Virus NAT for HIV, HBV, and HCV in Post-Mortal Blood Specimens over 48 h after Death of Infected Patients – First Results

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Keywords

Post-mortal tissue donation · Virus safety ·
NAT screening

Summary

Objective: According to EU regulations (EU directive 2006/17/EC), blood specimens for virologic testing in the context of post-mortal tissue donation must be taken not later than 24 h post mortem. **Methods:** To verify validity of NAT in blood specimens collected later, viral nucleic acid concentrations were monitored in blood samples of deceased persons infected with HIV (n = 7), HBV (n = 5), and HCV (n = 17) taken upon admission and at 12 h, 24 h, 36 h and 48 h post mortem. HIV and HCV RNA were quantified using Cobas TaqMan (Roche), HBV DNA was measured by in-house PCR. **Results:** A more than 10-fold decrease of viral load in samples taken 36 h or 48 h post mortem was seen in one HIV-infected patient only. For all other patients tested the decrease of viral load in 36-hour or 48-hour post-mortal samples was less pronounced. Specimens of 3 HIV- and 2 HBV-infected patients taken 24 h post mortem or later were even found to have increased concentrations (>10-fold), possibly due to post-mortem liberation of virus from particular cells or tissues. **Conclusion:** Our preliminary data indicate that the time point of blood collection for HIV, HBV and HCV testing by PCR may be extended to 36 h or even 48 h post mortem and thus improve availability of tissue donations.

Schlüsselwörter

Postmortale Gewebespende · Virussicherheit ·
NAT-Screening

Zusammenfassung

Hintergrund: Die Entnahme von Blutproben zur Virusdiagnostik im Rahmen der postmortalen Gewebespende muss nach EU Richtlinien (EU directive 2006/17/EC) spätestens 24 h nach dem Tod erfolgen. **Methoden:** Um die Validität von NATs in später entnommenen Proben zu überprüfen, wurde die Konzentration viraler Nukleinsäuren im Blut verstorbener Personen mit HIV- (n = 7), HBV- (n = 5) und HCV-Infektionen (n = 17) zum Zeitpunkt der Einlieferung sowie 12 h, 24 h, 36 h und 48 h post mortem bestimmt. HIV- und HCV-RNA wurde mittels Cobas TaqMan (Roche) gemessen; die Quantifizierung von HBV-DNA erfolgte mit einem In-house-PCR-Test. **Ergebnisse:** Eine mehr als zehnfache Abnahme der Viruskonzentration in 36 h oder 48 h post mortem entnommenen Proben wurde nur bei einem HIV-infizierten Patienten beobachtet. Bei allen anderen Patienten war die Viruslast 36 h oder 48 h post mortem weniger deutlich erniedrigt. In den Proben von 3 HIV- und 2 HBV-Infizierten, die 24 h post mortem oder später entnommen wurden, waren sogar deutlich höhere Konzentrationen (mehr als 10-fach) nachweisbar. Dies könnte auf einer postmortalen Freisetzung der Viren aus bestimmten Zellen oder Geweben beruhen. **Schlussfolgerung:** Unsere vorläufigen Ergebnisse deuten an, dass der Entnahmezeitpunkt für NAT-Analysen auf HIV, HBV und HCV im Rahmen der postmortalen Gewebespende auf 36 h oder 48 h post mortem ausgedehnt werden kann und so die Verfügbarkeit von Gewebespenden verbessert werden kann.

Introduction

Corneas as well as musculoskeletal and cardiovascular tissues from deceased persons continue to be an important source for treatment of corresponding tissue defects. In Germany about 30,000 of these allogeneic tissues are transplanted each year according to the Federal Ministry of Justice (www.bmg.bund.de/fileadmin/redaktion/pdf_misc/Bericht-Breg-Gewebe.pdf). Among the adverse events of cadaveric tissue transplantation, transmission of blood-borne viruses (HIV, HCV and HBV) represents the most important complication. Various precautions are required to avoid transplant-associated viral infections. These include assessment of medical history to ascertain risk factors for viral infections, limited autopsy of cadaveric donors, application of viral inactivation methods (irradiation, chemical and thermal inactivation), quarantine storage, and laboratory testing of post mortem blood samples for viral infection [1].

Rapid perusal of medical history often proves difficult, as information is only available from attending doctors or family members that soon after death are hard to address and may not be aware of particular risk behavior. Virus inactivation is not always possible without impairing quality of tissues. Therefore, laboratory testing of blood samples taken from the donor is essential for virus safety. According to EU directives 2006/17/EC and 2004/23/EC and the German Transplantation Act (Transplantationsgesetz; TPG-GewV), the minimum requirements for laboratory testing of tissue donors include anti-HIV-1/2, HBV surface antigen (HBsAg), anti-HBV core antigen (HBc), anti-HCV, and anti-*Treponema pallidum*, but NATs are not explicitly required. Although observed rarely, transmissions of HIV, HBV and HCV by transplantation of musculoskeletal, cardiovascular, and cornea tissues has been reported in the past [2–4]. Many of these cases were due to early infections not detected by serology, especially by using 1st- and 2nd-generation HIV tests unable to detect virus antigen. The risk of transmitting virus despite negative serology depends on the diagnostic window period, the time between infection and seropositivity. This window period can be reduced by NAT in particular for HCV, as HCV NATs become positive about 6 weeks earlier than HCV antibody tests [5, 6]. In HIV and HBV infections the gap between first positive NAT and first positive serology is shorter and usually amounts 4–5 days for HIV (in case of using 4th-generation HIV combo tests) and about 20 days for HBV, depending on the analytical sensitivity of the test [6–8].

Usefulness of NATs to reduce the risk of viral transmission by blood donations and organ transplantation has been shown in several studies [9–11]. In organ transplantation, testing of donors by NAT is recommended for HCV and, in case of high-risk donors, also for HIV [11, 12]. NAT screening for HIV and HCV of musculoskeletal tissue donors has recently been implemented in the USA [13], and some institutions like

the German Institute for Cell and Tissue Replacement also demand on virus NATs in addition to serology. Due to the blood content and the high donor-recipient ratio, testing by NAT has been recommended in particular for musculoskeletal tissues [1]. So far most commercial NATs are not licensed for post-mortal blood samples and must be validated for these specimens. Test sensitivity may be impaired by hemodilution due to pre-mortal blood transfusions, inhibition by products of blood cell lysis, degradation of viral nucleic acids, and interference with bacterial contaminations [14, 15]. Thus, as stipulated for serology, testing must not be performed later than 24 h after death. As the legal time of death may significantly diverge from discovery, the time period may be too short for blood sampling and to obtain consent of the next of kin. Here we report preliminary results of testing blood samples taken up to 48 h post mortem by NAT that would increase the number of possible donors.

Material and Methods

Deceased Individuals

A total of 27 deceased persons with proven HIV, HBV, and HCV infection were included as described recently [16]. Seven persons were infected with HIV-1, 3 with HBV, 15 with HCV, and 2 persons had HBV/HCV co-infection. Blood samples were taken only after obtaining consent of the next of kin. During the study duration, deceased individuals were stored at 2–8 °C in temperature-controlled cooling chambers at the Institute of Forensic Medicine, University Hospital Hamburg-Eppendorf (UKE). The study was approved by the Ethics Committee of the Ärztekammer Hamburg (local medical association, AZ: WF-024/09).

Blood Collection

Blood samples of deceased persons were taken upon admission to the Institute of Forensic Medicine of the UKE and, if possible, at 12, 24, 36 and 48 h post mortem. As described recently, blood samples (20 ml) were drawn from peripheral large vessels (Vena femoralis, subclavia) or, in individual cases, directly from the heart [16]. Samples were immediately centrifuged to obtain serum which was stored at 2–6 °C and used for serology and NAT within 24 h.

Laboratory Tests

Samples were tested by serology for HIV, HBV, and HCV to confirm infection as described by Edler et al. 2011 [16]. Serum HIV-1 and HCV RNA was quantified using Cobas® TaqMan® (Roche Diagnostics Deutschland GmbH, Mannheim, Germany) with detection limits of 20 copies/ml (HIV-1) and 15 IU/ml (HCV). Serum concentration of HBV DNA was measured by an in-house LightCycler PCR assay based on amplification of HBV core/pre-core gene sequences. DNA was extracted from 500 µl blood using NucliSENS® easyMAG® (bioMérieux Deutschland GmbH, Nürtingen, Germany) and eluted in 100 µl buffer of which 10 µl were subsequently used for amplification with primers HBV-1F (CCGTCTGTGCCTTCTCATCTG) and HBV-1R (AGTCCAAGACTYCTCTTATGYAAGACCTT) and Hot star Taq DNA-polymerase (Qiagen, Hilden, Germany). The PCR program consisted of 50 cycles of denaturation (95 °C, 10 s), annealing (57 °C, 4 s), and extension (72 °C, 10 s). Using PEI (Paul-Ehrlich-Institut) reference material HBV DNA #3620/05 (lyophilized human plasma HBV DNA-positive, genotype D subtype ayw2/3, calibrated against WHO standard HBV DNA #97/746), a detection limit of 20–40 IU/ml was determined. Each sample was run in duplicate with control DNA to test for PCR inhibitors.

Table 1. Monitoring of HIV-1 RNA levels post mortem

Patient	Age/gender	HIV-1 viral load, copies/ml				
		entry ^a	12 h post mortem	24 h post mortem	36 h post mortem	48 h post mortem
HIV 1	44 f	negative	nd	negative	negative	76
HIV 2	37 m	2,100	nd	280	nd	indeterminate ^b
HIV 3	40 m	nd	3,500	4,600	4,300	5,100
HIV 4	61 m	23	50	620	300	360
HIV 5	47 m	160,000	nd	200,000	6,800	9,800
HIV 6	47 m	nd	1,400	4,700	indeterminate ^b	indeterminate ^b
HIV 7	40 m	nd	negative	260	130	negative

f = Female; m = male; nd = not done.
^aIn case of nd at entry committal was after 12 h post mortem.
^bIndeterminate result: no amplification of internal control, indicating inhibition.

Table 2. Monitoring of HBV DNA levels post mortem

Patient	Age/gender	HBV viral load, IU/ml				
		entry ^a	12 h post mortem	24 h post mortem	36 h post mortem	48 h post mortem
HBV 2	32 m	900	8,000	10,000	20,000	20,000
HBV 3	57 m	nd	40,000	indeterminate ^b	indeterminate ^b	500,000
HBV 4	74 f	nd	8,000	30,000	20,000	30,000
HBV 7	52 m	nd	nd	negative	negative	negative
HBV 10	50 m	nd	nd	negative	negative	negative

f = Female; m = male; nd = not done.
^aIn case of nd at entry committal was after 12 h post mortem.
^bIndeterminate result: no amplification of external control, indicating inhibition.

Results

HIV

The PCR results of all 7 individuals infected with HIV (confirmed by HIV serology) are shown in table 1. In case of committal of deceased persons later than 12 h post mortem, the baseline sample at entry was dropped. All individuals were PCR-positive in at least one sample tested. Considering >10-fold changes as significant, 3 persons showed no significant change of viral load in specimens taken from different time points (patients HIV 2, HIV 3, and HIV 6). However, from 2 of these persons the samples taken 36 h and 48 h post mortem revealed indeterminate results since the internal control was not amplified, probably due to inhibition. Another 3 persons were found to have increased concentrations of HIV RNA (>10-fold) in specimens drawn 24 h post mortem or later (patients HIV 1, HIV 4, and HIV 7). Interestingly, patients HIV 1 and HIV 7 were PCR-negative at entry or 12 h post mortem and became PCR-positive in samples taken 24 h post mortem or later. Finally, in one HIV-infected person (HIV 5) the viral load of blood samples taken after 36 and 48 h was >10-fold lower compared to the 12-hour and 24-hour post mortem samples, but viral RNA was still detectable.

HBV

PCR results of all 5 individuals infected with HBV (confirmed by HBs antigen detection) are shown in table 2. Only 3 patients were positive by HBV PCR, in 2 patients no HBV DNA was detected in any of the samples tested. In one case viral DNA concentration was similar in all samples (patient HBV 4), while in 2 patients HBV DNA levels were significantly higher (>10-fold) in samples taken 24 h post mortem or later (patients HBV 2, HBV 3). In 2 samples from patient HBV 3 taken after 24 h and 36 h, the HBV PCR result was indeterminate because the external control was negative, probably due to PCR inhibition. However, a valid PCR result was obtained in the 48-hour post mortem sample of the same patient without any signs of inhibition.

HCV

PCR results of all 17 individuals infected with HCV (confirmed by HCV serology) are shown in table 3. In 6/17 infected individuals, HCV RNA was not detected in any of the samples tested. The remaining 11 had positive HCV PCR tests with no substantial changes of viral RNA concentrations in specimens taken at different points of time. That means all these patients had HCV RNA concentrations in samples taken 48 h post mortem that were not significantly

Table 3. Monitoring of HCV RNA levels post mortem

Patient	Age/gender	HCV viral load, IU/ml				
		entry ^a	12 h post mortem	24 h post mortem	36 h post mortem	48 h post mortem
HCV 1	50 m	nd	8,000	8,000	10,000	10,000
HCV 2	46 m	negative	nd	negative	negative	nd
HCV 3	32 m	negative	negative	negative	negative	negative
HCV 4	79 f	8,000	nd	10,000	5,000	20,000
HCV 7	29 f	nd	nd	800	3,000	800
HCV 8	50 m	nd	negative	negative	negative	negative
HCV 9	35 f	nd	40,000	nd	80,000	70,000
HCV 10	63 m	nd	40,000,000	20,000,000	30,000,000	20,000,000
HCV 11	59 f	nd	10,000	10,000	10,000	30,000
HCV 12	76 f	nd	100,000	60,000	100,000	20,000
HCV 13	26 m	nd	nd	700	4,000	900
HCV 14	51 m	nd	nd	negative	negative	negative
HCV 15	50 m	nd	nd	40,000	7,000	10,000
HCV 16	81 m	nd	7,000	10,000	3,000	2,000
HCV 17	47 m	30,000,000	nd	30,000,000	10,000,000	50,000,000
HCV 18	51 m	nd	negative	nd	negative	negative
HCV 20	46 m	nd	nd	nd	negative	negative

f = Female; m = male; nd = not done.
^aIn case of nd at entry committal was after 12 h post mortem.

lower than in samples taken at entry, 12 h, or 24 h post mortem. PCR inhibition was not detected in any of the samples tested for HCV.

Discussion

Viral safety is of utmost importance in post-mortal tissue donation. To reduce the risk of transmission of HIV, HBV, and HCV, it has been recommended to test blood specimens from potential donors by NATs for these viruses in addition to serology [1]. NATs provide higher sensitivity to detect acute infections because viral nucleic acids appear earlier in blood than antiviral antibodies. Pre-mortem blood samples suitable for virus diagnostic are rarely available, as they need to be collected less than 7 days before death and stored at 2–8 °C after centrifugation. Thus, testing is usually performed with blood samples obtained post mortem. However, some imponderability has to be considered when testing post-mortal blood. Multiple transfusions pre mortem may cause hemodilution that affect sensitivity, especially that of antibody testing. Furthermore, performance of diagnostic tests may be impaired in hemolytic or icteric samples as well as by bacterial contaminations [17–19]. For this reason blood of deceased persons must be collected no later than 24 h post mortem, as stipulated by EU directive 2006/17 EC and German TPG-GewV. Our preliminary data on NAT analysis in post-mortal blood samples of deceased persons with HBV and HCV infection indicate stability of viral nucleic acids up to 48 h. For all 14 HBV PCR- and HCV PCR-positive cases, the viral load measured in blood

samples collected 48 h post mortem was similar to that of samples taken 24 h post mortem or earlier (in no case >10-fold decrease was observed). In only one patient infected with HIV, the viral load in blood samples collected 36 h and 48 h post mortem was more than 10-fold lower compared to blood samples taken at entry and at 24 h post mortem. These samples, however, were still PCR-positive. Our preliminary data indicate reliable NAT analysis of blood samples taken up to 48 h post mortem when centrifuged immediately after collection. The expanded time slot would significantly improve availability of post-mortal tissues, as there is more time to obtain kinsmen's agreement on tissue donation. However, to extend the testing period for HIV, HBV, and HCV of potential tissue donors accordingly, our results need to be confirmed in future studies using robust NAT assays.

Indeterminate PCR results were obtained in 5 of 107 analyzed samples (4.7%). The rate is lower than in a previous study on blood of cadaveric cornea donors collected within 48 h post mortem, reporting 17.7% of indeterminate HIV or HCV PCR results [15]. The differences may relate to improved RNA extraction and amplification in our study, as in the prior study samples were stored at –80 °C and analyzed by the former Roche Amplicor® PCR test. Different vulnerability of individual NATs to inhibition may be analyzed in comparative studies using spiked post-mortal specimens. In one case of HBV infection, indeterminate results were observed in samples taken 24 h and 36 h post mortem, but not in a sample collected at 48 h, indicating that the effect of PCR inhibition may disappear possibly due to degradation or dilution of inhibitors. In any case, the findings indicate the importance to control for

PCR inhibition in generating true negative results. Accordingly, quality standards of NATs advise inhibition control to be part of every PCR test used for diagnostic purposes [20].

As a surprising finding an increase of viral load during the 48-hour period was found in 5 patients infected with HIV or HBV. In 3 HIV-infected patients the viral load was more than 10-fold higher in samples taken 36 h or 48 h post mortem as compared to samples taken at entry or 12 h post mortem. In one of these cases a positive PCR result was found only in the 48-hour post mortal sample, but not in blood samples collected at entry, 24 h, and 36 h post mortem. Nevertheless, this patient would have been excluded from tissue donation due to positive HIV serology. Similarly, in 2/3 PCR-positive, HBV-infected patients the viral load of the sample collected 48 h

post mortem was more than 10-fold higher than in samples taken at entry or 12 h post mortem. The increase of HBV DNA and HIV RNA blood concentrations may be related to post-mortal liberation or dissemination of virus particles from particular cells or tissues. Certainly, this observation must be verified by investigation of more cases, but if confirmed it would again support reliable results of NATs for HIV, HBV, and HCV in blood samples collected later than 24 h post mortem.

Disclosure Statement

The authors declare no conflict of interest with regard to this paper.

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