別紙様式第2-1

No. 32

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2008. 2. 22	該当なし	

一般的名称	新鮮凍結人血漿	研究報告の公表状況	ProMED 20080218.0645, 2008 Feb 18. 情報源:[1]G1 Globo.com, 2008 Feb 13. [2]Milenio.com, 2008 Feb 17.	公表国	[1]ブラジル[2]パラグアイ
販売名(企業名)	新鮮凍結血漿「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」(日本赤十字社)				

研究報告の概要 119

○南米における黄熱のアウトブレイク
[1]ブラジル
2008年1月21日、32歳の男性が黄熱のため死亡した。これは、ブラジルで発生した15人目の黄熱死亡患者である。保健当局の発表によると、この男性は2月13日に感染が確認されており、首都ブラジリア近郊のソブランディーノの病院で死亡した。ブラジリアで感染したと見られている。また、Mato Grossoでも1名の感染と死亡が確認された。
[2]パラグアイ
保健当局は2月16日に、首都アスンシオンの病院で集中治療を受けていた39歳の女性が死亡したと発表した。パラグアイではこれまでに、少なくとも6名が黄熱によって死亡した。多くの市民がワクチン投与を求めて病院に殺到している。政府は944,000人分のワクチンをブラジルから輸入した。その大半はブラジル政府から寄付されたものである。ドゥアルテ大統領は15日、黄熱感染対応のため非常事態宣言を発令した。

報告企業の意見	今後の対応
南米で黄熱の流行が拡大し、パラグアイで6名、ブラジルで15名の黄熱死亡患者が発生したの報告である。	日本赤十字社は、輸血感染症対策として献血時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。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

新鮮凍結血漿「日赤」
新鮮凍結血漿-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

(7)

Back

Archive Number 20080218.0645
Published Date 18-FEB-2008
Subject PRO/AH/EDR> Yellow fever - South America (02): Paraguay, Brazil

YELLOW FEVER - SOUTH AMERICA (02): PARAGUAY, BRAZIL
****
A ProMED-mail post
<http://www.promedmail.org>
ProMED-mail is a program of the
International Society for Infectious Diseases
<http://www.isid.org>

[1] Brazil
[2] Paraguay

******
[1] Brazil
Date: Wed 13 Feb 2008
Source: G1 Globo.com [in Portuguese, trans. Mod. TY, edited]
<http://g1.globo.com/Noticias/Brasil/0,,MUL297999-5598,00.html>

A 32-year-old man died in Brazilia of yellow fever (YF) on 21 Jan 2008. With this death, the number of deaths in the country due to this disease has increased to 15.

The Secretary of Health of the Federal District (DF) confirmed this additional death from yellow fever on Wednesday [13 Feb 2008]. The man died at the hospital in Sobradinho, a satellite city of Brasilia. The report confirming the cause of death was issued this past Wednesday [13 Feb 2008].

According to the Ministry of Health, the likely location of infection of the man was in the Federal District. That contradicts what the health authorities in Brasilia have previously expressed. According to them, prior to this announcement, the people who died of YF in the DF had all been infected in Goias [state]. Of the cases reported in the DF, 11 were confirmed, 3 are being investigated and 2 were discarded [based on] clinical [grounds] and laboratory [results].

Mato Grosso
The Ministry of Health, also confirmed on Wednesday [13 Feb 2008], the 1st YF case in Mato Grosso (MT). Laboratory tests performed by the Evandro Chagas Institute, in Para, indicated that a farmer from Novo Sao Joaquim, MT died of the disease.

According to the Ministry of Health, the state of Mato Grosso has 2 other suspected cases of the disease which are still under investigation.

--
Communicated by:
ProMED-mail <promed@promedmail.org>

[This worrisome report indicated that the man who died of YF acquired his infection in a satellite city of the DF, suggesting possible urban transmission. ProMED-mail requests more information concerning the probable location of infection and the travel history of the above mentioned fatality (in the DF), in order to have a better idea if this was another sylvan (jungle or forest) YF case or was truly a case of urban YF virus transmission. The Mato Grosso death is very likely a sylvan YF case.

An interactive ProMED health map of Brazil showing the location of Goias and Mato Grosso states and the Federal District can be accessed at: <http://healthmap.org/promed?v=-10.8,-53.1,4>. - Mod.TY]]

******
[2] Paraguay
Date: Sun 17 Feb 2008
Source: Milenio.com [in Spanish, trans. & summ. Mod. TY, edited]
<http://www.milenio.com:80/index.php/2008/02/17/194717/>

Health authorities reported this Sunday [17 Feb 2008] that a 39-year-old woman died Saturday night [16 Feb 2008], after a week of intensive therapy in a hospital in the capital [Asuncion].

At least 6 people have died in Paraguay as a result of the yellow fever (YF) outbreak which has the entire population on alert, and responding with a massive [influx going to] vaccination centers, the government announced. Thousands of citizens went to the health centers in the capital where massive vaccination is taking place.

This weekend, the country received 944 000 doses of [YF] vaccine from Brazil, of which 800 000 were donated by the government of the neighboring country and 144 000 were furnished by the Panamerican Health Organization.

Nicanor Duarte, the President of Paraguay, this past Friday [15 Feb 2008] declared a national state of emergency to address the YF outbreak, so that the [governmental] authorities can deal with this health emergency.

--
Communicated by:
ProMED-PORT <promed@promedmail.org>

[Given the massive vaccination campaign in the capital city, it appears that the previous urban YF cases that were acquired there have generated considerable concern (panic?) on the part of both the government and the citizens. ProMED-mail would be interested to know if similar vaccination campaigns are being carried out in other areas of Paraguay. Brazil, which had embargoed the export of the YF vaccine produced there, has shown remarkable public health citizenship by providing vaccine to Paraguay in a very timely way, despite continuing YF cases in Brazil.

A map of Paraguay can be accessed at:
<http://www.lib.utexas.edu/maps/americas/paraguay_pol98.jpg>. - Mod.TY]

[see also:
Yellow fever - South America: Paraguay, Brazil 20080217.0627
Yellow fever - Paraguay (03): (San Pedro): corr. 20080209.0533
Yellow fever - Paraguay (03): (San Pedro) 20080208.0511
Yellow fever - Paraguay (02): (San Pedro) alert 20080206.0475
Yellow fever - Paraguay (San Pedro) 20080205.0467
Yellow fever, monkeys - Argentina (02): conf. 20080212.0568
Yellow fever - Brazil (10): 20080205.0461
Yellow fever, monkeys - Argentina: (Misiones), susp. 20080205.0459
Yellow fever - Brazil (09): 20080203.0439
Yellow fever - Brazil (08): 20080124.0293
Yellow fever - Brazil (07): 20080119.0240
Yellow fever - Brazil (06): 20080116.0203
Yellow fever - Brazil (05): conf. 20080115.0194
Yellow fever - Brazil (04): susp. 20080111.0147
Yellow fever - Brazil (03) 20080110.0139
Yellow fever - Brazil (02): alert 20080109.0107
Yellow fever - Brazil: (Goias) susp. 2007 20080105.0056
2007

Yellow fever, monkeys - Brazil: (Goias), susp., RFI corr. 20071231.4196
Yellow fever, monkeys - Brazil: (Goias, Fed. Distr.): conf. 20071229.4173
Yellow fever, human, monkey - Brazil, Bolivia: 2007 20071224.4126
Yellow fever, monkey - Brazil (PI): susp 20071222.4119
Yellow fever, monkeys - Brazil (Goias): susp., RFI 20071217.4052
Yellow fever, monkeys - Brazil (RS): alert 20070910.2979
Yellow fever, human, monkey - Brazil (MG): not 20070508.1486
Yellow fever - Brazil (GO): alert 20070424.1335

Yellow fever, human, monkey - Brazil (MG) 20070421.1304]
.....mpp/ty/mpp

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	機構処理欄
				2007. 11. 22	該当なし	

一般的名称	(製造承認書に記載なし)	研究報告の公表状況	Ziemann M, Krueger S, Maier AB, Unmack A, Goerg S, Hennig H. Transfusion. 2007 Nov;47(11):1972-83.	公表国 米国
販売名(企業名)	合成血「日赤」(日本赤十字社) 照射合成血「日赤」(日本赤十字社) 合成血-LR「日赤」(日本赤十字社) 照射合成血-LR「日赤」(日本赤十字社)			

研究報告の概要

○セロコンバージョンと関連した供血者血漿検体中のサイトメガロウイルスDNAの高頻度陽性

背景:ヒトサイトメガロウイルス(CMV)は、血液細胞に潜伏感染すると考えられている。免疫不全患者の輸血感染(TT-CMV)は、CMV-血清反応陰性成分または白血球除去成分を使用しても発現する。

試験デザインおよび方法:過去にCMV血清反応陰性で、初めて抗CMV IgG陽性を示した供血者82名、1年以上血清反応陽性である供血者598名、血清反応陰性供血者150名を対象として、血漿中のCMV DNA陽性率を検討した。本試験後半では、供血血液31,745に基づく供血血液全体のCMV DNA陽性率を評価した。

結果:CMV DNAは、新たに血清反応陽性となった供血者の血漿検体の44%に反復的に検出された(直近前回の血清反応陰性成分供血までの期間に応じて12%～62%の範囲)。継続的な血清反応陽性または血清反応陰性供血者はいずれも、CMV DNA陰性であった。セロコンバージョンに関連したCMV DNAの検出は、ネオプテリンの有意な増加、ALT増加、白血球数減少と関連付けられたが、これら代替マーカーの感度はわずか71%であった。CMV初感染供血者による血液製剤中のCMV DNAの全体的な陽性率は0.13%以上であった。

結論:白血球除去の実施にもかかわらず、新規血清反応陽性供血者のウイルス血症はTT-CMV残存リスクの重大原因であると考えられる。本試験ではウインドウ期が検出可能で、再燃は検出できなかったため、血清反応陰性供血者由来の白血球除去血液の輸血には、1年以上血清反応陽性である供血者由来白血球除去血液の輸血と比較して、TT-CMVの高いリスクが示される可能性が考えられた。

報告企業の意見	今後の対応
新規CMV血清反応陽性供血者は血漿中のCMV DNA陽性率が高く、白血球除去を実施していてもTT-CMV残存リスクの重大原因であると考えられるとの報告である。	CMV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。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

High prevalence of cytomegalovirus DNA in plasma samples of blood donors in connection with seroconversion

Malte Ziemann, Sabine Krueger, Andrea B. Maier, Alexander Unmack, Siegfried Goerg, and Holger Hennig

BACKGROUND: Human cytomegalovirus (CMV) is considered to latently infect blood cells. Transfusion-transmitted infection (TT-CMV) of immunocompromised patients occurs despite the use of CMV-seronegative or leukoreduced units.

STUDY DESIGN AND METHODS: The prevalence of CMV DNA in plasma was investigated in 82 blood donors who had previously been seronegative for CMV and showed anti-CMV immunoglobulin G for the first time, 598 blood donors who were seropositive for at least 1 year, and 150 seronegative blood donors. In a second part of the study, the overall prevalence of CMV DNA in blood donations was assessed based on 31,745 donations.

RESULTS: CMV DNA was repeatedly detected in plasma samples of 44 percent of newly seropositive donors (12%-62%, depending on the interval to the last seronegative donation). All steadily seropositive or seronegative donors were negative for the presence of CMV DNA. Detection of CMV DNA in connection with seroconversion was accompanied by significantly increased neopterin, increased alanine aminotransferase, and reduced white blood cell counts, but the sensitivity of these surrogate markers was only 71 percent. The overall prevalence of CMV DNA in blood products due to primary CMV infection of donors was at least 0.13 percent.

CONCLUSION: Viremia of newly seropositive donors may be an important reason for the residual risk of TT-CMV despite leukoreduction. Furthermore, transfusion of WBC-reduced blood components from seronegative donors could imply a greater risk of TT-CMV than transfusion of WBC-reduced blood from donors who have been seropositive for at least 1 year, because window-phase donations but no reactivation could be detected in this study.

Human cytomegalovirus (CMV) is an ubiquitous β-herpesvirus causing mostly asymptomatic or mild mononucleosislike infections in immunocompetent subjects[1] with a prevalence of between 40 and 100 percent in adult populations.[2] Contrarily, infection of immunocompromised patients with CMV is a significant cause of morbidity and mortality. Symptoms of CMV infection in these patients cover a broad range from direct manifestations of viral replication like fever, leukopenia, thrombocytopenia, hepatitis, enteritis, and pneumonia to indirect sequelae like an elevated risk for renal allograft rejection or an impaired cellular immune response.[3,4]

An important route of infection for risk groups like seronegative recipients of marrow transplants or newborns is assumed to be transmission of CMV by blood products from latently infected blood donors (so called transfusion-transmitted CMV infection [TT-CMV]). Even if the exact sites and mechanisms of latency still remain to be clarified, CMV DNA has repeatedly been found in peripheral blood white blood cells (WBCs) of healthy, CMV-seropositive individuals, especially in cells of the myeloid lineage.[5,6]

Consequently, leukodepletion of blood products and inventories of seronegative blood donors have been employed to reduce the rates of TT-CMV since the 1980s.[7,8] Even after implementation of these strategies, however,

ABBREVIATIONS: CRP = C-reactive protein; TT-CMV = transfusion-transmitted cytomegalovirus.

From the Institute of Immunology and Transfusion Medicine, University of Lübeck, Lübeck, Germany.

Address reprint requests to: Holger Hennig, Institute of Immunology and Transfusion Medicine, University of Lübeck, Ratzeburger Allee 160, 23538 Lübeck, Germany; e-mail: hennig@immu.mu-luebeck.de.

Received for publication February 2, 2007; revision received March 29, 2007, and accepted April 28, 2007.

doi: 10.1111/j.1537-2995.2007.01420.x

TRANSFUSION 2007;47:1972-1983.

"break-through" infections persist with rates as high as 1 to 3 percent of transfused high-risk patients.[9-15] The impact of active CMV infection of donors (both primary and reactivated) with transmission of infectious virus as a reason for these break-through infections is discussed controversially.[16-18]

To our knowledge, infectious virus has never directly been detected in leukodepleted blood components. This may be due to the relatively poor sensitivity of commonly applied viral cultures or shell vial assays[19] even if more sensitive methods have been described recently.[20] CMV DNA in serum or plasma, on the other hand, is associated with active CMV infection[21] and used routinely for diagnosis and monitoring of CMV infections in risk groups like transplant recipients[22] or acquired immune deficiency syndrome (AIDS) patients.[23]

Drew and coworkers[17] found CMV DNA in the last seronegative sample of 1 of 192 donors (0.5%) and in the first seropositive sample of 2 donors (1.0%), both of whom were excluded from donation because of elevated alanine aminotransferase (ALT). This contrasts with the findings of Zanghellini and colleagues[24] who detected CMV DNA in plasma of 4 of 5 adolescents with CMV seroconversion, but so far no further study has addressed the prevalence of CMV DNA associated with seroconversion of donors.

Because the actual date of seroconversion can be any point between the last seronegative and the first seropositive donation, the prevalence of CMV DNA in the plasma of first-time seropositive donors would be expected to be higher if the interval since the last seronegative sample is relatively short. Nevertheless, there are no data about the correlation between interdonation interval and prevalence of CMV DNA in plasma of newly seroconverted donors or the variations in prevalences of CMV DNA between different donor collectives.

Therefore, we conducted a prospective study, grouping newly seropositive donors according to the interval since their last seronegative sample and measuring CMV DNA in plasma samples before and after seroconversion. Another objective of our study was to determine the sensitivity of surrogate markers for viral infections, like neopterin, ALT, or WBC count for the detection of CMV DNA in plasma in connection with seroconversion.

MATERIALS AND METHODS

Blood donors

Between August 2000 and June 2004, approximately 12,800 volunteer regular blood donors (47% female, 53% male) donated approximately 34,000 whole-blood donations per year (41% by female and 59% by male donors). They were between 18 and 67 years old and healthy and gave informed consent before the donation. Out of this blood donor collective, we investigated 82 well-defined CMV seroconversion cases, whereas the total number of CMV seroconversions during this period has not been determined. Donors were grouped according to the interval since the last seronegative sample, with intervals of less than 120, 120 to 729, and 730 days or more.

Additionally, 598 latently infected blood donors who had been seropositive for at least 1 year were included in this study, 148 of whom had been excluded from donation because of elevated ALT (more than 73 U/L or 112 U/L for female and male donors, respectively). Sampling dates from latently infected donors were distributed evenly throughout the year considering potential seasonal reactivations.[16] A total of 150 CMV-seronegative donors were tested for CMV DNA as controls.

In a further part of the study, all available samples from previously seronegative donors who were repeatedly reactive in the recombinant CMV immunoglobulin G (IgG) enzyme-linked immunosorbent assay (ELISA) between January and December 2006 were tested by polymerase chain reaction (PCR) to determine the minimum rate of CMV DNA–positive donations due to primary CMV infection of donors in our donor population.

Blood specimens

Whole-blood samples were collected in 5.5-mL tubes containing potassium-ethylenediaminetetraacetate (EDTA) at a concentration of 1.6 mg EDTA per milliliter of blood (Monovette, Sarstedt, Nümbrecht, Germany). Such samples were centrifuged at $3291 \times g$ for 4 minutes and EDTA plasma was separated within 24 hours. Plasma specimens were stored at 4 to 8°C for no longer than 72 hours or at less than –30°C until further processing.

Because of the impossibility of determining the actual seroconversion date, the date of the first seropositive sample from a previously seronegative donor was assumed to be the date of seroconversion.

Standard and control specimens

Human CMV quantitated viral DNA control, AD169 strain, Lot 110-018 (Advanced Biotechnologies Inc., Columbia, MD) was used to determine the detection limit of the CMV PCR (TaqMan, Applied Biosystems, Foster City, CA) described below and to quantify CMV DNA–positive samples. Lyophilized CMV DNA–positive cells from an external proficiency testing program (Instand e.V., Düsseldorf, Germany) were used as positive samples for the development and optimization of the TaqMan CMV PCR.

CMV serology

Anti-CMV screening was performed with an automated enzyme immunoassay to detect IgG antibodies against the autologous fusion proteins CG1 and CG2 (Biotest Anti-CMV recombinant IgG ELISA, Biotest AG, Dreieich,

Germany). Reactive samples were retested in duplicate and considered to be repeatedly reactive if at least one of the two repetitions also gave a positive result. In the first part of the study, repeatedly reactive samples were further confirmed by an automated ELISA with AD169-coated microparticles (AxSYM CMV IgG, Abbott GmbH, Wiesbaden, Germany).

Nucleic acid isolation

DNA from 1 mL of EDTA-plasma was prepared using the Extractor (NucliSens™, bioMérieux Deutschland GmbH, Nürtingen, Germany) according to the manufacturer's protocol for pooled plasma or serum samples up to 2.0 mL. To increase the nucleic acid yield, we added a first incubation step of samples together with the lysis buffer, which is based on guanidine thiocyanate at 60°C with horizontal shaking at 110 r.p.m. for 30 minutes. Total nucleic acids from 1 mL of plasma were eluted in 50 µL of elution buffer of which 20 µL was investigated in one PCR experiment to detect CMV DNA.

During the last part of the study between January and December 2006, DNA from 1 mL of EDTA-plasma was isolated with magnetic extraction reagents (NucliSens, bioMérieux, Boxtel, the Netherlands) according to the manufacturer's instructions.

TaqMan PCR

For amplification and simultaneous detection of PCR products, we developed a novel approach based on a quantitative PCR core kit (qPCR, Eurogentec, Seraing, Belgium) on a sequence detection system (ABI Prism 7700 SDS, Applied Biosystems). Primers and fluorogenic TaqMan probe for CMV DNA detection were chosen after comparative analysis of 68 sequences containing the glycoprotein B region of the CMV genome, which were available from the GenBank Nucleotide Database with computer software (OMIGA, Version 2.0, Oxford Molecular, Oxford, UK). In addition, we performed a nucleotide-nucleotide BLAST search via the Internet at http://www.ncbi.nlm.nih.gov/BLAST/ for the chosen oligonucleotides. For the forward primer, TaqMan probe, and reverse primer, we found 84, 80, and 84 hits, respectively, to CMV sequences that had been submitted to various databases. The sequence alignments ensured that the primers were homologous at the last 12 nucleotides at the 3′ end in all of the sequences and showed a maximum of one single-nucleotide polymorphism at the upper sequence. The TaqMan probe showed 100 percent homology or only one mismatch to all of the CMV hits. Seventeen non-CMV BLAST hits each to only one of the three CMV oligonucleotides ensured that no other organism could be detected with this method.

A sequence from the human C-reactive protein (CRP) gene, which was found to be detectable in human plasma was coamplified in each reaction as internal control.[25] The CMV probe was labeled with FAM as reporter and TAMRA as quencher and the CRP probe with VIC and TAMRA dyes. The CMV primers and probe were custom-synthesized by Eurogentec (Liege, Belgium), the CRP probe by Applied Biosystems (Weiterstadt, Germany), and the CRP primers by TIB Molbiol (Berlin, Germany). The sequences of all the oligonucleotides are provided in Table 1.

PCR experiments were carried out in special optical tubes (MicroAmp optical tubes/caps, PE Applied Biosystems, Foster City, CA) in a total volume of 50 µL. Concentrations of $MgCl_2$, CMV probe and primers were optimized by means of chessboard titrations. Final concentrations were 3.5 mmol per L for $MgCl_2$, 150 nmol per L for CMV forward primer, 300 nmol per L for the respective reverse primer, and 250 nmol per L for the CMV probe. The concentration of the CRP probe was 100 nmol per L, whereas those of the CRP primers were limited to 40 nmol per L each. Thermal cycler conditions were 10 minutes at 95°C followed by 40 cycles of 15 seconds at 95°C and 1 minute at 60°C. Threshold values were calculated as the upper 10-fold standard deviation (SD) of the background fluorescence signal measured over the baseline from Cycle 3 to Cycle 30. Results were interpreted as follows: a C_T of less than 40 is positive; a C_T of equal to 40 is negative.

To determine the 95 percent detection limit of the TaqMan CMV PCR, we investigated semilogarithmic dilutions of the CMV quantitated viral DNA control containing between 10^2 and $10^{-0.5}$ genome equivalents per µL (geq/µL) of CMV strain AD169. Twenty-eight samples of each concentration were processed in four consecutive TaqMan PCR procedures according to our protocol for

TABLE 1. Primer and probe sequences

Oligonucleotide	Sequence 5′→3′	Melting temperature (°C)
CMV		
Forward primer	CCCTCAAGTATGGAGATGTGGTG	59
TaqMan probe	FAM-AACACCACCAAGTACCCCTATCGCGTG-TAMRA	69
Reverse primer	AGCGAATAAGATCCGTACCCTG	58
CRP		
Forward primer	CCTGACCAGCCTCTCTCATGC	61
TaqMan probe	VIC-TTTGGCCAGACAGGTAAGGGCCACC-TAMRA	70
Reverse primer	TGCAGTCTTAGACCCCACCC	59

plasma samples. The 95 percent detection limit was calculated by means of probit analysis. Quantification of CMV DNA–positive samples was carried out by means of a standard curve derived from these validation experiments.

Diagnosis of CMV DNAemia

All samples were analyzed by TaqMan PCR in duplicate. Samples with invalid internal control or diverging results were retested twice. DNAemia was diagnosed by reproducibly positive results.

Surrogate markers for viral infections

As part of the routine blood donor screening, ALT levels were determined by the standard IFCC method at 37°C (GPT ALAT liquid IFCC, Medizintechnik Guder, Bad Oeynhausen, Germany) with an automated analyzer (COBAS Mira plus CC, Roche Diagnostics Instruments Center, Rotkreuz, Switzerland). ALT screening was mandatory in Germany until 2004 with limits for donor admission of no more than 73 and 112 U per L for female and male donors, respectively. WBC counts were measured with an automated hematology analyzer (Coulter Gen S, Beckman Coulter, Krefeld, Germany).

In a subgroup of 56 samples, the neopterin concentration was analyzed by an ELISA (neopterin ELISA RE59349, IBL Immuno Biological Laboratories, Hamburg, Germany). The cutoff value for elevated neopterin was set at 10 nmol per L representing the 98th percentile of a healthy asymptomatic population.[26]

Statistical analysis

Unless stated otherwise, means are calculated as arithmetic means ± SD. Confidence intervals (CIs) were calculated with a p value of 0.05. Differences between groups were examined with the U test. Calculations were assisted by database and statistical programs (Excel, Microsoft Corp., Redmond, WA; SPSS, SPSS Inc., Chicago, IL). The probability of appearance of CMV DNA in plasma of latently infected blood donors was calculated with the upper limits of $1 - \alpha$ confidence intervals of the binomical distribution for an α level of 0.05.

The sensitivity of surrogate markers for detection of CMV DNA–positive donations was calculated as the number of CMV DNA–positive donations with elevated markers related to the total number of CMV DNA–positive donations tested for this marker. For neopterin, for instance, this results in the formula

Sensitivity = (Number of CMV DNA–positive donations with elevated neopterin)/(Number of CMV DNA–positive donations tested for neopterin).

The percentage of patients potentially transfused with CMV DNA–positive blood components due to primary CMV infection of donors was calculated according to the formula

$$\%\text{Patients} = 100 \times [1 - (1 - p)^{\text{number of units transfused}}].$$

In this formula, p denotes the proportion of CMV DNA–positive donations related to all donations. Therefore, $(1 - p)$ is the probability of donations being negative for the presence of CMV DNA, and $(1 - p)^n$ is the probability of n units of blood from different donations all being negative for CMV DNA. So $1 - (1 - p)^n$ equals the probability of n units blood containing at least one CMV DNA–positive unit.

RESULTS

TaqMan PCR

Of 1055 plasma samples tested by TaqMan PCR, 1042 (98.8%) were clearly positive or negative, whereas only 13 (1.2%) showed ambiguous results. These were due to insufficient sample volume for repeated testing (6 samples) or equivocal results even of repeated testing (7 samples with 2 positive and 2 negative results each). All samples with ambiguous results were excluded from analysis.

The 95 percent detection limit of the TaqMan PCR was calculated to be 4.88 geq per PCR procedure (3.66-8.22 geq/PCR) with semilogarithmic dilutions of CMV quantitated viral DNA control (Table 2). For 1-mL plasma specimens, it would correspond to approximately 13.5 geq per mL, if an efficacy of DNA isolation of 90 percent is assumed. The mean CMV DNA concentration in positive samples was 166 geq per mL (SD, 395 geq/mL), with a maximum of approximately 3200 geq per mL.

CMV DNA in connection with seroconversion of blood donors

Eighty-two blood donors who were previously tested negative for the presence of CMV IgG antibodies at the

TABLE 2. Observed frequencies in TaqMan CMV PCR

Standard (geq/PCR procedure)	Number of subjects	Observed responses	Probit
100	28	28	1.000
31.6	28	28	1.000
10	28	28	0.999
3.16	28	21	0.784
1	28	15	0.384
0.316	27*	4	0.263

* One subject was excluded due to negative results for CRP DNA.

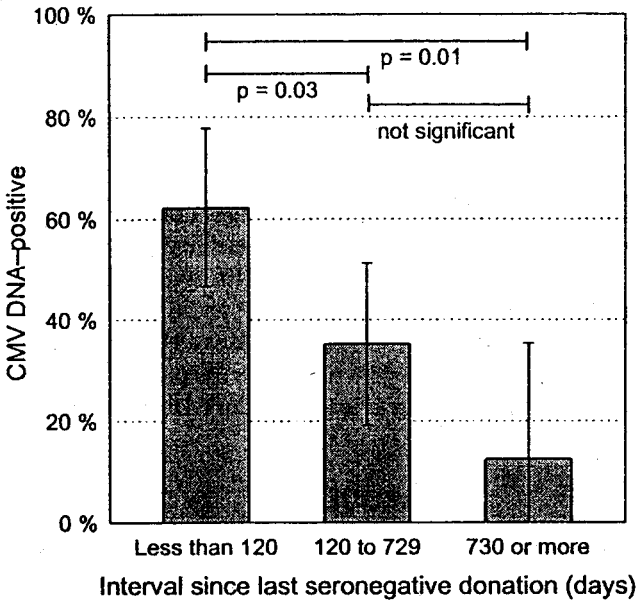

Fig. 1. Prevalence of CMV DNA in plasma of newly seropositive donors ($n = 79$) in dependence on the interval since the last seronegative sample. Prevalences are shown as percentages with 95 percent CI. The percentage of CMV DNA–positive donors was significantly greater after intervals of less than 120 days.

time of their last donation were repeatedly reactive in both anti-CMV assays and further investigated for the presence of CMV DNA. Thirty-six of 82 newly seropositive samples (44%) were repeatedly positive for the presence of CMV DNA by TaqMan PCR. The prevalence of CMV DNA was significantly higher if the interval since the last seronegative donation was less than 120 days ($p < 0.01$), whereas further differences between donors with longer intervals were not significant (Fig. 1).

In 68 (83%) of 82 seroconversion cases, we investigated the last CMV-seronegative donation before seroconversion. This way, we identified 2 reproducibly CMV DNA–positive window-phase donations (2.9%), 68 and 98 days before the first CMV-seropositive donation.

A second seropositive sample was available from 71 (87%) of 82 donors. Sixty-two of these samples (76%) were drawn within 1 year after the first seropositive sample. Samples from 4 donors were reproducibly CMV DNA–positive on Days 3, 5, 20, and 84 after the first seropositive sample, respectively (Table 3). Further plasma samples were available from only 2 of these donors. Both tested CMV DNA–negative on Day 97 and 207, respectively. So CMV DNA–negative samples were available from 59 of 82 donors (72%) within 1 year after the first seropositive sample, whereas no sample tested CMV DNA–positive 1 year or more after the first seropositive sample.

CMV DNA in latently infected and seronegative blood donors

All plasma samples of 150 seronegative and 450 latently infected donors who had been seropositive for at least 1 year were tested negative for the presence of CMV DNA. Additionally, 148 samples of latently infected donors with elevated ALT were available with mean ALT levels of 113 U per L (range, 76-906 U/L). These samples, too, all tested negative for the presence of CMV DNA. Based on the sample size, the proportion of CMV DNA–positive donors related to the total donor population (95% CI) was estimated to be less than 0.5 percent for latently infected donors and no more than 2 percent for latently infected donors with elevated ALT or seronegative donors.

Overall prevalence of CMV DNA in blood donations

In 2006, 102 previously seronegative donors tested repeatedly reactive in the recombinant IgG ELISA. This corresponds to an annual seroconversion rate of 0.8 percent relative to the total donor population.

Thirty-six donations from newly seropositive donors (41% of available samples) tested repeatedly positive for CMV DNA. Assuming the prevalence of CMV DNA in the first seropositive donation to be 41 percent for all 102 seroconversion cases results in a minimum rate of CMV DNA–positive units of 42 of 15,094 seropositive units (0.28%) or 42 of 31,745 units (0.13%), if the CMV serostatus is not taken into consideration (Fig. 2). These rates underestimate the actual prevalence of CMV DNA–positive units, because both window-phase donations and further seropositive donations containing CMV DNA have been ignored.

Surrogate markers for CMV DNAemia

The three common surrogate markers for subclinical viral infections, neopterin, ALT, and WBC count, have been tested in comparison to CMV DNA. Newly seropositive donors with CMV DNAemia had significantly higher neopterin and ALT values, as well as significantly lower WBC counts compared to newly seropositive donors without detectable CMV DNA in plasma. The best sensitivity was achieved by the neopterin ELISA, which detected 61 percent of CMV DNA–positive samples. The sensitivity of ALT was 42 percent if any values outside the normal range were considered. ALT values above the former German national limits for donor admission (>73 or 112 U/L for female and male donors, respectively) were detected in only 4 of 36 CMV DNA–positive donors and in no CMV DNA–negative donor. This equals a sensitivity of 11 percent.

WBC counts were slightly low in 6 of 36 CMV DNA–positive subjects (between 3.4×10^9 and 3.9×10^9/L),