資料２－２

Ａ　研究報告（詳細版）

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研究報告の概要

業界向け手引書（案）：血液および血液製剤を介した HIV 感染のリスク低減に関する勧告事項の改訂

本手引書は、血液又は原料血漿を含む血液成分を採取する血液事業者に、ヒト免疫不全ウイルス（HIV）感染高リスク者からの供血停止に関する FDA 勧告の改訂版を提示したものである。また、FDA は、この改訂に合わせて、血液事業者が供血者用教材、供血者の履歴問診票及び付随資料、並びに供血者の適格性確認事項及び製品管理法の改訂を勧める。本手引書には、1992 年 4 月 23 日付の血液事業者への覚書「Revised Recommendations for the Prevention of Human Immunodeficiency Virus (HIV) Transmission by Blood and Blood Products」（以下「1992 年血液に関する覚書」）に含まれる供血者用教材及び検査に関するその他の勧告も含まれる。最終決定された場合、本手引書が 1992 年血液に関する覚書より優先される。本手引書に含まれる勧告は、血液又は原料血漿を含む血液成分の採血に適用される。

報告企業の意見

ヒト免疫不全ウイルス（Human Immunodeficiency Virus：HIV）は、レトロウイルス科（retrovirus）レンチウイルス属（Lentivirus）に属し、直径約 110nm のエンベロープを持つ一本鎖 RNA ウイルスである。血清学的に HIV-1 と HIV-2 に分類され、HIV-1 は塩基配列により 4 群に分類され、グループ M（Major）、グループ O（Outlier）、グループ N（non-M/non-O）、グループ P（pending）に分けられるが、世界的に分布しているウイルスの多くがグループ M に属している。本剤の原料となる血液は抗 HIV-1 抗体，抗 HIV-2 抗体陰性であることを確認し、更に NAT スクリーニングを実施し、適合した血漿を使用している。万一、原料血漿に HIV が混入したとしても、HIV-1 を用いたウイルスクリアランス試験成績から、本剤の製造工程において不活化・除去されると考えている。

今後の対応

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

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。

Revised Recommendations for Reducing the Risk of Human Immunodeficiency Virus Transmission by Blood and Blood Products

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Draft Guidance for Industry

This guidance document is for comment purposes only.

Submit one set of either electronic or written comments on this draft guidance by the date provided in the *Federal Register* notice announcing the availability of the draft guidance. Submit electronic comments to http://www.regulations.gov. Submit written comments to the Division of Dockets Management (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Rm. 1061, Rockville, MD 20852. You should identify all comments with the docket number listed in the notice of availability that publishes in the *Federal Register*.

Additional copies of this guidance are available from the Office of Communication, Outreach and Development (OCOD), 10903 New Hampshire Ave., Rm. 3128, Silver Spring, MD 20993-0002, or by calling 1-800-835-4709 or 240-402-7800, or email ocod@fda.hhs.gov, or from the Internet at http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/default.htm.

For questions on the content of this guidance, contact OCOD at the phone numbers or email address listed above.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research
May 2015

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Contains Nonbinding Recommendations

Draft – Not for Implementation

Revised Recommendations for Reducing the Risk of Human Immunodeficiency Virus Transmission by Blood and Blood Products

Draft Guidance for Industry

This draft guidance, when finalized, will represent the Food and Drug Administration's (FDA's or Agency's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations. If you want to discuss an alternative approach, contact the FDA staff responsible for implementing this guidance using the contact information on the title page of this guidance.

I. INTRODUCTION

This guidance document provides you, blood establishments that collect blood or blood components, including Source Plasma, with FDA's revised donor deferral recommendations for individuals with increased risk for transmitting human immunodeficiency virus (HIV) infection. We (FDA) are also recommending that you make corresponding revisions to your donor education materials, donor history questionnaires and accompanying materials, along with revisions to your donor requalification and product management procedures. This guidance also incorporates certain other recommendations related to donor education materials and testing contained in the memorandum to blood establishments entitled, "Revised Recommendations for the Prevention of Human Immunodeficiency Virus (HIV) Transmission by Blood and Blood Products," dated April 23, 1992 (1992 blood memo) (Ref. 1). When finalized, it will supersede that 1992 blood memo. The recommendations contained in this guidance apply to the collection of blood and blood components, including Source Plasma.

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the FDA's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in FDA's guidances means that something is suggested or recommended, but not required.

II. BACKGROUND

The emergence of Acquired Immune Deficiency Syndrome (AIDS) in the early 1980s and the recognition that it could be transmitted by blood and blood products had profound effects on the United States (U.S.) blood system (Refs. 2, 3, 4). Although initially identified in men who have

sex with men (MSM) and associated with male-to-male sexual contact, AIDS was soon noted to be potentially transmitted by transfusion of blood components, and by infusion of clotting factor concentrates in individuals with hemophilia (Refs. 5, 6). Subsequently, AIDS was also found to be associated with heterosexual transmission through commercial sex work and with intravenous drug use (Refs. 7, 8). In 1983, AIDS was reported to be associated with the virus now known as human immunodeficiency virus (HIV). The historical understanding of HIV transmission at this point in 1983 informed the first blood donor deferral policy, which at the time was seeking to reduce the transmission of HIV through blood product transfusion.

A. History of Efforts to Reduce HIV Transmission by Blood Products

Beginning in 1983, the FDA issued recommendations for providing donors with educational material on risk factors for AIDS and for deferring donors with such risk factors in an effort to prevent transmission of AIDS (later understood to be caused by HIV) by blood and blood products (Refs. 2, 9, 10, 11). Providing donor education material and asking at-risk donors not to donate was demonstrated to have a significant impact on preventing HIV transmission prior to the availability of testing (Ref. 12). However, thousands of recipients of blood and blood components for transfusion and recipients of plasma-derived clotting factors became infected with HIV before the causative virus was identified and the first screening tests for HIV were approved in 1985 (Refs. 2, 4, 10).

Since September 1985, FDA has recommended that blood establishments indefinitely defer male donors who have had sex with another male, even one time, since 1977, due to the strong clustering of AIDS illness in the MSM community and the subsequent discovery of high rates of HIV infection in that population (Ref. 13). On April 23, 1992, FDA issued the 1992 blood memo, which contains the current recommendations regarding the deferral for MSM as well as the deferral recommendations for other persons with behaviors associated with high rates of HIV exposure, namely commercial sex workers, intravenous drug users, and certain individuals with other risk factors.

The use of donor education material, specific deferral questions, and advances in HIV donor testing (e.g., HIV antibody assays, p24 antigen assays, and nucleic acid tests (NAT)) have reduced the risk of HIV transmission from blood transfusion from about 1 in 2500 units prior to HIV testing to a current estimated residual risk of about 1 in 1.47 million transfusions (Refs. 14, 15). The development of pathogen inactivation procedures for products manufactured from pooled plasma in the 1980s improved the safety of these products by inactivating lipid-enveloped viruses. No transmissions of HIV, hepatitis B virus (HBV), or hepatitis C virus (HCV) have been documented through U.S.-licensed plasma derived products in the past two decades (Ref. 16).

Relating in large part to the development of more sensitive HIV testing methodologies, there have been calls in the social and scientific literature to revisit the blood donor deferral policies that were established about three decades ago, in particular, with regard

to the deferral of MSM. During the period from 1997 to 2010, FDA held a number of public meetings, including workshops and Blood Product Advisory Committee (BPAC) meetings to further review evidence and to discuss its blood donor deferral policies to help prevent the transmission of HIV (Refs. 17, 18, 19, 20). In June 2010, the Department of Health and Human Services (HHS) brought the issue of deferral of men who have had sex with another man, even one time, since 1977, for public discussion at a meeting of the Advisory Committee on Blood Safety and Availability (the Committee). The Committee heard presentations of currently available scientific data as well as comments from the public. The Committee recommended to the HHS Secretary "that the current MSM deferral policy, while suboptimal, should be retained pending the completion of targeted research studies that might support a safe alternative policy" (Ref. 21).

Based on these recommendations, in September 2010, an Interagency Blood, Organ & Tissue Safety Working Group on MSM (BOTS Working Group), consisting of representatives from the Centers for Disease Control and Prevention (CDC), Health Resources and Services Administration (HRSA), National Institutes of Health (NIH), HHS Office of Civil Rights, Office of the Assistant Secretary for Health (OASH), and FDA, was charged by the Assistant Secretary for Health with exploring the feasibility of a data and science-driven policy change. Subsequently, the BOTS Working Group designed and implemented one operational assessment and three research studies to gain more information to help inform a potential policy change. In addition, it considered the possibility of conducting a pilot study to assess the effect of a policy change. However, following review of comments received in response to a *Federal Register* notice titled, "Request for Information (RFI) on Design of a Pilot Operational Study To Assess Alternative Blood Donor Deferral Criteria for Men Who Have Had Sex With Other Men (MSM)" (77 FR 14801, March 13, 2012) (Ref. 22), requesting comment on potential pilot study designs, as well as further considerations regarding the significant statistical, financial and logistical challenges in implementing such a study, the BOTS Working Group decided that such a pilot study examining the potential effects of a policy change would not be feasible. Instead, the BOTS Working Group determined that resources at HHS could be used in more efficient ways to carefully review the studies that had been initiated (results of which are summarized in section II.C. below), to complete its review of the blood donation deferral criteria, and to establish a blood safety monitoring system.

B. Current Risk of HIV Infection Associated with Specific Behaviors

Recent data indicate that commercial sex work (CSW) and injection drug use (IDU) are behaviors that continue to place individuals both at a relatively high risk of HIV infection and at a relatively high risk of window period transmission of HIV (Ref. 23) and few data are available on the HIV risk in individuals who have discontinued CSW and IDU (Ref. 24). Deferral policies for CSW and IDU are also based on risks for transfusion transmitted infectious diseases, in addition to HIV, that are associated with these behaviors (Ref. 25).

Together, these findings continue to support an indefinite deferral of individuals currently or previously involved in CSW and IDU behaviors. Therefore, absent additional data, we have not proposed a change to current deferral policies for CSW or IDU.

Although MSM represent a small percentage of the U.S. male population (approximately 7% of men report that they have ever participated in MSM activity and approximately 4% of men report that they engaged in MSM activity in the last 5 years[1]) (Ref. 26), they comprise a large proportion of adults in the United States with existing and newly diagnosed HIV infections. Among persons living with HIV in 2011, CDC estimates that 57% were MSM (including MSM who were also IDU) (Ref. 27). MSM remain at increased risk of HIV infection. In 2010, male-to-male sexual contact accounted for 63% of newly diagnosed HIV infections among adults, and 78% of newly diagnosed HIV infections in men, indicating that male-to-male sexual contact remains associated with high risk of HIV exposure (Ref. 28).

C. Recent Data Relevant to the Deferral for MSM

The following results became available by mid-2014, from the operational assessment and all three of the research studies recommended by the BOTS Working Group.

1. Operational Assessment

The operational assessment examined quarantine release errors. Such errors occur when a blood establishment accidentally releases a unit of blood that should not have been released due to issues with donor qualification or testing. It became clear at an FDA workshop held in September 2011 that HIV risk from quarantine release errors has been minimized effectively by increased use of computerized inventory management, with a remaining small risk of human errors. Following the workshop, a White Paper was produced by AABB on this topic which describes a number of measures that could be taken to characterize and prevent such errors (Ref. 29). Quarantine release errors currently appear to contribute minimally to the risk of HIV transmission through the blood supply (Ref. 30).

2. Donor History Questionnaire Study

The Donor History Questionnaire (DHQ) Study involved cognitive interviews with potential donors. After receiving donor education materials, the potential donors completed the donor history questionnaire, and were then interviewed regarding their responses (Ref. 31). The key result of this study, which was

[1] Purcell et al., have reported that the estimation of the MSM population as a percent of all males over 13 years differ by recall period: Past 1 year = 2.9%; past 5 years = 3.9%; and ever = 6.9%.

highly consistent for both individuals who only have sex with partners of the opposite sex and MSM, was that individuals respond to questions posed by the questionnaire as if they were answering the more general and subjective question in the self-assessed context of "is my blood safe," rather than providing an answer to the literal questions as asked. In addition, the study found that potential donors might have benefited from shorter donor education materials and the ability to answer "I don't know" to questions that currently accept only "yes" or "no" responses.

3. Retrovirus Epidemiology Donor Study-II (REDS II) Transfusion-Transmitted Retrovirus and Hepatitis Virus Rates and Risk Factors Study

The REDS-II Transfusion-Transmitted Retrovirus and Hepatitis Virus Rates and Risk Factors Study 2011-2013 was a pilot blood donor surveillance study that evaluated four viral markers (HBV, HCV, human T cell lymphotrophic virus (HTLV), and HIV) in just over 50% of the nation's blood supply (Ref. 32). It also determined behavioral risk factors that were associated with donations of blood that tested positive for one of these viruses compared with control donations. In addition to demonstrating the feasibility of conducting such a surveillance program, there were several key findings. These included the finding that for each of these viral infections, the primary behavioral risk factors were consistent with the known epidemiology for each infection in the United States and validated the current blood donor deferral criteria. Sex with an HIV-positive partner and a history of male-to-male sexual contact remained the two leading independent risk factors for HIV infection in blood donors as originally observed in CDC-funded studies from the early 1990's. Sex with an HIV-positive partner was associated with a 132-fold increase in risk (multivariate adjusted odds ratio) for being HIV-positive, and a history of male-to-male sexual contact was associated with a 62-fold increase in risk. By comparison, the increase in risk for a history of multiple sexual partners of the opposite sex in the last year was 2.3-fold.

4. Recipient Epidemiology and Donor Evaluation Study-III (REDS-III) Blood Donation Rules Opinion Study (BloodDROPS)

BloodDROPS examined the opinions of MSM regarding the blood donor deferral policy (Ref. 33) through web-based surveys of the MSM community and non-compliant MSM who donated blood. A key finding of particular note was that MSM, who comprise approximately 7% (Ref. 26) of the U.S. male population represent an estimated 2.6% of male blood donors. Although the data were determined by different methodologies, they suggest an increase in the proportion of blood donors reporting MSM behavior from 0.7% in 1993 and 1.7% in 1998. The qualitative responses by both donating and non-donating groups of MSM revealed that these individuals view the current policy as discriminatory and

stigmatizing, and that some individuals knowingly donate despite the deferral. When asked about shortening the deferral period, since last male-to-male sexual contact, the most common response was that one year was "acceptable as a compromise," especially if shorter periods might be considered after confirming the safety of the new policy. The web-based community survey revealed that approximately 90% of MSM think the MSM blood donation deferral should change, and 59% of MSM reported they would comply with a change to a one-year deferral. Of the 83 blood donors who reported male-to-male sexual contact, 50.6% reported that they would adhere to a one-year deferral and 18.4% reported "don't know." Among the subset of the 30 MSM blood donors who had reported male-to-male sexual contact in the past year, 19 (62%) reported that they would adhere to a future one-year deferral and 3 (10%) indicated that they "don't know" if they would adhere to a one-year deferral (Ref. 32).

The prevalence of HIV infection in male blood donors who reported that they were MSM was determined to be 0.25%, which is much lower than the estimated 11-12% HIV prevalence in the population of individuals reporting regular MSM behavior (Ref. 33). This indicates that considerable self-selection likely took place in individuals who presented to donate.

5. Supportive Data on Australian MSM Policy Change

Some epidemiologic data are available from countries that have changed their deferral policy for MSM (Refs. 34, 35). The most robust data measuring the impact of these policy changes are available from Australia (Ref. 36). Australia also has a voluntary blood donor system and has a similar percentage of men reporting male-to-male sexual contact at some time during their lives (5% compared with 7% in the United States (Ref. 26). During the five years before and five years after a change from a lifetime deferral to a one-year deferral in Australia, there was no change in risk to the blood supply, defined by the number of HIV positive donations per year and the proportion of HIV-positive donors with male-to-male sex as a risk factor. In addition, the compliance rate with the one-year MSM deferral among male donors in Australia following the policy change was >99.7% (Ref. 37). Of note, unlike in the U.S., donors in Australia must sign a declaration in the presence of blood center staff that they understand that there are penalties, including fines and imprisonment, for providing false or misleading information.

D. Considerations of the BOTS Working Group

Over the course of its deliberations, the BOTS Working Group reviewed and discussed several different options for the MSM policy:

- no change,
- change to a five-year deferral,

- change to a one-year deferral,
- change to a deferral less than one year,
- pre-testing of potential donors, and
- deferral based upon individual risk assessment.

Although not making a change would maintain the current level of safety of the blood supply, as noted above, there is evidence that the deferral policy is becoming less effective over time. In addition, the policy is perceived by some as discriminatory. The data that a five-year deferral would be safer than a one-year deferral are not compelling. However, some have argued that a five-year deferral would, in theory, add a safeguard by allowing time for intervention against an emerging infectious disease that might spread rapidly among MSM and be transmitted through blood transfusion. Sufficient data are not available to assess the effectiveness of selecting MSM with low HIV risk based on deferral times of less than one year since last exposure. The individual risk-based options were not determined to be viable options for a policy change at this time for a number of reasons: pretesting would be logistically challenging, and would likely also be viewed as discriminatory by some individuals, and individual risk assessment by trained medical professionals would be very difficult to validate and implement in our current blood donor system due to resource constraints. Additionally, the available epidemiologic data in the published literature do not support the concept that MSM who report mutual monogamy with a partner or who report routine use of safe sex practices are at low risk for HIV. Specifically, the rate of partner infidelity in ostensibly monogamous heterosexual couples and same-sex male couples is estimated to be about 25%, and condom use is associated with a 1 to 2% failure rate per episode of anal intercourse (Refs. 38, 39, 40, 41). In addition, the prevalence of HIV infection is significantly higher in MSM with multiple male partners compared with individuals who have only multiple opposite sex partners (Ref. 28).

Change to a one-year deferral is also supported by other evidence, including the experience in countries that have already changed their policies to a one-year deferral (Argentina, Australia, Brazil, Hungary, Japan, Sweden and United Kingdom). In addition, this change would potentially better harmonize the deferral for MSM with the one-year deferral in place for both men and women who engage in certain other sexual behaviors associated with an increased risk of HIV exposure (e.g., sex with an HIV-positive partner, sex with a commercial sex worker). Thus, following careful review, the BOTS Working Group was supportive of a policy change to a one-year deferral for MSM.

E. Outcome of HHS and FDA Advisory Committee Meetings

Following deliberation of the BOTS Working Group, two advisory committee meetings were held. The HHS Advisory Committee on Blood and Tissue Safety and Availability (ACBTSA) met on November 13, 2014, to review the MSM deferral policy (Ref. 33). The scientific information described in sections II.C. and D. was presented to the

ACBTSA members along with the BOTS Working Group recommendation. Additionally, the meeting included an open public hearing session. The Committee voted 16 to 2 to recommend a policy change to a one-year MSM deferral. It also recommended that this change be accompanied by establishment of a robust system to monitor the safety of the blood supply and a communication plan on the policy change targeted to all stakeholders.

Subsequently, on December 2, 2014, the FDA BPAC met to consider measurement of HIV incidence in blood donors as an additional method to assess transfusion risk, and the potential value of laboratory tests to detect recently acquired HIV infections in seropositive donors as part of a Transfusion Transmissible Infections Monitoring System (TTIMS) (Ref. 42). An open public hearing was also held. At that meeting, FDA noted that it intended to establish a general program to monitor the safety of the blood supply in collaboration with the National Heart, Lung and Blood Institute (NHLBI), which could monitor for a number of different transfusion-transmitted viral infections. FDA also noted that it intended to engage in public discussions of issues such as enhancements to education about the donation of safe blood and further evaluation of the effectiveness of the blood donor history questionnaire. In their comments, some BPAC committee members indicated support for a change in MSM deferral policy to one year, and most members noted that they considered concomitant establishment of a blood donor monitoring program a prerequisite for any policy change. On the topic of testing for recency of HIV infection, several BPAC committee members commented that tests looking at how recently HIV infection had been (recency tests[2]) could potentially be very useful additions to the established measures of incidence for monitoring the safety of the blood supply.

F. Status of Other Deferral Categories

In addition to the behavioral deferrals noted for MSM, CSW and IDU, the 1992 blood memo addressed several other deferrals that had been recommended in order to reduce the risk of HIV transmission through the blood supply (Ref. 1). For most of these deferrals, directly applicable data are not available at this time to support a change in the existing deferral policies. In the case of the deferral for persons with hemophilia or related clotting disorders who have received clotting factor concentrates, the rationale for deferral has changed from prevention of HIV transmission to that of ensuring that donors are not harmed by the use of large bore needles used during the donation process. While

[2] HIV recency tests typically involve detailed assessment of the strength and characteristics of antibody profiles that develop and change over time in response to HIV infection. Thus, it appears to be technically feasible that a serologically-based HIV recency test, once validated in a blood donor setting, could reflect a high likelihood that an HIV infection occurred within a certain interval of time (e.g., in the past six months). While such tests are not yet FDA-approved for this purpose, this additional measure of new HIV infection may increase the statistical power to assess whether HIV incidence in the blood donor pool changed significantly after a change in the deferral recommendations.

21 CFR 640.3(c)(3) currently requires deferral for receipt of any derivative of human blood which the FDA has advised is a possible source of viral hepatitis, given the enhanced safety measures now used in the manufacture of clotting factor concentrates (Ref. 16), FDA does not consider the receipt of FDA-licensed clotting factor concentrates to be a risk factor for hepatitis. Further, FDA has not recommended a deferral for the receipt of other FDA-licensed plasma-derivatives because of HIV or hepatitis risk[3], and we intend to consider revisions to the current regulations.

III. RECOMMENDATIONS

The following sections summarize the revised recommendations related to blood donor deferral and requalification related to reducing the risk of HIV transmission by blood and blood products. Given the passage of time, and in order to simplify practical application of these criteria for donors and blood collection establishments, reference to "since 1977" present currently for some criteria has been dropped as the period of time during which individuals are assessed to be at risk of transmitting HIV.

A. Donor Education Material and Donor History Questionnaire

1. We recommend that donors be provided donor education material before each donation explaining the risk of HIV transmission by blood and blood products, certain behaviors associated with the risk of HIV infection, and the signs and symptoms associated with HIV infection, so that donors can self-defer. The donor education material should be presented to donors in a manner they will understand, which may include oral, written, or multimedia formats. The donor education material should instruct the donor not to donate when a risk factor for HIV infection or signs or symptoms of HIV infection are present. The donor education material should indicate that individuals who have engaged in any activity or who have any risk factor that would result in a deferral (see section III.B. of this guidance) should not donate blood or blood components.

2. We recommend that blood collection establishments update their donor education material, DHQ, including full-length and abbreviated DHQs, and accompanying materials (e.g., flow charts) and processes to incorporate the recommendations provided in this guidance.

3. We recommend that the updated DHQ include the following elements to assess donors for risk:

[3] Consistent with the donor history questionnaires and accompanying materials prepared by AABB and Plasma Protein Therapeutics Association (PPTA) and found acceptable by FDA, a voluntary donor deferral exists for the receipt of Hepatitis B Immune Globulin because the donor had been recently exposed to hepatitis B virus.

i. A history ever of a positive[4] test for HIV,

ii. A history ever of exchanging sex[5] for money or drugs,

iii. A history ever of non-prescription injection drug use[6],

iv. A history in the past 12 months of sex with a person with a positive test for HIV, a history of exchanging sex for money or drugs, or a history of non-prescription injection drug use,

v. A history in the past 12 months of receiving a transfusion of Whole Blood or blood components such as packed red blood cells, platelets, or plasma,

vi. A history in the past 12 months of contact with blood of another individual through percutaneous inoculation such as a needle stick or through contact with a donor's open wound or mucous membranes,

vii. A history in the past 12 months of a tattoo, ear or body piercing,

viii. A history in the past 12 months of syphilis or gonorrhea, or treatment for syphilis or gonorrhea,

ix. For male donors: a history in the past 12 months of sex with another man,

x. For female donors: a history in the past 12 months of sex with a man who has had sex with another man.

Note: In the context of the donor history questionnaire, male or female gender is taken to be self-identified and self-reported. In instances where a donor has asserted a change in gender identification, medical directors may exercise discretion with regard to donor eligibility.

[4] In this context, "positive" includes positive test results on an HIV diagnostic assay and repeatedly reactive or reactive results on antibody or NAT blood donor screening assays, respectively.

[5] Throughout this guidance the term "sex" refers to having anal, oral, or vaginal sex, regardless of whether or not a condom or other protection is used.

[6] Non-prescription injection drug use includes not only the injection of non-prescription drugs, but also includes the improper injection of legally-prescribed drugs, such as injecting a prescription drug intended for oral administration or injecting a prescription drug that was prescribed for another individual.

B. Donor Deferral

We recommend that you defer as follows:

1. Defer indefinitely an individual who has ever had a positive test for HIV.
2. Defer indefinitely an individual who has ever exchanged sex for money or drugs.
3. Defer indefinitely an individual who has ever engaged in non-prescription injection drug use.
4. Defer for 12 months from the most recent contact any individual who has a history of sex with a person who: has ever had a positive test for HIV, ever exchanged sex for money or drugs, or ever engaged in non-prescription injection drug use.
5. Defer for 12 months from the most recent transfusion any individual who has a history of receiving a transfusion of Whole Blood or blood components.
6. Defer for 12 months from the most recent exposure, any individual who has a history of contact with blood of another individual through percutaneous inoculation such as a needle stick or through contact with a donor's open wound or mucous membranes.
7. Defer for 12 months from the most recent tattoo, ear or body piercing, an individual who has a history of tattoo, ear or body piercing. However, individuals who have undergone tattooing within 12 months of donation are eligible to donate if the tattoo was applied by a state regulated entity with sterile needles and non-reused ink. Individuals who have undergone ear or body piercing within 12 months of donation are eligible to donate if the piercing was done using single-use equipment.
8. Defer for 12 months after completion of treatment, an individual with a history of syphilis or gonorrhea, or an individual with a history of diagnosis or treatment for syphilis or gonorrhea in the past 12 months.
9. Defer for 12 months from the most recent contact, a man who has had sex with another man during the past 12 months.
10. Defer for 12 months from the most recent contact, a female who has had sex during the past 12 months with a man who has had sex with another man.

We recommend that you defer indefinitely an individual with hemophilia or related clotting factor deficiencies requiring treatment with clotting factor concentrates for reasons of donor safety, rather than based upon the risk of HIV infection.

Note: Additional recommendations for donor deferral to reduce the risk of HIV transmission by blood and blood products have been established in other FDA guidance documents, including:

- "Guidance for Industry: Recommendations for Screening, Testing, and Management of Blood Donors and Blood and Blood Components Based on Screening Tests for Syphilis," dated September 2014;
- "Guidance for Industry - Recommendations for Management of Donors at Increased Risk for Human Immunodeficiency Virus Type 1 (HIV-1) Group O Infection," dated August 2009; and,
- "Memorandum to All Registered Blood Establishments - Recommendations for the Deferral of Current and Recent Inmates of Correctional Institutions as Donors of Whole Blood, Blood Components, Source Leukocytes, and Source Plasma," dated June 8, 1995.

Note: Collections from donors at risk of HIV infection must be approved by CBER consistent with the "Guideline for Collection of Blood or Blood Products from Donors with Positive Tests for Infectious Disease Markers ("High Risk" Donors)," dated September 1989.

C. Donor Requalification

1. Donors deferred because of a history of sex during the past 12 months with any of the following individuals: a person who has a positive test for HIV; a person with a history of exchanging sex for money or drugs; or a person with a history of non-prescription injection drug use, may be eligible to donate provided that 12 months since the last contact have passed and they meet all other donor eligibility criteria.
2. Donors deferred because of a history of receiving a transfusion of Whole Blood or blood components such as packed red blood cells, platelets, or plasma during the past 12 months may be eligible to donate if 12 months have passed since their last transfusion and they meet all other donor eligibility criteria.
3. Donors deferred because of a history of contact with blood of another individual through percutaneous inoculation such as a needle stick or through contact with a donor's open wound or mucous membranes during the past 12 months may be eligible to donate if 12 months have passed since their last exposure and they meet all other donor eligibility criteria.
4. Donors deferred because of a history of tattoo, ear or body piercing in the past 12 months may be eligible to donate if 12 months have passed since their last tattoo, ear or body piercing and they meet all other donor eligibility criteria.

5. Donors deferred because of a history of syphilis or gonorrhea, or treatment for syphilis or gonorrhea in the past 12 months may be eligible to donate if 12 months have passed since diagnosis and completion of treatment and they meet all other donor eligibility criteria.
6. Male donors previously deferred because of a history of sex with another man, even one time, since 1977, may be eligible to donate provided that they have not had sex with another man during the past 12 months and they meet all other donor eligibility criteria
7. Male donors deferred because of a history of sex with another man in the past 12 months may be eligible to donate provided they have not had sex with another man during the past 12 months and they meet all other donor eligibility criteria.
8. Female donors deferred because of a history of sex in the past 12 months with a man who has had sex with another man may be eligible to donate provided that during the past 12 months they have not had sex with a man who has had sex with another man and they meet all other donor eligibility criteria.

D. Product Retrieval and Quarantine; Notification of Consignees of Blood and Blood Components

If you collected blood or blood components from a donor who tests reactive for HIV on that donation, or when you are made aware of other reliable test results or information indicating evidence of HIV infection, you must follow the HIV "lookback" requirements in 21 CFR 610.46.

In addition, we recommend that you take the following actions if you determine that blood or blood components have been collected from a donor who should have been deferred according to the recommendations in section III.B. 2-10 of this guidance, for reasons other than a positive HIV test result.

1. If you collected blood or blood components from a donor who should have been deferred according to the recommendations in section III.B. of this guidance, we recommend that you quarantine and destroy any undistributed in-date blood or blood components collected from that donor.

2. If you distributed blood or blood components collected from a donor who should have been deferred according to the recommendations in section III.B. of this guidance, we recommend that you notify consignees of all blood and blood components. We recommend that the consignee retrieve and quarantine the in-date blood and blood components collected from that donor. We do not recommend retrieval and quarantine of plasma pooled for further

manufacturing into products that are manufactured under processes that include validated viral clearance steps, which have been shown to be robust in the clearance of lipid-enveloped viruses.

E. Product Disposition and Labeling

1. We recommend that you destroy or re-label blood or blood components that were collected from a donor who should have been deferred based on risk factors for HIV infection in accordance with the recommendations in section III.B. of this guidance. If you re-label the blood or blood components as described in this section, they may be released for research or for manufacture into noninjectable products or in vitro diagnostic reagents when no other suitable sources are available.

    a. You must use the following statement to prominently re-label the blood or blood components originally collected for transfusion in accordance with 21 CFR 606.121(f):

        "NOT FOR TRANSFUSION: Collected From a Donor Determined To Be At Risk For Infection With HIV"

        In addition, you should include one of the following cautionary label statements, as applicable:

        "Caution: For Laboratory Research Only"

        or

        "Caution: For Further Manufacturing into In Vitro Diagnostic Reagents For Which There Are No Alternative Sources"

        or

        "Caution: For Use in Manufacturing Noninjectable Products Only"

        And, for recovered plasma[7]:

        "Not for Use in Products Subject to License Under Section 351 of the Public Health Service Act"

[7] See FDA Compliance Policy Guide Sec 230.100 for the definition of recovered plasma. http://www.fda.gov/ICECI/ComplianceManuals/CompliancePolicyGuidanceManual/ucm073861.htm

b. You should use the following statements to prominently re-label the un-pooled blood or blood components originally collected or intended for further manufacture:

"Collected from a Donor Determined to be at Risk for Infection with HIV"

And

"Caution: For Laboratory Research Only"

or

"Caution: For Further Manufacturing into In Vitro Diagnostic Reagents For Which There Are No Alternative Sources"

or

"Caution: For Use in Manufacturing Noninjectable Products Only"

And, for recovered plasma:

"Not for Use in Products Subject to License Under Section 351 of the Public Health Service Act"

2. You must destroy or re-label blood or blood components, including Source Plasma, collected from a donor who currently tests reactive for HIV or collected from a donor deferred for reactive HIV testing (21 CFR 610.40(h)). If you re-label the blood or blood components, including Source Plasma, in accordance with 21 CFR 610.40(h) and 606.121, the blood or blood components may be released for research or for manufacture into noninjectable products or in vitro diagnostic reagents when no other suitable sources are available. You must label the reactive unit with the "BIOHAZARD" legend (21 CFR 610.40(h)(2)(ii)(B)), and:

a. You must use the following statement to prominently re-label the blood or blood components originally collected for transfusion (21 CFR 606.121(f)):

"NOT FOR TRANSFUSION: Collected From a Donor Determined To Be Reactive for HIV"

In addition, you should use one of the following cautionary label statements, as applicable:

"Caution: For Laboratory Research Only"

or

"Caution: For Further Manufacturing into In Vitro Diagnostic Reagents For Which There Are No Alternative Sources"

or

"Caution: For Further Manufacturing Use as a Component of a Medical Device For Which There Are No Alternative Sources"

b. You must use the following statement to prominently re-label the un-pooled blood or blood components, including Source Plasma, originally collected or intended for further manufacture (21 CFR 610.40(h)(2)(ii)(C)):

"Collected from a Donor Determined to be Reactive for Infection with HIV"

In addition, you should use one of the following cautionary label statements, as applicable:

"Caution: For Laboratory Research Only"

or

"Caution: For Further Manufacturing into In Vitro Diagnostic Reagents For Which There Are No Alternative Sources"

or

"Caution: For Further Manufacturing Use as a Component of a Medical Device For Which There Are No Alternative Sources"

F. Biological Product Deviation Reporting

If you have distributed blood or blood components for transfusion or for further manufacturing, collected from a donor who should have been deferred according to section III.B. of this guidance, you should report a biological product deviation as soon as possible, but you must report within 45 calendar days from the date you acquire the information reasonably suggesting that a reportable event has occurred (21 CFR 606.171).

G. Testing Requirements and Considerations

Section 610.40(a) (21 CFR 610.40(a)) requires establishments that collect blood or blood components to test each donation intended for use in preparing a product, for evidence of infection due to HIV type 1 (HIV-1) and HIV type 2 (HIV-2). In addition, 21 CFR 610.40(b) requires you to use one or more approved screening test as necessary to reduce adequately and appropriately the risk of transmission of HIV-1 and HIV-2. FDA has considered the use of approved donor screening tests for antibodies to both HIV-1 and HIV-2 as necessary to reduce adequately and appropriately the risk of transmission of HIV. In addition, FDA recommendations on the use of approved HIV-1 nucleic acid donor screening tests to meet the requirements under 21 CFR 610.40(b) are found in, "Guidance for Industry: Use of Nucleic Acid Tests on Pooled and Individual Samples from Donors of Whole Blood and Blood Components (including Source Plasma and Source Leukocytes) to Adequately and Appropriately Reduce the Risk of Transmission of HIV-1 and HCV," dated October 2004.

You must defer a donor who tests reactive by a donor-screening test for HIV-1 or HIV-2 (21 CFR 610.41), you must perform a supplemental (additional, more specific) test on donations that test reactive on a screening test (21 CFR 610.40(e)), and you must make reasonable attempts to notify a donor who has been deferred based on the results of tests for communicable diseases (21 CFR 630.6). Where appropriate, donors who are deferred because of reactive test results should be provided information about the need for medical follow-up and counseling.

Current FDA recommendations are found in "Guidance for Industry: Nucleic Acid Testing (NAT) for Human Immunodeficiency Virus Type 1 (HIV-1) and Hepatitis C Virus (HCV): Testing, Product Disposition, and Donor Deferral and Reentry, dated May 2010." In addition, for the purpose of donor counseling, if a donation tests repeatedly reactive for antibodies to HIV-1/HIV-2 or for HIV-2 on an approved donor screening test, but HIV-1 positivity is not confirmed on an approved supplemental test, further testing may be performed using licensed or approved tests to diagnose HIV-2 infection and clarify the donor's infection status.

IV. IMPLEMENTATION

You may implement the recommendations once you have revised your donor education material, DHQ, including full-length and abbreviated DHQs, and accompanying materials to reflect the new donor deferral recommendations. Licensed blood establishments must report the indicated revisions to FDA in the following manner (21 CFR 601.12):

1. Revision of your donor education materials, DHQ and accompanying materials must be submitted to FDA as a prior approval supplement (PAS) under 21 CFR 601.12(b). Revision of a previously FDA accepted DHQ and accompanying materials must be reported as a major change if you are revising the FDA accepted DHQ and accompanying materials to implement these new recommendations. Report such a change to FDA as a prior approval supplement (PAS) under 21 CFR 601.12(b).

2. If the current version of the donor educational materials, DHQ and accompanying materials prepared by the AABB Donor History Task Force or PPTA are revised to contain the recommendations in this guidance and are found acceptable by FDA, we would consider the implementation of the donor education materials, DHQ and accompanying materials to be minor changes, if implemented without modification and in their entirety as a complete process for administering questions to donors. Report such a change to FDA in your annual report under 21 CFR 601.12(d), noting the date the process was implemented.

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**2015年1月改訂（第9版）D10
*2014年9月改訂

日本標準商品分類番号
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血漿分画製剤 献血

* 特定生物由来製品
処方箋医薬品注)

ハプトグロビン静注2000単位「ベネシス」

生物学的製剤基準 人ハプトグロビン

Haptoglobin I.V. 2000 units-BENESIS

承認番号	22000AMX02342
薬価収載	1985年12月
販売開始	1986年6月
再審査結果	1992年12月

貯　法：凍結を避け10℃以下に保存
有効期間：検定合格の日から2年（最終有効年月日は瓶ラベル及び外箱に表示）
* 注）注意－医師等の処方箋により使用すること

本剤は，貴重な人血液を原料として製剤化したものである．原料となった血液を採取する際には，問診，感染症関連の検査を実施するとともに，製造工程における一定の不活化・除去処理を実施し，感染症に対する安全対策を講じているが，人血液を原料としていることによる感染症伝播のリスクを完全に排除することはできないため，疾病の治療上の必要性を十分に検討の上，必要最小限の使用にとどめること．（「使用上の注意」の項参照）

【禁忌】（次の患者には投与しないこと）
本剤の成分に対しショックの既往歴のある患者

【原則禁忌】（次の患者には投与しないことを原則とするが，特に必要とする場合には慎重に投与すること）
本剤の成分に対し過敏症の既往歴のある患者

【組成・性状】

有効成分〔1瓶(100mL)中〕	人ハプトグロビン	2,000単位※
添加物〔1瓶(100mL)中〕	塩化ナトリウム	0.9g
	水酸化ナトリウム	適量
	塩酸	適量
性状・剤形	本剤は1mL中に人ハプトグロビン20単位を含有する黄褐色の澄明な液剤である．	
pH	6.0～7.5	
浸透圧比	約1（生理食塩液に対する比）	
備考	人ハプトグロビンは，ヒト血液に由来する．（採血国：日本，採血の区別：献血）	

※1単位は1mgのヘモグロビンを結合する．
本剤は，製造工程（不溶化ヘパリンによる吸着処理）で，ブタ小腸粘膜由来成分（ヘパリン）を使用している．

【効能・効果】

熱傷・火傷，輸血，体外循環下開心術などの溶血反応に伴うヘモグロビン血症，ヘモグロビン尿症の治療

【用法・用量】

通常，成人では1回4,000単位を緩徐に静脈内に点滴注射するか，体外循環時に使用する場合は灌流液中に投与する．
症状により適宜反復投与する．
年齢，体重により適宜増減する．

（参考）小児に対する投与量は，通常1回2,000単位を目安とすること．

〈用法・用量に関連する使用上の注意〉
急速な注入により，血圧降下を起こすことがあるので，注射速度をできるだけ緩徐にすること．

【使用上の注意】

1．慎重投与（次の患者には慎重に投与すること）

(1) ハプトグロビン欠損症の患者〔過敏反応を起こすおそれがある．〕
(2) IgA欠損症の患者〔抗IgA抗体を保有する患者では過敏反応を起こすおそれがある．〕
(3) 肝障害のある患者〔ハプトグロビン-ヘモグロビン複合体は肝臓で処理されるため，肝臓に負担がかかるおそれがある．〕
(4) 溶血性・失血性貧血の患者〔ヒトパルボウイルスB19の感染を起こす可能性を否定できない．感染した場合には，発熱と急激な貧血を伴う重篤な全身症状を起こすことがある．〕
(5) 免疫不全患者・免疫抑制状態の患者〔ヒトパルボウイルスB19の感染を起こす可能性を否定できない．感染した場合には，持続性の貧血を起こすことがある．〕

2．重要な基本的注意

患者への説明：本剤の使用にあたっては，疾病の治療における本剤の必要性とともに，本剤の製造に際し感染症の伝播を防止するための安全対策が講じられているが，血液を原料としていることに由来する感染症伝播のリスクを完全に排除することができないことを，患者に対して説明し，理解を得るよう努めること．

(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．
1) 血漿分画製剤の現在の製造工程では，ヒトパルボウイルスB19等のウイルスを完全に不活化・除去すること

が困難であるため，本剤の投与によりその感染の可能性を否定できないので，投与後の経過を十分に観察すること．

2) 現在までに本剤の投与により変異型クロイツフェルト・ヤコブ病（vCJD）等が伝播したとの報告はない．しかしながら，製造工程において異常プリオンを低減し得るとの報告があるものの，理論的なvCJD等の伝播のリスクを完全には排除できないので，投与の際には患者への説明を十分行い，治療上の必要性を十分検討の上投与すること．

3．副作用

総症例数2,483例中3例(0.12%) 3件の副作用が報告されている．副作用は，血圧降下2件(0.08%)，嘔吐1件(0.04%)であった．(再審査終了時)

＊＊(1)重大な副作用

ショック(0.1%未満)，アナフィラキシー(頻度不明)：ショック，アナフィラキシーがあらわれることがあるので，観察を十分に行い，呼吸困難，喘鳴，胸内苦悶，血圧低下，脈拍微弱，チアノーゼ等が認められた場合には投与を中止し，適切な処置を行うこと．

(2)その他の副作用

下記のような症状があらわれることがあるので，観察を十分に行い，発現した場合には，適切な処置を行うこと．

種類＼頻度	0.1%未満	頻度不明
過敏症注)		発疹，蕁麻疹
消化器	嘔吐	

注)このような場合には投与を中止し，適切な処置を行うこと．

4．高齢者への投与

一般に高齢者では生理機能が低下しているので，患者の状態を観察しながら慎重に投与すること．

5．妊婦，産婦，授乳婦等への投与

妊婦又は妊娠している可能性のある婦人には，治療上の有益性が危険性を上回ると判断される場合にのみ投与すること．〔妊娠中の投与に関する安全性は確立していない．本剤の投与によりヒトパルボウイルスB19の感染の可能性を否定できない．感染した場合には胎児への障害(流産，胎児水腫，胎児死亡)が起こる可能性がある．〕

6．小児等への投与

低出生体重児，新生児に対する安全性は確立していない．

7．臨床検査結果に及ぼす影響

本剤には原料血漿由来のコリンエステラーゼが含まれており，投与後にコリンエステラーゼ活性値が上昇することがあるので，臨床診断に際しては注意すること．

8．適用上の注意

(1) 混濁しているものを投与してはならない．

(2) 輸液と混じて点滴する場合，輸液はpH5.0～10.5のものを使用すること．

(3) 使用後の残液は再使用しないこと．

【薬物動態】

〈参考〉動物における薬物動態(ラット)

Wistar系ラットにおける静脈内1回投与では，血中半減期は20時間，人ハプトグロビン-ラットヘモグロビン複合体は4.4時間であった．

5回連続投与では蓄積性は認められなかった．臓器内分布は，1回投与では，膵臓以外では血液と同様の減衰曲線を描いた．膵臓では初期いくぶん高い値を示すが，その後急激に低下し，24時間後には，肝，腎の1/2まで減少し，以後は他の臓器と同じ勾配で減少した．人ハプトグロビン-ラットヘモグロビン複合体投与群では，心臓，肺臓，脳，骨髄では血液と同じ減衰曲線を描いたが，肝臓，腎臓，膵臓では緩慢な減衰を示した[1]．

【臨床成績】

〈血色素尿に対する臨床成績〉

高度の溶血のために血色素尿を呈し，腎障害を併発する危険性のある症例159例，すなわち熱傷，火傷，輸血後溶血，溶血性疾患，体外循環下開心術など159例を対象とした臨床試験において，血色素尿の消失効果で判定した有効率は89.3%(142/159)であった[2]．なお，疾患別血色素尿に対する臨床成績は次のとおりであった．

	有効率
熱傷・火傷	83.9%(52/62)
体外循環	97.5%(78/80)
輸血及び溶血性疾患	100%(10/10)

投与量は成人(16歳以上)では大多数の症例が1回4,000単位を用いており，臨床効果も良好であった．15歳以下では乳児を含め1回2,000単位を用いた症例が多かった．なお，高度の溶血を伴う症例で，投与したハプトグロビンが消費され再度遊離ヘモグロビンが増加している場合には，必要に応じて反復投与がなされている症例もあった．また，市販後に実施された臨床研究会での成績は，重症熱傷[3]で90.5%(19/21)，体外循環下開心術[4]で84.6%(22/26)，食道静脈瘤硬化療法[5]で100%(21/21)の有効率であった．

【薬効薬理】

1．溶血モデルに対するハプトグロビンの効果(家兎)[6~8]

正常家兎に家兎^{59}Fe-ヘモグロビン(Hb)単独投与群，家兎^{59}Fe-ヘモグロビンと人ハプトグロビン(Hb-Hp)の混合液投与群を比較した結果，Hb-Hp投与群では，腎へのヘモグロビンの取り込み及び沈着が軽減され，病理所見においても異常が認められなかった．

また，Hb-Hp投与群では血色素尿の消失，尿量の確保，腎機能が保持されていた．

2．溶血液とエンドトキシンによる溶血モデルに対するハプトグロビンの効果(イヌ)[9]

イヌにエンドトキシンを投与し前処理を行った後，生理食塩液を投与した群(第1群)，溶血液と生理食塩液を同時に投与した群(第2群)，溶血液とハプトグロビンを同時に投与した群(第3群)について比較検討した．その結果，第2群では尿量やクレアチニンクリアランスなどを指標とした腎機能低下が顕著に認められたが，第3群では第2群に比べ，腎機能低下が抑制された．

【取扱い上の注意】

記録の保存：本剤は特定生物由来製品に該当することから，本剤を投与した場合は，医薬品名（販売名），その製造番号（ロット番号），投与した日，投与を受けた患者の氏名，住所等を記録し，少なくとも20年間保存すること．

【包　装】

ハプトグロビン静注2000単位「ベネシス」　100mL　1瓶

【主要文献及び文献請求先】

1．主要文献

1) 日本血液製剤機構：内部資料(ヒト・ハプトグロビンのラット静脈内投与後における体内分析，代謝および排泄)
2) 大城　孟　他：基礎と臨床 1984；18(11)：5913-5940
3) 太田宗夫　他：救急医学 1992；16(13)：1813-1819
4) 川島淳宏　他：基礎と臨床 1992；26(3)：1197-1205
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9) 矢野賢一　他：応用薬理 1987；33(6)：949-973

2．文献請求先

主要文献に記載の内部資料につきましても下記にご請求下さい．

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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
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販売名(企業名)	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) 赤血球液-LR「日赤」(日本赤十字社) 照射赤血球液-LR「日赤」(日本赤十字社)			オーストラリア

研究報告の概要

○輸血伝播ロスリバーウイルス(RRV)感染の初報告症例
2014年5月、オーストラリア赤十字血液センターは、2014年3月に供血した2日後、疲労と関節痛を発症し、急性RRV感染症と診断された供血者から献血後情報を入手した。
当該供血者からの赤血球製剤は、2014年3月12日に骨髄異形成症候群の患者に輸血されていた。5月28日に行われた受血者の血清学的検査では、間接蛍光抗体法によりRRV IgM抗体が検出され、赤血球凝集抑制試験で高力価(抗体価＞1:640)であることが分かった。これらの結果から、この数カ月間でのRRV感染であると考えられた。RT-PCRではRRV RNAは陰性であった。
当該供血者の供血時保管検体の解析を行ったところ、RRV IgM及びIgG抗体は陰性であったが、2領域のRT-PCRによりRRV RNAが検出され、配列決定によりRRVと確認された。これらの結果は、セロコンバージョン前の一時的なRRVウイルス血症期中における供血であったことを示している。
2013年7月1日～2014年6月30日の間、受血者の在住する地域におけるRRV感染症の報告は受血者以外になく、また、受血者は主に室内で過ごし、蚊に刺された覚えもないことから、受血者は輸血によりRRVに感染したと考えられる。これは、輸血伝播RRV感染の初の報告である。

報告企業の意見	今後の対応
輸血によりロスリバーウイルスが伝播した初の報告である。	日本赤十字社では、輸血感染症対策として受付時に海外滞在歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、発熱などの体調不良者を献血不適としている。さらに献血者に対し、献血後に何らかの病気や感染症に罹っていると分かった場合は連絡をいただけるよう記載したお願い文を配布している。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

赤血球濃厚液-LR「日赤」
照射赤血球濃厚液-LR「日赤」
赤血球液-LR「日赤」
照射赤血球液-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

First reported case of transfusion-transmitted Ross River virus infection

We describe the first documented case of Ross River virus (RRV) infection transmitted by blood transfusion. The recipient had a clinically compatible illness, and RRV infection was confirmed by serological tests. The implicated donation was positive for RRV RNA. We discuss the risk to blood recipients and the implications for blood donation in Australia.

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Clinical record

In May 2014, the Australian Red Cross Blood Service (the Blood Service) in Western Australia received a delayed notification from a donor who had developed fatigue and arthralgia 2 days after giving blood in March 2014 and was subsequently diagnosed with acute Ross River virus (RRV) infection (Box).

PathWest Laboratory Medicine WA detected RRV IgM antibodies using an inhouse indirect immunofluorescence antibody (IFA) test, but no RRV antibodies were detected using an inhouse haemagglutination inhibition (HI) antibody test 10 days after blood donation. RRV IgM antibodies are detected by IFA testing within a few days of onset of illness and routinely persist for several weeks or, occasionally, months or years. IFA tests are less prone to false-positive results compared with enzyme immunoassays. The HI antibody test primarily detects IgG antibodies, which appear within several weeks but after the IgM response.

Blood Service procedure stipulates that donors with a diagnosed RRV infection are unable to donate fresh components for 4 weeks after recovery. Moreover, fresh components donated from 4 weeks before illness onset to 4 weeks after recovery must be recalled.

The components from the implicated donation were identified: the red blood cell (RBC) component had been transfused to a patient on 12 March 2014, the plasma had been pooled for the manufacture of plasma-derived products and the platelet component had not been used. The treating clinician of the RBC recipient was notified as part of the recall procedure.

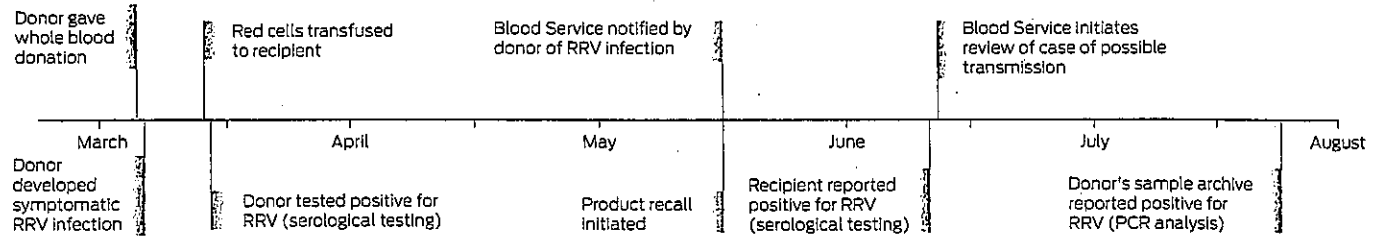
The recipient was having regular blood transfusions due to myelodysplastic syndrome that was associated with chronic fatigue and joint pains. The recipient reported a worsening of symptoms in the months after transfusion of the infected blood; however, there was not a clear exacerbation of these symptoms consistent with the incubation period of RRV.

"the Blood Service is taking steps to strengthen its messaging to donors regarding development of post-donation illnesses"

On notification from the Blood Service, the recipient's treating clinician requested serological testing for RRV on 28 May 2014, which found detectable IgM antibodies using the IFA assay and a high titre of antibodies by HI testing (antibody titre, >1:640). The detection of both IgM and HI antibodies indicates RRV infection in recent months. Previous testing for RRV IgM and HI antibodies in 2006 and August 2013 had been negative. Subsequent inhouse reverse transcriptase polymerase chain reaction (RT-PCR) analysis for RRV RNA performed on stored serum from 28 May gave a negative result. These results are consistent with RRV illness several months before 28 May, with resolution of the transient viraemic phase. No samples from the recipient in March 2014 were available for serological or PCR testing.

In response to this possible case of transfusion-transmitted RRV, the associated archived donor sample was retrieved and sent to the Victorian Infectious Diseases Reference Laboratory for RRV serological tests and RT-PCR analysis. This sample tested negative for RRV IgM and IgG but RRV RNA was detected by two inhouse RT-PCR tests and verified by sequencing. These results are consistent with the blood donation being collected

Timeline of major events related to the case of Ross River virus (RRV) transfusion transmission, 2014



PCR = polymerase chain reaction. ✦

during the pre-seroconversion but transient viraemic phase of RRV illness.

Discussion

Since the isolation of RRV from humans was first reported in 1972,[1] our understanding of the epidemiology of the disease has increased considerably. RRV is now known to be the most common mosquito-borne disease of humans in Australia,[2] and is endemic in several regions. An average of around 5000 cases of RRV disease are notified annually in Australia, with considerable yearly, seasonal and regional variability due to differences in environmental conditions that affect the mosquito vectors and native animal hosts of the virus.[2] The incubation period averages 7 to 9 days with a range of 2 to 21 days.[3] Symptoms of RRV most commonly include joint manifestations, which are usually symmetrical and acute in onset, with rash and fever being less common.[3] As many as 55% to 75% of RRV infections are asymptomatic.[3]

Arboviruses such as dengue viruses and West Nile virus are known to be transfusion transmissible,[4] and the potential of RRV to be transfusion transmissible was raised in this Journal in 1995.[5]

Although not previously documented, transfusion transmission of RRV has been considered theoretically possible, given a likely period of asymptomatic viraemia before the onset of symptoms.[4,6] This risk is supported by the observation of asymptomatic viraemia typically lasting 5 days after RRV infection in a mouse model.[7] On the assumption that transfused blood could transmit RRV, this study estimated the risk of RRV transfusion transmission during a 2004 outbreak in Cairns as one in 13542 donations. This risk was of the same order of magnitude as that estimated for dengue virus transmission by transfusion during a contemporaneous dengue fever outbreak in Cairns.[7]

The donor we describe developed an illness clinically compatible with RRV infection 2 days after donating blood and was shown to have a serological profile consistent with acute RRV infection 10 days after donating. The donated blood was subsequently shown to contain RRV RNA by two inhouse RT-PCR tests, and this was confirmed by sequencing.

While the exacerbation of the chronically ill recipient's fatigue and muscle and joint pains was not clearly consistent with the incubation period of RRV subsequent to the transfusion, the results of RRV serological tests performed about 2 months after transfusion were consistent with infection within this 2-month period. Unfortunately, there were no stored blood specimens collected from the recipient shortly after receiving the blood donation, and hence it was not possible to compare sequences with the donor virus to confirm transmission.

Surveillance by the WA Department of Health showed that the recipient was the only person for whom RRV infection was reported between 1 July 2013 and 30 June 2014 from the local government area in which she resided. The recipient also spent most of her time indoors and could not recall being bitten by mosquitoes. Taken together, these lines of evidence strongly support the likelihood that the recipient's RRV infection was transmitted by transfusion. Thus, this is the first report of transfusion-transmitted RRV.

Laboratory testing for RRV is not done for Australian blood donors during the donation process, and there is no validated blood screening test for RRV. To manage the risk of transfusion transmission, the Blood Service does not permit donors with symptoms compatible with RRV to donate until they are fully recovered. However, given that most RRV infections are asymptomatic and viraemia is present during the incubation period, excluding donors based on symptoms will not prevent all potentially infectious donations entering the blood supply. Provided infected donors report subsequent illness immediately to the Blood Service, the recall process should prevent the proportion of donations from symptomatic RRV infected donors from being used. Unfortunately, in this case, where notification was delayed for 2 months, the blood component had already been transfused. In response to this, the Blood Service is taking steps to strengthen its messaging to donors regarding development of post-donation illnesses.

In 2012, the Blood Service established a sample archive of every blood donation to meet regulatory standards and assist in investigation and lookback (tracing and notifying patients who may have received infected blood components and investigating donations and donors when a patient has a suspected transfusion-transmissible infection). This archive provides the ability to perform further testing on samples from past donations, as in this case, providing data on the actual risk associated with transfused donations from implicated donors and for investigations where an infection is reported in a recipient.

Transfusion transmission of RRV no longer appears to be only a theoretical risk. However, with about 5000 mosquito-related RRV notifications per year, transfusion transmission of RRV — or the related Barmah Forest virus, which has a lower incidence — is likely to remain a rare event. Any actions taken to prevent infected components entering the blood supply need to take into account the cost, the impact on supply and the severity of the infection in recipients. Laboratory screening is not a feasible option, given that RRV nucleic acid testing is not validated for blood donation screening or available for the large-scale nucleic acid detection equipment used by the Blood Service. In addition, the cost of individual testing is unlikely to be cost-effective and, although RRV can cause debilitating symptoms in some patients, most infections are either asymptomatic or mild and self-limiting.[8]

Identifying donors who are at risk of exposure and temporarily excluding them from donating fresh blood components in areas and times of RRV outbreaks is one potential risk-mitigation option. When this strategy was applied to dengue fever, it was estimated to cost the Blood Service around $1.0–$3.8 million.[9] However, irrespective

of the financial cost, this option is unlikely to be feasible, since RRV is endemic in many parts of Australia and such restrictions might have a critical impact on supply. Pathogen reduction technology (PRT) is an alternative risk-management option that would not have an impact on supply. The Blood Service is investigating the effectiveness of PRT for the prevention of arboviral transfusion transmission, including RRV, but further research is needed.[10]

The Australian blood supply is one of the safest in the world with respect to transfusion-transmitted infections. Yet, it is important to remember that blood transfusion is not without risk and should only be undertaken when the efficacy of the transfusion and improved clinical outcome outweigh the risks.[11]

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Competing interests: No relevant disclosures.

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別紙様式第2-1

No. 21

医薬品 研究報告 調査報告書

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販売名(企業名)	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) 赤血球液-LR「日赤」(日本赤十字社) 照射赤血球液-LR「日赤」(日本赤十字社)			

研究報告の概要

○ 2000～2008年にフランス国内ヘモビジランスネットワークに報告された、輸血伝播細菌感染の残存リスクと後方視的分析

背景: 輸血伝播細菌感染(TTBI)は、依然として最も高頻度な感染リスクである。同リスクを正確に把握するためには、TTBIの発生率を頻繁に評価、更新する必要がある。

研究デザインと方法: TTBIは、病院と血液センターの担当者によりフランスのヘモビジランスネットワークを通して報告された。地域のコーディネーターが調査を管理した。フランス国立医薬品・医療製品安全管理機構の多分野からなる専門家チームが、起因性と重症度の標準的スケールに従ってTTBIの各症例を分析した。可能性が高い、あるいは一定の起因性を持つTTBIの後方視的分析から、TTBIの発生率、重症度、関連する血液製剤の種類と関与する細菌について報告する。

結果: フランスでは、2000～2008年の期間に1,800万の赤血球(RBC)製剤、194万の血小板濃厚液(PC)、240万の新鮮凍結血漿が輸血された。TTBIの発生率は、全血液製剤(BC)、PC、RBCそれぞれに対して、100万当たり2.45件、24.7件、0.39件であった。PCに関しては、重症あるいは致死的なTTBIの発生率はそれぞれ100万当たり13.4件、5.14件であった。全TTBIの87%がPCで発生している。原因とされる細菌の66.7%がグラム陽性菌であり、通常の皮膚細菌叢に属していた。残る33.3%がグラム陰性菌である。

結論: フランスのヘモビジランスシステムは、初流血除去と皮膚消毒の改善は実施されたが、細菌検出スクリーニングは行っていない期間中のTTBIの発生率の正確な推定値を提供した。このヘモビジランスシステムは、細菌スクリーニングや病原体低減技術等の、追加の安全対策を評価するのに有用と考えられる。

報告企業の意見	今後の対応
フランスで2000～2008年にヘモビジランスシステムにより報告された輸血伝播細菌感染の87%は、PC輸血で発生していたとの報告である。なお、フランスのPCの有効期間は5日間(採血日=0日)である。	日本赤十字社では全献血者に問診を実施しているほか、輸血による細菌感染予防対策として全輸血用血液製剤を対象に保存前白血球除去を行っている。なお、PCの有効期間は採血後3日間である(採血日=0日)。また、医薬品医療機器等法に基づき及び関連法令に従い輸血副作用・感染症情報を収集し、医薬品医療機器総合機構を通じて国に報告している。今後も引き続き輸血副作用・感染症に関する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

赤血球濃厚液-LR「日赤」
照射赤血球濃厚液-LR「日赤」
赤血球液-LR「日赤」
照射赤血球液-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

Residual risk and retrospective analysis of transfusion-transmitted bacterial infection reported by the French National Hemovigilance Network from 2000 to 2008

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BACKGROUND: Regarding blood safety, transfusion-transmitted bacterial infection (TTBI) remains the most frequent infectious risk. The incidence of these episodes needs to be assessed and updated frequently to accurately manage this risk.

STUDY DESIGN AND METHODS: TTBIs were reported by the French network of local correspondents in each hospital and blood center. The regional coordinator managed the investigation. A multidisciplinary expert group from the French National Agency of Medicine and Health Products Safety (ANSM) analyzed each TTBI according to a standardized scale of imputability and severity. Only cases with likely or certain imputability are reported in this study.

RESULTS: In France, 18.0×10^6 red blood cell (RBC) products, 1.94×10^6 platelet concentrates (PCs), and 2.44×10^6 fresh-frozen plasma units were transfused throughout 2000 to 2008. The incidence of TTBI was 2.45, 24.7, and 0.39 per million blood components (BCs), PCs, and RBCs, respectively. For PCs, the incidences of severe (vital threat or death) and fatal TTBI were 13.4 and 5.14 per million, respectively. PCs were responsible for 87% of TTBIs. A total of 66.7% of the implicated bacteria were Gram positive, most of them belonging to the normal skin flora. A total of 33.3% of the other implicated bacteria were Gram negative.

CONCLUSION: The French hemovigilance system provides an accurate estimate of the TTBI incidence during a period with diversion and improving skin disinfection but without bacterial detection screening. This tool would be able to evaluate further additional safety procedures like bacterial screening and pathogen reduction technology.

Transfusion-transmitted bacterial infection (TTBI) remains one of the major complications of blood transfusion due to its potential severity and lethal threat. It is the most common infectious complication in transfusion.[1,2]

Irrespective of the preventive actions to consider, an accurate estimate of the incidence of TTBI is required to compare the incidence between the different studies performed. It is also necessary to estimate the efficacy of specific measures undertaken for their prevention. Reporting systems are essential to measure incidence in compiling scarce events. However, such organizations are heterogeneous among different countries.[3]

The French Hemovigilance Network was set up in 1994 to proceed toward an exhaustive declaration of any adverse event (AE) occurring in a recipient during or after the transfusion of a blood component (BC).[4] This network relies on local, regional, and national participants, allowing a standardized and stable reporting.[5]

ABBREVIATIONS: AE(s) = adverse event(s); ANSM = French National Agency of Medicine and Health Products Safety; APC(s) = apheresis platelet concentrate(s); BC(s) = blood component(s); EFS = French Blood Establishment; PC(s) = platelet concentrate(s); PPC(s) = pooled platelet concentrate(s); TTBI(s) = transfusion-transmitted bacterial infection(s); WB = whole blood.

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In this work, we describe the TTBI reporting system in France, the data compilation, and the validation procedures from 2000 to 2008 regarding the transfusion of a total of 18 million red blood cell (RBC) products, 1.94 million platelet concentrates (PCs), and 2.4 million units of fresh-frozen plasma (FFP). From a retrospective analysis of the TTBI with a likely or certain imputability, we report the incidence of TTBIs, their severity, the type of BCs concerned, and bacteria involved.

MATERIALS AND METHODS

Preparation of phlebotomy

The phlebotomy site during this period was prepared according to a two-stage procedure according to the French Blood Establishment (EFS) hygiene guidelines.[6] The site was first scrubbed with an antiseptic. Second, application of an antiseptic agent was performed. The procedure differs from center to center for the choice of the antiseptic agent. In most cases, the same antiseptic agent was used for the two-stage. For apheresis collection, either the two-stage or the four-stage method was used depending on the center.

Manufacturing procedure

Leukoreduction was introduced in France in April 1998 and thus covers all the survey period. Diversion of the 30 to 40 mL was fully applied to whole blood (WB) donation since September 2000 and was done for the majority of plateletpheresis procedures as early as 2001. It was then fully applied for any apheresis donation by the beginning of 2004. PCs were manufactured either by apheresis collection (APCs) for 80% of PC or by buffy coat method from WB (pooled PCs [PPCs]) for 20% of PC.

Briefly, after collection, the WB was stored between 2 and 20 hours before processing. When WB was collected from the bottom-and-top pack for buffy coat preparation, RBCs, FFP, and buffy coat were separated by centrifugation, and RBCs and plasma were then leukoreduced. Five buffy coats were pooled and subjected to a slow centrifugation to separate PC, which was then leukoreduced via filtration. In other cases, WB was leukoreduced by filtration before centrifugation to separate RBCs and FFP.

Between 2005 and 2008, APCs were collected and leukoreduced using Trima Accel (TA; Gambro BCT, Lakewood, CO) blood cell separator for more than 50%, and MCS+ (Haemonetics, Braintree, MA) and Amicus (AM; Baxter Transfusion Therapies, Deerfield, IL) blood cell separators for the remaining. Between 2000 and 2004, collection of apheresis platelets (PLTs) was marked by the modernization of apheresis devices, with replacement of Spectra by Trima for Gambro BCT, ACS 3000 by Amicus for Baxter, and MCP 2p and 3p by MCS+ for Haemonetics. Moreover, the proportion of collections performed on the Haemonetics separator decreased from approximately 55% to 25% in favor of the Trima separator (30 to 55%) and the proportion of collection performed on the Amicus separator increased slightly from 15% to 20%.

Each APC was transfused either as one single therapeutic dose or divided into two doses during the manufacturing process. The allowed storage time for PCs and RBCs with SAG-M were 5 and 42 days, respectively.

AE reporting system in France over the period analyzed

The reporting of AEs is compulsory for all health providers. A standard AE reporting form was created to harmonize and facilitate the reporting. The AE declaration procedures are specified in regulatory texts.[7] Each AE is classified according to the diagnostic orientation, severity grade, and imputability to transfusion. The severity before 2010 was scored on a scale of 1 to 4 and defined as follows: 1 = "absence of vital threat or long-term threat," 2 = "long-term morbidity," 3 = "immediate vital threat," and 4 = "death." In practice, Grade 2 is used for transfusion-transmitted viral infection and RBC alloimmunization. The imputability, that is, the causal relationship of blood transfusion in the occurrence of AE, was scored on a scale of 0 to 4 defined as follows: 0 = "excluded," 1 = "doubtful," 2 = "possible," 3 = "likely," and 4 = "unquestionable."

The hemovigilance system is organized in a network structured over three levels to guarantee the coverage of the entire territory (Fig. 1):

- The local level composed of hemovigilance correspondents, doctors, or pharmacists located in each health care settings (1500 hospitals or clinics) and in each 150 blood transfusion centers of EFS, in charge of the report of AEs and of carrying out local investigations.
- The regional level composed of 30 medical regional hemovigilance coordinators assigned to the directors of the Regional Health Agencies and 18 regional hemovigilance correspondents (doctors or pharmacists) of the regional EFS, in charge of monitoring the quality and reliability of the information.
- The national level including the French National Agency of Medicine and Health Products Safety (ex-Afssaps, now ANSM), the EFS, and the Centre de Transfusion Sanguine des Armées, in charge of the investigations concerning the bacterial infection reporting. In some cases, additional information or studies may be requested from all the network members concerned by a AE.

In 1996, a computer tool facilitating the exchanges between the different actors was set up (Gifit); it was replaced in 2004 by a data electronic filing system (e-fit).

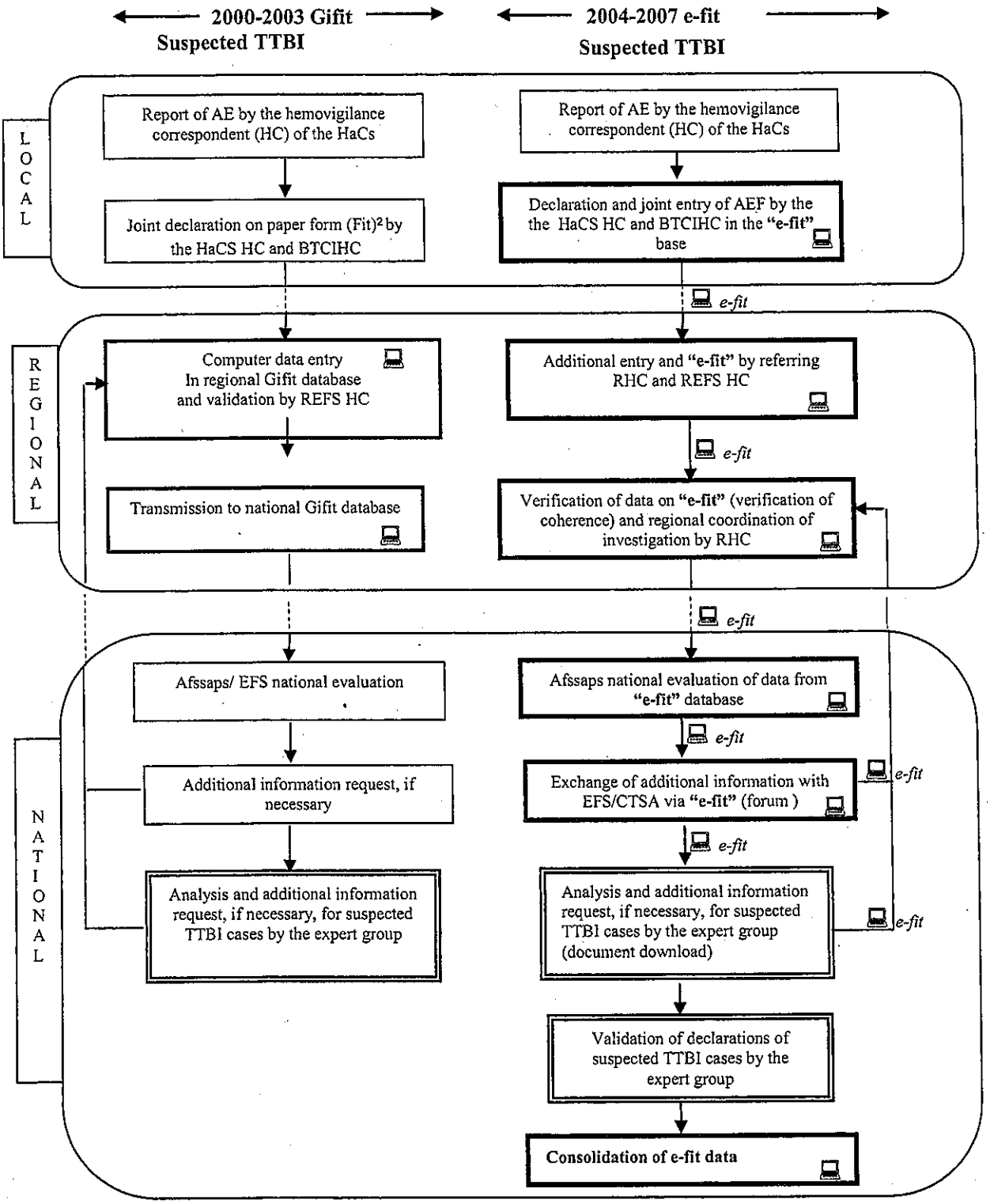

Fig. 1. Reporting and analysis procedures. Afssaps = French National Agency of Medicine and Health Products Safety; AEF = adverse event form; BTC = blood transfusion center; BTCIHC = BTC local hemovigilance correspondent; Gifit = transfusion incident report electronic data management; HaCs = hospital and clinics; HC = hemovigilance correspondent; REFS = Regional French Blood Establishment; RHC = regional hemovigilance coordinator.

Selection of cases for retrospective analysis

The reported cases that occurred between January 1, 2000, and December 31, 2008, were extracted from the national hemovigilance database e-fit according to the following criteria: AE diagnosed as a bacterial infection with a likely or certain imputability. Sixty-seven cases were extracted from the database according to this request. Among these cases, eight were excluded owing to insufficient data for subsequent analysis, three were reclassified as possible, and one case was reclassified as doubtful. The cases with Imputability 2 were extracted from the database and rechecked: no Imputability 2 case was reclassified as Imputability 3.[8] Finally, 55 cases were retained for analysis.

Bacteriologic explorations

Bacteriologic analysis

Owing to the difficulties in the performance and interpretation of the bacteriologic examinations revealed by a national survey, starting in 2005, the bacteriologic analyses were performed by approved laboratories after validation of the bacteria detection method according to a Ministry of Health circular dated December 15, 2003.[9]

Bacteriologic methods

The choice of the bacteriologic techniques was left to the initiative of each laboratory but the general procedures for blood culture were as follows. One aerobic and one anaerobic blood culture bottle were incubated at 37°C and kept for 10 days. At the same time, 2 × 0.1 mL of BCs were inoculated on two blood agar plates incubated for 37°C 2 days under aerobic conditions and for 37°C 5 days under anaerobic conditions, a different incubation time due to lower bacterial growth of anaerobic bacteria. If the BC was completely transfused, the bag would be rinsed with 20 mL of sterile saline and the same medium was inoculated as above.

Strains belonging to the same species and isolated from BCs, recipient blood cultures, and any sample of interest were analyzed phenotypically and genotyped in a certified laboratory or national reference center. The clusters of identical strains were left stored at −80°C at the ANSM Agency.

TTBI diagnostic criteria

Clinical criteria

The clinical criteria suggestive of TTBI were the following: temperature above 39°C or increase in temperature above 2°C occurring within 2 hours after the start of the transfusion associated with 1) at least two of the following symptoms—rigors, tachycardia > 120 beats/min or change greater than 40 from pretransfusion values, decrease of blood pressure by 30 mmHg, abdominal pain, or diarrhea; or 2) a major clinical sign—signs of shock or serious biologic signs including coagulation disorder and renal failure. However, subclinical forms did not exclude a TTBI diagnosis.

Microbiologic criteria

A positive BC and recipient blood culture with the same bacteria was the essential diagnostic criterion. A similar strain genotype allowed diagnosis of TTBI. When blood cultures were negative, especially in a patient under antibiotic treatment, the diagnosis was based on a set of arguments as clinical data, microbiologic results from other biological samples or microscopic direct examination of BC. This last criterion, when positive, was an important element for establishing the link between clinical symptomatology and bacterial contamination.

Imputability analysis grid

Due to the observed difficulties to establish the transfusion origin of a bacterial infection in the past, the experts drew up an analysis grid (Table 1) to help the members of the network to determine the imputability. Furthermore, the use of this grid allowed a standardization of this analysis.

Analysis of TTBI episodes by the national expert group

Since 2000, aiming to decrease the variability of data concerning TTBI, a multidisciplinary expert group was set up to analyze all the declared cases of suspected TTBI. It was composed of an infectious disease specialist, two microbiologists, a hospital or clinic hemovigilance correspondent, a regional hemovigilance coordinator, EFS representatives, and ANSM representatives. One of the missions of this group was to verify the quality of the data, to control and to validate the analysis carried out by the correspondents, and to request additional studies if necessary. This group also ruled on more complex cases in terms of diagnosis.

Statistical analysis

Calculation of incidence is as follows: number of TTBIs divided by issued BCs. In France, more than 99.5% of BCs issued were transfused, so figures are very similar. The statistical analysis was performed with computer software (SAS, Version 9.2, SAS Institute, Chicago, IL) using the following procedures: PROC MEANS and PROC FREQ (calculation of frequencies), PROC TTEST (comparison of incidences, t test, and p value).

RESULTS

Cases reported

Fifty-five cases were identified for the 9-year period. The sex ratio was 1.2 (30 males and 25 females) and the mean

TABLE 1. Imputability analysis grid

Case	Clinical signs compatible with a TTBI diagnosis	Microbiology results*		Genotyping of strains	Final diagnosis orientation	Imputability of transfusion
		BC cultures	Blood cultures			
1	Suggestive clinical signs†	Pos	Pos	Yes and genotype identification of strains	Bacterial infection (TTBI)	4
2		Pos	Pos	Genotype comparison not performed	Bacterial infection (TTBI)	DE‡ pos: 3 DE‡ neg or ND: 1 to 3 (according to clinical examination and sealed tubing attached BC result)
3		Pos§	Neg§ (ATB.T?)	Not applicable	Bacterial infection (TTBI)	1 to 3 (according to clinical examination, DE‡ and sealed tubing attached BC result)
4	Subclinical signs and/or existing infection	Pos§	Neg	Not applicable	TTBI or other diagnosis and "soiling" (according to sealed tubing attached BC and DE result)	DE‡ pos: according to expert opinions DE‡ neg or ND: other diagnosis
4 bis		Pos	Pos	Yes, if DE pos	TTBI or retrograde contamination argued (patient moved, pretransfusion blood cultures pos, etc.)	DE‡ pos: 2 to 3 DE‡ neg or ND: according to expert opinions
5	Yes	Neg	Neg or pos	Not applicable	Other diagnosis	Imputability 0
6	Yes	Does not comply	Pos	Not applicable	Not analyzable	

* The positivity concerns phenotypically similar strains. The demonstration of a positive direct examination of the BC (and/or sealed tubing attached BC) is a strong argument for a bacterial contamination of the BC.
† Circular DGS/DHOS/Afssaps no. 581 dated 15 December 2003.
‡ Direct examination of BC.
§ Dissociated microbiological results: When the BC culture is positive and the blood cultures of the receiver negative, the interpretation by the two hemovigilance correspondents and the microbiologist is compulsory and a case by case analysis is required.
ATB.T = antibiotic therapy; DE = direct examination; ND = not done; Neg = negative result; Pos = positive result.

age was 54 years. Forty-three of them (80%) exhibited a hematologic disease (20 acute leukemia, seven lymphomas, 16 miscellaneous blood diseases), and seven (12.7%) had a solid tumor. Two patients had undergone surgery and two were hospitalized for anemia assessment. Clinically, a fever was present in all cases, rigors in 39 cases (81%), and digestive symptoms in 17 cases (30%). Concerning the low-severity incidents (Grade 1) that represented in our study 45.8% of the TTBIs, the clinical signs were mainly rigors and/or hyperthermia with associated signs in 10 cases of 24: digestive signs (n = 5), transient hypotension (n = 2), tachycardia (n = 1), desaturation (n = 1), and anxiety (n = 1). Among the 48 TTBIs related to PCs, the age of the PC when it was transfused was available for 35 cases: two cases (5.7%) occurred with 1-day-stored PCs, seven (20%) with 2 days of storage, five (14.3%) with 3 days of storage, 15 (42.9%) with 4 days of storage, and six (17.1%) with 5 days of storage. Further details of cases are provided in the Appendix S1 (available as supporting information in the online version of this paper).

In our study, 96% of the TTBI cases observed with PCs occurred in subjects suffering from a blood disease (48/50) while they received only 7.7% of the totality of transfused PC as reported in a cross-sectional and nationwide survey.[10]

Annual variation

Figure 2A illustrates the variation of annual incidence of TTBIs between 2000 and 2008 for PCs. It varied between 45.7 and 13.5 per million. A decrease tendency occurred between 2000 and 2005 followed by a reincrease in 2006 and 2007 and decrease in 2008. Figure 2B illustrates the variation of annual incidence for all BCs.

Clinical severity of TTBI and incidence according to the type of BC

The severity of TTBI according to BC is presented in Table 2. An immediate life-threatening and death risk was observed in 35 and 22% of all the cases, respectively. TTBIs linked to RBCs were more severe than those linked to other BCs (71% of Grades 3 and 4 for RBCs vs. 54% for PCs).

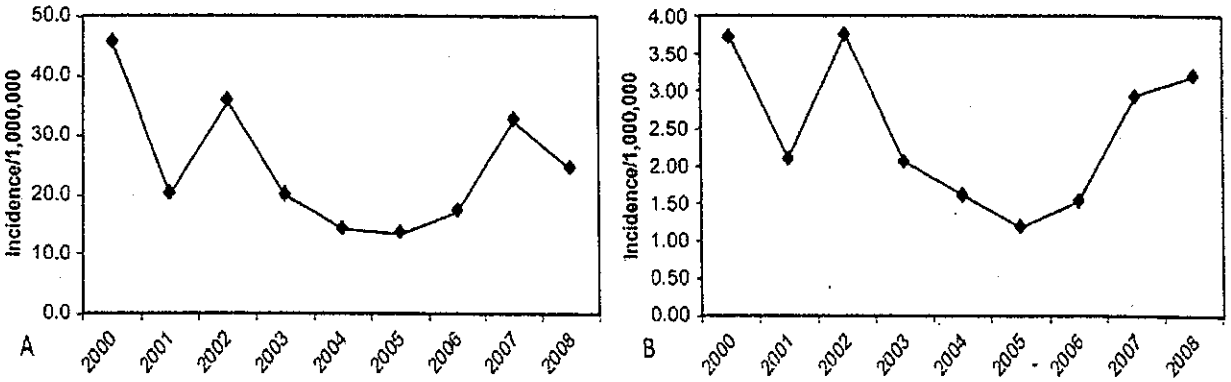

Fig. 2. Annual variation of the incidence of TTBIs related to PCs (A) or BCs (B) from 2000 to 2008.

TABLE 2. Distribution of TTBI severity levels depending on the type of blood components over the 2000-2008 period

	RBC	PC	*(PPC*	*APC)*	All blood components
Grade 1 (absence of life-threatening or long-term threat)	2 (28.6%)	22 (46%)	*6 (75%)*	*16 (40%)*	24 (43.6%)
Grade 3 (immediate life threatening)	3 (42.8%)	16 (33%)	*1 (12.5%)*	*14 (37.5%)*	19 (34.5%)
Grade 4 (death)	2 (28.6%)	10 (21%)	*1 (12.5%)*	*9 (22.5%)*	12 (21.9%)

RBC: red blood cells; PC: platelet concentrates; PPC: pool platelet concentrates; APC: apheresis platelet concentrates.

TABLE 3. Cumulative incidence (expressed in number of cases per million transfused BCs) TTBI over the 2000 through 2008 period according to the type of BC and severity*

	Number		Incidence		
BC type	Transfused BCs	TTBIs	All severity levels	Grades 3 and 4 severity	Grade 4 severity
All BCs	22.41×10^6	55	2.45	1.16	0.49
RBCs	18.0×10^6	7	0.388	0.28	0.11
PCs	1.94×10^6	48	24.7†	13.4	5.14
APCs	1.62×10^6	40	24.62	14.8	5.54
PPCs	0.32×10^6	8	25.03‡	6.26	3.13
FFP	2.44×10^6	0	0	0	0

* Grade 3 severity = immediate life threatening; Grade 4 severity = death.
† $p < 10^6$ by chi-square test by comparison to RBCs.
‡ Not significant by chi-square test by comparison to APCs.

Table 3 presents the cumulative incidence of TTBIs per million BCs, including all severity levels over the period 2000 through 2008 as a function of the nature of the BC. The incidence over the period was 2.45 per million BCs, 24.7 per million PCs, and 0.388 per million RBCs. No cases were described with FFP. PCs were responsible for 87% of TTBIs (48/55) with a 63-fold higher incidence than that of RBCs ($p < 10^{-9}$ by chi-square test).

The incidence between APCs and PPCs was very similar (not significant by chi-square test). If only TTBIs of Grades 3 and 4 severity are considered, the incidence per million is 13.4 for PCs (without difference between APCs and PPCs) and 0.28 for RBCs. The incidence of death related to TTBIs per million transfused units was 5.14 for PCs and 0.11 for RBCs (Table 3).

Microbiologic results

Table 4 presents the different bacteria that were isolated from BCs. A total of 58% were Gram-positive bacteria (including 75% staphylococci) and 42% were Gram-negative bacteria (including 78% of *Enterobacteriaceae*). All of the bacteria recovered from RBCs were Gram-negative bacilli.

The severity of TTBIs related to BCs is also presented in Table 4. For RBCs, *Escherichia coli, Klebsiella oxytoca,* and *Klebsiella pneumoniae* caused Grade 3 TTBIs. One strain of *Acinetobacter* spp. and one strain of *Yersinia enterocolitica* biovar 4, serovar 0:3, caused a Grade 4 TTBI.[11] In PCs, Gram-positive bacteria represented 66.7% of all the isolated bacteria, and Gram-negative bacteria, 33.3%. Most severe episodes related to PCs were due to

TABLE 4. Bacteria isolated from BCs associated with transfusion reactions according to severity

	Severity grade									
	RBCs (n = 7)			PPCs (n = 8)			APCs (n = 40)			Total
Bacteria	1	3	4	1	3	4	1	3	4	
Gram-positive bacteria										
S. aureus				1			5	3	4	13
Staphylococcus epidermidis				2			6			8
Other coagulase-negative staphylococci				1			2			3
E. faecalis								1	1	2
Streptococcus Group G				1	1					2
Streptococcus mitis								1		1
B. cereus								3		3
Total Gram-positive bacteria	0	0	0	5	1	0	13	8	5	32
Gram-negative bacteria										
Enterobacter cloacae							1			1
E. coli		1						3	2	6
K. oxytoca		1				1			1	3
K. pneumoniae	1	1							1	3
Proteus mirabilis	1									1
Serratia marcescens							1	2		3
Y. enterocolitica			1							1
Acinetobacter spp.			1							1
Acinetobacter haemolyticus				1						1
Acinetobacter lwoffi								1		1
Pseudomonas aeruginosa							1			1
Pseudomonas fluorescens/putida								1		1
Total Gram-negative bacteria	2	3	2	1	0	1	3	7	4	23

Staphylococcus aureus, *Enterococcus faecalis*, and *Bacillus cereus* for Gram-positive bacteria and to *Enterobacteriaceae* for Gram-negative bacilli.

DISCUSSION

TTBIs are the most common remaining infectious risks in transfusion. Their incidence should be accurately supervised, more particularly to monitor impact of additional safety procedures in terms of incidence. Many technical difficulties hinder TTBI incidences comparison: difficulties in the interpretation of observational data, variability of the AE monitoring systems, poor clinical recognition, and variability in laboratory practice.[12-15] In the French hemovigilance system, the network structure and compulsory nature of the reporting allow a nearly exhaustive determination of incidence. The reporting rate was stable over the considered period.[16]

The incidence of TTBI varied greatly according to the BC type (FFP, RBCs, PCs). No TTBI was described among the 2.4 million units of FFP. This was in accordance with previously published reports.[17-19] The incidence of TTBIs originated from RBCs was 0.38 per million, lower than the reported value from the German Hemovigilance System (0.93/million).[19] This incidence contrasted with the Bacthem study incidence of 5.8 per million units of RBCs between 1996 and 1998 in France, a value 15-fold higher; even methodologic aspects were different.[17] Similarly, a decrease in the incidence for RBC is seen since 1994 from the French hemovigilance data.[20] Since 1998, several measures were implemented in France. A guide for hygiene with consideration for blood collection was published in 2000 and a national guidelines document for managing TTBIs was produced in December 2003 to maintain a high level of sensitization of the staff in regard to TTBIs.[6,9,20] Major measures such as the systematic leukoreduction and diversion of the first 30 to 40 mL have shown their efficacy and were implemented in April 1998 for RBCs and between 2001 and 2003 for PCs.[20-24]

To date PCs are the major source of TTBIs in France (48 of 55 cases) and could be considered as a high-risk BC. The incidence of TTBI related to PCs was 24.7 per million PCs, all severity levels included. The incidences between the two types of PCs (PPCs and APCs) did not reveal any significant difference. In terms of health safety, the first concern is the life-threatening or death-inducing TTBI. The incidence of life-threatening and fatal TTBI, was 13.4 per million and the incidence of fatal TTBI alone was 5.14 per million. For comparison, the most suitable studies in terms of size and scope are the American Red Cross and German reports. In the United Kingdom, the incidence of TTBIs was not available due to lack of the denominator in the annual SHOT reports. The incidences of TTBI reported in a similar period including sample diversion but without bacteria screening by the American Red Cross for APCs (March 2003 to December 2003) and by the German Hemovigilance System for PCs (2006-2007) were 25 per million (4.2 fatalities per million) and 7.14 per million, respectively.[19,25] The incidence reported by German hemovigilance is significantly lower. The first explanation

could be that physicians do not systematically recognize a transfusion reaction as a septic incident. For instance, six febrile reactions with positive bacteriologic culture of PLTs were classified neither as possible septic transfusion nor as transfusion reaction by physicians.[26] Another hypothesis is that low-severity incidents, which represented in our study 45.8% of the TTBIs, are not all reported. Moreover, a lack of data on severity in these reports accounts for difficulties to make comparison between incidence and, particularly, what incidence of French study must be considered for comparison (24.7 per million with all severity or 13.4 per million with life-threatening and fatalities). The French hemovigilance system takes great attention to report all possible TTBIs including low-severity TTBIs to evaluate more accurately incidence of all clinically significant BC bacterial contaminations.

As observed for RBCs, the incidence reported here for all severity TTBIs related to PCs (24.7 per million) was lower than the previously published data from Bacthem study for the 1996 to 1998 period (32 per million PCs).[17] A decrease has also been observed in the follow-up of TTBI in the annual TTBI cases reported within SHOT data before and after 2002.[27] Similarly to RBC, this was considered as the main consequence of the same measures described above for RBC.

Variables such as type, bacterial load, and pathogenicity of bacteria involved are also important elements to explain the occurrence and the severity of TTBI.[28] In our work, few bacteria are concerned in RBC-transmitted bacterial infections over the studied period. Gram-negative bacilli clearly or exclusively predominate confirming previous results from BaCon studies in the United States, SHOT annual reports in the United Kingdom, and German hemovigilance data.[18,19,29,30] However, five of seven TTBIs are Grade 3 or 4. The hypothesis to explain the severity of these TTBIs could be the proliferation of cryophylic bacteria, suggested by storage conditions, which increase endotoxin production and thus the risk of severe sepsis.[28] In the hemovigilance data reported from 1994 to 1998 and in the French Bacthem study, Gram-positive bacteria represented 58 and 40% of cases, respectively, whereas no case was reported with Gram-positive bacteria in our study. This result suggests the efficacy of diversion and sensitization of staff in regard to skin antisepsis and hygiene.

The distribution of bacteria among TTBIs involving PCs was wider, mainly consisting of coagulase-negative staphylococci followed by enterobacteria. These data were in accordance with those of studies performed by others.[18,19,29,31] Except for *S. aureus*, TTBIs caused by other staphylococci species were responsible for low-grade AEs. Nevertheless, a fatal case has been previously described.[32] In our study the presence of skin flora bacteria was still significant in spite of the different preventive measures implemented in France.

Surprisingly the TTBI incidence due to skin flora bacteria between PPCs and APCs did not differ significantly, although PPCs were obtained from five collections and APCs from one collection. Similar data were found in prospective studies on the detection of bacteria in Europe where PPCs are produced using the buffy coat technique.[33,34] These results are in contradiction with the studies using the PLT-rich plasma method. The American Red Cross reported a 5.8-fold greater risk of confirmed contamination for PPCs and similar data were reported by others.[35,36] This difference could be ascribed to the WB prestorage stage and the leukoreduction by filtration.[37-41] The prestorage stage consisted of storage at 20°C of WB after collection for 2 to 20 hours before processing, allowing phagocytosis of bacteria by white blood cells (WBCs) and therefore the bactericidal effect of WB. This prestorage stage with WBCs did not exist for apheresis PLT collection. Furthermore, the leukoreduction of WB, RBCs, and plasma was carried out by filtration. This one can reduce bacterial load even its effect varied dramatically depending on bacterial species.[38-40,42,43] Leukoreduction for apheresis PLTs was carried out via a different mechanism, during the cell separation phase (elutriation).

Another explanation could be that the contamination of the punctured zone may be more difficult to prevent for apheresis blood collection because of a collection time greater than 45 minutes. A possible explanation for this might be that venipuncture results in skin flaps, from which skin bacteria might be rinsed and eluted during prolonged flow.[22]

Enterococcus were more rarely involved in TTBIs. However, the *E. faecalis* infections observed in this study were severe. These enteric bacteria were usually considered weakly virulent but were known to cause bacteremia, endocarditis, and local infections especially in deficiency patients. No related cases were notified in the BaCon and SHOT studies.[18,29] *Clostridium perfringens*, a Gram-positive anaerobic bacilli, is another enteric bacteria that could cause serious sepsis among recipients.[44,45] *Enterococcus* and *Clostridium* could also be isolated in the environment or on the skin or be transmitted by the hands of the collection personnel or technicians hygiene conditions are deficient.[44]

The isolation of *B. cereus* often led to interpretation problems. These bacilli and their spores were broadly spread in nature and often considered as air or skin contaminants. However, they should be considered as significant on strict bacteriologic criteria. Our study added three new cases to the 11 previously reported.[17,18,28,29,46,47]

Among enterobacteria, the *Klebsiella-Enterobacter-Serratia* group predominated with respect to *E. coli* in the present work as in the BaCon, SHOT, and Bacthem studies. Indeed *S. marcescens* could proliferate under poor nutritional conditions both in RBCs stored at +4°C and in PCs stored at 22°C.[48]

A question raised by these data regards the improvement of blood safety from a bacteriologic point of view after sample diversion and continued improved donor arm disinfection. The first intervention to be considered is bacterial screening for PC as a high-risk BC. The American Red Cross reported an incidence of 9.4 per million for APCs and 0.98 for fatalities with screening detection. This points out a reduction of 30% for TTBIs and 50% for fatalities, respectively.[49,50] Since its implementation in the United Kingdom in February 2011, no transmission has yet been reported.[18] In the case of bacterial screening, some issues however must be considered such as timing (immediate or delayed) or volume of sampling, culture methods with aerobic bottle alone or aerobic and anaerobic bottles, or methods with rapid detection system. These issues are important because bacterial concentration is very low after production of PCs, bacterial growth kinetics can vary considerably, detection is especially difficult if the bacteria are in lag phase, and all TTBIs are not prevented by early bacterial screening strategies.[50-52] For the bacteriologic diagnostic of our study, the standardization of the processing of positive culture for diagnostic has allowed a uniform, controlled quality of the detection and recovery of pathogens. A similar approach in early bacterial screening could be of interest for managing blood culture bottles inoculated at the storage stage to reduce the risk of false-positive results. A new approach in bacterial screening is the detection of contaminated units at the time of issue when there is a high burden of bacteria and when detection could be best achieved. Altogether, the challenge of bacterial screening is to overcome the sensitivity limitations to continue decreasing risk of bacterial sepsis as well as specificity limitations to limiting the waste of a limited resource, as blood is. In any case, TTBI events are so uncommon that an exhaustive hemovigilance system, like that in France, could evaluate accurately the impact of these methods in term of incidence.

Due to the remaining risk of bacterial sepsis in spite of screening detection, an alternative option would be the pathogen inactivation of PCs for preventing the transmission of bacterial infections and many other pathogens.[53] While pathogen-reduced plasma is already used routinely, there are persistent concerns about the impact of pathogen reduction on the integrity of BCs and on toxicity.[54] Furthermore, there are conflicting data regarding the therapeutic efficacy of pathogen-reduced PCs.[55,56] In France, a multicenter, randomized, controlled, double-blind, therapeutic trial (named EFFIPAP, ClinicalTrials.gov Identifier NCT01789762) including 810 patients was beginning in 2013 to evaluate PC treated by amotosalen and UVA with regard to bleeding as primary objective and with regard to transfusion needs, outcomes, and safety as secondary objective. Therefore, the need for careful assessment of the risks and benefits of pathogen reduction technologies must be regularly considered.

In conclusion, this retrospective study illustrates the importance of hemovigilance as an important tool to evaluate safety of BC transfusion. Furthermore, the incidence of the TTBI throughout 2000 to 2008 provides a baseline to evaluate the impact of further additional safety procedures like bacterial screening and pathogen reduction technology.

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CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's Web site:

Appendix S1. Description of cases.

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
				2015. 4. 4	該当なし	

一般的名称	人赤血球液	研究報告の公表状況	Kamitsukasa H, Iri M, Tanaka A, Nagashima S, Takahashi M, Nishizawa T, Okamoto H. J Med Virol. 2015 Apr;87(4):589-600. doi: 10.1002/jmv.24115. Epub 2015 Jan 21.	公表国 日本
販売名(企業名)	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) 赤血球液-LR「日赤」(日本赤十字社) 照射赤血球液-LR「日赤」(日本赤十字社)			

研究報告の概要

○HBVが消失した、またはオカルトHBV感染患者における自然発生的なHBVの再活性化

HBVの再活性化は、免疫抑制療法、免疫不全疾患、臓器移植、抗ウイルス剤の中止等により引き起こされるが、これらのリスク因子のない、HBVが消失した、またはオカルトHBV感染の高齢患者2人に、HBVの再活性化が自然発生した。

患者1)2008年10月に冠動脈バイパスグラフト術を受けた74歳男性。手術時、HBs抗原は陰性だがHBs抗体は陽性であった。2009年7月、肝機能値の急激な上昇及び血清中のHBs抗原、HBe抗原が陽性であり、ウイルス量は6.4 logコピー/ml、ジェノタイプCであった。HBc IgM抗体は陰性であった。これらの所見に基づき、HBVの再活性化と診断された。同患者のHBVの全ゲノム配列からはHBe抗原産生を妨げるコアプロモーター及びプレコア領域の変異は観察されなかった。

患者2)2002年に食道癌のため内視鏡による粘膜切除術歴があり、2009年に糖尿病と診断された際の検査ではHBs抗原陰性であった76歳男性。2012年9月、食道癌の再発に伴う切除術の事前検査で、HBV量は少ないものの(2.1 logコピー/ml)、HBs抗原陽性であった。手術後、化学療法はせず外来で経過観察していたが、3カ月後にHBV DNA(ジェノタイプB)量が5.1 logコピー/mlに上昇した(HBc IgMは陰性)。同患者はオカルトHBVキャリア状態からの再活性化と診断された。患者の分離株からは、プレコア領域のG1896A変異が観察された。

HBVが消失した、またはオカルトHBV感染患者において、事前の免疫抑制療法がないHBV再活性化の報告はこれまでになく、本研究が初の報告である可能性がある。加齢、手術のストレス、癌及び動脈硬化や糖尿病などの生活習慣病が、HBVの再活性化を自然に誘発する可能性があることを示唆している。

使用上の注意記載状況・その他参考事項等

赤血球濃厚液-LR「日赤」
照射赤血球濃厚液-LR「日赤」
赤血球液-LR「日赤」
照射赤血球液-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

報告企業の意見	今後の対応
HBV再活性化の既知のリスク因子のない高齢患者2人(HBVが消失した、あるいはオカルトHBV感染者)において、HBVが再活性化したとの報告である。	日本赤十字社では、化学発光酵素免疫測定法(CLEIA)によりHBs抗原、HBc抗体検査を実施することに加え、20プールでスクリーニングNATを行っていたが、更なる安全対策を目的に2012年8月より抗体検査の判定基準を強化し、さらに2014年8月よりNATシステムを変更し、全検体に対し個別検体によるNAT(個別NAT)スクリーニングを開始している。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。

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Spontaneous Reactivation of Hepatitis B Virus (HBV) Infection in Patients With Resolved or Occult HBV Infection

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Reactivation of a former hepatitis B virus (HBV) infection can be triggered by immunosuppressive therapy, diseases associated with an immunocompromised state, organ transplantation or the withdrawal of antiviral drugs. Despite the absence of such risk factors, a spontaneous reactivation of HBV replication occurred in two elderly patients with resolved or occult HBV infection. A 73-year-old male underwent coronary artery bypass grafting in October 2008, and was negative for HBsAg but positive for anti-HBs. In July 2009, his serum became positive for HBsAg, HBeAg and HBV DNA (6.4 log copies/ml; genotype C), but negative for anti-HBc IgM, with abrupt elevation of the liver enzymes. The entire genomic sequence of HBV recovered from this patient revealed no mutations in the core promoter and precore regions that interfere with HBeAg production. A 76-year-old male with a history of endoscopic mucosal resection for esophageal cancer in 2002 and an initial diagnosis of diabetes mellitus in 2009, at which time he was negative for HBsAg. He was found to be positive for HBsAg in September 2012 during a laboratory examination performed prior to the resection of recurrent esophageal cancer, despite a low HBV load (2.1 log copies/ml). Three months later, without the administration of any anticancer drugs, the HBV DNA (genotype B) level increased to 5.1 logcopies/ml. A precore G1896A variant with high quasispecies diversity was recovered from thepatient. Aging, surgical stress and complication of disease(s) associated with compromised immunity, such as cancer, arteriosclerosis and diabetes mellitus may trigger spontaneous HBV reactivation. ***J. Med. Virol. 87:589–600, 2015.*** © 2015 Wiley Periodicals, Inc.

KEY WORDS: hepatitis viruses; entire genome; phylogenetic analysis; precore mutant

INTRODUCTION

Approximately two billion people worldwide have been exposed to HBV, which affects an estimated 350 million persistently infected individuals worldwide [Lavanchy, 2004]. HBV causes a spectrum of liver diseases, including acute hepatitis, chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma [Liang, 2009]. Patients with acute HBV infection sometimes develop fulminant hepatitis B. HBV is a circular, partially double-stranded DNA virus of approximately 3,200 nucleotides (nt) that belongs to the prototype group of the *Hepadnaviridae* family [Mason et al., 2012]. The HBV genome is classified into 10 genotypes, designated A–J [Okamoto et al., 1988; Norder et al., 1994; Stuyver et al., 2000; Arauz-Ruiz et al., 2002; Tran et al., 2008; Tatematsu et al., 2009]. With the exception of genotypes E, G, H, and J, the genotypes are divided into several subgenotypes, such as B1-B9 in genotype B and C1-C16 in genotype C [Mulyanto et al., 2012]. In Japan, subgenotypes B1 and C2 are prevalent among patients with chronic infections [Tanaka et al., 2005].

The presence of HBV DNA in the liver (with or without detectable HBV DNA in the serum) of HBsAg-negative individuals tested with the currently available serum assays is defined as occult HBV infection [Raimondo et al., 2008]. The clearance of HBsAg and appearance of anti-HBs, with normalization of the liver function, is generally accepted as

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evidence of a clinical and serological recovery from acute hepatitis B. However, HBV replication has been shown to persist at low levels in the liver for decades [Michalak et al., 1994]. Reactivation of HBV in chronic HBV carriers or individuals with an occult or resolved HBV infection during or after immunosuppressive treatment, such as chemotherapy or transplantation chemotherapy, which is characterized by an increased level of serum HBV DNA, abnormal liver function and hepatic failure, has been reported [Lok et al., 1991; Seth et al., 2002; Shouval and Shibolet, 2013; Zachou et al., 2013]. Although a spontaneous reactivation of chronic hepatitis B presenting as acute-on-chronic liver failure has been described in the literature since the 1980 s [Davis et al., 1984; Tong et al., 1987; Okuno et al., 1989], the occurrence of HBV reactivation without prior immunosuppressive treatment, based on the status of an occult or resolved HBV infection that had been contracted earlier, has not been reported.

Recently, two cases of spontaneous HBV reactivation were treated at a national hospital in Japan, both of which occurred in patients in their 70 s with occult or resolved HBV infections. In the present study, the entire genomic sequences of the HBV isolates obtained from these two patients were analyzed to characterize the HBV that was spontaneously reactivated in these elderly patients.

MATERIALS AND METHODS

Serum Samples

Serum samples were periodically collected from two patients (No. 1 and No. 2) who suffered from spontaneous reactivation of previously occult or resolved HBV. The presence of immunoglobulin (Ig) M (IgM) antibodies against the hepatitis A virus (HAV) (anti-HAV IgM), hepatitis B surface antigens (HBsAg), the corresponding antibodies (anti-HBs), IgM class antibodies against the HBV core antigens (anti-HBc IgM), whole antibodies against HBV core (anti-HBc), hepatitis B e antigens (HBeAg), the corresponding antibodies (ant-HBe) and antibodies to HCV (anti-HCV) was assayed using a chemiluminescence enzyme-immunoassay (CLEIA) with commercial kits (Fujirebio, Tokyo, Japan) or by chemiluminescence immunoassay (CLIA) with commercial kits (Abbott Japan, Tokyo, Japan). The IgM and IgA classes of antibodies to the hepatitis E virus (HEV) (anti-HEV IgM and anti-HEV IgA, respectively) were detected with an in-house enzyme-linked immunosorbent assay (ELISA) using a purified recombinant ORF2 protein of HEV genotype 4 that had been expressed in the pupae of a silkworm as the antigen probe, as described previously [Takahashi et al., 2005]. The IgG class antibodies against hepatitis delta antigens (HDAg) were also determined via an in-house ELISA using a purified recombinant HDAg protein that had been expressed in the pupae of a silkworm as the antigen probe, as described previously [Inoue et al., 2005]. The presence of antibodies against human immunodeficiency viruses 1 (HIV-1) and 2 (HIV-2) (anti-HIV 1/2) and antibodies to human T-cell leukemia virus type 1 (HTLV-1) was assayed by the particle agglutination method (Fujirebio).

Quantitation of the HBV DNA was also performed using the serum samples from the patients according to the COBAS TaqMan HBV Test v2.0 (Roche Diagnostics, Tokyo, Japan) or an in-house real-time PCR as described previously [Akahane et al., 2002]. The serological detection of the HBV genotypes was conducted using an *ELISA* with monoclonal antibodies against preS2 epitopes [Usuda et al., 1999], and subgenotypes were determined by a phylogenetic analysis as described below.

Informed consent was obtained from the patients for participation in the present investigation.

Amplification of the Entire HBV Genome

The complete nucleotide sequences of four HBV genomes obtained from the two patients were determined according to the previously described method [Mulyanto et al., 2010]. Briefly, nucleic acids were extracted from 100 µl of serum using a commercially available kit (SMITEST EX-R&D; G&G Science, Fukushima, Japan), and were subjected to nested PCR using primers derived from the well-conserved areas in the HBV genomes of all 10 genotypes (A–J) reported to date [Okamoto et al., 1988; Norder et al., 1994; Stuyver et al., 2000; Arauz-Ruiz et al., 2002; Tran et al., 2008; Tatematsu et al., 2009] and TaKaRa *Ex Taq* polymerase (TaKaRa Bio, Shiga, Japan). PCR was subsequently performed for 35 cycles in the first round and 25 cycles in the second round under the same cycling conditions (94°C, 30 sec; 55°C, 30 sec; 72°C, 2 min), targeting three overlapping regions of HBV DNA (the primer sequences at both ends were excluded) spanning nt 2333–3215/1–667 (1,550 base pairs [bp]), nt 480–1795 (1,316 bp) and nt 1699–2380 (682 bp), respectively. The nucleotide numbers are defined in accordance with the genotype C HBV strain of 3,215 nt (AB033550).

Determination of the Nucleotide Sequences and Phylogenetic Analyses

The amplification products were sequenced directly on both strands using a BigDye Terminator v3.1 Cycle Sequencing kit (Life Technologies, Carlsbad, CA) on an Applied Biosystems 3130xl Genetic Analyzer (Life Technologies). The sequence analysis was performed using the Genetyx software program (version 12.0.6; Genetyx, Tokyo, Japan), and multiple alignments were generated by the CLUSTAL Omega software program (version 1.2.0) [Goujon et al., 2010]. Phylogenetic trees were constructed according to the neighbor-joining method [Saitou and Nei, 1987] with the Kimura two-parameter model and 1,000 replicates of bootstrap resampling, as

implemented in the MEGA6 software program (version 6.0.6) [Tamura et al., 2013].

RESULTS

Patient 1

A 74-year-old male (Patient 1) had underwent coronary artery bypass grafting (CABG) at a city hospital on October 10, 2008, at which time he was negative for HBsAg but positive for anti-HBs (12.7 mIU/ml) (Table I). Although the patient's liver enzyme levels were within the normal limits until April 2009, slightly elevated levels of AST (58 IU/L) and ATL (79 IU/L) were noted on May 28, 2009. Due to abrupt elevations of the AST (449 IU/L) and ALT (273 IU/L) levels and positivity for HBsAg on July 9, 2009, he was transferred to Tokyo National Hospital and hospitalized with a clinical diagnosis of HBV-associated hepatic injury 2 weeks later.

The laboratory tests performed on admission showed that he had hepatic dysfunction with elevated ALT (436 IU/L) and AST (318 IU/L) and total bilirubin (2.0 mg/dl) levels. His serum was positive for HBsAg, HBeAg and HBV DNA (6.4 log copies/ml; genotype C), with concurrent positivity for anti-HBs, but was negative for anti-HBc IgM (CLIA, 0.2 S/CO) and anti-HAV IgM, anti-HCV, anti-HDV, and anti-HEV IgM/IgA (Table II). Based on these findings, the patient was diagnosed with reactivation of HBV infection. Although his consciousness was normal, the possibility of deterioration could not be ruled out, because of the persistence of ALT elevation for more than one month, a decrease in the serum albumin level and elevation of the serum bilirubin level. Therefore, immediately after admission, the patient received treatment with entecavir (0.5 mg/day). The HBsAg and HBV DNA became undetectable on August 10, 2009, and continued to be negative up through the end of the observation period of April 9, 2012.

Patient 2

On February 24, 2009, a 73-year-old male was admitted to Tokyo National Hospital due to drug misuse, at which time HBsAg was negative in his serum (anti-HBs and anti-HBc were not examined). The patient reported receiving endoscopic mucosal resection for esophageal cancer in 2002 and cholecystectomy for cholelithiasis in 2004. He was first diagnosed with diabetes mellitus during admission, and thereafter, was followed-up as outpatient at the hospital. On September 3, 2012, the patient was found to be positive for HBsAg on a laboratory examination performed prior to surgery for recurrent esophageal cancer, although the titer was low (Table III). There were no abnormalities on a physical examination, and no signs of chronic liver dysfunction, such as ascites, leg edema or vascular spiders, and the HBV DNA titer was low at 2.2 log copies/ml. After the resection of the esophageal cancer, the patient was followed-up in the outpatient clinic without chemotherapy.

On December 6, 2012, although biochemical tests revealed no elevated levels of AST (14 IU/L), ALT (11 IU/L), or γ-glutamyl transpeptidase (γ-GT) (39 IU/L), the HBsAg and HBV DNA (genotype B) levels were

TABLE I. Changes in Liver Function Test Results and HBV-Related Markers in Patient 1

									HBV DNA	
Date of sampling (yr/mon/day)	AST (IU/L)	ALT (IU/L)	T-Bil (mg/dl)	HBsAg (COI)	Anti-HBs (mIU/ml)	Anti-HBc (COI)	HBeAg (S/CO)	Anti-HBe (%)	TaqMan (log copies/ml)	In-house (copies/ml)
2008/10/10	29	22		−	+ (12.7)					
2009/02/23	22	20		−	+ (16.3)					
2009/05/28	58	79								
2009/07/09	449	273		+ (>2000)			+ (1590)	−	+ (8.0)	
2009/07/23[a]	436	318	2.0	+ (2000)	+ (118.0)	+ (100.0)	+ (1600)	−	+ (6.4)	
2009/07/25[b,c]				+						+ (1.4×10^7)
2009/07/27[b]	.302	254	2.1	+						+ (2.2×10^5)
2009/07/30	316	245	1.9							
2009/08/03	254	246	1.6							
2009/08/06	77	125	1.3							
2009/08/10[b]	28	49	1.1	−						−
2009/09/14[b]	16	9	0.8	−			+ (5.6)	−	−	−
2009/11/16	19	8	0.8				+ (1.2)	−	−	
2009/12/21[b]	17	11	0.7	−			−	−	−	−
2010/01/26	23	12	0.9	−			−	−		
2010/03/29[b]	27	15	0.8			+ (100.0)	−	+ (93.8)	−	−
2010/04/27	20	13	0.7	−	+ (28.0)		−	+ (71.1)	−	
2011/09/26[b]	18	9	1.0	−			−	+ (75.1)	−	−
2012/04/09	16	9	0.8	−			−	+ (66.6)	−	

[a]On admission.
[b]Stored serum samples were available.
[c]The entire genomic sequence of HBV was determined.

TABLE II. The Laboratory Data on Admission for the Two Patients With Spontaneous HEV Reactivation

	Patient 1	Patient 2
Hematology		
WBC	5,300/μl	5,500/μl
RBC	360×10^4/μl	324×10^4/μl
Hemoglobin	11.9 g/dl	10.0 g/dl
Hematocrit	35.9%	31.2%
Platelets	14.1×10^4/μl	24.6×10^4/μl
Blood Chemistry		
Total protein	8.0 g/dl	5.5 g/dl
Albumin	3.5 g/dl	2.2 g/dl
Total bilirubin	2.0 mg/dl	0.6 mg/dl
AST	436 IU/L	14 IU/L
ALT	318 IU/L	11 IU/L
ALP	860 IU/L	540 IU/L
γ-GT	219 IU/L	39 IU/L
BUN	19.1 mg/dl	9.9 mg/dl
Creatinine	1.1 mg/dl	1.0 mg/dl
Total cholesterol	96 mg/dl	72 mg/dl
Coagulation		
PT%	34%	74%
APTT	45.5 sec	31.0 sec
Viral Markers		
Anti-HAV IgM	(−)	(−)
Anti-HCV	(−)	(−)
Anti-HDV	(−)	(+)
Anti-HEV IgM/IgA	(−)/(−)	(−)/(−)
HBsAg	2,000.0 COI (+)	9.9 COI (+)
Anti-HBs	118.0 IU/ml (+)	<5.0 IU/ml (−)
Anti-HBc (whole)	100 COI (+)	9.1 COI (+)
Anti-HBc IgM	0.2 S/CO (−)	0.1 S/CO (−)
HBeAg	1,600.0 S/CO (+)	<1.0 S/CO (−)
Anti-HBe	<5.0% (−)	92.7% (+)
HBV DNA	6.4 log copies/ml	5.1 log copies/ml
HBV genotype	C	B
Anti-HIV1/2	(−)	(−)
Anti-HTLV1	(−)	(−)
Immunology		
IgA	386 mg/dl	354 mg/dl
IgG	2,577 mg/dl	1,394 mg/dl
IgM	76 mg/dl	92 mg/dl
ANA	40x	80x

WBC, white blood cells; RBC, red blood cells; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; γ-GT, gamma-glutamyl transpeptidase; LDH, lactate dehydrogenase; BUN, blood urea nitrogen; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen; ANA, anti-nuclear antigen; PT, prothrombin time; APTT, activated partial thromboplastin time.

elevated to 9.9 COI and 5.1 log copies/ml, respectively, without positivity for anti-HBc IgM (Table III). His serum was positive for anti-HDV, but negative for HDV RNA, suggesting that there had been a past HDV infection. Based on these findings, a diagnosis of reactivation from occult HBV carrier status was established, and he was admitted to receive treatment with entecavir (0.5 mg/day). One month after admission, his HBV DNA level declined to 2.6 log copies/ml (Table III), and he started to receive chemotherapy with cisplatin (110 mg/day, once every 3 weeks) and fluorouracil (1000 mg/day for 5 days a week). However, due to a gradual deterioration of his general condition, the patient died on February 17, 2013.

Characteristics of the Entire HBV Genomes Obtained From Patients 1 and 2

In order to characterize the HBV implicated in the reactivation of the resolved HBV infection in Patient 1, a nucleotide sequence analysis was performed using viral DNA extracted from the serum sample obtained from Patient 1 on July 25, 2009, which had an HBV DNA titer of 1.4×10^7 copies/ml. The P1–090725 isolate recovered from Patient 1 had a genomic length of 3,215 bp, and was most similar to genotype C and subgenotype C2 HBV strains (Fig. 1), with the highest similarity (99.5%) to the reported HBV strain of Non-HCC_pt_No.10 (AB670283) over the entire genome (Table IV). The P1–090725 isolate possessed no mutations in the core promoter or precore region, including those at nt 1762/1764 and nt 1896, which are known to abrogate HBeAg production or interfere with the transcription of precore mRNA and to reduce the expression of the HBeAg precursor [Okamoto et al., 1990; Moriyama et al., 1996]. Of note, the P1–090725 isolate was 98.6% similar to the pNDR260 isolate that was obtained from an HBeAg-positive asymptomatic carrier in Japan, and was 99.3% identical to a consensus sequence of 18 wild-type subgenotype C2 HBV strains in Japan, including Non-HCC_pt_No.10 and pNDR260, over the entire genome (Table IV), suggesting that the P1–090725 isolate was a wild-type virus. The P1–090725 isolate shared 100% identity with the consensus sequence in the C gene product, and differed by only 0.5–1.3% from the consensus sequence in the amino acid sequences of the other three genetic regions, with the conversion at aa 615 (Ile for P1–090725 and Leu for the consensus sequence) and 805 (Leu/Pro) in the P gene, aa 167 (Leu/Ser) in the S gene and aa 49 (Try/His) in the X gene. Since P805L in the P gene product and S167L in the S gene product were not identified in the reported subgenotype C2 HBV strains (deposited in the DDBJ/EMBL/GenBank databases as of July 2014), these two amino acid substitutions are likely to be unique to the P1–090725 isolate obtained in the present study.

In Patient 2, a serum sample obtained before HBV reactivation was available, and the HBV isolate (P2–120831) recovered from the serum sample with an HBV DNA titer of 2.1×10^2 copies/ml was compared with the isolates obtained from serum samples with an HBV DNA titer of 1.9 or 2.5×10^6 copies/ml collected during HBV reactivation (P2–121210 and P2–121214). All three HBV isolates obtained from Patient 2 had a genomic length of 3,215 bp and were segregated into subgenotype B2 within genotype B (Fig. 2). These three HBV isolates possessed a common precore mutation (G1896A), unaccompanied by core promoter double mutations at nt 1762/1764, thus indicating that Patient 2 was infected with a precore mutant, which was unable to produce the HBeAg protein. Of interest, P2–120831 had 31

TABLE III. Changes in Liver Function Test Results and HBV-Related Markers in Patient 2

Date of sampling (yr/mon/day)	AST (IU/L)	ALT (IU/L)	T-Bil (mg/dl)	HBsAg (COI)	Anti-HBs (mIU/ml)	Anti-HBc (COI)	HBeAg (S/CO)	Anti-HBe (%)	HBV DNA TaqMan (log copies/ml)	HBV DNA In-house (copies/ml)
2009/02/24	24	18		–						
2009/08/10	34	25		–						
2010/07/30	29	30								
2011/08/04	32	30								
2012/08/02	30	28	0.6							
2012/08/23	43	34	1.7	–						
2012/08/27	55	48								
2012/08/31[a,b]				–	–					+ (2.1×10^2)
2012/09/03				+ (1.5)		+ (12.4)			+ (2.2)	
2012/09/04	43	19	2.8							
2012/11/05	22	21	0.5							
2012/12/06[c]	14	11	0.6	+ (9.9)	–	+ (9.1)	–	+ (92.7)	+ (5.1)	
2012/12/10[a,b]				+ (43.5)	–		–			+ (1.9×10^6)
2012/12/14[a,b]	15	9								+ (2.5×10^6)
2012/12/23	15	10	0.5							
2013/01/16[a]				+ (310.1)		+ (50.0)	–	+ (89.3)	+ (2.6)	+ (7.0×10^3)
2013/01/17	61	28	0.6							
2013/01/23	19	14	0.7							
2013/02/04	123	110	0.7							

[a]Stored serum samples were available.
[b]The entire genomic sequence of HEV was determined.
[c]On admission.

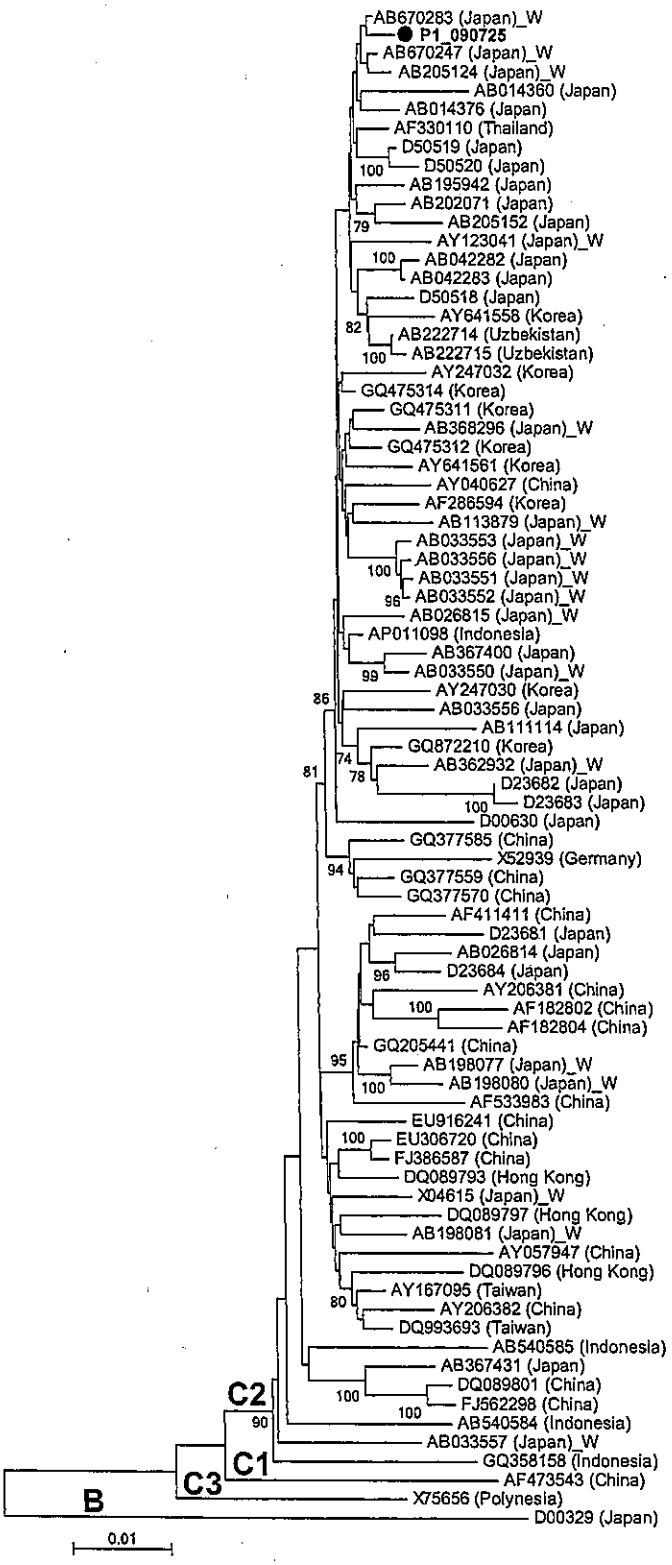
nucleotide positions that were a mixture of two nucleotides (A/C, T/C, A/G, A/T, G/T, or G/C), while P2–121210 and P2–1214 had 36 and 35 such nucleotide positions, respectively. Of these, 24 nucleotide positions (nt 288, 339, 406, 456, 480, 482, 553, 554, 555, 648, 657, 810, 831, 949, 1053, 1569, 2183, 2235, 2525, 2588, 2753, 2775, 2925, and 3080) with a mixture of two nucleotides were common to the three HBV isolates. As listed in Table V, four nucleotide positions (nt 463, 479, 930, and 1800) with a mixture of two nucleotides were found exclusively in P2–120831, while nine nucleotide positions (nt 494, 1726, 1846, 1961, 1962, 2155, 2239, 2303, and 2962) with a mixture of two nucleotides were restricted to both P2–121210 and P2–121214. Based on a comparison with the consensus sequence of 40 reported subgenotype B2 HBV isolates, it was speculated that Thr at aa 114 in the S gene (S_114-Thr), P_468-Asn, X_118-Thr, C_21-Pro/Asn, C_113-Glu, C_135-Pro, preS1_39-Gln and P_219-Thr were associated with the reactivation of HBV replication.

The nucleotide sequence data determined in this study have been assigned DDBJ/EMBL/GenBank accession numbers AB981580-AB981583.

DISCUSSION

The present study revealed that reactivation of HBV infection can occur in elderly people with resolved or occult HBV infection, despite the absence of reported triggers for reactivation, including immunosuppressive therapy, diseases associated with an immunocompromised state, organ transplantation or the withdrawal of antiviral drugs, and suggested that either wild-type or precore G1896A mutants may be associated with HBV reactivation. Of note, when the HBV genomes in two patients with spontaneous reactivation were analyzed by direct sequencing in the present study, a wild-type HBV genome with a single sequence was recovered from Patient 1 who had been negative for HBsAg but positive for anti-HBs approximately four months before reactivation, while G1896A variants having a mixture of two nucleotides at 35 or 36 nucleotide positions across the entire genome were obtained from Patient 2, who had been negative for both HBsAg and anti-HBs but had harbored low-titer HBV DNA approximately three months before reactivation.

The clearance of HBsAg and appearance of anti-HBs, with normalization of the liver function, is generally accepted as evidence of a clinical and serological recovery from acute hepatitis B. However, HBV replication has been shown to persist at low levels in the liver for decades [Michalak et al., 1994; Raimondo et al., 2013]. Although it is unknown whether Patient 1 in the present study had anti-HBc and low-titer HBV DNA, the presence of anti-HBs, in the absence of detectable HBsAg, indicates the resolution of the HBV infection (Table I). The presence of HBV DNA in the serum or liver of HBsAg-negative individuals tested with the currently available serum assays is defined as occult HBV infection [Raimondo et al., 2008]. Occult HBV infections are associated with significantly lower HBV DNA levels compared with HBsAg-positive chronic HBV infections [Stramer et al., 2011]. In some cases, the serum HBV DNA levels may be near the cut-off for the assay or undetectable in patients with occult hepatitis B,


AB670283 (Japan)_W
P1_090725
AB670247 (Japan)_W
AB205124 (Japan)_W
AB014360 (Japan)
AB014376 (Japan)
AF330110 (Thailand)
D50519 (Japan)
100
D50520 (Japan)
AB195942 (Japan)
AB202071 (Japan)
79
AB205152 (Japan)
AY123041 (Japan)_W
100
AB042282 (Japan)
AB042283 (Japan)
D50518 (Japan)
AY641558 (Korea)
82
AB222714 (Uzbekistan)
100
AB222715 (Uzbekistan)
AY247032 (Korea)
GQ475314 (Korea)
GQ475311 (Korea)
AB368296 (Japan)_W
GQ475312 (Korea)
AY641561 (Korea)
AY040627 (China)
AF286594 (Korea)
AB113879 (Japan)_W
AB033553 (Japan)_W
100
AB033556 (Japan)_W
AB033551 (Japan)_W
96
AB033552 (Japan)_W
AB026815 (Japan)_W
AP011098 (Indonesia)
AB367400 (Japan)
99
AB033550 (Japan)_W
86
AY247030 (Korea)
AB033556 (Japan)
AB111114 (Japan)
74
GQ872210 (Korea)
81
78
AB362932 (Japan)_W
D23682 (Japan)
100
D23683 (Japan)
D00630 (Japan)
GQ377585 (China)
94
X52939 (Germany)
GQ377559 (China)
GQ377570 (China)
AF411411 (China)
D23681 (Japan)
AB026814 (Japan)
96
D23684 (Japan)
AY206381 (China)
100
AF182802 (China)
AF182804 (China)
GQ205441 (China)
95
AB198077 (Japan)_W
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AB198080 (Japan)_W
AF533983 (China)
EU916241 (China)
100
EU306720 (China)
FJ386587 (China)
DQ089793 (Hong Kong)
X04615 (Japan)_W
DQ089797 (Hong Kong)
AB198081 (Japan)_W
AY057947 (China)
DQ089796 (Hong Kong)
AY167095 (Taiwan)
80
AY206382 (China)
DQ993693 (Taiwan)
AB540585 (Indonesia)
AB367431 (Japan)
DQ089801 (China)
100
FJ562298 (China)
100
C2
90
AB540584 (Indonesia)
AB033557 (Japan)_W
C1
GQ358158 (Indonesia)
C3
AF473543 (China)
B
X75656 (Polynesia)
D00329 (Japan)
0.01

TABLE IV. Comparison of the Nucleotide Sequence Identities Between the HBV Isolate Obtained From Patient 1 and Reported Wild-type HBV Isolates of the Same Subgenotype (C2) in Japan

HBV isolates compared	Nucleotide (amino acid) sequence identity%				
	Entire genome (3,215 nt)	P gene [2,529 nt (843 aa)]	preS1/preS2/S gene [1,200 nt (400 aa)]	X gene [462 nt (154 aa)]	C gene [549 nt (183 aa)]
P1_090725 versus AB033550[a]	98.6	98.5 (98.5)	99.1 (98.2)	97.8 (96.1)	98.6 (100)
P1_090725 versus AB670283[b]	99.5	99.6 (99.7)	99.8 (99.5)	99.1 (99.3)	99.8 (100)
P1_090725 versus Cons[c]	99.3	99.4 (99.5)	99.7 (99.3)	98.4 (98.7)	99.6 (100)

[a]A representative subgenotype C2 HBV isolate (pNDR260) obtained from an HBeAg-positive asymptomatic carrier in Japan.
[b]A representative subgenotype C2 HBV isolate (Non-HCC_pt_No.10) that was most similar to the P1_090725 isolate among those deposited in the DNA databases as of July 2014. The Non-HCC pt No. 10 isolate had no core promoter or precore mutations.
[c]The consensus sequences of 18 presumably wild-type subgenotype C2 HBV strains (AB026815, AB033550-AB033553, AB033556-AB033557, AB113879, AB198077, AB198080-AB198081, AB205124, AB362932, AB368296, AB670247, AB670283, AY123041, and X04615) isolated in Japan that had no core promoter or precore mutations.

although replicative HBV DNA can still be detected in the liver tissue [Raimondo et al., 2008]. In Patient 2 in the present study, although HBsAg had been continuously negative for at least 3 years since 2009, HBV DNA was found to be positive at a low titer three months before HBV reactivation (Table III). Therefore, it is reasonable to consider that Patient 2 had occult HBV infection.

The anti-HBc IgM antibody titer is used to distinguish between acute and chronic HBV infections [Rodella et al., 2006]. It has previously been reported that a titer of anti-HBc IgM greater or less than 10.0 S/CO, detectable according to the CLIA method, is useful for differentiating between acute and acute-on-chronic hepatitis B [Nakao et al., 2006]. In the present study, both patients were negative for anti-HBc IgM (Table II), supporting the notion that these two patients developed hepatitis on a background of resolved or occult HBV infection, and exclude the possibility of acute HBV infection.

Reactivation of HBV infection is known to occur not only in chronic HBV carriers, but also in individuals with an occult or resolved HBV infection [Lok et al., 1991; Seth et al., 2002; Shouval and Shibolet, 2013; Zachou et al., 2013]. The cases of HBV reactivation that have been reported are characterized by a wide range of clinical manifestations, from an increased level of serum HBV DNA without clinically relevant manifestations to fulminant life-threatening hepatitis [Hoofnagle, 2009]. It is well documented that chronic HBV infection may be reactivated in patients with disease-related or therapeutically-induced conditions of strong immune suppression [Loomba et al., 2008]. Reactivation of occult HBV infection following immunosuppressive therapy or chemotherapy has been reported, although it occurs more rarely than in HBsAg-positive patients [Shouval and Shibolet, 2013; Zachou et al., 2013]. It has also been reported that reactivation in patients with occult HBV infection may lead to the development of fulminant hepatitis [Marzano et al., 2007; Kusumoto et al., 2009].

As noted above, HBV replication has been shown to persist for decades at low levels in the nuclei of the hepatocytes of the patients as HBV cccDNA, an intermediate form of the virus life cycle that serves as a template for gene transcription [Michalak et al., 1994; Levrero et al., 2009; Raimondo et al., 2013], which may explain the recent increase in the rate of HBV reactivation leading to fulminant hepatic failure, known as de novo hepatitis B, in patients with resolved infection during or after chemotherapy and transplantation [Lok et al., 1991; Seth et al., 2002]. Of interest, the studies evaluating the histology in apparently healthy individuals who had recovered from acute hepatitis B have revealed that a mild necroinflammation persists for many years after the resolution of the acute hepatitis [Blackberg and Kidd-Ljunggren, 2000; Yuki et al., 2003].

Other than reactivation due to disease-related or therapeutically-induced conditions of strong immunosuppression, spontaneous reactivation of chronic hepatitis B presenting as acute-on-chronic liver failure or acute exacerbation of hepatitis in individuals chronically infected with HBV, in association with subjective symptoms and abnormalities on liver function tests, has rarely been reported [Davis et al., 1984; Tong et al., 1987; Okuno et al., 1989; Fattovich

Fig. 1. Phylogenetic tree constructed according to the neighbor-joining method based on the full-length genomic sequences of 80 HBV isolates of genotype C (subgenotypes C1–C3), using a genotype B HBV (D00329) as an outgroup. In addition to the P1–090725 isolate obtained in the present study which is indicated in bold type with a closed circle for visual clarity, 79 reported HBV isolates of genotype C, including 76 subgenotype C2 isolates and one isolate each of subgenotypes C1 and C3, whose entire genomic sequences were known, were included for comparison. The accession numbers are shown, followed by the name of the country in which the strain was isolated. W denotes a wild-type HBV isolate without mutations in the core promoter and precore region that can interfere with the production of HBeAg. Bootstrap values of >70% are indicated for the nodes as a percentage obtained from 1,000 resampling analyses of the data.

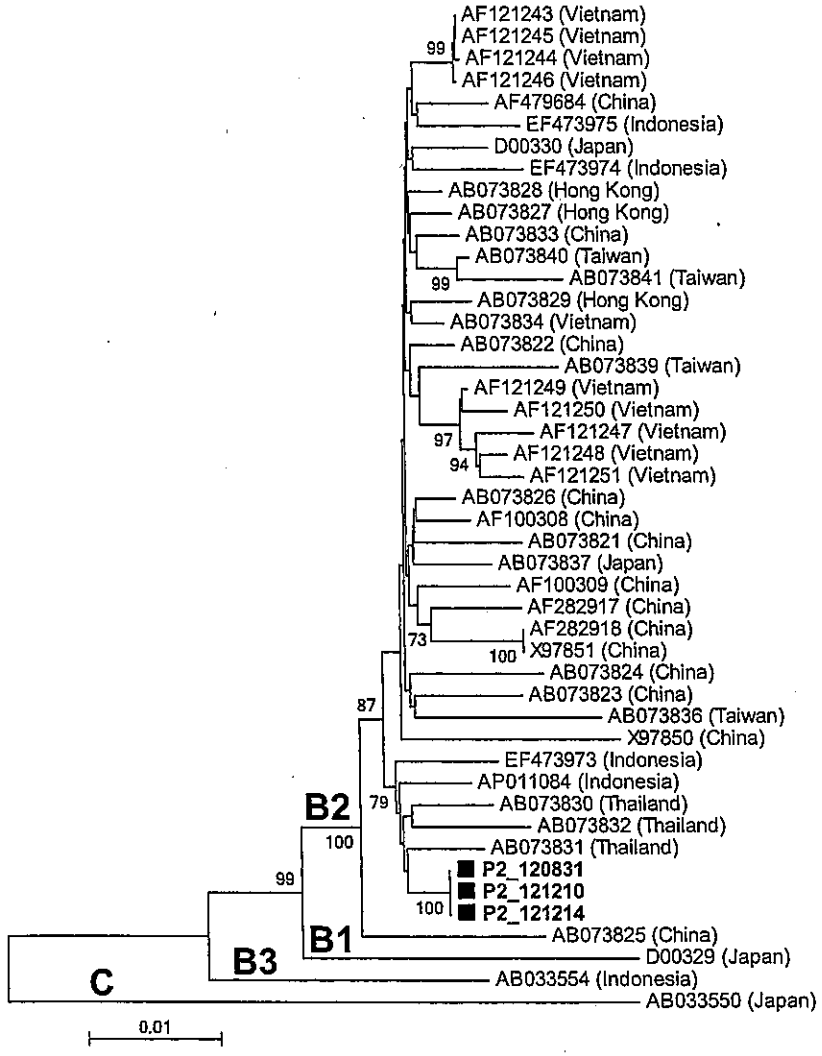

Fig. 2. Phylogenetic tree constructed by the neighbor-joining method based on the full-length genomic sequences of 42 HBV isolates of genotype B (subgenotypes B1-B3), using a genotype C HBV (AB033550) as an outgroup. In addition to the P2–120831, P2–121210, and P2–121214 isolates obtained in the present study, which are indicated in bold type with closed boxes for visual clarity, 39 reported HBV isolates of genotype B, including 37 subgenotype B2 isolates and one each of subgenotypes B1 and B3, whose entire genomic sequences were known, were included for comparison. The accession numbers are shown, followed by the name of the country in which the strain was isolated. Bootstrap values of >70% are indicated for the nodes as a percentage obtained from 1,000 resampling analyses of the data.

et al., 1990; Gupta et al., 1990; Meyer and Duffy, 1993; Laskus et al., 1994; Zhang et al., 2014]. However, there have been no reports of spontaneous reactivation in individuals with resolved or occult HBV infection, probably due to the rare occurrence of reactivation in HBsAg-negative individuals with latent HBV infection, even in cases in an immunosuppressed state. Therefore, the present report may be the first to describe a spontaneous reactivation of HBV in individuals with resolved or occult HBV infection.

The risk of HBV reactivation has been reported to be closely related to the level of immune suppression achieved, which changes significantly based on the immunomodulatory agents used during the treatment and the type of immunosuppressive drug(s) administered (steroids, traditional immunosuppressants or biological agents). Stronger immunosuppression may lead to increased viral replication; consequently, a more severe clinical reactivation may occur when immunosuppressive therapy is discontinued [Perrillo, 2001]. In the present study, no such immunosuppressive agents had been administered to either of the patients before HBV reactivation.

The most common causes of compromised immunity that favor the development of tuberculosis are

TABLE V. Comparison of the Nucleotide and Amino Acid Sequences of the P2_120831 Isolate Obtained Before HBV Reactivation With the P2_121210 and P2_121214 Isolates During Reactivation and the Consensus Sequence of Reported Subgenotype B2 HBV Strains

Nucleotide position	HBV genomic region	Amino acid position	Codon position	Nucleotide			Amino acid changes (from P2_120831 to P2_121210 and P2_121214)[c]	Cons[d]	Amino acids in HBV that emerged during reactivation
				P2_120831[a]	P2_121210[b]	P2_121214[b]			
463	S	103	3	R (A/G)	G	G	Met/Ile to Met	Met	
	P	458	1	R (A/G)	G	G	Val/Ile to Val	Val	
479	S	109	1	M (A/C)	C	C	Leu/Ile/Lys/Gln to Leu/Gln	Leu	
	P	463	2	M (A/C)	C	C	Ser/Tyr/stop to Ser	Ser	
494	S	114	1	T	W (A/T)	W (A/T)	Ser to Ser/Thr	Ser	Thr
	P	468	2	T	W (A/T)	W (A/T)	Ile to Ile/Asn	Ile	Asn
930	P	613	3	M (A/T)	A	A	Gln/His to Gln	Gln	
1726	X	118	2	A	M (A/C)	M (A/C)	Asn to Asn/Thr	Thr	Thr
1800	X	143	1	Y (T/C)	T	T	Cys/Arg to Cys	Cys	
1846	preC	11	3	A	W (A/T)	W (A/T)	Ser (no change)	Ser	
1961	C	21	1	C	M (A/C)	M (A/C)	His to His/Pro/Asn	Ser	Pro/Asn
1962	C	21	2	A	M (A/C)	M (A/C)	His to His/Pro/Asn	Ser	Pro/Asn
2155	C	85	3	C	M (A/C)	M (A/C)	Val (no change)	Val	
2239	C	113	3	C	M (A/C)	M (A/C)	Asp to Asp/Glu	Glu	Glu
2303	C	135	1	T	Y (T/C)	Y (T/C)	Ser to Ser/Pro	Pro	Pro
2962	preS1	39	1	G	S (G/C)	S (G/C)	Glu to Glu/Gln	Glu	Gln
	P	219	2	G	S (G/C)	S (G/C)	Arg to Arg/Thr	Arg	Thr

[a]The HBV isolate obtained before HBV reactivation (August 31, 2012).
[b]The HBV isolates obtained during HBV reactivation (December 10 and 14, 2012).
[c]Amino acid that is identical to the amino acid at the corresponding position of the consensus sequence is underlined.
[d]The consensus sequence of 40 reported subgenotype B2 HBV strains (see Fig. 2 for Accession numbers).

reported to be HIV/AIDS, malnutrition, aging, and most recently, smoking and diabetes mellitus [Restrepo and Schlesinger, 2014]. Spontaneous reactivation of HBV replication in an HIV-co-infected patient with isolated anti-HBc antibodies has been reported [Pei et al., 2014]. However, both patients in the present study tested negative for anti-HIV antibodies (Table II). Patient 1 had a history of cerebral infarction (8 years ago) and underwent coronary artery bypass grafting for coronary artery stenosis (nine months before the reactivation). Patient 2 had a history of resection of esophageal cancer (10 years ago) and an initial diagnosis of diabetes mellitus 3 years before the reactivation, and underwent resection of recurrent esophageal cancer three months prior to the reactivation, although no anticancer drugs were administered. The patient was also malnourished, typified by low levels of albumin (2.2 g/dl) and total cholesterol (72 mg/dl). Surgery has been shown to cause various immunological disturbances [Lennard et al., 1985; Szczesny et al., 2005]. Aging, cancer (hematological malignancy and solid tumor), stress due to surgery, malnutrition and lifestyle-related diseases, such as arteriosclerosis and diabetes mellitus, may have been the causes of the compromised immunity in these two patients who experienced reactivation of HBV without the administration of immunosuppressive drugs.

A variety of mutations in the HBV genomes are known to be associated with their sensitivity to antiviral therapy and the pathogenesis of liver diseases [Kosaka et al., 1991; Liang et al., 1991; Omata et al., 1991; Sato et al., 1995; Rodriguez et al., 2013; Kim et al., 2014]. Predominant reactivation of G1896A variants has been observed frequently in HBsAg-positive carriers who developed fatal viral reactivation under immunosuppressive conditions [Steinberg et al., 2000]. It has been reported recently that the clinical course and outcome of patients with reactivation from occult HBV infection results commonly in severe liver dysfunction and fatal acute liver failure, with most fatal cases predominantly containing G1896A precore variants [Hui et al., 2006; Umemura et al., 2008]. In addition, wild-type or G1896A variants recovered from patients with reactivation from occult HBV infection have been characterized by low genetic heterogeneity [Inuzuka et al., 2014].

In the present study, a wild-type HBV without genetic heterogeneity was recovered from Patient 1 with spontaneous reactivation from a resolved HBV infection. It is unclear whether occult HBV carriers with the G1896A precore variant have an increased risk of developing HBV reactivation and fatal acute liver failure [Inuzuka et al., 2014]. However, the favorable outcome after treatment with entecavir in Patient 1, without the development of fatal liver failure, may be ascribable to the reactivation of wild-type HBV. Contrary to the report by Inuzuka et al. [2014] G1896A mutants with marked genetic heterogeneity, possessing a mixture of two nucleotides at 36 nucleotide positions, were isolated from the serum of Patient 2 with spontaneous reactivation from occult HBV infection. Exactly the same result was obtained from the serum of the patient four days later, confirming the reproducibility. Further studies are needed to establish whether the nucleotide alterations that emerged after reactivation (Table V) were associated with the heightened replication ability of the virus.

In conclusion, the present study indicated that spontaneous reactivation of HBV can occur in elderly patients with resolved or occult HBV infection, and suggests that aging, stress due to surgery, cancer (hematological malignancies and solid tumors), malnutrition and lifestyle-related diseases, such as arteriosclerosis and diabetes mellitus, may lead to compromised immunity that can result in the spontaneous reactivation of latent HBV infection.

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別紙様式第2-1

No. 15

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
				2015. 4. 4	該当なし	

一般的名称	人赤血球液	研究報告の公表状況	Emerging Infectious Disease Journal, Vol.21 No.3; Available from: http://wwwnc.cdc.gov/eid/article/21/3/14-1662	公表国 日本
販売名(企業名)	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) 赤血球液-LR「日赤」(日本赤十字社) 照射赤血球液-LR「日赤」(日本赤十字社)			

研究報告の概要

○国内で発生したデング熱症例、2014年東京
日本では近年約200例の輸入デング熱症例が報告されているが、国内症例は70年間確認されていなかった。しかし、2014年8月26日、海外渡航歴のない患者におけるデング熱症例が東京で報告され、2014年10月31日までに合計160例の国内症例が確認されている。
国立国際医療研究センターでは、160例中19例(12%)を確定診断した。これら19例は平均年齢33歳(6－64歳)で、10人が男性であった。患者は高血圧(2人)及び喘息(1人)を除き、基礎疾患を有していなかった。1人の患者は2006年にフィリピン滞在中、デング熱への感染歴があった。19例中15例は発症日近くに代々木公園を訪問し、蚊に刺されていた。残り4例も、本アウトブレイクの影響が及んだ場所を訪れていた。19例のうち16例は入院、3例は外来で治療され、後遺症なく回復した。
デング熱の既往歴がある患者は、7日間続く熱、胸水、点状出血と血小板減少を呈し、WHOガイドラインに沿ってデング出血熱と診断された。同患者は、発症日の血清学的検査でデングウイルス(DENV) 非構造蛋白質(NS)1抗原及びIgG抗体陽性であったが、DENV IgM抗体は陰性であり、二度目のDENV感染であることが証明された。
19例中18例の患者サンプルはNS1抗原陽性であり、リアルタイムPCRによりDENV-1と確認された。残りの1症例はELISAによりDENV IgM及びIgG抗体陽性であることが確認された。3人の患者の血清におけるDENVエンベロープタンパク質ゲノムの系統発生解析で、本アウトブレイクの初症例患者からのDENV-1株の配列と100%の相同性を有することが証明された。この結果は、本アウトブレイクにおけるDENV症例の多くが1つの株に起因することを示唆している。

報告企業の意見	今後の対応
2014年8月、70年ぶりに国内発生のデング熱症例が発生し、2014年10月31日までに計160症例が報告された。国立国際医療研究センターで確定診断された19例について分析したところ、18例がDENV-1と確認され、3人の患者の血清におけるDENV-1エンベロープタンパク質ゲノムの系統発生解析で、同アウトブレイクの初症例患者から得られた株と100%の相同性を有することが明らかとなったとのことである。	日本赤十字社では、輸入感染症対策としては受付時に海外滞在歴の有無を確認し、帰国(入国)後4週間献血不適としている。また、国内においてデング熱感染が確認された場合は、感染が確認された地区への訪問歴を確認し、最後の訪問から4週間献血不適とするとともに、献血前後の発熱等に関する情報収集を強化することとしている。今後も引き続き、新興・再興感染症の発生状況等に関する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

赤血球濃厚液-LR「日赤」
照射赤血球濃厚液-LR「日赤」
赤血球液-LR「日赤」
照射赤血球液-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

Autochthonous Dengue Fever, Tokyo, Japan, 2014

Satoshi Kutsuna, Yasuyuki Kato, Meng Ling Moi, Akira Kotaki, Masayuki Ota, Koh Shinohara, Tetsuro Kobayashi, Kei Yamamoto, Yoshihiro Fujiya, Momoko Mawatari, Tastuya Sato, Junwa Kunimatsu, Nozomi Takeshita, Kayoko Hayakawa, Shuzo Kanagawa, Tomohiko Takasaki, Norio Ohmagari

After 70 years with no confirmed autochthonous cases of dengue fever in Japan, 19 cases were reported during August–September 2014. Dengue virus serotype 1 was detected in 18 patients. Phylogenetic analysis of the envelope protein genome sequence from 3 patients revealed 100% identity with the strain from the first patient (2014) in Japan.

Although ≈200 imported cases of dengue fever have recently been reported in Japan (*1*), an autochthonous case had not been confirmed there for 70 years (*2*). However, on August 26, 2014, an autochthonous case of dengue fever in a patient with no history of overseas travel was reported in Tokyo, and as of October 31, 2014, a total of 160 autochthonous cases in Japan had been confirmed (*3*).

The Cases

We report 19 cases of confirmed autochthonous dengue fever treated at the National Center for Global Health and Medicine in Tokyo, Japan, during August 26–September 22, 2014 (Tables 1, 2; online Technical Appendix Table, http://wwwnc.cdc.gov/EID/article/21/3/14-1662-Techapp1.pdf). Because the National Center for Global Health and Medicine is located close to the epicenter of this outbreak, 19 (12%) of the 160 cases of this outbreak were confirmed at this Center. Informed consent for participation in this study was obtained from all 19 patients. Of these 19 patients, the median age was 33.0 years (range 6–64 years), and 10 (55.6%) were men. None of the patients had any underlying illness except for hypertension (2 patients) and asthma (1 patient). One patient had a history of having contracted dengue fever while in the Philippines in 2006. None of the patients had traveled overseas during the 3 months before the outbreak of dengue virus type 1 (DENV-1) in Japan.

Places of exposures were assessed for all patients; 15 patients had recently visited Yoyogi Park and were bitten by mosquitoes while there; the remaining 4 patients had visited Shinjuku Central Park, Meiji Jingu Shrine, Meiji-ingu Gaien, and Ueno Park. All of these parks have been reported as affected regions in this outbreak (*3*) (Figure 1). The day of exposure was estimated for 9 patients for whom the day of visitation and mosquito bites while in the parks could be confirmed. Among these 9 patients, the median incubation period was 6 (range 3–9) days. For the other 10 patients, the incubation period was not determined because they had visited the parks over several days or because they lived near these parks. The dates of symptom onset ranged from August 12, 2014, through September 22, 2014; peak incidence occurred in the beginning of September.

Of the 19 patients, 16 were admitted to the National Center for Global Health and Medicine and discharged without sequelae; the other 3 received outpatient treatment and recovered. The patient with a history of dengue fever (patient 19 in the online Technical Appendix Table) experienced fever lasting 7 days, pleural effusion, spontaneous petechiae, and thrombocytopenia (15×10^3 cells/μL on day 8 after illness onset); dengue hemorrhagic fever was diagnosed for this patient by using the World Health Organization guidelines (*4*). On the day of illness onset for this patient, serum was positive for DENV nonstructural protein 1 (NS1) antigen and IgG but negative for DENV IgM. These results demonstrated that this DENV infection was secondary. Epidemiologic studies have also shown that the risk for dengue hemorrhagic fever is significantly higher

Table 1. Clinical characteristics of 19 patients with dengue fever, Tokyo, Japan, August 26, 2014–September 22, 2014

Sign or symptom	Patients, no. (%)
Fever*	19 (100)
Headache	17 (89.5)
Arthralgia	7 (36.8)
Myalgia	7 (36.8)
Nausea	5 (26.3)
Vomiting	2 (10.5)
Diarrhea	1 (5.3)
Rash at first visit	4 (21.1)
Rash during course of illness	15 (78.9)
Sore throat	2 (10.5)
Cough	4 (21.1)
Sputum	1 (5.3)

*Median duration of fever >38°C = 7 (range 4–11) days.

Author affiliations: National Center for Global Health and Medicine, Tokyo, Japan (S. Kutsuna, Y. Kato, M. Ota, K. Shinohara, T. Kobayashi, K. Yamamoto, Y. Fujiya, M. Mawatari, T. Sato, J. Kunimatsu, N. Takeshita, K. Hayakawa, S. Kanagawa, N. Ohmagari); National Institute of Infectious Diseases, Tokyo (M.L. Moi, A. Kotaki, T. Takasaki)

DOI: http://dx.doi.org/10.3201/eid2103.141662

Table 2. Laboratory findings for 19 patients with dengue fever, Tokyo, Japan, August 26, 2014–September 22, 2014

Laboratory finding at first visit	Reference range	Patient median (interquartile range)
Leukocytes, cells/mm^3	3,500-8,500	2,600 (2,385–3,540)
Hematocrit, %	M 40–50, F 35–45	41.8 (38.5–42.9)
Platelets, $\times 10^3/\mu L$	150–350	115 (79–150)
Aspartate transaminase, IU/L	13–33	35 (22–41)
Alanine transaminase, IU/L	8–42	20 (14–26)
Lactate dehydrogenase, IU/L	119–229	227 (166–261)
C-reactive protein, mg/L	0–0.3	6.1 (2.2–16.1)

for patients with secondary rather than primary DENV infection (*5*).

Of the 19 cases, 18 were confirmed as DENV-1 infection by real-time PCR (TaqMan; Life Technologies, Grand Island, NY, USA) (*6*), and samples were positive for NS1 antigen (Platelia Dengue NS1 Antigen assay; Bio-Rad Laboratories, Marnes-la-Coquette, France). The remaining case (case 11) was confirmed positive for IgM and IgG against DENV by dengue IgM ELISA (Focus Diagnostics, Inc., Cypress, CA, USA) and dengue IgG ELISA (Vircell, Granada, Spain), respectively. The serotype of the DENV in the other 18 patients was confirmed to be serotype 1. Nucleotide sequences were determined by using BigDye Terminator version 3.1 (Applied Biosystems, Foster City, CA, USA). Phylogenetic analysis of the DENV envelope (E) protein genome sequence obtained from serum from patient 2 (GenBank accession no. LC006123) demonstrated that the E protein shared 100% homology with the sequence of a DENV-1 strain from the first patient in this outbreak in Japan (GenBank accession no. LC002828). The sequence from patient 2 shared 99.7% identity with the sequence of a DENV strain isolated in Guangzhou, China, in 2013 (GenBank accession no. KJ545459) and 99.3% identity with the sequence of a DENV strain isolated in Indonesia in 2010 (GenBank accession no. JN415489) (Figure 2). The sequence of the E protein from another 2 patients (patients 4 and 10) shared 100% homology with that of patient 2.

Conclusions

Our results suggest that a single strain may have caused most of the DENV cases in Tokyo. A similar outbreak of dengue fever had been reported in Ningbo, China (68 cases) (*7*), and in Hawaii, USA (122 cases) (*8*).

DENV is transmitted mainly through the bite of the *Aedes aegypti* mosquito, which is distributed in tropical and subtropical regions. In Japan, the distribution of *Ae. aegypti* mosquitoes is limited, and as of 2013, these mosquitoes had been found only at the Narita International Airport (*9*), which is located ≈60 km from the site of the DENV outbreak in Tokyo. In contrast, the distribution of *Ae. Albopictus* mosquitoes, another vector of DENV, extends from western regions to northern regions of

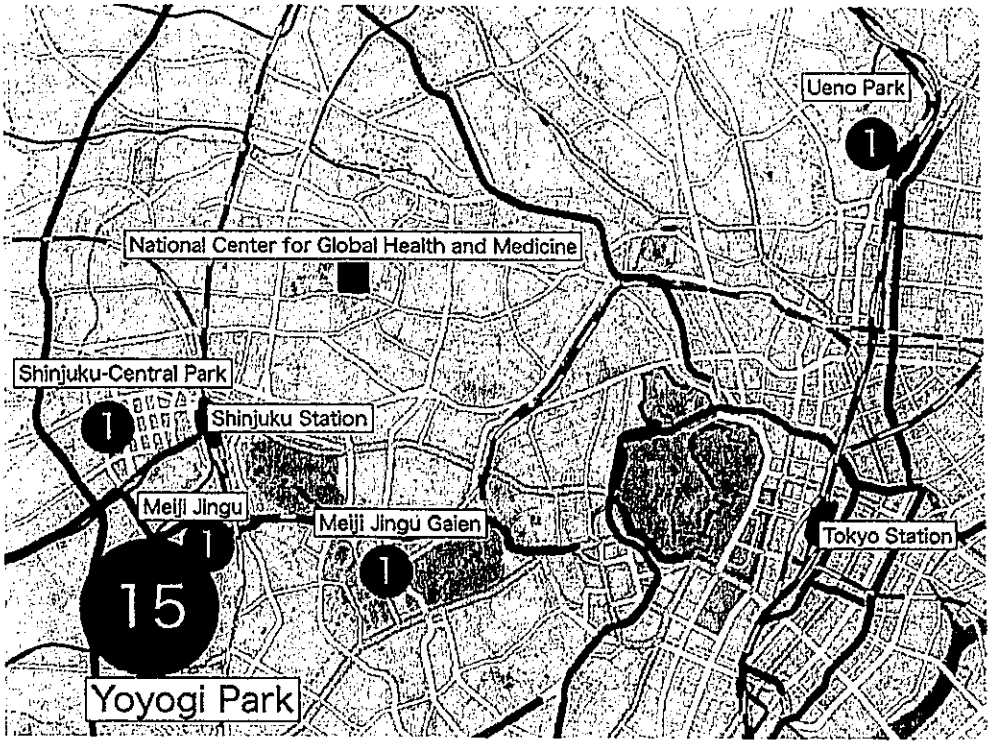

Figure 1. Locations of presumptive exposure to dengue virus mosquito vectors for 19 patients, Tokyo, Japan, August 26–September 22, 2014. Numbers in circles indicate numbers of cases contracted at each location.

Japan, including Tokyo. *Ae. albopictus* mosquitoes are also expanding into the northern regions of the main island because of global warming (*10*). Tokyo is one of the most heavily populated cities in the world, and Yoyogi Park is located at the center of the Shinjuku-Shibuya area in Tokyo.

The population density of *Ae. albopictus* mosquitoes in Tokyo is higher than that in suburban areas (*11*). It is possible that high human and mosquito population densities contributed to this outbreak. All 19 patients had been bitten by a mosquito while in Tokyo, mainly in Yoyogi Park, where most of the 160 patients with autochthonous dengue cases had also been (*12*).

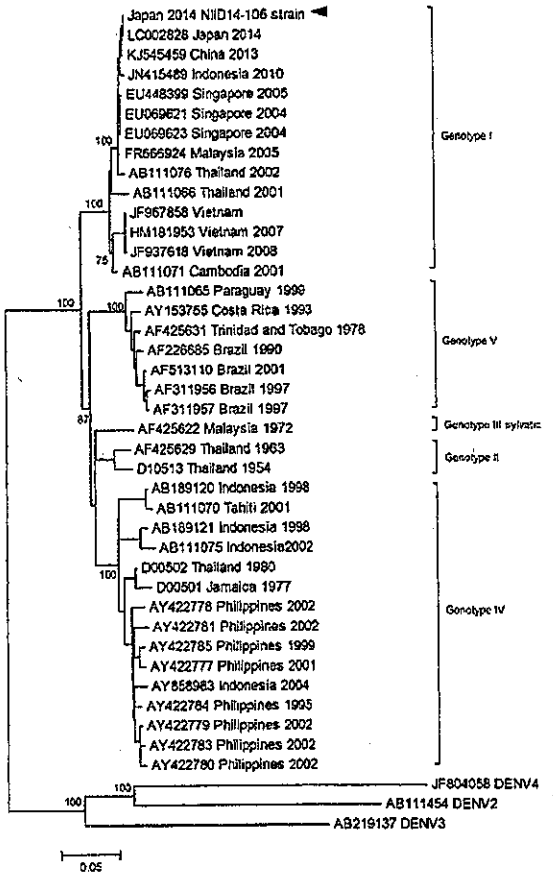

Figure 2. Phylogenetic analysis of a dengue virus (DENV) sequence derived from a patient with confirmed autochthonous dengue fever (patient 2), Tokyo, Japan, contracted during August 26–September 22, 2014. Phylogenetic tree is based on the envelope protein genome sequence of selected dengue virus type-1 (DENV-1) strains. DENV-2, DENV-3, and DENV-4 serotypes were used as outgroups. Percentages of successful bootstrap replication are indicated at the nodes. DENV-1 genotypes are indicated on the right. The DENV-1 National Institute of Infectious Diseases (NIID) strain 14-106 (GenBank accession no. LC006123) is indicated with an arrowhead. Virus strains are indicated by GenBank accession number, place, and date of isolation. Scale bar indicates number of nucleotide substitutions per site.

Recently, a case of dengue fever imported to England from Japan was found to be associated with this outbreak (*13*). Previous investigators speculated that the virus may have been spread from infected visitors by mosquitoes in the park (*13*). The outbreak, however, coincides with a period during which several tropical-themed festivals and activities were hosted, July–August 2014. These activities attracted a high number of local and international visitors to the park.

Before this 2014 outbreak, dengue fever was diagnosed for a German traveler who had returned from Japan in 2013 (*14*). Neutralization tests confirmed that the traveler's infection was caused by DENV-2. She was reportedly bitten by mosquitoes when in Fuefuki, but she had also traveled to Tokyo and Kyoto during her trip to Japan. No local DENV cases were reported in 2013, although cases may have been underreported because of the lack of local dengue outbreaks in Japan for the past 70 years.

Because adult *Ae. albopictus* mosquitoes cannot survive the winter in Japan, only eggs overwinter (*15*). Thus, the outbreak of autochthonous dengue fever is expected to end as mosquito activity decreases during autumn. The Japanese government is currently strengthening vector control measures and increasing awareness among residents to prevent similar outbreaks.

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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 2015年5月25日	新医薬品等の区分 該当なし。	総合機構処理欄
一般的名称	別紙のとおり。		研究報告の公表状況	EID;21(2):359-361, 2015	公表国 タヒチ	
販売名（企業名）	別紙のとおり。					

研究報告の概要

問題点：タヒチで、血精液症の患者の精液からジカウイルスが分離された。

2013年12月、仏領ポリネシアでのジカウイルスのアウトブレイク中にタヒチで血精液症の患者が受診し、患者の精液からジカウイルスが分離された。以前から性行為によるジカウイルスの伝搬が示唆されていたが、今回の事例はこれを裏付けるものである。

ジカウイルスは、フラビウイルス科フラビウイルス属の蚊媒介性アルボウイルスである。1947年にウガンダのジカの森のアカゲザルから初めて分離された。1960年代には、アジア及びアフリカでヒトの孤発症例が報告された。2007年には、ミクロネシア連邦のヤップ島での最初の大流行が報告された。最大規模のジカウイルス大流行報告は、2013年10月に南太平洋のフランス領ポリネシア（居住者のいる67の島からなるフランス領）で始まったもので、約28,000人（人口の11%）が病気のため受診した。ジカ熱に最も多い症状は、発疹、発熱、関節痛、及び結膜炎である。患者の大半は軽微であったが、重度の神経学的合併症がフランス領ポリネシアの患者で見られている。

報告企業の意見	今後の対応
別紙のとおり。	今後とも関連情報の収集に努め、本剤の安全性の確保を図っていきたい。

使用上の注意記載状況・その他参考事項等

記載なし。

別紙

一般的名称	①人血清アルブミン、②人血清アルブミン、③人血清アルブミン*、④人免役グロブリン、⑤人免役グロブリン、⑥乾燥ペプシン処理人免疫グロブリン、⑦乾燥スルホ化人免疫グロブリン、⑧乾燥スルホ化人免疫グロブリン、⑨乾燥スルホ化人免疫グロブリン、⑩乾燥スルホ化人免疫グロブリン、⑪乾燥スルホ化人免疫グロブリン*、⑫乾燥濃縮人活性化プロテインC、⑬乾燥濃縮人血液凝固第Ⅷ因子、⑭乾燥濃縮人血液凝固第Ⅷ因子、⑮乾燥濃縮人血液凝固第Ⅷ因子、⑯乾燥濃縮人血液凝固第Ⅸ因子、⑰乾燥濃縮人血液凝固第Ⅸ因子、⑱乾燥濃縮人血液凝固第Ⅸ因子、⑲乾燥抗破傷風人免疫グロブリン、⑳抗HBs人免疫グロブリン、㉑抗HBs人免疫グロブリン、㉒抗HBs人免疫グロブリン、㉓トロンビン、㉔トロンビン、㉕トロンビン、㉖フィブリノゲン加第ⅩⅢ因子*、㉗フィブリノゲン加第ⅩⅢ因子、㉘乾燥濃縮人アンチトロンビンⅢ、㉙ヒスタミン加人免疫グロブリン製剤、㉚人血清アルブミン*、㉛人血清アルブミン*、㉜乾燥ペプシン処理人免役グロブリン*、㉝乾燥濃縮人アンチトロンビンⅢ、㉞乾燥濃縮人血液凝固第Ⅹ因子加活性化第Ⅶ因子
販売名（企業名）	①献血アルブミン20“化血研”、②献血アルブミン25“化血研”、③人血清アルブミン“化血研”*、④ガンマーグロブリン筋注450mg/3mL「化血研」、⑤ガンマーグロブリン筋注1500mg/10mL「化血研」、⑥献血グロブリン注射用2500mg「化血研」、⑦献血ベニロンーⅠ静注用500mg、⑧献血ベニロンーⅠ静注用1000mg、⑨献血ベニロンーⅠ静注用2500mg、⑩献血ベニロンーⅠ静注用5000mg、⑪ベニロン*、⑫注射用アナクトC2,500単位、⑬コンファクトF注射用250、⑭コンファクトF注射用500、⑮コンファクトF注射用1000、⑯ノバクトM静注用400単位、⑰ノバクトM静注用800単位、⑱ノバクトM静注用1600単位、⑲テタノセーラ筋注用250単位、⑳ヘパトセーラ筋注200単位/mL、㉑ヘパトセーラ筋注200単位/1mL、㉒ヘパトセーラ筋注1000単位/5mL、㉓トロンビン“化血研”、㉔献血トロンビン経口・外用5千「化血研」、㉕献血トロンビン経口・外用1万「化血研」、㉖ボルヒール*、㉗ボルヒール組織接着用、㉘アンスロビンP500注射用、㉙ヒスタグロビン皮下注用、㉚アルブミン20%化血研*、㉛アルブミン5%化血研*、㉜静注グロブリン*、㉝アンスロビンP1500注射用、㉞バイクロット配合静注用、㉟ノバクトM静注用500単位、㊱ノバクトM静注用1000単位、㊲ノバクトM静注用2000単位
報告企業の意見	ジカウイルスは、日本脳炎ウイルス、デングウイルス、ウエストナイルウイルスなどと同属のフラビウイルス属の蚊媒介性感染症ウイルスとして知られており、一本鎖RNA及びエンベロープを有するウイルスである。今回、報告された症例では、蚊による感染経路以外の新たな感染経路が示唆される。 上記製剤の製造工程には、冷エタノール分画工程、ウイルス除去膜ろ過工程、加熱工程といった原理の異なるウイルスクリアランス工程が複数導入されており、各工程のウイルスクリアランス効果は「血漿分画製剤のウイルスに対する安全性確保に関するガイドライン（医薬発第1047号、平成11年8月30日）」に基づく、モデルウイルスを用いたウイルスプロセスバリデーションにより確認されている。今回報告したジカウイルスのモデルウイルスには、エンベロープの有無、核酸の種類等から、ウシウイルス性下痢ウイルス（BVDV）が該当すると考えられるが、上記工程のBVDVクリアランス効果については上記バリデーションにより確認されている。また、これまでに上記製剤によるジカウイルスへの感染報告例は無い。 以上の点から、上記製剤はジカウイルスに対する安全性を確保していると考える。

*：現在製造を行っていない

Potential Sexual Transmission of Zika Virus

Didier Musso, Claudine Roche, Emilie Robin, Tuxuan Nhan, Anita Teissier, Van-Mai Cao-Lormeau

In December 2013, during a Zika virus (ZIKV) outbreak in French Polynesia, a patient in Tahiti sought treatment for hematospermia, and ZIKV was isolated from his semen. ZIKV transmission by sexual intercourse has been previously suspected. This observation supports the possibility that ZIKV could be transmitted sexually.

Zika virus (ZIKV) is a mosquitoborne arbovirus in the family *Flaviviridae*, genus *Flavivirus*. It was first isolated in 1947 from a rhesus monkey in the Zika forest of Uganda (*1*). Sporadic human cases were reported from the 1960s in Asia and Africa. The first reported large outbreak occurred in 2007 on Yap Island, Federated States of Micronesia (*2*). The largest known ZIKV outbreak reported started in October 2013 in French Polynesia, South Pacific (*3*), a territory of France comprising 67 inhabited islands; an estimated 28,000 persons (11% of the population) sought medical care for the illness (*4*). The most common symptoms of Zika fever are rash, fever, arthralgia, and conjunctivitis. Most of the patients had mild disease, but severe neurologic complications have been described in other patients in French Polynesia (*5*).

The Study

In early December 2013, during the ZIKV outbreak, a 44-year-old man in Tahiti had symptoms of ZIKV infection: asthenia, low grade fever (temperature from 37.5°C to 38°C) and arthralgia. Symptoms lasted 3 days. Eight weeks later, he described a second episode of symptoms compatible with ZIKV infection: temperature from 37.5°C to 38°C, headache on days 1–3, and wrist arthralgia on days 5–7. The patient did not seek treatment, thus biological samples were not collected during the first 2 periods of illness. The patient fully recovered from the second episode, but 2 weeks later he noted signs of hematospermia and sought treatment. Because the patient had experienced symptoms of ZIKV infection some weeks before, he was referred to our laboratory in the Institut Louis Malardé, Papeete, Tahiti for ZIKV infection diagnostic testing. The medical questionnaire revealed no signs of urinary tract infection, prostatitis, urethritis, or cystitis, and the patient stated that he did not had any recent physical contact with persons who had acute ZIKV infection. We collected blood and semen samples. Direct and macroscopic examinations of the semen confirmed hematospermia. We extracted RNA using the NucliSENS easyMAG system (bioMérieux, Marcy l'Etoile, France) from 200 µL of blood and from 500 µL of semen and urine; both were eluted by 50 µL of elution buffer. We used 5 µL of RNA extracted for amplification. We tested blood and semen RNA extracts using real-time reverse transcription PCR (rRT-PCR) as described using 2 primers/probe amplification sets specific for ZIKV (*3*). The rRT-PCR results were positive for ZIKV in semen and negative in blood, and confirmed by sequencing of the genomic position 858–1138 encompassing the prM/E protein coding regions of ZIKV. The generated sequence (GenBank accession no. KM014700) was identical to those previously reported at the beginning of the ZIKV outbreak (*3*). Three days later, we collected a urine sample, then a second set of blood and semen samples. Semen and urine from this second collection were not found to contain traces of blood by both direct and macroscopic examinations. rRT-PCR detected ZIKV RNA in the semen and urine, but not in the blood sample.

We quantified ZIKV RNA loads using an RNA synthetic transcript standard that covers the region targeted by the 2 primers/probe sets. RNA loads were: 2.9×10^7 copies/mL and 1.1×10^7 copies/mL in the first and second semen samples, respectively, and 3.8×10^3 copies/mL in the urine sample.

We cultured semen and urine as described for dengue virus cultured from urine (*6*). Briefly, 200 µL of each sample diluted in 200 µL of 1% fetal calf serum (FCS) minimum essential medium (MEM) were inoculated onto Vero cells and incubated for 1 h at 37°C; inoculum was then removed and replaced by 1 mL of culture medium. We also inoculated a negative control (200 µL of 1% FCS-MEM) and a positive control (5 µL of a ZIKV-positive serum diluted in 200 µL of 1% FCS-MEM). The cells were then incubated at 37°C in 5% CO_2 for 6 days. The presence of ZIKV in the culture fluids was detected by rRT-PCR as described.

Replicative ZIKV particles were found in the 2 semen samples but none were detected in the urine sample. This finding does not exclude the possibility that ZIKV particles were present in urine. Positive samples were not titered.

Conclusions

The ZIKV natural transmission cycle involves mosquitoes, especially *Aedes* spp. (*7*), but perinatal transmission (*8*) and potential risk for transfusion-transmitted ZIKV infections has also been demonstrated (*9*). Moreover, ZIKV

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transmission by sexual intercourse has been suggested by Foy et al. (*10*), who described a patient who was infected with ZIKV in southeastern Senegal in 2008. After returning to his home in Colorado, United States, he experienced common symptoms of ZIKV infection and symptoms of prostatitis. Four days later, he observed signs of hematospermia, and on the same day, his wife had symptoms of ZIKV infection. Because the wife of the patient had not traveled out of the United States during the previous year and had sexual intercourse with him 1 day after he returned home, transmission by semen was suggested. ZIKV infection of the patient and his wife was confirmed by serologic testing, but the presence of ZIKV in the semen of the patient was not investigated.

Infectious organisms, especially sexually transmitted microorganisms including viruses (human papillomavirus or herpes simplex virus), are known to be etiologic agents of hematospermia (*11*). To our knowledge, before the report of Foy et al. (*10*) and this study, arbovirus infections in humans had not been reported to be associated with hematospermia, and no arboviruses had been isolated from human semen.

We detected a high ZIKV RNA load and replicative ZIKV in semen samples, but ZIKV remained undetectable by rRT-PCR in the blood sample collected at the same time. These results suggest that viral replication may have occurred in the genital tract, but we do not know when this replication started and how long it lasted. The fact that the patient had no common symptoms of ZIKV acute infection concomitantly to hematospermia suggests that the viremic phase occurred upstream, probably during the first or second episode of mild fever, headache, and arthralgia.

The detection of ZIKV in both urine and semen is consistent with the results obtained in a study of effects of Japanese encephalitis virus, another flavivirus, on boars. The virus was isolated from urine and semen of experimentally infected animals, and viremia developed in female boars that artificially inseminated with the infectious semen (*12*).

Flaviviruses have also been detected in urine of persons infected with West Nile virus (WNV). WNV RNA was detected in urine for a longer time and with higher RNA load than in plasma (*13*). WNV antigens were detected in renal tubular epithelial cells, vascular endothelial cells, and macrophages of kidneys from infected hamsters (*14*), suggesting that persistent shedding of WNV in urine was caused by viral replication in renal tissue. Dengue virus (DENV) RNA and DENV nonstructural protein 1 antigen were also detected in urine samples for a longer time than in blood, but infectious DENV has not been isolated in culture. Hirayama et al. concluded that the detection of DENV by rRT-PCR was useful to confirm DENV infections after the viremic phase (*6*). Also, yellow fever virus RNA was isolated from the urine of vaccinated persons (*15*), and Saint Louis encephalitis viral antigens, but not infective virus, have been detected in urine samples from infected patients (*10*).

Our findings support the hypothesis that ZIKV can be transmitted by sexual intercourse. Furthermore, the observation that ZIKV RNA was detectable in urine after viremia clearance in blood suggests that, as found for DENV and WNV infections, urine samples can yield evidence ofZIKV for late diagnosis, but more investigation is needed.

We obtained written informed consent from the patient for publication of this report, and publication of data related to ZIKV infections have been approved by the Ethics Committee of French Polynesia under reference 66/CEPF.

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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 2015年5月25日	新医薬品等の区分 該当なし。	総合機構処理欄
一般的名称	別紙のとおり。		研究報告の公表状況	EID;17(5):880-882, 2011	公表国 米国	
販売名（企業名）	別紙のとおり。					

研究報告の概要

問題点：セネガルでジカウイルスに感染したアメリカ人科学者が、帰国後、妻に性行為によりウイルスを感染させた。

米国で、二人のアメリカ人の科学者が2008年にセネガルでジカウイルスに感染した。一人は帰国後妻にこのウイルスを感染させた。直接の接触が感染経路として示唆されたが、可能性として考えられるのは性行為による感染である。

ジカウイルス（蚊が媒介するフラビウイルス）が、アフリカ及び東南アジアのsentinel monkeys、蚊、及び病人から分離されている。ジカウイルス感染がセネガルの東南部及びアフリカのその他の地域の人に比較的多いが、ジカウイルス関連の疾患は実際より少なく報告されている又は誤診されている可能性があることが血清学的調査から示唆される。2007年、西南太平洋のヤップ島でジカウイルス感染が流行し、島民の70%が感染した。このことから、このウイルスが新興の病原体であることが浮き彫りになった。本試験の目的は、2008年コロラド州で起こったアルボウイルス病の稀な症例3件を調査報告することであった。

報告企業の意見	今後の対応
別紙のとおり。	今後とも関連情報の収集に努め、本剤の安全性の確保を図っていきたい。

使用上の注意記載状況・その他参考事項等

記載なし。

別紙

一般的名称	①人血清アルブミン、②人血清アルブミン、③人血清アルブミン*、④人免役グロブリン、⑤人免役グロブリン、⑥乾燥ペプシン処理人免疫グロブリン、⑦乾燥スルホ化人免疫グロブリン、⑧乾燥スルホ化人免疫グロブリン、⑨乾燥スルホ化人免疫グロブリン、⑩乾燥スルホ化人免疫グロブリン、⑪乾燥スルホ化人免疫グロブリン*、⑫乾燥濃縮人活性化プロテインC、⑬乾燥濃縮人血液凝固第Ⅷ因子、⑭乾燥濃縮人血液凝固第Ⅷ因子、⑮乾燥濃縮人血液凝固第Ⅷ因子、⑯乾燥濃縮人血液凝固第Ⅸ因子、⑰乾燥濃縮人血液凝固第Ⅸ因子、⑱乾燥濃縮人血液凝固第Ⅸ因子、⑲乾燥抗破傷風人免疫グロブリン、⑳抗 HBs 人免疫グロブリン、㉑抗 HBs 人免疫グロブリン、㉒抗 HBs 人免疫グロブリン、㉓トロンビン、㉔トロンビン、㉕トロンビン、㉖フィブリノゲン加第ⅩⅢ因子*、㉗フィブリノゲン加第ⅩⅢ因子、㉘乾燥濃縮人アンチトロンビンⅢ、㉙ヒスタミン加人免疫グロブリン製剤、㉚人血清アルブミン*、㉛人血清アルブミン*、㉜乾燥ペプシン処理人免役グロブリン*、㉝乾燥濃縮人アンチトロンビンⅢ、㉞乾燥濃縮人血液凝固第Ⅹ因子加活性化第Ⅶ因子
販売名(企業名)	①献血アルブミン 20“化血研”、②献血アルブミン 25“化血研”、③人血清アルブミン“化血研”*、④ガンマーグロブリン筋注 450mg/3mL「化血研」、⑤ガンマーグロブリン筋注 1500mg/10mL「化血研」、⑥献血グロブリン注射用 2500mg「化血研」、⑦献血ベニロンーⅠ静注用 500mg、⑧献血ベニロンーⅠ静注用 1000mg、⑨献血ベニロンーⅠ静注用 2500mg、⑩献血ベニロンーⅠ静注用 5000mg、⑪ベニロン*、⑫注射用アナクトC 2,500 単位、⑬コンファクトF注射用 250、⑭コンファクトF注射用 500、⑮コンファクトF注射用 1000、⑯ノバクトM静注用 400 単位、⑰ノバクトM静注用 800 単位、⑱ノバクトM静注用 1600 単位、⑲テタノセーラ筋注用 250 単位、⑳ヘパトセーラ筋注 200 単位/mL、㉑ヘパトセーラ筋注 200 単位/1mL、㉒ヘパトセーラ筋注 1000 単位/5mL、㉓トロンビン“化血研”、㉔献血トロンビン経口・外用5千「化血研」、㉕献血トロンビン経口・外用1万「化血研」、㉖ボルヒール*、㉗ボルヒール組織接着用、㉘アンスロビンP500 注射用、㉙ヒスタグロビン皮下注用、㉚アルブミン 20%化血研*、㉛アルブミン 5%化血研*、㉜静注グロブリン*、㉝アンスロビンP1500 注射用、㉞バイクロット配合静注用、㉟ノバクトM静注用 500 単位、㊱ノバクトM静注用 1000 単位、㊲ノバクトM静注用 2000 単位
報告企業の意見	ジカウイルスは、日本脳炎ウイルス、デングウイルス、ウエストナイルウイルスなどと同属のフラビウイルス属の蚊媒介性感染症ウイルスとして知られており、一本鎖 RNA 及びエンベロープを有するウイルスである。今回、報告された症例では、蚊による感染経路以外の新たな感染経路が示唆される。 上記製剤の製造工程には、冷エタノール分画工程、ウイルス除去膜ろ過工程、加熱工程といった原理の異なるウイルスクリアランス工程が複数導入されており、各工程のウイルスクリアランス効果は「血漿分画製剤のウイルスに対する安全性確保に関するガイドライン(医薬発第 1047 号、平成 11 年8月 30 日)」に基づく、モデルウイルスを用いたウイルスプロセスバリデーションにより確認されている。今回報告したジカウイルスのモデルウイルスには、エンベロープの有無、核酸の種類等から、ウシウイルス性下痢ウイルス(BVDV)が該当すると考えられるが、上記工程の BVDV クリアランス効果については上記バリデーションにより確認されている。また、これまでに上記製剤によるジカウイルスへの感染報告例は無い。 以上の点から、上記製剤はジカウイルスに対する安全性を確保していると考える。

*:現在製造を行っていない

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Probable Non–Vector-borne Transmission of Zika Virus, Colorado, USA

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Clinical and serologic evidence indicate that 2 American scientists contracted Zika virus infections while working in Senegal in 2008. One of the scientists transmitted this arbovirus to his wife after his return home. Direct contact is implicated as the transmission route, most likely as a sexually transmitted infection.

Zika virus (ZIKV), a mosquito-transmitted flavivirus, has been isolated from sentinel monkeys, mosquitoes, and sick persons in Africa and Southeast Asia (*1*,*2*). Serologic surveys indicate that ZIKV infections can be relatively common among persons in southeastern Senegal and other areas of Africa, but that ZIKV-associated disease may be underreported or misdiagnosed. In 2007, a large outbreak of ZIKV infection occurred on Yap Island in the southwestern Pacific that infected ≈70% of the island's inhabitants (*3*), which highlighted this virus as an emerging pathogen. The purpose of this study was to investigate and report 3 unusual cases of arboviral disease that occurred in Colorado in 2008.

The Study

Two American scientists (patients 1 and 2) lived and worked in the village of Bandafassi in southeastern Senegal in August 2008, while performing a mosquito-sampling project in surrounding villages. Patients 1 and 2 were men (36 and 27 years of age, respectively), and both had received the yellow fever 17D vaccine before their travel to Senegal. During their project, both patients reported being bitten often by wild *Aedes* spp. mosquitoes in the evenings while they worked. Patients 1 and 2 left Bandafassi on August 21, stayed in Dakar for 2 days, and then returned to their homes in northern Colorado on August 24. Both patients became ill 6–9 days after their return.

Symptoms in patient 1 began on August 30 and consisted of swollen ankles, a maculopapular rash on his torso, and extreme fatigue and headache, but no fever was recorded. On August 31, he experienced the same symptoms and light-headedness and chills, wrist and ankle arthalgia, and symptoms of prostatitis (perineal pain and mild dysuria). However, he remained afebrile. Fatigue and rash decreased on September 1; only residual wrist arthralgia, headache, and prostatic symptoms persisted. On September 2, two aphthous ulcers appeared on his lip. On September 3, he and his wife observed signs of hematospermia (red–brown fluid in his ejaculate) that lasted until September 7. Patient 2 experienced his symptoms during August 29–September 1, which included a maculopapular rash on his torso, extreme fatigue, headache, and swelling and arthralgia in his wrists, knees, and ankles. However, symptoms of prostatitis or hematospermia did not devlop. Acute-phase blood samples were obtained from both patients on September 2.

In patient 3 (a nurse and the wife of patient 1) similar clinical symptoms developed on September 3, including malaise, chills, extreme headache, photophobia, and muscle pain that continued through September 6. She did not have detectable fever. On September 7, a maculopapular rash developed on her torso (Figure) that expanded to her neck and thighs on the following day, and an aphthous ulcer developed on her inside lip. On September 8, arthralgia in her wrists and thumbs and conjunctivitis developed. Her acute symptoms waned over the next several days. Patient 3 had an acute-phase blood sample drawn on September 8. On September 11, she visited her primary care physician, who performed a complete blood count test and studies of hepatic function; all results were within reference ranges. Patient 2 reported wrist

arthralgia for 1 month after his acute illness, and patients 1 and 3 have had recurring wrist or thumb joint arthralgia since their acute illness. Convalescent-phase blood samples were drawn on September 22 from patients 1 and 2 and on September 26 from patient 3.

Acute-phase and convalescent-phase paired serum specimens from the 3 patients were tested independently by several different laboratories. Results of virus isolation were negative for all samples when tested in Vero and *Aedes albopictus* mosquito (C6/36) cell cultures and by intracerebral inoculation of acute-phase serum of patient 3 into suckling mice. Likewise, reverse transcriptase–PCRs with 16 different sets of arbovirus-specific primers did not detect arboviral RNA in any of the samples. Serologic analyses (Appendix Table) of samples from patients 1 and 2 showed matching results. Hemagglutination inhibition antibody titers and virus neutralizing titers were highly elevated above background levels for ZIKV and yellow fever virus (YFV) compared with other viruses tested. These titers most often increased in the time between obtaining acute-phase and convalescent-phase serum samples. Complement fixation tests against ZIKV and YFV antigens confirmed these interpretations. Hemagglutination inhibition, complement fixation, and virus neutralizing titers against ZIKV alone developed only in the convalescent-phase sample of patient 3.

Conclusions

Evidence suggests that patients 1 and 2 were infected with ZIKV, probably in southeastern Senegal, by bites from infected mosquitoes. The village of Bandafassi is located in a disease-endemic area where ZIKV has been isolated from humans, nonhuman primates, and mosquitoes (*4*,*5*). Serologic results suggest an anamnestic response to ZIKV infection, likely stemming from their vaccination with YFV. The time between infection and the onset of clinical manifestations can be inferred to be ≥9 days, given the patients' travel history. Their clinical symptoms are consistent with reported symptoms of ZIKV-associated disease (*3*,*6*–*9*). Exceptions are aphthous ulcers in patient 1 (also reported by patient 3), prostatitis, and hematospermia. Whether these exceptions are typical but unreported symptoms or clinical anomalies is not clear.

Results also support ZIKV transmission from patient 1 to patient 3. Patient 3 had never traveled to Africa or Asia and had not left the United States since 2007. ZIKV has never been

reported in the Western Hemisphere. Circumstantial evidence suggests direct person-to-person, possibly sexual, transmission of the virus. Temperatures and mosquito fauna on the northern Front Range in Colorado when transmission occurred do not match known mosquito transmission dynamics of ZIKV by tropical *Aedes* species. Patient 3 had ZIKV disease 9 days after the return of her husband from Senegal. However, the extrinsic incubation period of ZIKV in *Ae. aegypti* mosquitoes was >15 days at 22°C–26°C (*10*). Area temperatures during the week of return of patient 1 fluctuated between 10°C and 31°C, only *Ae. vexans* mosquitoes of the subgenus *Aedimorphus* are commonly captured on the northern Colorado Front Range, and known tropical ZIKV vectors are mostly from the subgenus *Stegomyia* (*4*). Mosquito sampling around the home of patients 1 and 3 at the time yielded only 7 *Ae. vexans* mosquitoes and 11 other mosquitoes of the *Culex* and *Culiseta* genera.

Furthermore, patients 1 and 3 reported having vaginal sexual intercourse in the days after patient 1 returned home but before the onset of his clinical illness. It is reasonable to suspect that infected semen may have passed from patient 1 to patient 3 during coitus. Another possibility is that direct contact and exchange of other bodily fluids, such as saliva, could have resulted in ZIKV transmission, but illness did not develop in the 4 children of patients 1 and 3 during this time.

To the best of our knowledge, human sexual transmission of an arbovirus has not been documented. However, Japanese encephalitis virus was discharged into the semen of experimentally infected boars and could infect female pigs by artificial insemination (*11*). Furthermore, West Nile virus RNA and St. Louis encephalitis virus antigen have been detected in urine of humans (*12,13*), and viruria has occurred in hamsters infected with West Nile virus (*14*) and Modoc virus (*15*). If sexual transmission could be verified in subsequent studies, this would have major implications toward the epidemiology of ZIKV and possibly other arthropod-borne flaviviruses.

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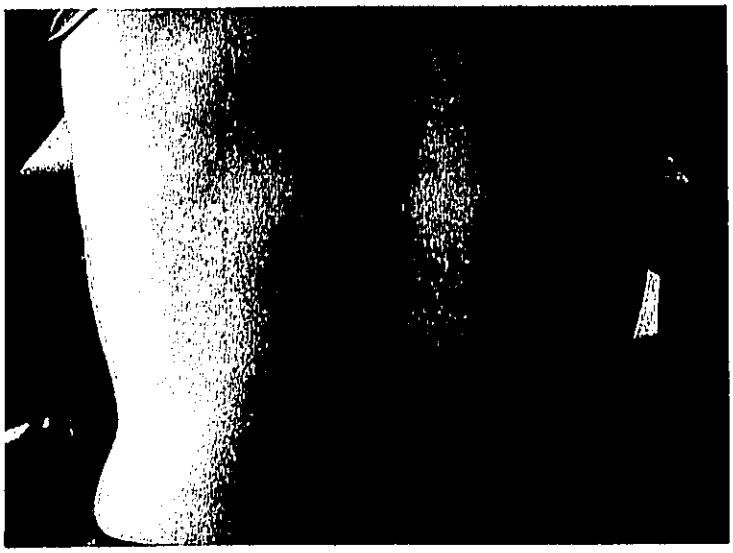

Figure. Maculopapular rash on patient 3 infected with Zika virus, Colorado, USA.

Appendix Table. Serologic results for 3 patients in whom symptoms of arboviral illness developed, northern Colorado, USA, 2008*

Serum sample	Viruses/viral antigens tested														
	GETV	CHIKV	SINV	ZIKV	DENV1	DENV2	DENV3	DENV4	YFV	WNV	UGSV	SLEV	TAHV	JCV	LACV
Hemagglutination inhibition test															
Pt1a	0	0	NT	5,120	2,560	640	2,560	2,560	5,120	2,560	1,280	NT	0	NT	NT
Pt1c	0	0	NT	5,120	1,280	320	1,280	1,280	2,560	1,280	1,280	NT	0	NT	NT
Pt2a	0	0	NT	1,280	160	80	320	320	1,280	160	160	NT	0	NT	NT
Pt2c	0	0	NT	2,560	160	80	320	320	1,280	160	160	NT	0	NT	NT
Pt3a	0	0	NT	0	0	0	0	0	0	0	0	NT	0	NT	NT
Pt3c	0	0	NT	320	0	0	0	0	0	0	0	NT	0	NT	NT
Plaque-reduction neutralization test															
Pt1a	NT	<10	<10	**10,240**	80	**1,280**	320	160	**≥20,480**	<10	NT	80	<10	NT	NT
Pt1c	NT	NT	NT	**≥40,960**	160	230	320	320	**≥40,960**	160	NT	NT	NT	NT	NT
Pt2a	NT	NT	NT	**10,240**	<20	80	80	40	**5,120**	NT	NT	NT	NT	NT	NT
Pt2c	NT	NT	NT	**40,960**	80	160	160	160	**20,480**	80	NT	NT	NT	NT	NT
Pt3a	NT	NT	NT	10	<10	<10	<10	<10	<10	<10	NT	<10	NT	<10	<10
Pt3c	NT	NT	NT	**1,280**	<10	<10	<10	<10	<10	<10	NT	NT	NT	NT	NT
Complement fixation test															
Pt1a	NT	NT	NT	64/8	NT	NT	NT	NT	64/8	NT	NT	NT	NT	NT	NT
Pt1c	NT	NT	NT	128/32	NT	NT	NT	NT	64/8	NT	NT	NT	NT	NT	NT
Pt2a	NT	NT	NT	16/8	NT	NT	NT	NT	16/8	NT	NT	NT	NT	NT	NT
Pt2c	NT	NT	NT	32/32	NT	NT	NT	NT	16/8	NT	NT	NT	NT	NT	NT
Pt3a	NT	NT	NT	0	NT	NT	NT	NT	0	NT	NT	NT	NT	NT	NT
Pt3c	NT	NT	NT	16/8	NT	NT	NT	NT	0	NT	NT	NT	NT	NT	NT

*_Alphaviridae_: GETV, Getah virus; CHIKV, chikungunya virus; SINV, Sindbis virus; _Flaviviridae_: ZIK, Zika virus; DENV, dengue virus; YFV, yellow fever virus; WNV, West Nile virus; UGSV, Uganda S virus; SLEV, St. Louis encephalitis virus; _Bunyaviridae_: TAHV, Tahyna virus; JCV, Jamestown Canyon virus; LAC, La Crosse virus; pt1, 2, and 3, patients 1, 2, and 3, respectively; a and c, acute-phase and convalescent-phase serum samples, respectively; NT, not tested. Hemagglutination test, numbers are reciprocal of serum dilution inhibiting 4 units of antigen; 0 = negative reaction at a 1:20 dilution. Plaque-reduction neutralization test, numbers refer to neutralizing antibody titers with a 90% cutoff value: titers ≥ positive control serum samples are indicated in **boldface**. Complement fixation tests, numbers refer to reciprocal of serum titer/repicocal of the antigen titer; 0, negative reaction at <8/<8.

別紙様式第2-1

No. 11

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
				2015. 4. 4	該当なし	

一般的名称	人赤血球液	研究報告の公表状況		公表国
販売名(企業名)	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) 赤血球液-LR「日赤」(日本赤十字社) 照射赤血球液-LR「日赤」(日本赤十字社)		Sonnenberg P, Field N. Clin Infect Dis. 2015 Mar 15;60(6):974-5. doi: 10.1093/cid/ciu981. Epub 2014 Dec 11.	英国

研究報告の概要

エボラウイルスは、回復後の血液、糞便、嘔吐物等から検出されなくなった後も、精液、膣分泌物、母乳からは検出される。精巣や乳腺等にウイルスが持続する理由としては、これらの組織が免疫的に隔離された部位であり、ウイルス排除が遅れるというものである。
世界保健機構は、エボラの発症から3か月間はコンドームを使用するよう推奨しているが、我々の知る限り、精液中のウイルス残存に関しては12名について、膣分泌物に関しては1名の患者の検体から得られた所見に過ぎない。これらの患者に関しては、精液では最長101日、膣分泌物では33日間のウイルス残存が報告されているが、それ以降の検体は得られず、十分な数の被験者の泌尿生殖器検体を、長期に渡って系統的に調べた研究はない。また、回復期の患者に向けた授乳のガイドラインは作成されていない。ウイルスは、授乳期の女性1名にて、発症から15日後の母乳で検出されている(それ以降の検体は得られていない)。疾病管理予防センターのガイダンスは、安全な代替法がある場合は授乳を避けるべきであるが、授乳を行わないリスクについても同時に検討すべきとしている。
安全な授乳の再開に関する確実なデータはなく、伝播の可能性(ウイルス量を含む)、ウイルス残存期間、宿主免疫反応の違いは数値化されていない。回復期にエボラの性的伝播が起きた場合、人口寄与割合(PAF)は低い。一方、母乳が乳児栄養の中心である場合は特に、授乳に関するPAFは高いとみられる。
現在のエボラ流行の規模、生存者の増加、上述の経路によって家族内感染が持続する可能性を考えれば、精液、膣分泌物、母乳におけるエボラウイルスの持続を調べる研究が早急に必要である。そのような研究によって、回復期後にウイルス伝播を防止するための適切な公衆衛生上の情報を提供し、流行を予測する伝播動態の数理モデルに関する厳格なパラメータが得られる。

報告企業の意見	今後の対応
エボラウイルスは、回復後の血液、糞便、嘔吐物等から検出されなくなった後も、精液、膣分泌物、母乳からは検出されるとの報告である。	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

赤血球濃厚液-LR「日赤」
照射赤血球濃厚液-LR「日赤」
赤血球液-LR「日赤」
照射赤血球液-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

Sexual and Mother-to-Child Transmission of Ebola Virus in the Postconvalescent Period

To the Editor—During the exponential phase of the current Ebola epidemic in West Africa, public health and infection control guidelines are necessarily being developed in real time using available evidence. This means making recommendations using data from previous smaller outbreaks of Ebola or other viral hemorrhagic fevers, and from selected patients who may not be representative of outbreak populations. We consider the evidence base for recommendations in the postconvalescent period and implications for infection control.

Ebola virus has been detected in semen [1–5], vaginal secretions [2], and breast milk [5] after patients no longer have detectable virus in other bodily fluids, such as blood, feces, and vomitus. The most likely explanation for viral persistence (eg, in testes, mammary glands) is that these are immunologically protected sites with delayed viral clearance.

The World Health Organization recommends using condoms during any sexual intercourse for 3 months after onset of symptoms. Yet, our understanding about the frequency and duration of, and risk associated with, viral persistence in semen is limited to findings from just 12 patients and for vaginal samples to just 1 patient (Table 1). Viral persistence has been shown up to 101 days for semen and 33 days for vaginal samples, without later samples in these individuals. However, no study with sufficient sample size has systematically tested consecutive urogenital samples over an extended time period. There is currently no specific guidance on breastfeeding for convalescing patients. Ebola virus has been detected in breast milk at 15 days after disease

Table 1. Cases With Documented Ebola Virus in Vaginal Secretions, Semen, and Breast Milk in the Postconvalescent Period

Patient	Sample Type	Latest Day After Disease Onset: Positive Sample	Earliest Day After Disease Onset: Negative Sample	Assay	Reference
1	Semen	61	76	Guinea pig infective units	Edmond et al [1]
2	Vaginal	33	Not done	RT-PCR	Rodriguez et al [2]
3	Semen	82	704	RT-PCR	Rodriguez et al [2]
4	Semen	101	Not done	RT-PCR	Rodriguez et al [2]
5	Semen	97	700	RT-PCR	Rodriguez et al [2]
6	Semen	63	707	RT-PCR	Rodriguez et al [2]
7	Semen	82	697	RT-PCR	Rowe et al [3]
8	Semen	91	Not done	RT-PCR	Rowe et al [3]
9	Semen	63	97	RT-PCR	Rowe et al [3]
10	Semen	63	701	RT-PCR	Rowe et al [3]
11	Semen	62	Not done	RT-PCR	Rowe et al [3]
12	Semen	19	Not done	Not stated	Richards et al [4]
13	Semen	40	45	RT-PCR	Bausch et al [5]
14	Breast milk	15	Not done	RT-PCR	Bausch et al [5]

Abbreviation: RT-PCR, reverse transcription polymerase chain reaction.

onset in 1 lactating woman (again without later samples) [5]. If safe alternatives exist, Centers for Disease Control and Prevention guidance states that mothers should avoid breastfeeding, but risks of not breastfeeding must also be considered.

There are no definitive data on which to base advice on safe resumption of breastfeeding. The transmission probabilities (including viral load), duration of risk, and differences in host immune response have not been quantified. If it occurs, the population attributable fraction (PAF) for sexual transmission of Ebola during convalescence may be low, given the physical toll of illness and associated stigma. The PAF for breastfeeding, especially in settings where this is a main source of infant nutrition, may be higher.

Given the scale of the current epidemic, the increasing numbers of survivors, and the possibility that transmission may be sustained within family settings through these routes, well-designed studies on persistence of Ebola virus in semen, vaginal secretions, and breast milk are urgently needed. These will inform appropriate public health messages to interrupt transmission during the postconvalescent period and provide robust parameters for mathematical models of the transmission dynamics of Ebola for epidemic projections.

Note

Potential conflict of interest. Both authors: No reported conflicts.

Both authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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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 2015 年 1 月 20 日	新医薬品等の区分 該当なし	機構処理欄
一般的名称	ﾃｸﾈﾁｳﾑ人血清ｱﾙﾌﾞﾐﾝ（^{99m}Tc）		研究報告の公表状況	International Journal of Infectious Diseases 33 (2015) 120–122	公表国 ロシア	
販売名（企業名）	テクネアルブミンキット（富士フイルムＲＩファーマ株式会社）					

研究報告の概要

【要約】
我々はここでクリミア・コンゴ出血熱（CCHF）が検査で確認された死亡例と、その患者に治療を提供した 8 名の医療従事者間で院内感染を引き起こしたことについて報告する（医療従事者は全員生存）。
本報告は、少なくとも CCHF 患者が人工呼吸器を使用している時、CCHF の空気感染が現実のリスクであることを実証する。CCHF 患者のエアロゾルを発生させる医療処置中の空気感染予防策として、具体的には気道保護 N95 マスクまたは同等の標準的マスク、眼の保護、単独の空気感染予防措置室または良好な換気設定等の措置が、常に標準的予防措置として追加されなければならない。

使用上の注意記載状況・その他参考事項等

特になし

報告企業の意見	今後の対応
クリミア・コンゴ出血熱（CCHF）ウイスルの感染経路については、ダニ刺されやダニとの直接接触、感染した家畜の組織や血液への暴露、医療関係者を通じて感染患者の血液や体液との接触により感染することが知られていた。 本報告は、2011年5月にロシアで感染患者の治療を行った8名の医療従事者間で院内感染が発生し、うち2名については患者との直接接触または潜在的間接的接触がなく、エアロゾルを発生させる医療処置中の患者の部屋に短時間滞在している間に空気伝播によって感染したことが疑われるという初めての報告であり、CCHFウイルスの空気感染の可能性を示唆するものである。 本報告は重大な感染症かつ新規感染経路に該当すると考えられ、感染症定期報告の対象と判断する。	本研究報告は、ヒト血液を原料とする血漿分画製剤とは直接関連がないことから、現時点で当該生物由来製品に関し、措置等を行う必要はないと判断する。

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Perspective

Probable Crimean-Congo hemorrhagic fever virus transmission occurred after aerosol-generating medical procedures in Russia: nosocomial cluster

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SUMMARY

We report here a fatal case of laboratory confirmed Crimean-Congo hemorrhagic fever (CCHF), which caused nosocomial infection in eight health care workers (HCWs), who had provided medical care for the patient. All the HCWs survived.

The report demonstrates that airborne transmission of CCHF is a real risk, at least when the CCHF patient is in a ventilator. During performance of any aerosol-generating medical procedures for any CCHF patient airborne precautions should always be added to standard precautions, in particular, airway protective N95 mask or equivalent standard, eye protection, single airborne precaution room, or a well-ventilated setting.

1. Introduction

Human cases of Crimean-Congo hemorrhagic fever (CCHF) most frequently occur among agricultural workers or inhabitants of rural areas after bites of infected Hyalomma ticks or direct contact with ticks, for instance, by removing ticks by unprotected hands, and more rarely among slaughterhouse workers exposed to the blood and tissues of infected livestock and medical personnel through contact with the body fluids of infected patients.[1–4]

The South-Western regions of Russia (Astrakhan, Rostov and Volgograd, Krasnodar and Stavropol regions, Kalmykia, Dagestan and Ingushetia Republics) are endemic for CCHF.[5,6] 1,654 CCHF cases with 73 fatalities (CFR 4,4%) were recorded from 1999 to 2013.[7] More than 400 cases of CCHF were diagnosed in the Rostov region alone from 2000 to 2013.

Nosocomial cases of CCHF among health workers (HCWs) in Russia are rare, and are connected with direct contact between infected blood and unprotected skin or eye mucosa. In the Rostov region nosocomial cases were reported in 1961 (1 person), 1966 (2 persons) and in 1999 (5 persons)[8]; isolated cases have also been identified in 2003, 2006 and 2007. Between 1999 and 2005 6 nosocomial CCHF cases among HCWs were recorded in the Stavropol region.[9]

In May 2011 a nosocomial cluster was registered among eight HCWs who had provided medical care for a patient with CCHF in the central district hospital in Salsk district of the Rostov region. The peculiarity of this cluster was that not only the direct but also airborne transmission of CCHF probably took place. The cluster was described in an official letter of Federal Service for Surveillance on Consumer Rights Protection and Wellbeing of the Russian Federation (Rospotrebnadzor)[10] and mentioned in a number of publications in Russian.[8,11,12]

A nosocomial CCHF cluster with possible airborne transmission has not been described previously; its description is provided below according to Russian sources.[8,10–12] We also took part in the investigation of this cluster and treatment of patients.

1.1. Index case

A 23-year-old pregnant woman (22 weeks of pregnancy) was admitted to the infectious diseases department of the central district hospital in the Salsk district of the Rostov region on 6 May 2011 (1st day of illness) with initial suspicion of influenza-like illnesses (ILI) and pyelonephritis. She lived in a rural area endemic for CCHF, and denied any tick bites or contacts with ticks within the 2 weeks before onset of the disease. She was on a countryside picnic 2 - 3 days before the debut of symptoms. An X-ray

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examination showed a left-side lobar pneumonia, and proteinuria and leukocyturia were found. Urine culture tests were not performed. The patient was started on antibiotics and symptomatic therapy. On the 8th of May she became hypoxic and needed nasal oxygen. Increased bleeding from the catheter and hematomas in places of intramuscular injections were observed May 8-9.

CCHF was clinically diagnosed and laboratory confirmed by PCR and anti-CCHF specific IgM both being positive on the 5th day of disease (May 9, 2011). Ribavirin was not administered to the patient because of the pregnancy, anemia (Hb below 10 g/dl) and late stage of the disease. The patient's condition started to deteriorate from May 10 and she developed severe double sided pneumonia, hematemesis, hemoptysis, metrostaxis, and haemorrhage from the subclaviar catheter.

The patient continued to receive antibiotics, plasma, thrombocyte concentrates, erythrocyte concentrates and other support therapy. The patient also received mucolytics and broncholytics through compression inhaler NEBULFLAEM on May 9-10. During this period of time she was not on ventilation. Due to a low oxygen saturation the patient was intubated on May 10 and mechanically ventilated until her death on May 11, 2011. (7th day of illness).

During the medical care of the patient 3 physicians, 4 nurses and 1 hospital attendant contracted CCHF. All 8 HCWs developed CCHF between May 12 and May 15, 2011 and were hospitalized between May 14-16, 2011 with influenza-like illnesses (ILI) and suspicion of CCHF. All patients had fever (38—39.5°C), fatigue, headache, chills, body aches; 3 persons had hemorrhages at the site of injection; 2 persons had sore throat. CCHF was confirmed by RT-PCR and serological assays (anti-CCHF specific IgM ELISA in high titers 1/1800-1/6400) in all 8 cases.

More information about secondary cases is provided below.

Secondary cases:

#1. Nurse from infectious diseases department, who performed intravenous injections through a catheter, and monitored inhalation use hourly;
#2. Nurse from the infectious diseases department, who assisted in central venous catheterization, performed intravenous injections through a catheter, and monitored inhalation use hourly;
#3. Nurse from the intensive care unit (ICU) department, who installed the equipment for artificial lung ventilation (ALV) within a 20 minute period. She had no direct or potential indirect contact with the patient's body or her biological fluids, and did not participate in inhalation or ventilation of the patient;
#4. Hospital attendant from the infectious diseases department, who performed change of linen, cleaning room, and disinfection of the bedpan;
#5. Anesthetist from the ICU, who was in the ICU ward not more than 10 min, while the patient was in the ventilator. She had no direct or potential indirect contact with the patient or her biological fluids, and did not participate in inhalation or ventilation of the patient;
#6. Clinician from the infectious diseases department who attended the patient, doing rounds when she was in the ventilator. This physician also treated 2 more CCHF patients at the same time.
#7. Anesthesiologist from the ICU who took care of the ventilator treatment (ALV) of the patient in ICU;
#8. Obstetrician from the gynecology department who examined the patient from the first day of admission to the hospital until the death of the index patient.

All HCWs survived.

2. Discussion

Epidemiological investigation established that all HCWs worked in the patient's room during potential exposure period without goggles and particulate respirator, and used only gloves, disposable surgical masks and gowns. This set of personal protective equipment (PPE) does not protect skin, conjunctiva and upper respiratory tract against aerosols which could contain the particles of sputum streaked with infected blood, from the patient, who throughout her stay in the hospital was located in an isolation room without negative pressure. We cannot exclude that HCWs (#1,#2, #4,##6-8), who had direct contact with body or biological fluids of the patient, could have used their PPE in an inappropriate manner, for example, incorrectly put on and/or taken off their PPE. But 2 HCWs (#3 and #5) had no direct or indirect contact with body or fluids of the patients and also used the above mentioned PPE. Only 3 HCWs (#1, #2 and #7) in this cluster were directly involved in the performance of the aerosol-generating procedures (inhalation, intubation, ventilation); the other 5 HCWs provided medical care for the patient or supervised the equipment.

We found only two previous reports indicating the possibility of aerosol transmission of CCHF[13,14] and descriptions of 2 cases in a recent report from Turkey which described possible aerosol transmission of CCHF to HCWs while they were performing intubation and ventilation of a child and the resuscitation of an adult patient.[15]

In this nosocomial cluster which took place in Russia 2 HCWs out of 8 secondary cases (cases #3 and #5) probably became infected through airborne transmission during their short stay in the patient's room, where at that time aerosol-generating procedures were being performed.

We believe that four core factors are important for this nosocomial train of transmission:

1. probably infected aerosols which could spread in the ward during performance of aerosol-generating medical procedures (AGMPs) - inhalation and ILV;
2. high level of viremia on the 5-6th day of disease without antiviral treatment (ribavirin);
3. inappropriate usage of PPE when AGMPs were performing;
4. poor ventilation of the patient's ward;

which could all lead to aerosol transmission of CCHF.

According to the official statement of Rospotrebnadzor[10] in 6 secondary cases, there was a high risk of combined direct and airborne transmission, in 2 secondary cases direct CCHF transmission was not proven.

This case of airborne transmission of CCHF demonstrates that during performance of any AGMPs for any CCHF patient, airborne precautions should always be added to standard precautions (particulate respirator protective to N95 or equivalent standard, eye protection, single airborne precaution room or well-ventilated setting, etc.) according to WHO guidelines[16] for all HCWs who are in a patient's room. Access to any room where the aerosol-generating procedures are performed should be extremely limited.

Conflict of Interest/Funding: None

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＊＊2014年１月改訂（第８版）
＊2011年１月改訂

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再審査結果	1987年９月

放射性医薬品／心疾患診断薬

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テクネ®アルブミンキット

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放射性医薬品基準テクネチウム人血清アルブミン(99mTc)注射液 調製用

本剤は、貴重な血液を原料として製剤化されたものです。問診、感染症関連の検査等の安全対策を講じていますが、血液を原料としていることに由来する感染症の伝播等の危険性を完全に排除することはできないことから、疾病の診断上の必要性を十分に検討の上、必要最小限の使用にとどめるようお願いします。

【組成・性状】

１バイアル中

人血清アルブミン		50mg
添加物	塩化スズ（Ⅱ）二水和物	0.45mg
	塩酸	適量
	水酸化ナトリウム	適量
外観		凍結乾燥された白色粉末
調製後注射液 テクネチウム人血清アルブミン（99mTc）注射液		
外観		無色～淡黄色澄明の液
pH		2.0～3.0
浸透圧比（0.9%生理食塩液に対する比）		約１

本剤の成分である「テクネチウム人血清アルブミン（生物由来製品指定成分名）」には人血清アルブミン（採取国：日本、献血）が使用されている。

【効能又は効果】

RIアンギオカルヂオグラム及び心プールシンチグラムによる心疾患の診断

【用法及び用量】

１．テクネチウム人血清アルブミン（99mTc）注射液の調製
① 冷蔵庫から本品を取り出し、約５分間放置して室温に戻す。
② 放薬基「過テクネチウム酸ナトリウム（99mTc）注射液」１～９mLを本品バイアルに加える。
③ よく振盪して内容物を溶解した後、室温で５分間放置することにより、テクネチウム人血清アルブミン（99mTc）注射液が調製される。

２．RIアンギオカルヂオグラフィー及び心プールシンチグラフィー
上記によって得られたテクネチウム人血清アルブミン（99mTc）注射液370～740MBqを肘静脈より急速注入し、ディテクターを患者の胸部に指向させたシンチカメラを用いて、注入直後から撮影を始めることにより、RIアンギオカルヂオグラムを得、またRIアンギオカルヂオグラフィー終了後に撮影することにより心プールシンチグラムを得る。
また、同じく上記によって得られたテクネチウム人血清アルブミン（99mTc）注射液185～370MBqを静注し、ディテクターを患者の胸部に指向させたシンチカメラ及びシンチスキャナーを用いて、注入数分後に撮影することにより心プールシンチグラムを得る。

[注] 注意－医師等の処方せんにより使用すること。

【使用上の注意】

１．重要な基本的注意
(1) 診断上の有益性が被曝による不利益を上回ると判断される場合にのみ投与することとし、投与量は最小限度にとどめること。
(2) 髄液腔内に投与しないこと。

２．慎重投与（次の患者には慎重に投与すること）
薬物過敏症又はアレルギー性体質の患者。

３．副作用
承認前の臨床試験では、総症例333例中、副作用は認められなかった。
承認後の使用成績調査では、3,218症例中、副作用は２例（0.06%）に徐脈、血圧低下、湿疹各１件認められた。
（再審査終了時）
以下の副作用は、上記調査で認められたものである。
その他の副作用

	0.1%未満
循環器	徐脈、血圧低下
皮膚	湿疹

４．高齢者への投与
一般に高齢者では生理機能が低下しているので、患者の状態を十分に観察しながら慎重に投与すること。

５．妊婦、産婦、授乳婦等への投与
妊婦又は妊娠している可能性のある婦人及び授乳中の婦人には、原則として投与しないことが望ましいが、診断上の有益性が被曝による不利益を上回ると判断される場合にのみ投与すること。

６．小児等への投与
小児等に対する安全性は確立していない（現在までのところ、十分な臨床試験成績が得られていない）。

７．適用上の注意
(1) 調製時：標識に使用する過テクネチウム酸ナトリウム（99mTc）注射液の量は必要最小限度にとどめること。
(2) 調製後：本品は調製後６時間以内に投与すること。

８．その他の注意
(1) 本品は血液凝固因子を含まない。
(2) (社)日本アイソトープ協会医学・薬学部会放射性医薬品安全性専門委員会の「放射性医薬品副作用事例調査報告」において、まれに血管迷走神経反応（気分不快、嘔吐など）があらわれることがあると報告されている。

【薬物動態】

テクネチウム人血清アルブミン（99mTc）注射液は静注した場合、人血清アルブミンとほぼ同じ挙動を示す。静注後７分までは循環血液によって次第に希釈されその後平衡に達すると考えられる[1]。また、分子量が大きく血管外への漏出が少ないため、組織液や貯溜液への移行は少なく、１時間程度は安定した血液プールを示す[1,2]。しかし長時間となると貯溜液中に滲出することもある[2]。心プールシンチグラフィの場合、心外膜液貯溜等があると、この部分には血液が入らず、貯溜液によるγ線の吸収により、放射能を欠くいわゆるHaloを示す[3]。また、粘液腫[4,5]、血栓[6]、悪性腫瘍転移[7]等では心プール内に欠損像を示す。

【臨床成績】

本品の有効性についてのモニター調査の結果、読影できたものを有効例とした場合の有効率（有効例数／症例数）は次のとおりであった。

疾患名	有効例数／症例数	有効率
心筋梗塞	478／481	99.4%
狭心症	76／76	100%
心筋症	78／79	98.7%
僧帽弁閉鎖不全症	44／44	100%
心不全	49／50	98.0%
虚血性心疾患	6／6	100%
高血圧症	108／108	100%
動脈瘤	65／65	100%

【吸収線量】

MIRD法により計算した吸収線量は次のとおりである。

臓器	吸収線量（mGy/37MBq）
全身	0.07
肺	0.06
肝臓	0.12
脾臓	0.11
腎臓	0.52
性腺	0.21

（自社データ）

【有効成分に関する理化学的知見】

1．人血清アルブミン
・分子量　約７万

2．^{99m}Tcの核物理学的特性
(1) 物理的半減期　6.015時間
(2) 主なγ線エネルギー　141keV（89.1%）
(3) 減衰表

経過時間（時間）	残存放射能（%）	経過時間（時間）	残存放射能（%）
−3	141.3	11	28.2
−2	125.9	12	25.1
−1	112.2	13	22.4
0	100	14	19.9
1	89.1	15	17.8
2	79.4	16	15.8
3	70.8	17	14.1
4	63.1	18	12.6
5	56.2	19	11.2
6	50.1	20	10.0
7	44.6	21	8.9
8	39.8	22	7.9
9	35.4	23	7.1
10	31.6	24	6.3

【取扱い上の注意】

(1) 本品の調製は無菌的に行い、また適当な鉛容器で遮蔽して行うこと。
(2) 本品の調製の際、バイアル内に空気を入れないこと、またバイアル内を陽圧にしないこと。

【包　　装】**

２バイアル

【主要文献】

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【文献請求先】*

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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 2015年5月25日	新医薬品等の区分 該当なし。	総合機構処理欄
一般的名称	別紙のとおり。		研究報告の公表状況	KRQE NEWS 13 , May 19, 2015	公表国 米国	
販売名（企業名）	別紙のとおり。					

研究報告の概要

問題点：米国で、コウモリにみられる狂犬病ウイルスの新種が報告された。

保健当局によると、ニューメキシコ州南部で女性1名を咬んだキツネは狂犬病の新種の株に感染していたことが検査で示唆された。78歳の女性は、2015年4月20日にリンカーン郡で咬まれた後、狂犬病ワクチンの接種を受け、狂犬病の発症を免れた。州の保険省によると，アトランタの米CDCでウイルスの遺伝子検査が行われた。ニューメキシコ州の保健当局者によると，新種の株はコウモリにみられる狂犬病株の近縁である。

報告企業の意見	今後の対応
別紙のとおり。	今後とも関連情報の収集に努め，本剤の安全性の確保を図っていきたい。

使用上の注意記載状況・その他参考事項等

記載なし。

MedDRA/J ver. 18.0

別紙

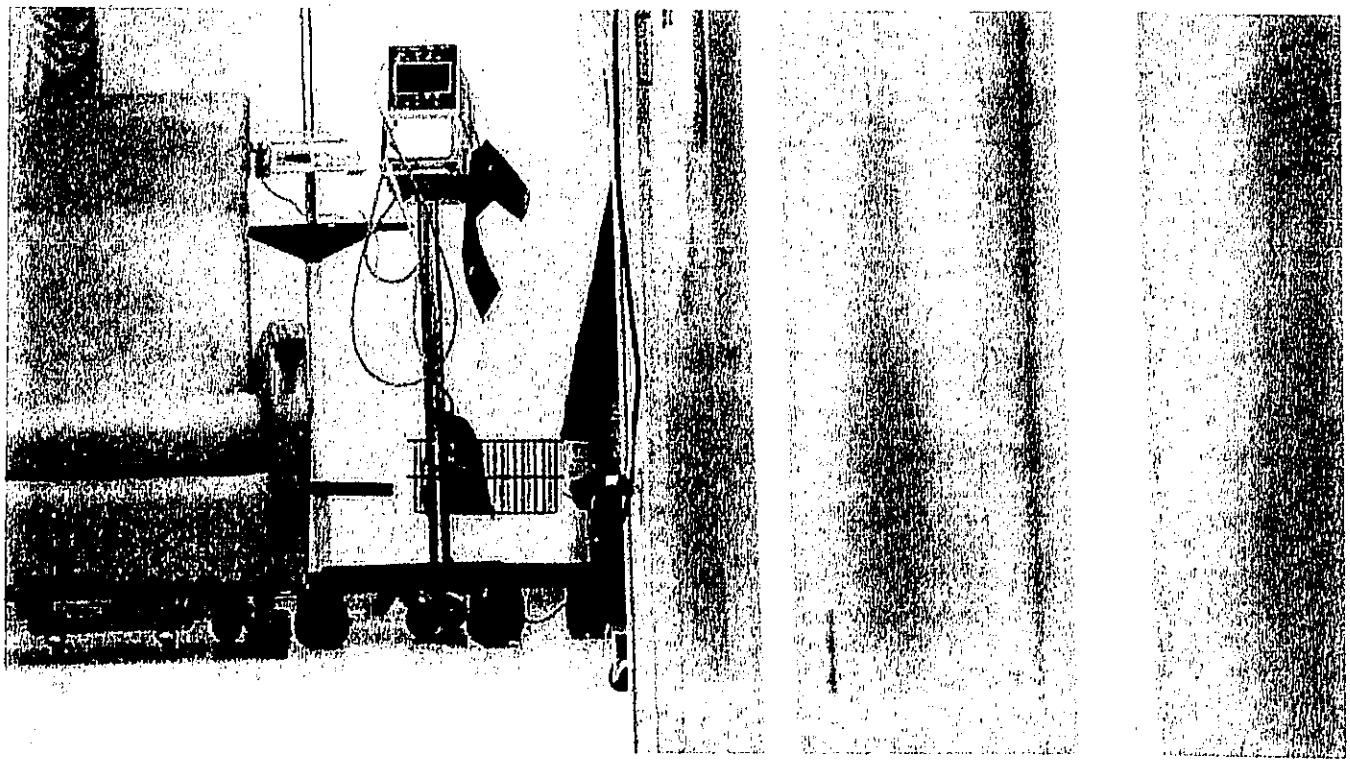
一般的名称	①人血清アルブミン、②人血清アルブミン、③人血清アルブミン*、④人免役グロブリン、⑤人免役グロブリン、⑥乾燥ペプシン処理人免疫グロブリン、⑦乾燥スルホ化人免疫グロブリン、⑧乾燥スルホ化人免疫グロブリン、⑨乾燥スルホ化人免疫グロブリン、⑩乾燥スルホ化人免疫グロブリン、⑪乾燥スルホ化人免疫グロブリン*、⑫乾燥濃縮人活性化プロテインC、⑬乾燥濃縮人血液凝固第Ⅷ因子、⑭乾燥濃縮人血液凝固第Ⅷ因子、⑮乾燥濃縮人血液凝固第Ⅷ因子、⑯乾燥濃縮人血液凝固第Ⅸ因子、⑰乾燥濃縮人血液凝固第Ⅸ因子、⑱乾燥濃縮人血液凝固第Ⅸ因子、⑲乾燥抗破傷風人免疫グロブリン、⑳抗HBs人免疫グロブリン、㉑抗HBs人免疫グロブリン、㉒抗HBs人免疫グロブリン、㉓トロンビン、㉔トロンビン、㉕トロンビン、㉖フィブリノゲン加第ⅩⅢ因子*、㉗フィブリノゲン加第ⅩⅢ因子、㉘乾燥濃縮人アンチトロンビンⅢ、㉙ヒスタミン加人免疫グロブリン製剤、㉚人血清アルブミン*、㉛人血清アルブミン*、㉜乾燥ペプシン処理人免役グロブリン*、㉝乾燥濃縮人アンチトロンビンⅢ、㉞乾燥濃縮人血液凝固第Ⅹ因子加活性化第Ⅶ因子
販売名（企業名）	①献血アルブミン20“化血研”、②献血アルブミン25“化血研”、③人血清アルブミン“化血研”*、④ガンマーグロブリン筋注450mg/3mL「化血研」、⑤ガンマーグロブリン筋注1500mg/10mL「化血研」、⑥献血グロブリン注射用2500mg「化血研」、⑦献血ベニロンーⅠ静注用500mg、⑧献血ベニロンーⅠ静注用1000mg、⑨献血ベニロンーⅠ静注用2500mg、⑩献血ベニロンーⅠ静注用5000mg、⑪ベニロン*、⑫注射用アナクトC2,500単位、⑬コンファクトF注射用250、⑭コンファクトF注射用500、⑮コンファクトF注射用1000、⑯ノバクトM静注用400単位、⑰ノバクトM静注用800単位、⑱ノバクトM静注用1600単位、⑲テタノセーラ筋注用250単位、⑳ヘパトセーラ筋注200単位/mL、㉑ヘパトセーラ筋注200単位/1mL、㉒ヘパトセーラ筋注1000単位/5mL、㉓トロンビン“化血研”、㉔献血トロンビン経口・外用5千「化血研」、㉕献血トロンビン経口・外用1万「化血研」、㉖ボルヒール*、㉗ボルヒール組織接着用、㉘アンスロビンP500注射用、㉙ヒスタグロビン皮下注用、㉚アルブミン20%化血研*、㉛アルブミン5%化血研*、㉜静注グロブリン*、㉝アンスロビンP1500注射用、㉞バイクロット配合静注用、㉟ノバクトM静注用500単位、㊱ノバクトM静注用1000単位、㊲ノバクトM静注用2000単位
報告企業の意見	狂犬病ウイルスは、ラブドウイルス科のリッサウイルス属に属するウイルスであり、一本鎖RNA及びエンベロープを有するウイルスである。 今回、米国で、キツネに咬まれたヒトからコウモリにみられる狂犬病ウイルス株の近縁の新種の株が報告された。 上記製剤の製造工程には、冷エタノール分画工程、ウイルス除去膜ろ過工程、加熱工程といった原理の異なるウイルスクリアランス工程が複数導入されており、各工程のウイルスクリアランス効果は「血漿分画製剤のウイルスに対する安全性確保に関するガイドライン（医薬発第1047号、平成11年8月30日）」に基づく、モデルウイルスを用いたウイルスプロセスバリデーションにより確認されている。今回報告した狂犬病ウイルスのモデルウイルスには、エンベロープの有無、核酸の種類等から、ウシウイルス性下痢ウイルス（BVDV）が該当すると考えられるが、上記工程のBVDVクリアランス効果については上記バリデーションにより確認されている。また、これまでに上記製剤による狂犬病ウイルスへの感染報告例は無い。 以上の点から、上記製剤は狂犬病ウイルスに対する安全性を確保していると考える。

*：現在製造を行っていない

KRQE NEWS 13

Health Department: Fox that bit woman had new rabies strain

By The Associated Press
Published: May 19, 2015, 1:47 pm



SANTA FE (AP) – Health officials say tests indicate that a fox that bit a woman in southern New Mexico had a new strain of rabies.

The 78-year-old woman was bitten in Lincoln County on April 20. She then got rabies vaccinations that prevented her from developing rabies, which is often fatal.

The state Department of Health says genetic testing of the virus was done at the federal Centers for Disease Control and Prevention in Atlanta.

New Mexico Health Secretary Retta Ward says the new strain is related to other rabies strains found in bats.

Ward says state and federal officials are going to collect dead foxes and bats in Lincoln County to test them for rabies.

Health officials advise that children should be advised to never touch a bat or other wild animal.

※※2014年９月改訂（第25版）
※2012年４月改訂

日本標準商品分類番号
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血漿分画製剤
静注用人免疫グロブリン製剤

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処方箋医薬品注）
注）注意-医師等の処方箋により使用すること

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生物学的製剤基準　乾燥スルホ化人免疫グロブリン

Kenketsu Venilon®-I

貯　　法：30℃以下に凍結を避けて保存
有効期間：国家検定合格の日から２年
（最終有効年月日は容器及び外箱に表示）

承認番号	500mg製剤	22100AMX01040000
	1,000mg製剤	22100AMX01041000
	2,500mg製剤	22100AMX01042000
	5,000mg製剤	22100AMX01043000
薬価収載	2009年９月	
販売開始	500mg製剤	1992年１月
	1,000mg製剤	
	2,500mg製剤	
	5,000mg製剤	2001年11月
※※再審査結果	2013年12月	
再評価結果	2001年８月	
効能追加	2010年１月	
用量追加	2003年７月	

本剤は、貴重なヒト血液を原材料として製剤化したものである。有効成分及び添加物としてヒト血液由来成分を含有しており、原材料となったヒト血液を採取する際には、問診、感染症関連の検査を実施するとともに、製造工程における一定の不活化・除去処理などを実施し、感染症に対する安全対策を講じているが、ヒト血液を原材料としていることによる感染症伝播のリスクを完全に排除することはできないため、疾病の治療上の必要性を十分に検討の上、必要最小限の使用にとどめること。（「使用上の注意」の項参照）

【禁　忌（次の患者には投与しないこと）】
本剤の成分に対しショックの既往歴のある患者

【原則禁忌（次の患者には投与しないことを原則とするが、特に必要とする場合には慎重に投与すること）】
本剤の成分に対し過敏症の既往歴のある患者

【組成・性状】

1. 組成

本剤は、スルホ化人免疫グロブリンＧを含む凍結乾燥製剤で、１バイアル中に各々下記の成分を含有する。

成分		500mg製剤	1,000mg製剤	2,500mg製剤	5,000mg製剤
有効成分	スルホ化人免疫グロブリンＧ	500mg	1,000mg	2,500mg	5,000mg
添加物	グリシン	225mg	450mg	1,125mg	2,250mg
	人血清アルブミン	25mg	50mg	125mg	250mg
	D-マンニトール	100mg	200mg	500mg	1,000mg
	塩化ナトリウム	90mg	180mg	450mg	900mg
添付溶剤：日本薬局方注射用水		10mL	20mL	50mL	100mL

本剤の有効成分であるスルホ化人免疫グロブリンＧ及び添加物の人血清アルブミンは、ヒトの血液（採血国：日本、採血方法：献血）を原材料としている。また、本剤は製造工程でブタの腸粘膜由来成分（ヘパリン）を使用している。

2. 製剤の性状

本剤は白色の凍結乾燥製剤であり添付の溶剤で溶解するとき、微黄色の澄明又はわずかに白濁した液剤となり、肉眼的にほとんど沈殿を認めない。

pH	6.4～7.2
浸透圧比	約２（生理食塩液に対する比）

【効能・効果】

1. 低又は無ガンマグロブリン血症
2. 重症感染症における抗生物質との併用
3. 特発性血小板減少性紫斑病（他剤が無効で著明な出血傾向があり、外科的処置又は出産等一時的止血管理を必要とする場合）
4. 川崎病の急性期（重症であり、冠動脈障害の発生の危険がある場合）
5. ギラン・バレー症候群（急性増悪期で歩行困難な重症例）
6. 次の疾患における神経障害の改善（ステロイド剤が効果不十分な場合に限る）
   チャーグ・ストラウス症候群
   アレルギー性肉芽腫性血管炎

〈効能・効果に関連する使用上の注意〉
(1) 重症感染症において抗生物質との併用に用いる場合は、適切な抗菌化学療法によっても十分な効果の得られない重症感染症を対象とすること。
(2) 川崎病に用いる場合は、発病後７日以内に投与を開始することが望ましい。
(3) チャーグ・ストラウス症候群又はアレルギー性肉芽腫性血管炎の神経障害の治療に用いる場合は、ステロイド剤による適切な治療（原則として、副腎皮質ステロイドをプレドニゾロン換算で40mg/日を４週間以上投与）によっても十分な効果の得られない患者を対象とすること〔【臨床成績】(6)の項参照〕。

【用法・用量】

本剤は、添付の日局注射用水（500mg製剤では10mL、1,000mg製剤では20mL、2,500mg製剤では50mL、5,000mg製剤では100mL）に溶解して、以下のとおり効能又は効果に応じて投与する。直接静注する場合は、極めて緩徐に行う。

(1) **低又は無ガンマグロブリン血症**
通常、１回にスルホ化人免疫グロブリンＧ200～600mg（４～12mL）/kg体重を３～４週間隔で点滴静注又は直接静注する。なお、患者の状態に応じて適宜増減する。

(2) **重症感染症における抗生物質との併用**
通常、成人に対しては、１回にスルホ化人免疫グロブリンＧ2,500～5,000mg（50～100mL）を、小児に対しては、１回にスルホ化人免疫グロブリンＧ50～150mg（１～３mL）/kg体重を点滴静注又は直接静注する。なお、年齢及び症状に応じて適宜増減する。

(3) **特発性血小板減少性紫斑病**
通常、１日にスルホ化人免疫グロブリンＧ200～400mg（４～８mL）/kg体重を点滴静注又は直接静注する。なお、５日間投与しても症状の改善が認められない場合は以降の投与を中止すること。年齢及び症状に応じて適宜増減する。

(4) **川崎病**
通常、１日にスルホ化人免疫グロブリンＧ200mg（４mL）/kg体重を５日間点滴静注又は直接静注、若しくは2,000mg（40mL）/kg体重を１回点滴静注する。なお、年齢及び症状に応じて５日間投与の場合は適宜増減、１回投与の場合は適宜減量する。

(5) **ギラン・バレー症候群**
通常、１日にスルホ化人免疫グロブリンＧ400mg（８mL）/kg体重を５日間点滴静注又は直接静注する。

(6) **チャーグ・ストラウス症候群又はアレルギー性肉芽腫性血管炎における神経障害の改善**
通常、１日にスルホ化人免疫グロブリンＧ400mg（８mL）/kg体重を５日間点滴静注する。

〈用法・用量に関連する使用上の注意〉
(1)急速に注射すると血圧降下を起こす可能性がある。
(特に低又は無ガンマグロブリン血症の患者には注意すること。)
(2)投与速度[1]:
1)初日の投与開始から30分間は0.01～0.02mL/kg/分で投与し、副作用等の異常所見が認められなければ、0.03～0.06mL/kg/分まで徐々に投与速度を上げてもよい。2日目以降は、前日に耐容した速度で投与することができる。
2)川崎病に対し2,000mg(40mL)/kgを1回投与する場合には、基本的には1)の投与速度を遵守することとするが、目安としては12時間以上かけて点滴静注すること。
(3)低又は無ガンマグロブリン血症の用法・用量は、血清IgGトラフ値を参考に、基礎疾患や感染症などの臨床症状に応じて、投与量、投与間隔を調節する必要があることを考慮すること。
(4)チャーグ・ストラウス症候群又はアレルギー性肉芽腫性血管炎の神経障害の治療において、本剤投与後4週間は再投与を行わないこと(4週間以内に再投与した場合の有効性及び安全性は検討されていない)。

【使用上の注意】
1. 慎重投与(次の患者には慎重に投与すること)
(1)IgA欠損症の患者〔抗IgA抗体を保有する患者では過敏反応を起こすおそれがある。〕
(2)腎障害のある患者〔腎機能を悪化させるおそれがある。〕
(3)脳・心臓血管障害又はその既往歴のある患者〔大量投与による血液粘度の上昇等により脳梗塞又は心筋梗塞等の血栓塞栓症を起こすおそれがある。〕
(4)血栓塞栓症の危険性の高い患者〔大量投与による血液粘度の上昇等により血栓塞栓症を起こすおそれがある。〕
(5)溶血性・失血性貧血の患者〔ヒトパルボウイルスB19の感染を起こす可能性を否定できない。感染した場合には、発熱と急激な貧血を伴う重篤な全身症状を起こすことがある。〕
(6)免疫不全患者・免疫抑制状態の患者〔ヒトパルボウイルスB19の感染を起こす可能性を否定できない。感染した場合には、持続性の貧血を起こすことがある。〕
(7)心機能の低下している患者〔大量投与により、心不全を発症又は悪化させるおそれがある。〕
2. 重要な基本的注意
[患者への説明]
本剤の使用にあたっては、疾病の治療における本剤の必要性とともに、本剤の製造に際しては感染症の伝播を防止するための安全対策が講じられているものの、ヒトの血液を原材料としていることに由来する感染症伝播のリスクを完全に排除することができないことを、患者に対して説明し、その理解を得るよう努めること。
(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抗体及び抗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。さらに、プールした試験血漿については、HIV、HBV、HCV、HAV及びヒトパルボウイルスB19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。その後の本剤の製造工程であるCohnの低温エタノール分画、スルホ化処理及びウイルス除去膜処理は、HIVをはじめとする各種ウイルスの除去・不活化効果を有することが確認されているが、投与に際しては、次の点に十分注意すること。
1)血漿分画製剤の現在の製造工程では、ヒトパルボウイルスB19等のウイルスを完全に不活化・除去することが困難であるため、本剤の投与によりその感染の可能性を否定できないので、投与後の経過を十分に観察すること。
2)現在までに本剤の投与により変異型クロイツフェルト・ヤコブ病(vCJD)等が伝播したとの報告はない。しかしながら、製造工程において異常プリオンを低減し得るとの報告があるものの、理論的なvCJD等の伝播のリスクを完全には排除できないので、投与の際には患者への説明を十分行い、治療上の必要性を十分検討の上投与すること。
(2)ショック等重篤な副作用を起こすことがあるので注意して使用し、経過を十分観察すること。特に小児等に使用する場合には投与速度に注意するとともに、経過を十分に観察すること。(〈用法・用量に関連する使用上の注意〉の項参照)
(3)本剤は抗A及び抗B血液型抗体を有する。したがって、血液型がO型以外の患者に大量投与したとき、溶血性貧血を起こすことがある。
(4)本剤による特発性血小板減少性紫斑病の治療は原因療法ではなく、対症療法であることに留意すること。
(5)小児の急性特発性血小板減少性紫斑病は多くの場合自然寛解するものであることを考慮すること。
(6)川崎病の患者における追加投与は、本剤投与における効果不十分(発熱の持続等)で症状の改善が見られない等、必要と思われる時のみに行うこと(本剤の追加投与に関しては有効性、安全性は確立していない)。
(7)ギラン・バレー症候群においては、筋力低下の改善が認められた後、再燃することがあるので、その場合には本剤の再投与を含め、適切な処置を考慮すること。
(8)チャーグ・ストラウス症候群又はアレルギー性肉芽腫性血管炎の神経障害において、本剤投与後に明らかな臨床症状の悪化や新たな神経症状の発現等が認められた場合には、治療上の有益性と危険性を十分に考慮した上で、本剤の再投与を判断すること(本剤を再投与した場合の有効性及び安全性は確立していない)。
3. 相互作用
[併用注意](併用に注意すること)

薬剤名等	臨床症状・措置方法	機序・危険因子
非経口用生ワクチン 麻疹ワクチン おたふくかぜワクチン 風疹ワクチン これら混合ワクチン 水痘ワクチン等	本剤の投与を受けた者は、生ワクチンの効果が得られないおそれがあるので、生ワクチンの接種は本剤投与後3カ月以上延期すること。また、生ワクチン接種後14日以内に本剤を投与した場合は、投与後3カ月以上経過した後に生ワクチンを再接種することが望ましい。 なお、特発性血小板減少性紫斑病(ITP)、川崎病、ギラン・バレー症候群、チャーグ・ストラウス症候群又はアレルギー性肉芽腫性血管炎に対する大量療法(200mg/kg以上)後に生ワクチンを接種する場合は、原則として生ワクチンの接種を6カ月以上(麻疹感染の危険性が低い場合の麻疹ワクチン接種は11カ月以上)延期すること[注1)]。	本剤の主成分は免疫抗体であるため、中和反応により生ワクチンの効果が減弱されるおそれがある。

※※4. 副作用
ベニロンの承認時まで及びベニロン、献血ベニロン－Ⅰの使用成績調査等の総症例14,523例中545例(3.75%)に副作用が認められており、効能・効果別の副作用発現状況は次のとおりである。
1)低又は無ガンマグロブリン血症[注]:
264症例中11例(4.17%)に副作用が認められた。主な副作用は、発熱3件(1.14%)、悪寒2件(0.76%)、血圧低下2件(0.76%)等であった。(承認時及び使用成績調査終了時)
注「通常、成人に対しては、1回にスルホ化人免疫グロブリンG2,500～5,000mg(50～100mL)を、小児に対しては、1回にスルホ化人免疫グロブリンG50～150mg(1～3mL)/kg体重を点滴静注又は直接静注する。なお、年齢及び症状に応じて適宜増減する。」に従って投与された際の副作用発現状況である。
2)重症感染症における抗生物質との併用:
10,881症例中37例(0.34%)に副作用が認められた。主な副作用は、発疹8件(0.07%)、悪寒6件(0.06%)、発熱6件(0.06%)、麻疹5件(0.05%)、呼吸困難5件(0.05%)、悪心5件(0.05%)等であった。(承認時及び使用成績調査終了時)
3)特発性血小板減少性紫斑病:
709症例中53例(7.48%)に副作用が認められた。主な副作用は、頭痛18件(2.54%)、発熱17件(2.40%)、そう痒症5件(0.71%)、悪心5件(0.71%)、嘔吐4件(0.56%)、発疹4件(0.56%)等であった。(承認時及び再審査終了時)

4)川崎病：
1,389症例中15例(1.08%)に副作用が認められた。主な副作用は、チアノーゼ3件(0.22%)、蕁麻疹3件(0.22%)、ALT(GPT)上昇3件(0.22%)、AST(GOT)上昇3件(0.22%)、振戦2件(0.14%)、悪寒2件(0.14%)、発熱2件(0.14%)、血圧低下2件(0.14%)等であった。(承認時及び再審査終了時)

※※5)ギラン・バレー症候群：
1,249症例中410例(32.8%)に副作用が認められた。主な副作用は、ALT(GPT)上昇98件(7.8%)、肝機能異常89件(7.1%)、AST(GOT)上昇84件(6.7%)、白血球数減少43件(3.4%)、頭痛35件(2.8%)、好中球数減少27件(2.2%)等であった。(承認時及び再審査終了時)

6)チャーグ・ストラウス症候群、アレルギー性肉芽腫性血管炎：
31症例中19例(61.3%)に副作用が認められた。主な副作用は、頭痛5件(16.1%)、ALT(GPT)上昇3件(9.7%)、血小板減少症2件(6.5%)、倦怠感2件(6.5%)、白血球数減少2件(6.5%)等であった。(承認時)

なお、川崎病の急性期を対象とした使用成績調査における副作用の発現率は1.14%(12例/1,053例)で、そのうちショック0%(0例0件)、ショック又はショックが疑われる症状(チアノーゼ、血圧低下等)0.28%(3例4件)であり、重篤な副作用の発現率は0%(0例0件)であった。また、川崎病の急性期の再審査期間中に報告された自発報告において、出荷量あたりの重篤な副作用の発現例数は2.8例/1,000kg(7例12件)で、そのうちショック1.6例/1,000kg(4例4件)、ショック又はショックが疑われる症状(チアノーゼ、血圧低下等)1.6例/1,000kg(4例4件)であった。

(1)重大な副作用

1)**ショック、アナフィラキシー**(0.1%未満)：ショック、アナフィラキシーがあらわれることがあるので、観察を十分に行い、呼吸困難、頻脈、不安感、胸内苦悶、血圧低下、チアノーゼ等が認められた場合には、直ちに投与を中止し、適切な処置を行うこと。

2)**肝機能障害、黄疸**(頻度不明)：AST(GOT)、ALT(GPT)、Al-P、γ-GTP、LDHの著しい上昇等を伴う肝機能障害、黄疸があらわれることがあるので、観察を十分に行い、異常が認められた場合には、適切な処置を行うこと。

3)**無菌性髄膜炎**(頻度不明)：大量投与により無菌性髄膜炎(項部硬直、発熱、頭痛、悪心・嘔吐あるいは意識混濁等)があらわれることがあるので、このような場合は投与を中止し、適切な処置を行うこと。

4)**急性腎不全**(頻度不明)：急性腎不全があらわれることがあるので、投与に先立って患者が脱水状態にないことを確認するとともに、観察を十分に行い、腎機能検査値(BUN, 血清クレアチニン等)の悪化、尿量減少が認められた場合には投与を中止し、適切な処置を行うこと。なお、急性腎不全の危険性の高い患者においては、適宜減量し、できるだけゆっくりと投与することが望ましい。

5)**血小板減少**(頻度不明)：血小板減少を起こすことがあるので、観察を十分に行い、このような場合には、適切な処置を行うこと。

6)**肺水腫**(頻度不明)：肺水腫があらわれることがあるので、呼吸困難等の症状があらわれた場合には投与を中止し、適切な処置を行うこと。

7)**血栓塞栓症**(頻度不明)：大量投与例で、血液粘度の上昇等により、脳梗塞、心筋梗塞、肺塞栓症、深部静脈血栓症等の血栓塞栓症があらわれることがあるので、観察を十分に行い、中枢神経症状(めまい、意識障害、四肢麻痺等)、胸痛、突然の呼吸困難、息切れ、下肢の疼痛・浮腫等の症状が認められた場合には、投与を中止し、適切な処置を行うこと。
なお、血栓塞栓症の危険性の高い患者においては、適宜減量し、できるだけゆっくりと投与することが望ましい。〔「慎重投与」(3)(4)および「高齢者への投与」(2)の項参照〕

8)**心不全**(頻度不明)：主として川崎病への大量投与例で、循環血漿量過多により心不全を発症又は悪化させることがあるので、観察を十分に行い、呼吸困難、心雑音、心機能低下、浮腫、尿量減少等が認められた場合には、投与を中止し、適切な処置を行うこと。
なお、心機能の低下している患者においては、適宜減量し、できるだけゆっくりと投与することが望ましい。〔「慎重投与」(7)の項参照〕

(2)その他の副作用

	頻度不明	0.1～5%未満	0.1%未満
過敏症[注]	発赤、腫脹、水疱、汗疱	発疹	熱感、蕁麻疹、そう痒感、局所性浮腫等
循環器	血圧低下、血圧上昇		
肝臓		AST(GOT)、ALT(GPT)等の上昇	
消化器			悪心、嘔吐、食欲不振、腹痛
血液	白血球減少、好中球減少、好酸球増多、溶血性貧血、貧血		
その他	胸痛、体温低下、CK(CPK)上昇、喘息様症状	頭痛、発熱、悪寒、戦慄	倦怠感

注)このような場合には投与を中止し、適切な処置を行うこと。

5. 高齢者への投与

(1)一般に高齢者では生理機能が低下しているので、患者の状態を観察しながら慎重に投与すること。

(2)一般的に高齢者では脳・心臓血管障害又はその既往歴のある患者がみられ、血栓塞栓症を起こすおそれがあるので、患者の状態を観察しながら慎重に投与すること。

6. 妊婦、産婦、授乳婦等への投与

妊婦又は妊娠している可能性のある婦人には、治療上の有益性が危険性を上回ると判断される場合にのみ投与すること。
〔妊娠中の投与に関する安全性は確立していない。本剤の投与によりヒトパルボウイルスB19の感染の可能性を否定できない。感染した場合には胎児への障害(流産、胎児水腫、胎児死亡)が起こる可能性がある。〕

7. 小児等への投与

低出生体重児、新生児に対する安全性は確立していない。

8. 臨床検査結果に及ぼす影響

本剤には各種感染症の病原体又はその産生物質に対する免疫抗体が含まれており、投与後の血中にこれら免疫抗体が一時検出されることがあるので、臨床診断には注意を要する。

9. 適用上の注意

投与時：

1)溶解時に不溶物の認められるものは使用しないこと。また、一度溶解したものはできるだけ速やかに使用を開始すること。なお、使用後の残液は、細菌汚染のおそれがあるので再使用しないこと(本剤は細菌の増殖に好適なたん白であり、しかも保存剤を含有していないため)。

2)他の製剤との混注は避けること。

【薬物動態】

1. 低又は無ガンマグロブリン血症患者7例にスルホ化人免疫グロブリンGを100mg/kg体重投与した場合の平均血中濃度(投与前149mg/dL)は投与24時間後では313mg/dL、2週間後では206mg/dLであった[4]。
2. 健康成人及び低又は無ガンマグロブリン血症の患者における投与試験から、スルホ化人免疫グロブリンGの血中半減期は約25日であることが確認されている[4,5]。

【臨床成績】

1. 臨床効果

(1)低又は無ガンマグロブリン血症：
免疫グロブリン補充療法を受けたX連鎖無ガンマグロブリン血症患者29例を対象としたレトロスペクティブな研究において、高用量の静注用人免疫グロブリン(IVIG)(3週間ごとに350～600mg/kg)の治療を受け、血清IgGトラフ値が500mg/dL以上となった患者の感染症の発症頻度及び入院期間は1.04回/年及び0.70日/年であったが、未治療、筋注用人免疫グロブリンもしくは低用量IVIG(3週間ごとに200mg/kg未満)で治療され、血清IgGトラフ値が151mg/dL以上500mg/dL未満だった患者では、1.75回/年及び9.00日/年であったとの報告がある[6]。

(2)重症感染症における抗生物質との併用：
再評価に対する市販後臨床試験において、広範囲抗生物質を3日間投与しても感染主要症状の十分な改善が認められない重症感染症の患者682例を対象として、抗生物質と静注用人免疫グロブリン5g/日、3日間との併用群(IVIG群)又は抗生物質単独投与群(対照群)に割り付けた非盲検群間比較試験を行った。

解熱効果、臨床症状の改善効果又は検査所見(炎症マーカーであるCRP値の推移)を評価基準として有効性を評価した結果、IVIG群はいずれにおいても対照群に比べ有意に優れており、有効率はIVIG群61.5%(163/265)、対照群47.3%(113/239)であった[7]。

(3)特発性血小板減少性紫斑病(ITP):
ITP患者で副腎皮質ステロイド剤が無効な症例及び摘脾後再発し、薬剤が無効な症例又は主治医が適当と認めた症例の計177症例(成人75例、小児102例)のうち400mg/kg/日の5日間投与された93例(成人33例、小児60例)での成績の概要は以下の通りであった。
1)有効率は68.8%(64例/93例)であった。
2)血小板数は、投与前2.7万/mm³、投与1日後3.8万/mm³、投与2日後6.6万/mm³、投与3日後7.8万/mm³、投与4日後10.2万/mm³、投与5日後13.2万/mm³、投与7日後12.8万/mm³と増加した。
3)93例のうち、副腎ステロイド剤が無効であった60例に対する有効率は63.3%(38例/60例)であった。また、摘脾の効果が一過性あるいは無効であった12例に対する有効率は66.7%(8例/12例)であった。

(4)川崎病:
1)200mg/kg/日、5日間投与された151症例のうち冠動脈障害が認められなかった有効以上の症例は127例であり、有効率は84.1%(127例/151例)であった。
上記川崎病に対する効果はベニロンとアスピリンを併用した100症例(有効率84.0%)、ベニロン単独の51症例(有効率84.3%)から得られたものである。
2)信頼の出来る学術雑誌に掲載された科学的根拠となり得る論文の試験成績では、2g/kgを1回投与された原田スコア4以上の急性期ハイリスク患児72例のうち冠動脈障害が認められなかった症例は69例(95.8%)であった[8]。

(5)ギラン・バレー症候群:
1)400mg/kg体重/日、5日間投与された亜症成人患者23例において、Hughesの運動機能尺度が1段階以上改善した治療開始4週目の改善率は60.9%(14例/23例)であった[9]。
2)重症小児患者11例では、同じく治療開始4週目の改善率は81.8%(9例/11例)であった。

(6)チャーグ・ストラウス症候群、アレルギー性肉芽腫性血管炎:
ステロイド抵抗性(ステロイド剤を寛解導入療法としてプレドニゾロン換算で40mg/日以上を4週間以上投与、漸減後に維持療法としてプレドニゾロン換算で5～20mg/日の一定用量を4週間以上投与)で、MMTスコア合計が130以下、かつMMTスコアが3以下となる神経障害箇所を有する患者23例に本剤400mg/kg/日を5日間投与した。その結果、本剤投与開始2週間後の徒手筋力検査(MMT)スコア合計変化量が本剤投与前に比し有意に改善した(表1)。なお、第1期でのMMTスコア合計変化量は本剤群でプラセボ群の変化量を上回った(本試験では、本剤のプラセボに対する優越性を検証するための検出力は考慮されていない)(表2)[10]。

表1　本剤投与開始2週間後のMMTスコア合計の変化量

本剤投与開始直前	本剤投与開始2週間後	変化量	p値*
113.37±16.02	120.50±11.91	7.13±9.76	0.002

平均値±標準偏差(23例)
＊対応のあるt検定

表2　第1期の本剤及びプラセボ投与開始2週間後のベースラインからのMMTスコア合計変化量

第1期 投与薬剤	ベースライン	投与開始2週間後	変化量	群間差 [95%信頼区間]
本剤 (A群、8例)	110.00±12.56	118.13±11.15	8.13±9.49	4.99 [-0.64, 10.63]
プラセボ (B＋C群、15例)	109.97±16.95	113.10±17.42	3.13±3.52	

平均値±標準偏差
本試験では、3つの投与期を設定し、A、B及びC群の各投与期における投与薬剤は以下のとおり設定した。
A群:第1期 本剤、第2期 プラセボ、第3期 プラセボ
B群:第1期 プラセボ、第2期 本剤、第3期 プラセボ
C群:第1期 プラセボ、第2期 プラセボ、第3期 本剤

2. 反復投与
本剤を1年以上にわたってくり返し投与した症例においても本剤に対する特異的な抗体産生は見られていない[11]。

【薬効薬理】
1. 抗体活性
10,000人以上の健康成人血漿から精製濃縮された高純度の免疫グロブリンGを原料としているため、種々の細菌、毒素、ウイルス等に対する抗体を有している[12]。
2. オプソニン効果
大腸菌を用いて検討した結果、スルホ化人免疫グロブリンGは生体本来の免疫グロブリンGと同様、食細胞の貪食能、殺菌能の増強効果等のオプソニン効果が認められている[13][14]。
3. 溶菌活性
スルホ化人免疫グロブリンGは正常な補体の活性化にもとづく溶菌活性能を有している[15][16]。
4. 血小板減少抑制効果
抗血小板抗血清を投与したラットの実験的血小板減少症において、スルホ化人免疫グロブリンGを投与することにより、血小板減少抑制作用が認められている[17]。
5. 冠動脈障害抑制効果
離乳期ウサギに馬血清をくり返し投与することによって作成した冠動脈障害モデルに対して冠動脈障害抑制効果が認められている[18]。
6. 末梢神経障害抑制効果
ウシ末梢神経抗原の免疫により惹起されたラットアレルギー性神経炎モデルにおいて、ラット免疫グロブリン又はスルホ化人免疫グロブリンGを投与することにより末梢神経障害の抑制作用が認められている[19][20]。

【取扱い上の注意】
[記録の保存]
本剤は特定生物由来製品に該当することから、本剤を使用した場合は、医薬品名(販売名)、その製造番号又は製造記号(ロット番号)、使用年月日、使用した患者の氏名、住所等を記録し、少なくとも20年間保存すること。

【承認条件】
1. 急性期川崎病治療における2,000mg/kg体重1回投与での副作用発現、臨床効果等に関するデータを収集する目的で、適切な市販後調査を実施すること。
2. チャーグ・ストラウス症候群又はアレルギー性肉芽腫性血管炎における神経障害の改善について、国内での治験症例が極めて限られていることから、製造販売後、一定数の症例に係るデータが集積されるまでの間は、原則として全症例を対象に使用成績調査を実施することにより、本剤使用患者の背景情報を把握するとともに、本剤の安全性及び有効性に関するデータを早期に収集し、本剤の適正使用に必要な措置を講じること。

【包　装】
献血ベニロン－I静注用500mg:1バイアル
溶剤(日本薬局方注射用水)10mL添付
献血ベニロン－I静注用1000mg:1バイアル
溶剤(日本薬局方注射用水)20mL、薬液調整用針(溶解移注針)、通気針添付
献血ベニロン－I静注用2500mg:1バイアル
溶剤(日本薬局方注射用水)50mL、薬液調整用針(溶解液注入針)、通気針添付
献血ベニロン－I静注用5000mg:1バイアル
溶剤(日本薬局方注射用水)100mL、薬液調整用針(溶解液注入針)、通気針添付

【主要文献】

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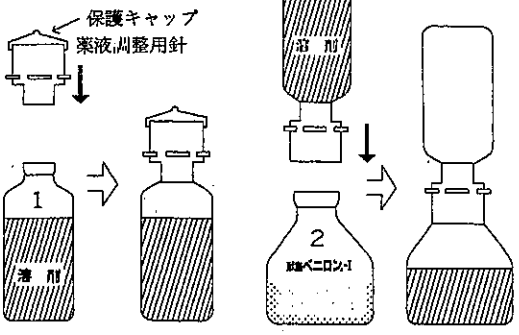
【文献請求先】
主要文献に記載の社内資料につきましても下記にご請求下さい。

帝人ファーマ株式会社　学術情報部
〒100-8585　東京都千代田区霞が関３丁目２番１号
フリーダイヤル　0120-189-315

〈溶解方法〉
冷蔵保存をしていた場合は製剤及び添付の溶剤(日本薬局方注射用水)バイアルを冷蔵庫から取り出し、室温にもどす。製剤バイアルは陰圧となっているため、必ず下記の順序に従って溶解すること。
500mg製剤
1. 製剤及び溶剤バイアルのゴム栓を消毒する。
2. 注射器で溶剤を全量抜き取り、製剤バイアルに突き刺し、溶剤の全量を壁面に沿ってゆっくり注入する。
3. 製剤バイアルをできるだけ泡をたてないようゆるやかに回転振盪しながら完全に溶解する(激しく振盪しないこと)。

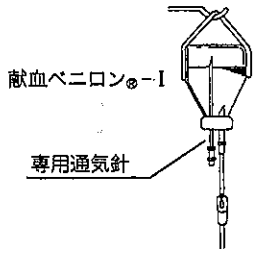
1,000mg、2,500mg、5,000mg製剤
1. 製剤及び溶剤バイアルのゴム栓を消毒する。
2. 添付の薬液調整用針の保護キャップのついている側を上にし、針に指を触れないようにして溶剤バイアル１にまっすぐにさし込む(図１)。
3. 薬液調整用針の保護キャップを外した後、薬液調整用針を溶剤バイアルにさしたまま逆さまにし、針に指を触れないようにして製剤バイアル２にまっすぐにすばやくさし込む(図２)。
4. 溶剤が全量注入されたら、製剤バイアルから薬液調整用針を溶剤バイアルとともに抜き去る。
5. 製剤バイアルをできるだけ泡をたてないようゆるやかに回転振盪しながら完全に溶解する(激しく振盪しないこと)。



(図１)　　(図２)

注：なお、誤って、先に製剤バイアルに薬液調整用針を取り付けると、製剤バイアル内の陰圧が解除され、溶剤を移注出来ません。この場合は500mg製剤の溶解方法に準じて、注射器を使用して移注して下さい。

〈通気針の使用方法〉
(1,000mg、2,500mg、5,000mg製剤)
1. 製剤バイアルに点滴チューブの針を刺し、バイアルを逆さまにつるしておく。
2. 通気針のフィルターの部分を指で蓋をした状態のまま、通気針を製剤バイアルに差し込み、先端が液面上に出たことを確認してからフィルター部分の指を放す。



＊1,000mg、2,500mg、5,000mg製剤に添付している通気針は、献血ベニロン－Ｉを点滴静注する時に気泡が生じないように、特別に軸が長く設計されたものです。
＊市販の輸液セットに組み込まれた通気針は針が短く、液面から出ないために点滴の際気泡が生じますので、添付の通気針をご使用下さい。

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研究報告の概要

イヌ－ヒト感染およびヒト－ヒト感染の可能性があるヒトの肺ペストのアウトブレイク－コロラド州、2014 年 6 月～7 月

2014 年 6 月 28 日、患者 A（中年男性）は発熱および咳嗽を来たし肺炎と診断され入院した。7 月 8 日、コロラド公衆衛生環境部（CDPHE）の検査室にて、患者 A から採取された血液検体においてペスト菌が特定され、患者 A は肺ペストと診断された。3 群保健所（TCHD）が実施する調査により、患者 A のイヌが最近喀血で死亡していたことが判明した。患者 A の診断後に、検査されたイヌの肝臓および肺組織がペスト菌に対して陽性であった。また、2014 年 6 月 24 日～25 日に同じイヌと接触し後日発熱などを呈した 2 例（患者 B および患者 C）について、後日ペスト菌検査陽性およびペスト菌抗体価の上昇がそれぞれ確認された。2014 年 7 月 4 日、発熱、呼吸困難などを呈し、肺炎と診断され入院した患者 D は、6 月 25 日に死亡した同じイヌに接触しており、6 月 29 日～30 日に患者 A と接触していた。後日、患者 D の血清検体においてペスト菌の抗体価の上昇が認められた。本症例は、患者 A からヒト－ヒト感染した可能性があり、米国においてこのような事象が報告されるのは 1924 年以降初めてである。

報告企業の意見	今後の対応
ペスト菌（Yersinia pestis）は、腸内細菌科に属する通性嫌気性のグラム陰性桿菌で、ノミやエアロゾルを介して伝播する。組織内及び培養菌などの新鮮な菌では、約 1.5×0.7μm の両端の丸い楕円形の短桿菌で、単染色法では特徴ある明瞭な極小体が観察される。発育適温は 28～30℃で、1～45℃で発育する。万一、原料血漿にペスト菌が混入したとしても、製造工程中の各種除菌ろ過処理及びウイルス除去膜によるろ過処理により、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

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。

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Outbreak of Human Pneumonic Plague with Dog-to-Human and Possible Human-to-Human Transmission — Colorado, June–July 2014

Janine K. Runfola, MS[1], Jennifer House, DVM[2], Lisa Miller, MD[2], Leah Colton, PhD[2], Donna Hite[1], Alex Hawley[1], Paul Mead, MD[3], Martin Schriefer, PhD[3], Jeannine Petersen, PhD[3], Colleen Casaceli, MPH[4], Kristine M. Erlandson, MD[5], Clayton Foster, MD[5], Kristy L. Pabilonia, DVM, PhD[6], Gary Mason, DVM, PhD[6], John M. Douglas, Jr., MD[1] (Author affiliations at end of text)

On July 8, 2014, the Colorado Department of Public Health and Environment (CDPHE) laboratory identified *Yersinia pestis*, the bacterium that causes plague, in a blood specimen collected from a man (patient A) hospitalized with pneumonia. The organism had been previously misidentified as *Pseudomonas luteola* by an automated system in the hospital laboratory. An investigation led by Tri-County Health Department (TCHD) revealed that patient A's dog had died recently with hemoptysis. Three other persons who had contact with the dog, one of whom also had contact with patient A, were ill with fever and respiratory symptoms, including two with radiographic evidence of pneumonia. Specimens from the dog and all three human contacts yielded evidence of acute *Y. pestis* infection. One of the pneumonia cases might have resulted through human-to-human transmission from patient A, which would be the first such event reported in the United States since 1924. This outbreak highlights 1) the need to consider plague in the differential diagnosis of ill domestic animals, including dogs, in areas where plague is endemic; 2) the limitations of automated diagnostic systems for identifying rare bacteria such as *Y. pestis;* and 3) the potential for milder plague illness in patients taking antimicrobial agents. Hospital laboratorians should be aware of the limitations of automated identification systems, and clinicians should suspect plague in patients with clinically compatible symptoms from whom *P. luteola* is isolated.

Investigation and Results

Patient A, a previously healthy middle-aged man, developed fever and cough on June 28. Over the next 24 hours his condition worsened with increasing cough and the production of bloody sputum. He was admitted to a local hospital where he was diagnosed with pneumonia (Figure). Blood cultures collected on June 30 grew a gram-negative rod that was initially identified as *P. luteola* using an automated identification system. Over the next 6 days patient A's respiratory status deteriorated, and he was transferred to another facility where he required intubation. Because of the severity of his illness and previous reports of misidentification of *Y. pestis* as *P. luteola* (*1*,*2*), the isolate was sent to the CDPHE laboratory for further testing. On July 8 the specimen was correctly identified as *Y. pestis*, and patient A received a diagnosis of pneumonic plague. Patient A was treated with broad-spectrum antibiotics, including levofloxacin and streptomycin, and recovered after hospitalization for 23 days.

TCHD initiated an investigation, consisting of interviews with patient A's family, evaluation of potential exposures to the patient, and an environmental assessment to determine the risk for further disease transmission. The investigation revealed

INSIDE

Continuing Education examination available at http://www.cdc.gov/mmwr/cme/conted_info.html#weekly.

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that patient A's dog, a male American pit bull terrier aged 2 years, became ill with fever, jaw rigidity, drooling, and right forelimb ataxia on June 24 (Table). The dog was kept overnight at a veterinary clinic and humanely euthanized the following day after developing dyspnea and bloody sputum. Patient A had close contact with the dog during euthanasia. Necropsy revealed gastric and pulmonary hemorrhage. Samples tested negative for evidence of rabies virus infection and anticoagulants; histopathologic examination of the tissues was declined by patient A. Following patient A's diagnosis with plague, liver and lung tissues from the dog were tested for *Y. pestis*, and results were positive by both polymerase chain reaction assay and culture. Archived formalin-fixed tissues from the dog were processed for histopathology, revealing severe acute bronchopneumonia with intra-alveolar bacteria. The investigation also identified three other persons who had been in close contact with the ill dog, one of whom who also had contact with patient A. All three subsequently received diagnoses of plague, and all three recovered (Table, Figure).

On June 30, 2 days after patient A became ill, patient B, a female veterinary clinic employee, developed a fever and cough and visited an urgent care facility, where bronchitis was diagnosed. She reported close contact with the ill dog on June 24–25. After her symptoms failed to improve with self-initiated amoxicillin/clavulanic acid, patient B visited an emergency department on July 5, received a diagnosis of pneumonia, and was treated with azithromycin, with improvement over the next several days. After notification on July 10 of her exposure to plague, she visited a health care provider and was treated with oral levofloxacin. A polymerase chain reaction test on a sputum specimen was positive for *Y. pestis*. Subsequent testing of paired acute and convalescent serum specimens demonstrated a fourfold increase in antibody titers to *Y. pestis*, indicative of recent infection (Table).

Patient C, a female veterinary clinic employee, also had close contact with the dog on June 24–25 and self-initiated a 6-day course of oral doxycycline on June 25. On July 4, she experienced fever, chills, myalgia, and fatigue; symptoms progressed to chest tightness and cough. Following notification of the exposure to plague on July 9, patient C self-initiated a second course of doxycycline and was medically evaluated later that day. Crackles were heard during chest auscultation; however, results of a chest radiograph were normal. A full course of oral doxycycline was continued with resolution of symptoms. Initial and follow-up serum specimens tested positive for antibody to *Y. pestis*, with a greater than fourfold decrease in antibody titers at follow-up 6 months later (Table).

On July 4, patient D, a woman who was a close contact of patient A, experienced chest tightness, dyspnea, and fever. She was evaluated at an emergency department, received a diagnosis of pneumonia, and was treated with oral levofloxacin. Patient D handled the body of the dog on June 25 after it died, at one point getting blood on her hands. She also had extended close contact with patient A on June 29–30 while he was coughing bloody sputum. On July 8, after patient A was identified with pneumonic plague, patient D was hospitalized and treated with

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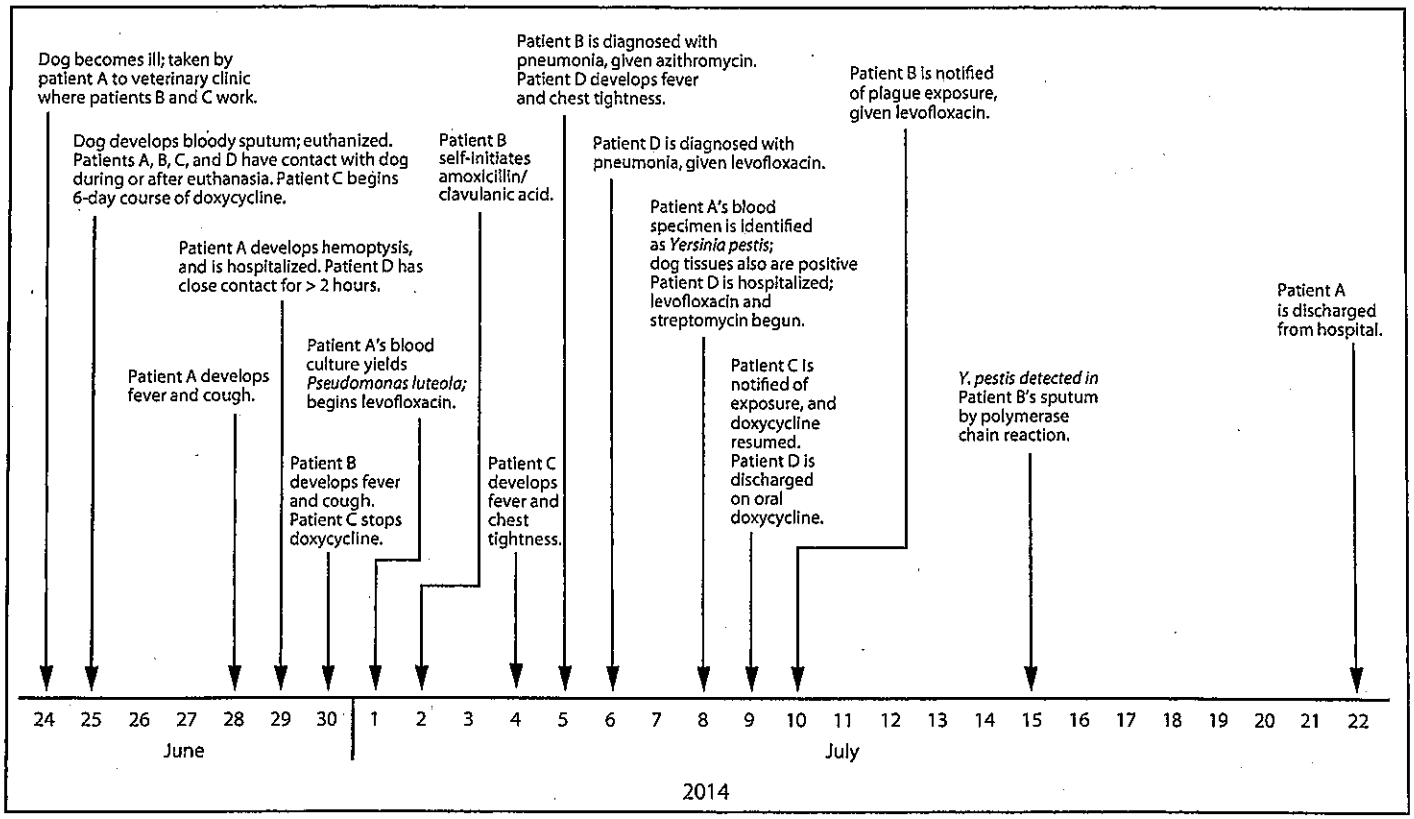
Suggested citation: [Author names; first three, then et al., if more than six.] [Report title]. MMWR Morb Mortal Wkly Rep 2015;64:[inclusive page numbers].

FIGURE. Timeline of diagnoses and treatment for patients identified in a pneumonic plague outbreak — Colorado, 2014



levofloxacin and streptomycin. Paired acute and convalescent serum specimens for patient D demonstrated a greater than fourfold increase in antibody titers to *Y. pestis* (Table).

Public Health Response

TCHD evaluated potential exposures from each patient and conducted an environmental assessment to determine the risk for further disease transmission. Case status was assigned according to case definitions developed by the Council of State and Territorial Epidemiologists for CDC's National Notifiable Diseases Surveillance System.*

Medical personnel and personal contacts of all four patients were notified of their possible exposure to plague. A total of 114 persons had close contact with the dog or one or more of the human patients: 36 in veterinary settings, 58 in human health care settings, and 20 as close personal contacts. Antimicrobial prophylaxis was recommended for 88 persons interviewed within 7 days of exposure. The remaining 26 were advised to monitor for fever for 7 days and to seek medical attention immediately if symptoms occurred.

On July 9, TCHD surveyed patient A's property for evidence of plague. Live rabbits were observed on the property but no other wildlife. Inactive prairie dog burrows were present; however, it was reported that the prairie dog colony had been intentionally eradicated in October 2013.

CDPHE issued press releases for public awareness and Health Alert Network notifications to health care providers and veterinarians on July 9, 10, and 18. Medical facilities were instructed to use droplet precautions for persons with suspected plague. TCHD staff members distributed information on plague symptoms and transmission risk to homes in the vicinity of the index patient. No further cases have been identified.

Discussion

Plague is a rare but life-threatening zoonosis caused by *Y. pestis*. A median of eight cases of human plague are reported annually in the United States (*3*), primarily among residents of semirural areas in New Mexico, Arizona, Colorado, and California. Normally a pathogen of rodents, *Y. pestis* is transmitted to humans through the bite of infected rodent fleas or direct contact with the tissues or secretions of infected animals. Bubonic plague, characterized by fever and painful regional

* Available at http://wwwn.cdc.gov/NNDSS/script/casedef.aspx?CondYrID=800&DatePub=1/1/1996.

TABLE. Dates of exposure and illness onset and test results for patients identified in a pneumonic plague outbreak — Colorado, June–July 2014

Patient	Date of exposure (source)	Onset of illness	Chest radiograph findings	Hospitalized	Laboratory test results: Polymerase chain reaction: Specimen	Date	+/-	Culture: Specimen	Date	+/-	Serologic testing: Initial: Specimen	Date	Titer	Follow-up: Date	Titer
Dog	Unknown	June 24	PNA	Yes	Liver/lung tissue	June 26	+	Liver/lung tissue	June 26	+	NT	NT	NT	NT	NT
A	June 25	June 28	PNA	Yes	Blood	June 29	+	Blood	June 29	+	NT	NT	NT	NT	NT
B	June 25	June 30	PNA	No	Sputum	July 10	+	Sputum, blood	July 10	-	Blood	July 10	1:64	July 24	1:64
														Jan 12 2015	1:256
C	June 25	July 4	no evidence of PNA	No	Blood	July 9	-	Blood	July 9	-	Blood	July 9	1:32	July 24	1:32
														Jan 12 2015	-
D	June 25 (dog)	July 5	PNA	Yes	Blood	July 6	-	Blood	July 6	-	Blood	July 6	-	July 12	1:32
	June 29 (patient A)													July 23	1:32

Abbreviations: PNA = pneumonia; + = positive test result; - = negative test result; NT = not tested.

lymphadenopathy, results from percutaneous exposure and accounts for approximately 85% of reported cases. Pneumonic plague occurs as either a complication of untreated bubonic plague (10%–13% of all cases) or as a primary pneumonia following inhalation of infectious droplets (2% of all cases) (*4*). Untreated pneumonic plague has a fatality rate of ≥93% and can be spread from person to person through aerosols generated during coughing. A third clinical form, septicemic plague, is characterized by fever and shock without localizing signs or symptoms. Laboratory diagnosis of plague is based on culture or polymerase chain reaction assays of blood, sputum, or lymph node aspirates, or on serology. Effective therapy includes aminoglycosides and doxycycline. In addition, the fluoroquinolones levofloxacin and ciprofloxacin have been approved recently by the Food and Drug Administration based on animal studies.[†] The advent of antimicrobial therapy has reduced overall plague mortality from >60% to approximately 16% (*3,5,6*).

In this outbreak, all four patients had laboratory-confirmed plague, including three patients (A, B, and D) with clinical and radiographic evidence of pneumonia. The fourth patient (C) had an atypical presentation with respiratory symptoms but no radiographic evidence of pneumonia, possibly as a result of partial treatment immediately after exposure. Three patients (A, B and C) became ill shortly after exposure to an ill infected dog. The source of infection for patient D is less certain because she had exposure to both the dog on June 25–26 (an incubation period of 9–10 days) and to patient A on June 29–30 while he had hemoptysis (an incubation period of 5–6 days). The shorter incubation period is more typical of plague and therefore supports human-to-human transmission (*6*). Nevertheless, transmission from the dog cannot be excluded given the animal's role in the other three infections and because incubation periods of up to 10 days have been reported, although rarely (*7*). Primary pneumonic plague is rare in the United States with only 74 cases reported during 1900–2012, and this event represents the largest outbreak and the first instance of possible human-to-human transmission since an outbreak in Los Angeles in 1924 (*3,5*).

Y. pestis infection in dogs generally is either asymptomatic or the cause of only a mild, self-limiting febrile illness (*8*). Dogs can play a role in human infection through transport of rodent fleas into the home (*8,9*). This outbreak began with illness in a pet dog, a previously unrecognized source of plague exposure in the United States. The only previously published case of direct transmission of plague from a dog to a human was reported from China in 2009 (*10*). Although symptomatic plague in dogs is rare, veterinarians should consider the possibility of *Y. pestis* infection in ill dogs with wildlife exposure in areas where plague is endemic.

This outbreak is notable for the several factors that delayed its recognition. First, patient A's bacterial isolate initially was identified as *P. luteola* by an automated blood culture system, and the correct identification of *Y. pestis* was only made 7 days later. This delay resulted in the exposure of numerous medical personnel. Misidentification and a resulting delayed diagnosis have been previously reported, reinforcing the need

[†] Available at http://www.fda.gov/downloads/Drugs/DrugSafety/ucm088619.pdf (levofloxacin) and http://www.fda.gov/downloads/Drugs/DrugSafety/ucm246794.pdf (ciprofloxacin).

What is already known on this topic?

Rapid identification of plague is critical in patients who live in, or who have recently traveled to, regions where plague is endemic, including the western United States. The three most common forms of plague are bubonic, pneumonic, and septicemic, with the majority of cases presenting as bubonic. Although the rarest form of plague (approximately 2% of reported cases), primary pneumonic plague has a high (≥93%) mortality rate when left untreated.

What is added by this report?

The outbreak in Colorado represents the largest outbreak of pneumonic plague in the United States since 1924. The source of the outbreak was a dog with pneumonic plague, an atypical occurrence because dogs infected with *Yersinia pestis* generally are either asymptomatic or exhibit mild self-limiting febrile illness and are not considered a direct source of human infection. Four persons developed plague after exposure to the ill dog; one of the patients also had close contact with the index patient after he developed plague pneumonia, supporting possible human-to-human transmission. Diagnosis in the index case was delayed because of misidentification of a bacterial isolate as *Pseudomonas luteola* by an automated blood culture system. The spectrum of disease in this outbreak was broader than usual for pneumonic plague, with two of the four patients not requiring hospitalization, possibly as a result of self-administration of antibiotics or medical prescription of azithromycin, an antibiotic not recommended for plague.

What are the implications for public health practice?

Plague should be considered in the differential diagnosis of dogs with respiratory illness in areas where plague is endemic. The results of automated blood culture systems should be evaluated critically when rare diseases are suspected. Patients with suspected pneumonic plague should be isolated before laboratory confirmation and treated with appropriate antibiotics. Blood or sputum cultures should be sent to state public health laboratories for confirmation.

for critical evaluation of results from automated systems and education of hospital microbiologists regarding this limitation (*1*,*2*). Among 12 *Y. pestis* isolates obtained from U.S. patients during 2010–2013, at least three (25%) were originally misidentified by automated systems (Division of Vector-borne Diseases, National Center for Emerging and Zoonotic Infectious Diseases, CDC, unpublished data, 2015). Second, the spectrum of disease was broader than usual for pneumonic plague (*7*), with two of the four patients not requiring hospitalization. The clinical course of the milder cases might have been modified by self-administration of antibiotics or medical prescription of azithromycin, an antibiotic not recommended for plague. Pneumonia is the only form of plague with the potential for human-to-human transmission. Delayed recognition because of inaccurate laboratory test results and atypical clinical presentations can lead to high numbers of potential exposures to health care workers, laboratory workers, and other close contacts.

Although human plague is rare in North America, it remains a public health concern in the western United States where *Y. pestis* circulates among wild rodent populations. The risk for plague can be minimized by avoidance of possibly infected rodents (e.g., prairie dogs) and their fleas. All suspected or confirmed plague cases and rodent die-offs in areas where plague is endemic should be reported immediately to public health officials so that exposures can be minimized to prevent additional transmission. Once plague is suspected, appropriate precautions and treatment should be initiated immediately, and clinical specimens should be collected and tested as soon as possible. Early recognition of plague, especially the pneumonic form, is critical to effective clinical management and a timely public health response. Veterinarians should consider plague in the differential diagnosis of ill domestic animals, including dogs, in areas where plague is endemic.

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感染症定期報告に関する今後の対応について

平成１６年度第５回
運営委員会確認事項
（平成１６年９月１７日）

１ 基本的な方針

運営委員会に報告する資料においては、

(1) 文献報告は、同一報告に由来するものの重複を廃した一覧表を作成すること。

(2) 8月の運営委員会において、国内の輸血及び血漿分画製剤の使用した個別症例の感染症発生報告は、定期的にまとめた「感染症報告事例のまとめ」を運営委員会に提出する取り扱いとされた。これにより、感染症定期報告に添付される過去の感染症発生症例報告よりも、直近の「感染症報告事例のまとめ」を主として利用することとすること。

２ 具体的な方法

(1) 感染症定期報告の内容は、原則、すべて運営委員会委員に送付することとするが、次の資料概要を作成し、委員の資料の確認を効率的かつ効果的に行うことができるようにする。

① 研究報告は、同一文献による重複を廃した別紙のような形式の一覧表を作成し、当該一覧表に代表的なものの報告様式（別紙様式第2）及び該当文献を添付した**「資料概要A」**を事務局が作成し、送付する。

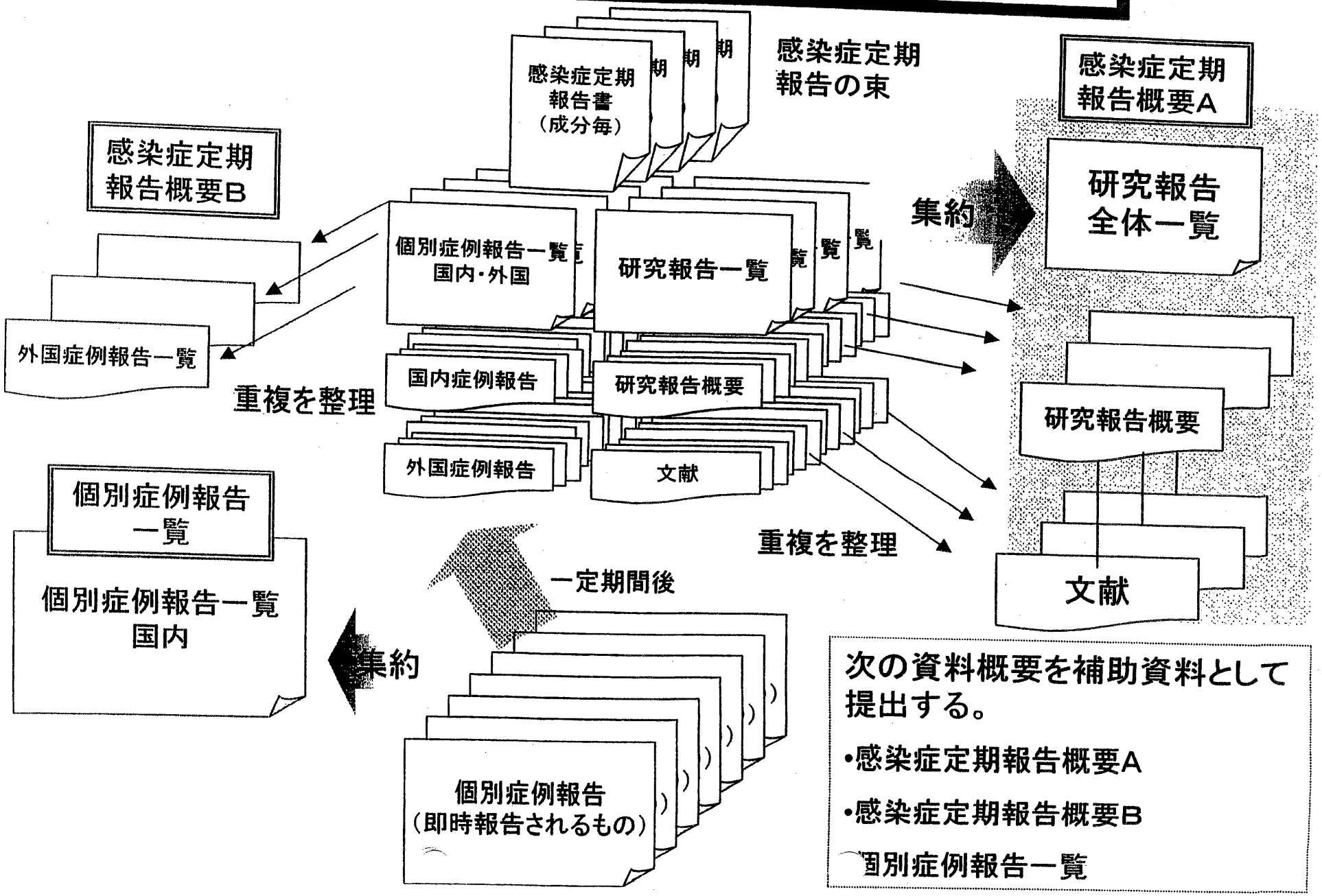
② 感染症発生症例報告のうち、発現国が「外国」の血漿分画製剤の使用による症例は、同一製品毎に報告期間を代表する感染症発生症例一覧（別紙様式第4）をまとめた**「資料概要B」**を事務局が作成し、送付する。

③ 感染症発生症例報告のうち、発現国が「国内」の輸血による症例及び血漿分画製剤の使用による感染症症例については、「感染症報告事例のまとめ」を提出することから、当該症例にかかる「資料概要」は作成しないこととする。ただし、運営委員会委員から特段の議論が必要との指摘がなされたものについては、別途事務局が資料を作成する。

(2) 発現国が「外国」の感染症発生症例報告については、国内で使用しているロットと関係がないもの、使用時期が相当程度古いもの、因果関係についての詳細情報の入手が困難であるものが多く、必ずしも緊急性が高くないと考えられるものも少なくない。また、国内症例に比べて個別症例を分析・評価することが難しいものが多いため、緊急性があると考えられるものを除き、その安全対策への利用については、引き続き、検討を行う。

(3) 資料概要A及びBについては、平成16年9月の運営委員会から試験的に作成し、以後「感染症的報告について（目次）」資料は廃止することとする。

感染症定期報告・感染症個別症例報告の取り扱い



次の資料概要を補助資料として提出する。

- 感染症定期報告概要A
- 感染症定期報告概要B
- 個別症例報告一覧