×20)	Negative	< 100	≥ 100	≥ 100	NT
HBsAg (AxSYM)	I	Original Concentration (×8)	Negative Negative	34	0	0	8	5
	II	Original Concentration (×8)	Negative Positive	3	1	4	11	11

NT, not tested.

Of the 40 samples (Table 4; lanes b–e) that were HBV DNA-positive either before or after concentration, 13 were HBsAg-negative even following HBsAg concentration. Of these 13 samples, 5 (Table 4; lane I-e) had HBV DNA loads exceeding 100 copies/ml by conventional individual NAT, and eight (Table 4; lane I-d) were quantitatively less than 100 copies/ml on the non-concentrated sample but became NAT positive (≥ 100 copies/ml) following concentration. Of the 77 samples, 30 (Table 4; lane II) had detectable HBsAg following HBsAg concentration. Of these 30 samples, 27 were NAT positive but three (lane II-a) remained NAT-negative even after concentration. Thirty-four of the 77 samples (Table 4; lane I-a) remained negative for both HBsAg and HBV DNA following concentration for both markers.

Discussion

We have previously reported that HBV DNA could be detected in the HBsAg-negative phases of HBV infection (early window period and occult HBV infections) [2–4,18]. However, the use of HBV NAT remains limited, because the HBV viral loads seen in HBsAg-negative infected donors (occult HBV infection) are generally low [19–22]. Although the infectivity of occult HBV is low compared to that in the window phases of early infection [17], we have encountered post-transfusion HBV infection caused by both HBsAg and mini-pool NAT-negative, but individual NAT-positive donations [16].

It has previously been reported that NAT sensitivity can be increased by reducing the number of donations in the mini-pool [23], increasing the input volume of serum, and by addition of an ultracentrifugation step [24]. From the viewpoint of cost-effectiveness, an inexpensive and easy method to increase sensitivity is desirable. We have previously reported a virus concentration method using polyethyleneimine [25]. However, HBV DNA and HBsAg were not concentrated qualitatively by the method, because the combination of extracted nucleic acids of viruses and magnetic beads is difficult to dissociate in the presence of protein-degenerative reagents. We have solved this problem with the use of poly-L-lysine that coagulates with viruses in the presence of bivalent metal ions (zinc acetate).

Owing to the low concentrations of HBV DNA present in early acute infection when both mini-pool NAT and HBsAg are non-reactive, individual NAT would be the best option giving a much higher yield, an increased window period closure, and consequently greater benefit. It is also much debated whether the most sensitive HBsAg detection method is superior to mini-pool NAT, but inferior to individual NAT [21,23]. If 20-pool NAT samples are concentrated 20 times, the sensitivity of 20-pool NAT might be equal to that of individual NAT.

It is important to determine whether HBV could be concentrated in the presence of anti-HBs. In this study, HBV was much more efficiently concentrated in the presence of anti-HBs than without (Table 2). The results showing that the efficacy of concentration was more than 1·0 might be a result of the easy coagulation of antigen antibody-reacted materials with poly-L-lysine beads. However, in the case of HBsAg concentration, it is difficult to measure the efficacy of HBsAg concentration in the presence of anti-HBs, because anti-HBs inhibits the detection of HBsAg by EIA. The coexistence of other viruses would not affect the concentration of HBsAg/HBV DNA, as shown in Table 3. Moreover, the procedure is useful for concentrating coinfected enveloped viruses as HCV, although it will be difficult to concentrate non-enveloped viruses as parvovirus B19. HCV that is difficult to concentrate by ultracentrifugation because of its low density is easily concentrated quantitatively by our method.

We succeeded in concentrating HBsAg from occult HBV infection. The theoretical plasma HBsAg concentration was eightfold (2 ml of plasma/0·25 ml of elution); however, from the parallel translation of the linear line (vertical axis – s/n

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and horizontal axis – dilution folds of samples), the relative efficacies of concentration were 0·56–0·64. The reason for the low efficacy of HBsAg concentration compared to the efficacy of HBV DNA concentration (0·49–0·76) might be due to HBsAg (22 nm) being smaller than HBV (45 nm) and thus the efficacy of agglutination with poly-L-lysine being different.

In countries where NAT is not available or feasible, the use of a highly sensitive HBsAg assay is crucial in ensuring blood safety. Although individual NAT is the golden standard, at later stages of infection, low concentrations of infectious viruses, which may not be detectable by NAT, might be found in some HBsAg-positive blood donations [19,20]. HBsAg tests with high sensitivity are predicted to have a comparable yield to mini-pool NAT [21]. If the sensitivity of HBsAg detection would be increased by several times, NAT might not always be necessary in late-stage HBV infection. In our study, five samples with low-level HBsAg, detectable only after concentration, were not detected by conventional individual NAT (Table 4; lanes b, c). Twenty-seven of the 40 cases in which HBV DNA was detected were shown to have HBsAg after concentration. The remaining 13 cases (Table 4; lane I-d, e) could not be detected by HBsAg concentration, demonstrating the limitation of our method.

Although HBsAg-negative subjects may retain a low infectivity and have a low risk for progressive liver damage [17], HBV DNA testing or an HBsAg detection method with the highest sensitivity should be implemented to decrease the risk of post-transfusion HBV infection [26;27]. Our new HBV/HBsAg concentration method could contribute to increasing the sensitivity of HBV DNA/HBsAg detection. The concentration method could be combined with either Chemoluminescent Immunoassay (CLIA; PRISM, Abbott) or individual donation NAT to further increase the overall sensitivity of HBV detection. Alternatively, if a high-sensitivity method such as the CLIA was combined with our method, then it might be possible to undertake screening using pooled samples. Our concentration method would potentially be capable of replacing individual NAT by mini-pool NAT, although the present efficacy of concentration is not 1·0 but about 0·7 (Table 1).

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研究報告の概要

○20プールNAT導入後、初めて確認された輸血によるHCV感染の一例
症例は新規に最重症再生不良性貧血と診断された54歳の女性で、2007年6月20日に初回輸血が実施され、初回輸血前感染症検査はHCV抗体陰性、HCVコア蛋白陰性であった。10月1日の輸血後感染症検査でHCVコア蛋白の陽性化【28,183.1 fmol/L (＜20.0)】が明らかとなったため、血液センターに連絡し遡及調査を開始した。初回輸血前感染症検査残余の保存血清でHCV-RNAが陰性であることを確認した(PCR)。患者には6月20日から10月1日の間に合計54本の赤血球濃厚液または濃厚血小板輸血があり、保管54検体についてHCV個別NAT(核酸増幅法)を施行したところ、2007年8月17日輸血の赤血球濃厚液からHCV-RNAを検出した。患者と献血者のHCV Core-E1-E2領域(1,279bp)の塩基配列をdirect sequence法で決定し、比較した結果両者は一致した。この結果、本症例は輸血によるHCV感染である可能性が極めて高いと結論した。
日本では1999年7月から献血血液の感染症検査に500プールNATを導入し、2000年には50プール、2004年には20プールとしてきた。世界で最も先進的かつ高感度システムといえる。20プールNAT陰性献血血液由来の血液製剤からのHCV感染の報告は本報告が初であり、NAT陰性献血血液からでもHCV感染が成立しうることが示された。
また、患者はHCV混入血の輸血から肺炎で死亡されるまでの約7ヵ月間、HCV抗体価が陽性になることはなく、10月24日以降HCVコア蛋白値は一貫して施設測定可能上限50,000.0以上であった。免疫抑制状態の患者に対するHCV感染については、輸血前後のスクリーニング検査としてHCVコア蛋白が必要である。

使用上の注意記載状況・その他参考事項等

人全血液-LR「日赤」
照射人全血液-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

自発報告:
2007年10月19日付1-07000104

報告企業の意見	今後の対応
日本において、プールNAT導入後3例の輸血によるHCV感染症例があるが、本症例は20プールNAT導入後初めて確認された輸血によるHCV感染の報告である。	日本赤十字社では、HCV抗体検査を実施することに加えて、HCVについて20プールでスクリーニングNATを行い、陽性血液を排除している。また、これまでの凝集法と比べて、より感度の高い化学発光酵素免疫測定法(CLEIA)及び精度を向上させた新NATシステムを導入した。HCV感染に関する新たな知見等について今後も情報の収集に努める。

OS-1-40 血液疾患患者における末梢血細菌・真菌 PCR 検査の有用性の検討

PCR analysis of blood for diagnosis of bacterial and fungal infection in hematological patients

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桝屋 正浩[1]、中瀬 一則[4]、松島 佳子[2]、和田 英夫[2]、登 勉[2]、片山 直之[1]（三重大学 血液腫瘍内科[1]、
三重大学医学部附属病院 中央検査部[2]、三重大学医学部附属病院 輸血部[3]、三重大学 がんセンター[4]）

【目的】血液疾患の感染症治療における末梢血の細菌・真菌 PCR 検査の有効性につき前向きに検討した。【方法】2007 年 4 月より当院で化学療法あるいは造血幹細胞移植を受けた白血病患者のうち、同意が得られた延べ 8 人に対して、定期的(1 週間毎)にまたは発熱時に末梢血の細菌・真菌 PCR 検査と血液培養を施行した。PCR 結果は原則的に非開示とした。【結果】全例経過中に発熱がみられた。PCR 検査は延べ 14 回陽性(細菌 13 回、真菌 1 回)、血液培養は延べ 6 回陽性で(すべて細菌)、そのうち 3 回で両方陽性となった。なお、連続陽性は 1 回とカウントした。培養でのみ陽性となった 3 回すべてで検出されたのは皮膚常在菌であり臨床的にも contamination と考えられた。培養と PCR の両方で細菌が検出された 3 回のうち、1 回は同時期の血液で、2 回は培養陽性となる 2、9 日前の血液ですでに PCR 陽性であった。細菌 PCR のみ陽性であった 10 回のうち 8 回は臨床経過から感染の原因菌と考えられたが、経験的抗生剤治療により多くは解熱が得られていた。しかし、Stenotrophomonas maltophilia が同定された 1 回では全身状態が増悪したため結果を開示し、抗生剤の変更により改善がみられた。真菌 PCR のみ陽性の 1 回では、臨床的に侵襲性肺アスペルギルス症と診断される 20 日前から Aspergillus fumigatus が検出されていた。【結論】細菌感染の多くは、血液培養の結果あるいは経験的抗生剤投与により治療可能であった。しかし、血液培養が陽性となる前から PCR 陽性となっていたケースや、血液培養では検出されず PCR でのみ陽性のケースもみられ、細菌 PCR の結果を参考に、より早期から確実に原因菌を想定した抗生剤治療が開始できていた可能性がある。また、真菌感染症においても、血液 PCR の結果が臨床経過の改善に有用な症例があることが示唆された。今後さらに多くの症例で、細菌・真菌 PCR 検査の臨床的有効性を前向きに検討することが必要であると考えられた。

OS-1-41 Levofloxacin と Polymyxin B を消化管殺菌として好中球減少期に投与された血液悪性疾患 119 例での感染症合併

Infections in neutropenic patients who received prophylactic Levofloxacin or Polymyxin B

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小池 隆夫[2]（北海道大学病院造血細胞治療センター[1]、北海道大学病院 第 2 内科[2]、北海道大学病院 高度先進医療支援センター[3]）

【背景】Giampaolo らは血液悪性疾患を含む担癌患者への化学療法において、プラセボと比較して Levofloxacin（LVFX）が細菌感染予防に有用である、と報告した。(NEJM, 2006) このような報告を受け、血液悪性疾患治療における好中球減少期の消化管殺菌として、非吸収性の Polymyxin B (PMB) に代わり、LVFX が用いられることが多くなったが、この二剤の感染予防効果の差については不明な点が多い。当科では消化管殺菌として、1999 年 4 月から 2005 年 6 月までは PMB を、その後現在までは LVFX を使用してきた。この二剤投与下での感染症などについて比較検討した。【患者と方法】対象は当科で血液悪性疾患に対する治療を受けた 119 例で、PMB 群 66 例、LVFX 群 53 例。年齢・性別に差はなく、疾患は PMB 群が NHL46 例、MM13 例、HL3 例、その他 4 例、LVFX 群が AML15 例、ALL12 例、NHL12 例、MDS5 例、MM3 例、その他 6 例。治療は PMB 群が自家移植 64 例、同種移植 2 例、LVFX 群が化学療法 21 例、自家移植 11 例、同種移植 21 例。移植前疾患状態は PMB 群が CR または PR 61 例、その他 5 例、LVFX 群が CR または PR 35 例、その他 18 例。好中球減少期に 38 度以上の発熱が生じた際には各種培養を行うと共に、発熱性好中球減少症のガイドラインに基づいて点滴抗生剤や抗真菌剤の投与を行った。【結果】好中球 1000/μl 以下の期間は PMB 群 11 ± 4 日、LVFX 群 18 ± 12 日と有意に LVFX 群で長かった。血液培養陽性は PMB 群 7 例（グラム陽性菌 3 例、陰性菌 4 例）、LVFX 群 5 例（グラム陽性菌 4 例、陰性菌 1 例）、感染により PMB 群でのみ 2 例が死亡した。38 度以上の発熱期間、点滴抗生剤の使用、最大 CRP 値などには二群間で差を認めなかった。【考察】今回の検討は患者背景も異なり、直接の比較ではないが、LVFX 群で感染に不利と思われる因子が多いにも関わらず、検討したパラメータでは少なくとも同等ないしは勝っており、LVFX の血液悪性疾患における消化管殺菌としての有用性が示唆された。

OS-1-42 20 プール NAT 導入後、初めて確認された輸血による HCV 感染の一例

The first case of transfusion-transmitted HCV infection slipping through the 20-member-pool NAT

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田中 靖人[5]、溝上 雅史[5]、飯田 真介[1]、上田 龍三[1]（名古屋市立大学 腫瘍免疫内科学[1]、名古屋市立大学 輸血部[2]、
愛知県赤十字血液センター[3]、日本赤十字社中央血液研究所[4]、名古屋市立大学 臨床分子情報医学[5]）

症例は新規に最重症再生不良性貧血と診断された 54 歳女性。初回輸血前感染症検査で HCV 抗体陰性、HCV コア蛋白陰性。6 月 20 日初回輸血。2007 年 10 月 1 日の輸血後感染症検査で HCV コア蛋白の陽性化【28183.1 fmol/L（＜ 20.0)】が明らかとなった。直ちに血液センターに報告し遡及調査を開始。はじめに患者の初回輸血前感染症検査残余の保存血清で HCV-RNA が陰性であることを確認した (PCR)。初回輸血から 10 月 1 日の間に合計 54 本の RCC または PC 輸血があった。それら対象の保管 54 検体についてそれぞれ HCV 個別 NAT(核酸増幅法) を施行、うち 1 検体 (2007 年 8 月 17 日輸血 RCC) から HCV-RNA を 検出した。患者 HCV と献血者の HCV Core-E1-E2 領域 (1279bp) の塩基配列を direct sequence 法で決定し、比較した結果両者は一致した。この結果、本症例は輸血による HCV 感染である可能性が極めて高いと結論した。日本では 1999 年 7 月から献血血液の感染症検査に 500 プール NAT を導入し、2000 年には 50 プールに、2004 年からは 20 プール NAT とし、そのスクリーニング感度を上げてきた。世界で最も先進的かつ高感度システムといえる。20 プール NAT 陰性献血血液由来の血液製剤からの HCV 感染の報告は本報告が初である。本発表の第 1 のメッセージは【NAT 陰性献血血液由来の血液製剤からでも HCV 感染が成立しうる】ことである。また、本症例は 2007 年 10 月 17 日に同種骨髄移植を施行し、2008 年 3 月 30 日に肺炎のため死亡された。HCV 混入血の輸血から約 7 ヶ月の全経過で HCV 抗体価が陽性になることはなく、10 月 24 日からは HCV コア蛋白値は一貫して施設測定可能上限 50000.0 以上であった。すなわち、免疫抑制状態の患者に対する HCV 感染については HCV 抗体検査のみでは不十分であることを意味する。これらの事実から、第 2 のメッセージは【輸血前後のスクリーニング検査として HCV コア蛋白が必要である】ことである。本症例をふまえ、発表当日は【血液製剤の安全性】について議論したい。

別紙様式第2-1

No. 37

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
				2008. 9. 18	該当なし	

一般的名称	解凍人赤血球濃厚液	研究報告の公表状況	Aaron S, McMahon JM, Milano D, Torres L, Clatts M, Tortu S, Mildvan D, Simm M. Clin Infect Dis. 2008 Oct 1;47(7):931-4.	公表国
販売名(企業名)	解凍赤血球濃厚液「日赤」(日本赤十字社) 照射解凍赤血球濃厚液「日赤」(日本赤十字社) 解凍赤血球-LR「日赤」(日本赤十字社) 照射解凍赤血球-LR「日赤」(日本赤十字社)			米国

研究報告の概要

○C型肝炎ウイルスの鼻腔内伝播:ウイルス学的および臨床的エビデンス

汚染した薬物吸引器具によるC型肝炎ウイルス(HCV)の鼻腔内伝播の可能性が考えられてはいるが、ウイルス感染源として確定されていない。ニューヨーク市のコミュニティ・クリニックから18歳以上で血液中のHCV PCR陽性の吸引用麻薬常用者38名をリクルーティングした。鼻汁検体を採取したほか、被験者が通常薬物を使用する時のようにストローを使用し、このストローを回収して、血液及びHCV RNAの存在を調べた。鼻汁検体28(74%)、ストロー3(8%)で血液が検出された。HCV RNAは鼻汁検体5(13%)、ストロー2(5%)で検出された。被験者のうち11名では、鼻中隔穿孔など慢性的薬物吸引と関連する鼻の異常が見られた。鼻汁検体と薬物吸引器具に血液とHCV RNAが存在することから、HCV鼻腔内伝播のウイルス学的妥当性が示された。

使用上の注意記載状況・その他参考事項等

解凍赤血球濃厚液「日赤」
照射解凍赤血球濃厚液「日赤」
解凍赤血球-LR「日赤」
照射解凍赤血球-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

報告企業の意見	今後の対応
汚染した薬物吸引器具によるC型肝炎ウイルスの鼻腔内伝播のウイルス学的妥当性を示したとの報告である。	HCV感染の新たな伝播ルート等について、今後も情報の収集に努める。