Table 1 Laboratory codes and assay protocols used by participants

Laboratory code	Method	Type
1	Roche COBAS TaqMan HBV test with use of HPS viral nucleic acid kit	Quantitative
2A	Artus HBV LC PCR kit	Quantitative
2B	In-house real-time PCR	Quantitative
3A	Roche COBAS AmpliScreen HBV test	Qualitative
3B	In-house real-time PCR	Qualitative
3C	In-house real-time PCR	Qualitative
4	In-house real-time PCR	Quantitative
5	In-house PCR	Qualitative
6	Roche COBAS AmpliScreen HBV test	Qualitative

PCR, polymerase chain reaction; HBV, hepatitis B virus.
In-house assay details for the following laboratories; 2B, the assay was based on a previously published amplification method [6] targeting the HBs gene with detection using the Roche LightCycler; 3B, qualitative real-time PCR assay amplifying the core region of the HBV genome with detection using the Roche LightCycler; 3C as for 3B, with an initial ultracentrifugation step prior to extraction; 4 real-time PCR amplifying the X region of the HBV genome [7] and detection using the Roche LightCycler; 5, qualitative PCR assay amplifying the HBV core region and detection using capillary electrophoresis.

Table 2 Estimated IU/ml ($\log_{10}$) from quantitative assays

Laboratory number	Sample	
	Sample 1	Sample 2
1	5·99	5·97
2A	6·08	5·99
2B	6·06	5·92
4	5·94	5·86
Mean[a]	6·00	5·93

[a]Results combined for laboratory 2 to give a single mean prior to calculating overall mean of laboratories.

Table 3 Estimated polymerase chain reaction (PCR)-detectable units/ml ($\log_{10}$) for qualitative assays

Laboratory number	Sample	
	Sample 1	Sample 2
3A	6·48	6·58
3B	6·90	6·68
3C	6·56	6·35
5	6·49	6·25
6	6·51	6·59

Results

For the analysis of the results, a code number was allocated at random for each laboratory (Table 1), and does not reflect the numbers assigned to laboratories that participated in the original collaborative study to establish the 1st IS (97/746). Where individual laboratories returned data from more than one assay method, or repeat assays by different operators, the results were analysed separately, and referred to as, for example, laboratories 3A and 3B. Each participating laboratory performed four separate assay runs on the two preparations as requested in the study protocol. The types of assays used by participants are recorded in Table 1; these cover a range of in-house (n = 5) and commercially available tests (n = 4). Where they have been disclosed, details of the assay and region of the HBV genome amplified are indicated (Table 1). Three laboratories (1, 2A, 2B, and 4) returned data from quantitative assays, with results expressed in IU/ml. All calculations were based on the estimates of $\log_{10}$ IU/ml, to give overall mean figures for each laboratory. Three laboratories (3A, 3B, 5 and 6) returned data from end-point dilution series, produced using qualitative assays. These were analysed to determine the polymerase chain reaction (PCR)-detectable units/ml for each sample, using the statistical methods described in the publication of the original collaborative study to establish the 1st IS for HBV DNA [1].

The estimated IU/ml ($\log_{10}$) from the quantitative assays and PCR-detectable units/ml ($\log_{10}$) from the qualitative assays are shown in Tables 2 and 3, respectively. For both quantitative and qualitative assays, the results for Samples 1 and 2 are extremely close. For the quantitative assays, combining the results from laboratory 2 to give a single laboratory mean, the overall estimate for the 1st IS, Sample 1, is 6·00 $\log_{10}$ IU/ml, exactly the assigned unitage, and 5·93 $\log_{10}$ IU/ml for Sample 2. If the results of the assays from laboratory 2 are considered separately (2A and 2B), then the overall means are 6·02 and 5·94 $\log_{10}$ IU/ml for Samples 1 and 2, respectively. There is also very close agreement between the results from the individual laboratories. One set of results submitted by laboratory 3C was returned as crossing point (Ct) values; these were not included in the main analysis, as it was not possible to convert them to either IU or PCR-detectable units. However, these results were in line with all other assay methods (i.e. demonstrating equivalence of Samples 1 and 2). Calculating the pairwise difference in $\log_{10}$ estimates between Samples 1 and 2 for each laboratory that provided quantitative data, there was a small, but marginally significant (P = 0·044) difference of 0·08. When the results from laboratory 2 are combined to give a single laboratory mean, the difference between Samples 1 and 2 is similar (0·07), but no longer significant. Laboratory 4 also measured the Eurohep reference sample R1. Samples 1 and

© 2008 The Author(s)
Journal compilation © 2008 Blackwell Publishing Ltd., *Vox Sanguinis* (2008)

Table 4 Estimated IU/ml (log_{10}) for accelerated degradation samples

Storage temperature	Sample	
	Sample 1	Sample 2
−20 °C	6·02	5·92
4 °C	5·92	5·91
20 °C	5·94	6·03

The accelerated thermal degradation samples were stored at 4 °C and 20 °C for a period of 51 and 56 months; these samples were compared to vials of 97/746 that were stored continuously at −20 °C. Four vials of each sample stored at 4 °C and 20 °C were analysed on four separate occasions, each sample extract was tested in triplicate on each occasion. The data were pooled for the two different storage times and mean values shown for the estimated IU/ml (log_{10}).

2 were originally prepared from R1 following a 1 in 500 dilution in human plasma. The titre of R1 was determined to be 8·73 log_{10} IU/ml, which is in very good agreement with the expected titre of 8·70 log_{10} IU/ml. The difference between Samples 1 and 2 was not significant when estimates from all laboratories were included. This was the case whether treating the different assay methods of laboratory 3 as three separate laboratories ($P = 0·099$) or combining their estimates into a single laboratory mean ($P = 0·124$).

Stability studies

A total of four separate assay runs were performed by a single laboratory. The overall mean estimated IU/ml (log_{10}) for the different samples and storage temperatures are shown in Table 4. From analysis of the raw data, no degradation was evident for any of the test samples when compared with baseline samples stored at −20 °C; as a consequence the results were combined for the samples stored for 51 months, and those stored for 56 months. The results summarized in Table 4 clearly demonstrate that no degradation has occurred. Performing a formal significance test, there was no significant difference in estimated IU/ml across the temperatures for either sample. It should be noted that the formal test allowed for any possible differences between the samples stored for 51 months and those stored for 56 months. It is not possible to obtain precise predictions of expected loss per year, because no observed degradation has taken place and, thus, it was not possible to apply the Arrhenius model of accelerated degradation [8,9]. However, if it were assumed that the degradation rate would double with every 10 °C increase in storage temperature, the lack of any detectable degradation at 20 °C for over 4 years would equate to no detectable degradation at −20 °C for 64 years. Real-time stability, of the 1st IS (Sample 1) and Sample 2, as effectively detemined in the present collaborative study, indicates no loss of potency of these two preparations since time of manufacture, as evidenced by the values reported by the participants.

Conclusions

The results of this collaborative study are in good agreement with the results of the original study [1]. Using only the results of the quantitative assays, which are expected to be more precise than the qualitative assays, there was a difference of around 0·07 to 0·08 log_{10} between the estimated IU/ml for the 1st IS and the candidate replacement, Sample 2. If assays from two differing methods used by laboratory 2 are treated as if from separate laboratories, this difference is marginally significant ($P = 0·044$). However, if the results for laboratory 2 are first combined, the difference is no longer significant. Including the results from all participants, using both quantitative and qualitative assays, there is no significant difference between the 1st IS and the candidate replacement, Sample 2. This lack of significant difference is in contrast to a recently completed study to establish the 3rd IS for hepatitis C virus (HCV) RNA [10]. Here two lyophilized preparations, derived from the same bulk, were evaluated by 33 laboratories that calibrated them against the 2nd HCV IS, using a wide range of commercial and in-house quantitative and qualitative assays. The relative potencies of the two new lyophilized HCV RNA preparations were 5·19 and 5·41 log_{10} IU/ml, while the unprocessed bulk material had a relative potency of 5·70 log_{10} IU/ml. These differences in relative potencies between the two lyophilized HCV RNA preparations were statistically significant ($P < 0·0001$), with a clear loss of potency on processing. This is in contrast to the HBV study presented here. From the original collaborative study and data from this new study, there is no significant difference between the potencies of the two HBV DNA Samples 1 and 2, nor was there any detectable loss of titre of the preparations following lyophilization [1].

The results of the accelerated degradation studies have also demonstrated that both Samples 1 and 2 are extremely stable and suitable for long-term use, with no detectable degradation for either preparation after storage at 20 °C for more than 4 years. This stability is in contrast to the 1st and 2nd IS for HCV RNA (96/790 and 96/798, respectively), which showed an average decrease of log_{10} 1·9 for samples stored at 20 °C for more than 5 years [11]. This difference in the observed stability may be due to the nature of the viral nucleic acid, which in the case of HBV is DNA, in contrast to the RNA genome of HCV, which is likely to be more unstable and susceptible to degradation. However, it is possible that further unknown factors influence the stability.

On the basis of this study, Sample 2 (97/750) was established as the 2nd IS for HBV DNA for NAT-based assays by the WHO

© 2008 The Author(s)

ECBS in October 2006. This preparation has a potency of 10^6 IU/ml. Each vial contains the equivalent of 0·5 ml of material, and the content of each vial is 5×10^5 IU per vial. Vials of 97/750 are available from NIBSC.

References

1 Saldanha J, Gerlich W, Lelie N, Dawson P, Heermann K, Heath A, the WHO Collaborative Study Group: an international collaborative study to establish a World Health Organization international standard for hepatitis B virus DNA nucleic acid amplification techniques. *Vox Sang* 2001; 80:63–71

2 Heermann KH, Gerlich WH, Chudy M, Schaefer S, Thomssen R, the Eurohep Pathobiology Group: Quantitative detection of hepatitis B virus DNA in two international reference plasma preparations. *J Clin Microbiol* 1999; 37:68–73

3 World Health Organization: *WHO Expert Committee on Biological Standardization*, technical report series, 904; Fiftieth report, 2002:23–24

4 Baylis SA, Davis C, Holmes H: Minutes of the eighteenth meeting of the Scientific Working Group on the Standardisation of Genome Amplification Techniques for the Safety Testing of Blood, Tissues and Organs (SoGAT). National Institutes of Health, Bethesda MD, USA, 24–25 May 2005

5 Baylis SA, Shah N, Minor PD: Evaluation of different assays for the detection of parvovirus B19 DNA in human plasma. *J Virol Methods* 2004; 121:7–16

6 Weinberger KM, Wiedenmann E, Böhm S, Jilg W: Sensitive and accurate quantitation of hepatitis B virus DNA using a kinetic fluorescence detection system (TaqMan PCR). *J Virol Methods* 2000; 85:75–82

7 Jursch CA, Gerlich WH, Glebe D, Schaefer S, Marie O, Thraenhart O: Molecular approaches to validate disinfectants against human hepatitis B virus. *Med Microbiol Immunol* 2002; 190:189–197

8 Kirkwood TBL: Predicting the stability of biological standards and products. *Biometrics* 1977; 33:736–742

9 Tydeman MS, Kirkwood TBL: Design and analysis of accelerated thermal degradation tests for the stability of biological standards I. Properties of maximum likelihood estimators. *J Biol Stand* 1984; 12:195–206

10 Baylis SA, Heath A, the collaborative Study Group: WHO collaborative study to establish a replacement WHO International Standard for hepatitis C virus RNA nucleic acid amplification technique (NAT)-based assays. WHO ECBS Report 2007; WHO/BS/07.2055

11 Saldanha J, Heath A, Aberham C, Albrecht J, Gentili G, Gessner M, Pisani G: World Health Organization collaborative study to establish a replacement WHO international standard for hepatitis C virus RNA nucleic acid amplification technology assays. *Vox Sang* 2005; 88:202–204

© 2008 The Author(s)
Journal compilation © 2008 Blackwell Publishing Ltd., *Vox Sanguinis* (2008)

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		機構処理欄
				2008. 2. 18	該当なし		
一般的名称	新鮮凍結人血漿		研究報告の公表状況	沼尾宏, 渡辺泰宏, 立花直樹. 第37回日本肝臓学会西部会; 2007 Dec 7-8; 長崎.		公表国 日本	
販売名(企業名)	新鮮凍結血漿「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」(日本赤十字社)						

研究報告の概要

○輸血によりHBs抗体エスケープ変異株に感染した一例
【症例】40歳代女性。平成16年8月より発熱あり、白血病の疑いにて平成16年10月21日入院。混合型急性白血病として10月25日より化学療法を施行。入院時HBsAg−、HCVAb−であった。10月末より11月にかけてALT387IU/Lまで上昇した。11月10日にはHBsAg−、HBsAb+、HbcAb+、HBeAg−、HBeAb−、HBV-DNAポリメラーゼ0CPM。一時肝機能は正常化したものの、平成18年2月中旬より再びトランスアミナーゼの上昇を軽度認めた。白血病が血液学的寛解となり4月17日退院。外来で化学療法を施行していたが、5月30日AST 947IU/L、ALT 1683IU/Lと上昇し再度入院。HBeAg+、HBeAb+、HBcAb・IgM+で、他の肝炎ウイルスマーカーが陰性であったためHBV感染を疑い、6月5日よりラミブジンを100mg/日で投与開始した。6月6日HBV-PCR 3.4LC/mLであった。その後ALT 2357IU/L、T-Bil 7.41mg/dlまで上昇し、肝炎の改善傾向はなかった。ラミブジンの継続と肝庇護療法にて肝機能は改善した。6月23日の肝生検では小葉内肝細胞壊死を伴った高度の炎症細胞浸潤を認め、急性肝炎の所見であった。AST17IU/L、ALT27IU/Lとなり7月22日退院。HBV-PCR陽性となるまでに患者に投与されたすべての血液製剤について個別HBV-NATを実施した結果、平成16年11月に輸血したFFPがHBV陽性であった。製剤と患者のHBVはいずれもGenotype C/Subtype ayrでS抗原のN末端から145番目のアミノ酸がGlyからSerに置換しており、エスケープミュータントであった。また、両者のα領域(PreS/S領域を含むP領域の前半部)の塩基配列は一部の塩基の共存を除き、完全に一致した。HBV-DNAはいずれも定量限界(100copies/mL)未満であった。患者はその後もラミブジンの投与を継続し、骨髄移植を行った。肝炎の再燃は認めなかったが、白血病のため平成18年10月永眠された。【考察】核酸増幅検査を含む献血者のスクリーニングを行っているにもかかわらず、本邦では年間10-20例のHBV感染が報告されている。その原因の一つがHBs抗体エスケープミュータントであるが、本症例のように献血者、受血者ともに塩基配列の解析を行い感染が証明された例はきわめて稀と考えられ報告する。

使用上の注意記載状況・その他参考事項等

新鮮凍結血漿「日赤」
新鮮凍結血漿-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:2006年3月16日付1-05000059

報告企業の意見	今後の対応
輸血によりHBs抗体エスケープ変異株に感染し、献血者、受血者の塩基配列の解析を行って感染が証明された症例の報告である。	日本赤十字社では、HBs抗原検査及びHBc抗体検査を実施することに加えて、HBV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。HBV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。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)の導入を順次進めている。NATの精度向上についても評価・検討している。

A522 肝 臓 48巻 suppl. (3) (2007)

O-9 初診時よりHBs抗体陽性であったB型急性肝炎の一例

○石田素子，松本修一，筋浦立成
福岡徳洲会病院総合内科

【症例】33歳男性【主訴】全身関節痛【現病歴】来院5日前起床時に頚部痛を自覚し日毎に全身関節に拡大，症状の改善がなく当科外来を初診した．関節痛は時間帯，安静労作に関係なく，食欲低下を認める以外は発熱，かぜ症状などは認めない．【生活歴】MSM（men who have sex with men）で最終性交は半年前，パートナーは固定していない．刺青・輸血歴・海外渡航歴なし．常用薬なし．喫煙は20本/日，機会飲酒．【家族歴】肝疾患なし．【来院時現症】眼瞼結膜に黄疸なし．胸部異常所見なし．肝脾触知せず．疼痛のある関節に腫脹，発赤，熱感なし．【検査所見】AST 1430IU/l，ALT 2630 IU/l，PT50%，T.bil0.8mg/dl，IgM-HA抗体陰性，HCV-RNA陰性，HBs抗原（2000）陽性，HBs抗体（1000）陽性，IgMHBc抗体（31.5）陽性，HBV-DNA>7.6LGE/ml，HBVgenotypeA【経過】安静のみで採血データは徐々に改善し，入院時認めた食欲低下や関節痛もそれとともに軽快した．HBs抗体陽性ではあったがIgMHBc抗体高濃度陽性でB型急性肝炎と診断した．感染経路としては性感染症と予想され，その他の感染症も検査した結果，2期梅毒を認めたが，HIVは陰性であった．【まとめ】診断初期よりHBs抗原抗体が共に高値を示しその判断に苦慮したB型急性肝炎を経験した．HBs抗原抗体の共存について文献的な報告を含め考察する．

O-10 輸血によりHBs抗体エスケープ変異株に感染した一例

○沼尾 宏[1]，渡辺泰宏[2]，立花直樹[3]
青森県立中央病院消化器内科・化学療法科[1]，わたなべ内科クリニック[2]，青森県立中央病院輸血部[3]

【症例】40歳代女性．主訴：肝機能障害．家族歴：特記すべきことなし．既往歴：平成元年切迫早産．現病歴：平成16年8月より発熱あり近医受診．白血病の疑いにて平成16年10月21日当院リウマチ・血液内科紹介入院．経過：混合型急性白血病として10月25日より化学療法を施行．入院時HBsAg－(0.00)，HCVAb－（0.1）であった．10月末より11月にかけてALT 387 IU/Lまでの上昇を認めた．11月10日の採血ではHBsAg－(<0.05IU/mL)，HBsAb＋（69.0mIU/mL)，HbcAb＋（1.82 S/CO)，HBeAg－(0.4)，HBeAb－(16%)，HBV-DNAポリメラーゼ0 CPM．その後一時肝機能は正常化したものの平成18年2月中旬より再びトランスアミナーゼの上昇を軽度認めた．白血病が血液学的寛解となり4月17日退院．外来でプレドニゾロンを含む化学療法を施行していたが，5月30日AST 947 IU/L，ALT 1683IU/Lと上昇しリウマチ・血液内科入院．HBeAg＋(1.52S/CO)，HBeAb＋(65.2)，HBcAb-IgM＋(20.9 S/CO)で，かつ他の肝炎ウイルスマーカーが陰性であったためHBV感染を疑い6月5日よりラミブジンを100mg/日で投与開始した．6月6日HBV-PCR 3.4LC/mLであった．その後ALT 2357IU/L，T-Bil 7.41mg/dlまで上昇し，肝炎の改善傾向がないため当科転科となった．ラミブジンの継続と肝庇護療法にて肝機能は改善した．6月23日の肝生検では小葉内肝細胞壊死を伴った高度の炎症細胞浸潤を認め，急性肝炎の所見であった．AST 17IU/L，ALT 27IU/Lとなり7月22日退院．HBV-PCR陽性となるまでに患者に投与されたすべての血液製剤について個別HBV-NATを実施した結果，平成16年11月に輸血したFFPがHBV陽性であった．この製剤中と患者のHBVはいずれもGenotype C/Subtype ayrでS抗原のN末端から145番目のアミノ酸がGlyからSerに置換しており，エスケープミュータントであった．また，両者のα領域（PreS/S領域を含むP領域の前半部）の塩基配列は一部の塩基の共存を除き，完全に一致した．HBV-DNAはいずれも定量限界(100copies/mL)未満であった．患者はその後もラミブジンの投与を継続し，骨髄移植を行った．肝炎の再燃は認めなかったが，白血病のため平成18年10月永眠された．【考察】核酸増幅検査を含む献血者のスクリーニングを行っているにもかかわらず，本邦では年間10～20例のHBV感染(occult HBVによる感染)が報告されている．その原因の一つがHBs抗体エスケープミュータントであるが，本症例のように献血者，受血者ともに塩基配列の解析を行い感染が証明された例はきわめて稀と考えられ報告する．

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
				2007. 11. 22	該当なし		
一般的名称	（製造承認書に記載なし）		研究報告の公表状況	Dhalla S, Tenner CT, Aytaman A, Shukla NB, Villanueva G, Punla G, Patterson C, Comas J, Bini EJ. American Society for the Study of Liver Diseases; 2007 Nov 2-6; Boston.		公表国 米国	
販売名（企業名）	合成血「日赤」（日本赤十字社） 照射合成血「日赤」（日本赤十字社） 合成血-LR「日赤」（日本赤十字社） 照射合成血-LR「日赤」（日本赤十字社）						

研究報告の概要

○入れ墨とC型肝炎ウイルス感染の強い相関：患者3871名による多施設共同研究

背景：HCV感染のリスク要因として静注薬物使用と1992年以前の輸血がよく知られてきたが、入れ墨に関しては議論があった。さらに、複数の先行する研究では、HCV感染のリスク要因としての入れ墨は静注薬物使用と混同されてきた可能性がある。本研究の目的は、典型的なHCV感染リスクのない患者多数における入れ墨とHCV感染の関係を明らかにすることである。

方法：慢性HCV感染の患者（HCV RNA陽性）とコントロール群（HCV抗体陰性）が3つの研究施設での外来診療時、詳細なアンケートに回答した。患者の人口統計学的データとHCVリスク要因を含むデータを収集した。

結果：合計3871名の患者のうち1930名が慢性HCV感染群、1941名が陰性のコントロール群だった。平均年齢（55.2±9.0 vs. 55.6±11.3年、p=0.34）と男女比（80.3% vs 81.4%、p=0.39）に有意差はなかった。しかし、感染群は人種的／民族的マイノリティが多かった(78.5% vs 56.5%、p<0.001)。予想されたとおり、静注薬物使用(65.9% vs 17.8%、p<0.001)と1992年以前の輸血(22.3% vs 11.1%、p<0.001)は、HCV感染群の方がコントロール群よりも多かった。感染群は2つ以上の入れ墨がある傾向が強く(35.2% vs 12.5%；OR=3.81；95% CI、3.24-4.49；p<0.001)、これは年齢、性別、人種/民族についての補正後も有意だった（OR=4.57；95% CI、3.83-5.45；p<0.001）。静注薬物使用歴と1992年以前の輸血歴を持つ患者を除外した後に残った1887名を分析した(466名が感染群、1421名がコントロール群)。陽性群の患者は入れ墨歴がある傾向が強く（34.1% vs. 11.9%；OR=3.84；95% CI、2.99-4.93；p<0.001）、年齢、性別、人種／民族についての補正後も統計的に有意だった（OR=4.47；95% CI, 3.42-5.83；p<0.001）。

結論：入れ墨は、静注薬物使用や1992年以前の輸血などの典型的なHCV感染リスク要因のない患者群においても、HCV感染と強く相関している。入れ墨のある患者にはHCV検査が推奨されるべきである。

使用上の注意記載状況・その他参考事項等

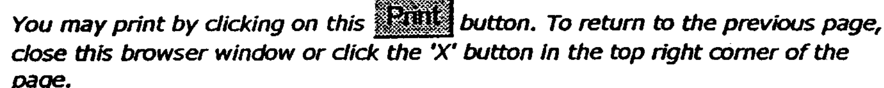
合成血「日赤」
照射合成血「日赤」
合成血-LR「日赤」
照射合成血-LR「日赤」

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
入れ墨は、静注薬物使用や1992年以前の輸血などの典型的なHCV感染リスク要因のない患者群においても、HCV感染と強く相関しているとの報告である。	日本赤十字社は、輸血感染症対策として問診時に過去1年以内に入れ墨を入れた人は献血不適としている。今後も引き続き情報の収集に努める。

JRC2007T-085

AASLD 2007



ID# 136
Location: Auditorium (Hynes)
Time of Presentation: Nov 05 5:30 PM - 5:45 PM
Category: Q04. HCV: Epidemiology

Strong Association between Tattoos and Hepatitis C Virus Infection: A Multicenter Study of 3,871 Patients

E. J. Bini[5]; S. Dhalla[1]; C. T. Tenner[2]; A. Aytaman[3]; N. B. Shukla[4]; G. Villanueva[4]; G. Punla[5]; C. Patterson[4]; J. Comas[3]
1. Department of Medicine, NYU School of Medicine, New York, NY, USA.
2. Division of General Internal Medicine, VA Medical Center & NYU School of Medicine, New York, NY, USA.
3. Division of Gastroenterology, VA Medical Center, Brooklyn, NY, USA.
4. Division of Gastroenterology, Bellevue Hospital Center & NYU School of Medicine, New York, NY, USA.
5. Division of Gastroenterology, VA Medical Center & NYU School of Medicine, New York, NY, USA.

Background: Although injection drug use and blood transfusions prior to 1992 are well-accepted risk factors for hepatitis C virus (HCV) infection, the evidence for tattoos as a risk factor for HCV is conflicting. Furthermore, several prior studies that have evaluated tattoos as a risk factor for HCV infection were potentially confounded by injection drug use. The aim of this study was to determine the association between tattoos and HCV infection in a large population of patients without traditional risk factors for HCV infection.
Methods: Patients with chronic HCV infection (HCV RNA positive) and controls (HCV antibody negative) completed a detailed questionnaire at the time of their scheduled visit to the outpatient primary care or GI clinic at 3 study sites. Data collected included patient demographics and information on HCV risk factors.
Results: A total of 3,871 patients were enrolled, including 1,930 with chronic HCV infection and 1,941 HCV negative controls. There were no differences in the mean age (55.2 ± 9.0 vs. 55.6 ± 11.3 years, $p = 0.34$) or the proportion who were male (80.3% vs. 81.4%, $p = 0.39$) between HCV-infected patients and controls. However, HCV positive patients were more likely to be racial/ethnic minorities (78.5% vs. 56.5%, $p < 0.001$). As expected, injection drug use (65.9% vs. 17.8%, $p < 0.001$) and blood transfusions prior to 1992 (22.3% vs. 11.1%, $p < 0.001$) were more common in HCV-infected patients than in control subjects. Patients with HCV infection were significantly more likely to have had one or more tattoos (35.2% vs. 12.5%; OR = 3.81; 95% CI, 3.24 – 4.49; $p < 0.001$) and this remained highly significant after adjustment for age, sex, and race/ethnicity (OR = 4.57; 95% CI, 3.83 – 5.45; $p < 0.001$). After excluding all patients with a history of ever injecting drugs and those who have had a blood transfusion prior to 1992, a total of 1,887 subjects remained for analysis (466 HCV positive and 1,421 controls). Among these 1,887 patients without traditional risk factors for HCV infection, we found that HCV positive patients were still significantly more likely to have a history of tattoos (34.1% vs. 11.9%; OR = 3.84; 95% CI, 2.99 – 4.93; $p < 0.001$) and this remained highly statistically significant after adjustment for age, sex, and race/ethnicity (OR = 4.47; 95% CI, 3.42 – 5.83; $p < 0.001$).
Conclusions: Tattoos are strongly associated with HCV infection, even among those without traditional HCV risk factors such as injection drug use and blood transfusions. All patients with tattoos should be offered HCV testing.

Abstract Central® (patent pending). © ScholarOne, Inc., 2007. All Rights Reserved.
Abstract Central and ScholarOne are registered trademarks of ScholarOne, Inc.
Terms and Conditions of Use

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
				2008. 1. 21	該当なし	
一般的名称	(製造承認書に記載なし)		研究報告の公表状況	Spada E, Abbate I, Sicurezza E, Mariano A, Parla V, Rinnone S, Cuccia M, Capobianchi MR, Mele A. J Med Virol. 2008 Feb;80(2):261-7.	公表国 イタリア	
販売名(企業名)	合成血「日赤」(日本赤十字社) 照射合成血「日赤」(日本赤十字社) 合成血-LR「日赤」(日本赤十字社) 照射合成血-LR「日赤」(日本赤十字社)					

研究報告の概要	使用上の注意記載状況・その他参考事項等
○イタリアの血液透析施設におけるC型肝炎集団感染の分子疫学 血液透析患者は、C型肝炎ウイルス(HCV)感染のリスクが高い。本試験の目的は、疫学的、分子学的手法を用いて、血液透析施設におけるHCV集団感染について検討することである。2003年4月～2003年10月に、当該施設を利用する患者4名にHCV抗体セロコンバージョンが認められた。この4名を、当該施設来院時にすでにHCV抗体陽性であった患者10名に加え、14名全員の抗HCV抗体陽性患者のHCV RNAおよびHCV遺伝子型を検査した。HCV RNA 陽性患者のNS5BおよびHVR1/ E2遺伝子領域を増幅し、配列を決定し、系統発生解析を行った。さらに、患者全員から得られた臨床疫学的記録を調べた。新たに感染した患者4名はいずれも遺伝子型2cであった。来院時にすでにHCV抗体陽性であった患者10名のうち2名でも遺伝子型2cが検出された。系統発生解析は、新規HCV感染患者全員が、2c慢性感染患者2名中1名から検出された2c分離ウイルスと群生した分離ウイルスと近縁であることを示した。いずれのHCV-2c感染患者にも血液透析以外のリスク因子はなかった。新規HCV-2c感染患者4名中3名と当該集団感染に関与したHCV-2c慢性感染患者1名は、同日の同一シフト時に透析を受けたが、装置は別のものを使用していた。残りのHCV-2c新規感染患者と前述の3名中1名は、同日の別のシフト時に同一の装置を使用して透析を受けた。当該集団感染は、おそらく感染制御手段の不備によるものであると考えられるが、1症例においては関連装置による伝播が除外できない。	合成血「日赤」 照射合成血「日赤」 合成血-LR「日赤」 照射合成血-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
2003年4月～2003年10月に、イタリアの血液透析施設でHCVの集団感染が発生し、感染制御手段の不備と装置による伝播が疑われたとの報告である。	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。

Journal of Medical Virology 80:261–267 (2008)

Molecular Epidemiology of a Hepatitis C Virus Outbreak in a Hemodialysis Unit in Italy

Enea Spada,[1]* Isabella Abbate,[2] Elvia Sicurezza,[3] Andrea Mariano,[4] Vincenza Parla,[3] Sebastiano Rinnone,[5] Mario Cuccia,[5] Maria Rosaria Capobianchi,[2] and Alfonso Mele[1]

[1]*Istituto Superiore di Sanità, National Centre of Epidemiology, Surveillance and Health Promotion, Rome, Italy*
[2]*Istituto Nazionale di Malattie Infettive Lazzaro Spallanzani, Laboratory of Virology, Rome, Italy*
[3]*Azienda Ospedaliera Universitaria, Vittorio Emanuele II, Ferrarotto S. Bambino, Unità Operativa di Nefrologia-Dialisi, Catania, Italy*
[4]*Istituto Nazionale di Malattie Infettive Lazzaro Spallanzani, Clinical Research Department, Rome, Italy*
[5]*Azienda Unità Sanitaria Locale 3 Catania, Dipartimento di Prevenzione, Servizio Epidemiologia e Prevenzione, Catania, Italy*

Hemodialysis patients are at increased risk of hepatitis C virus (HCV) infection. The aim of this study was to investigate a HCV outbreak in a hemodialysis unit using epidemiological and molecular methods. Between April 2003 and October 2003, anti-HCV seronconversion was detected in four patients attending the unit. These cases were added to 10 patients already anti-HCV positive upon admission in the unit. All 14 anti-HCV patients were tested for HCV RNA and HCV genotype. NS5B and HVR1/E2 genomic regions were amplified and sequenced in all HCV RNA positive patients and phylogenetic analysis was performed. Furthermore, clinical-epidemiological records obtained from all patients were examined. All four patients newly infected harbored genotype 2c. Genotype 2c was also detected in 2 of 10 patients already anti-HCV positive upon admission. Phylogenetic analysis showed that all newly HCV infected patients harbored very closely related viral isolates that clustered together with the 2c isolate found in one of the two 2c chronic infected patients. All HCV-2c infected patients had no other risk factors except hemodialysis. Three of four newly HCV-2c infected patients and the one HCV-2c chronically infected involved in the outbreak received dialysis on the same day and same shift but used different machines. The remaining HCV-2c newly infected patient and one of the above cited three received dialysis on the same day during different shifts but used the same machine. The outbreak was probably due to breaks of infection control procedures although a related-machine transmission cannot be excluded in one of the cases. ***J. Med. Virol. 80:261–267, 2008.***
© 2007 Wiley-Liss, Inc.

KEY WORDS: epidemiological investigation; genotype; hemodialysis; hepatitis C virus; nosocomial infection; phylogenetic analysis

INTRODUCTION

Patients on hemodialysis are recognized as a group at increased risk of infection with hepatitis C virus (HCV). The prevalence and incidence of HCV infection among patients receiving hemodialysis varies widely between countries and also within the same country [Fabrizi et al., 2002]. In Italy, the prevalence of HCV among hemodialysis patients ranges between 10% and 50%, and the incidence is around 1–2 cases per 100 person-years [Petrosillo et al., 2001; Di Napoli et al., 2006].

The risk of HCV transmission by blood transfusion to patients receiving hemodialysis has been considerably reduced since screening of blood donors for HCV antibodies (anti-HCV) was introduced and recombinant erythropoietin for treatment of anemia became available [Di Napoli et al., 2006]. However, HCV transmission in hemodialysis units still occurs, and occasionally it is responsible for large outbreaks [Le Pogam et al., 1998; Delarocque-Astagneau et al., 2002; Fabrizi et al., 2002; Kokubo et al., 2002;

Grant sponsor: "Sorveglianze speciali" of the Italian Ministry of the Health (partial support).

*Correspondence to: Dr. Enea Spada, Istituto Superiore di Sanità, National Centre of Epidemiology, Surveillance and Health Promotion, Clinical Epidemiology Unit, Via Giano Della Bella, 34-00162 Rome, Italy. E-mail: enea.spada@iss.it

Accepted 30 October 2007
DOI 10.1002/jmv.21088
Published online in Wiley InterScience
(www.interscience.wiley.com)

© 2007 WILEY-LISS, INC.

WILEY InterScience
DISCOVER SOMETHING GREAT

Savey et al., 2005]. Several studies, by using molecular biology techniques, provided evidence of a nosocomial patient-to-patient mode of transmission in most of these HCV infection occurring in hemodialysis settings, despite rigorous preventive measures [Le Pogam et al., 1998; Delarocque-Astagneau et al., 2002; Kokubo et al., 2002; Savey et al., 2005]. Important risk factors for acquiring nosocomial HCV infection in patients on hemodialysis seems to be particularly a longer duration of hemodialysis, a high HCV prevalence in the unit and a low personnel/patient ratio (<1/3 or at least 1/4) [Petrosillo et al., 2001].

However, the exact mechanisms of the patient-to-patient transmission of HCV within hemodialysis units have not been clearly identified and they may be different in relation to the different policies followed in each hemodialysis unit for the management of patients [Petrosillo et al., 2001; Fabrizi et al., 2002]. Most authors currently believe that most cases of HCV patient-to-patients transmission can be attributed to lack of implementation of or breaks in infection control procedures [Le Pogam et al., 1998; Petrosillo et al., 2001; Delarocque-Astagneau et al., 2002; Fabrizi et al., 2002; Kokubo et al., 2002; Savey et al., 2005]. The possibility of HCV transmission between patients through the dialysis machines is controversial. However, this possibility cannot be entirely excluded especially in case of contamination of internal components of the machine not accessible to routine disinfection, and in the hemodialysis units in which the disinfection of the machines between treatments is not routinely performed or those in which dialysers and/or dialysis tubing sets are reused [Le Pogam et al., 1998; Delarocque-Astagneau et al., 2002; Fabrizi et al., 2002; Savey et al., 2005].

This study describes an outbreak of acute HCV type 2c infection involving four patients attending an outpatient hemodialysis unit in southern Italy. Molecular analysis of viral isolates in association with an epidemiological investigation was performed to trace the source and the possible routes of transmission of HCV during this outbreak.

PATIENTS AND METHODS

Hemodialysis Setting and Procedures

At the time of the outbreak, the unit consisted in a room with 8 dialysis machines in which 32 outpatients regularly underwent maintenance hemodialysis three times weekly (Monday-Wednesday-Friday or Tuesday-Thursday-Saturday) on 1 of the 2 shifts per day (either morning or evening shift). Thus, every machine was used by two persons per day. Normally patients were dialysed on the same shift, but not always on the same machine. No dedicated areas or machines or personnel were used for HCV infected patients. Hemodialysis was carried out using Hospal-INTEGRA® dialysis machine. The machines were disinfected with chlorine dioxide (ISTRUMET, Hospal®) between each shift and dialysers and tubing sets were disposable and were never reused. Two nurses took care of eight patients in each shift, but they could also move from patient to patient if needed. No multidose vials were used among patients.

HCV Infection Monitoring

HCV infection was monitored in all dialysis patients by performing testing for serum alanine aminotranserase (ALT) monthly and for anti-HCV upon admission and then every 6 months. Anti-HCV test was also performed in case of ALT elevation. Prior to the beginning of the outbreak, the prevalence of anti-HCV among the 32 patients attending the unit was 31.2% (10 patients).

Case Definition, Case Finding and Data Collection

During the routine screening for HCV infection conducted from April 2003 to October 2003 four incident cases of HCV infection were identified in the unit. That the four cases had occurred in a relatively brief period of time led to suspect a nosocomial outbreak. A potential outbreak case-patient was defined as any patient who had showed seroconversion between October 2002 and October 2003 and who had received dialysis in the unit at least 6 months before the detection of the first seroconversion case.

Since in the unit, at the time of the outbreak, the monitoring of HCV infection was based on the detection of anti-HCV only, to identify retrospectively other cases of new infections and the potential source of the outbreak on June 2004 blood samples for anti-HCV and HCV RNA testing were obtained from all the patients who had received dialysis in the unit since April 2002 and from all their household contacts. All healthcare workers employed in the unit underwent periodical testing for blood-borne viruses. A blood sample was also obtained from the one healthcare worker (a doctor) who was known to be anti-HCV positive.

From the medical records, kept constantly for all patients, data on medical and dialysis history, blood transfusion, recent surgical, or medical invasive procedures, intravenous drug use and other parenteral exposure, such as tattoos and piercing, were obtained. Furthermore, the dialysis schedule (day and shift) seating arrangements, type of vascular access, type of dialyser membrane, hemodialysis machine, bleeding episodes, nurse-patient assignment, dialysis equipment maintenance as well as infection control measures were all recorded.

Virological Analysis

The seroconversions of the patients involved in the outbreak were detected during the routine screening for anti-HCV performed in all patients attending the unit. In the unit, anti-HCV antibodies were detected by using a third generation enzyme immunoassay (Cobas Core Anti-HCV EIA II, Roche Diagnostic Systems, Basel,