Is rapid hepatitis C virus testing from corpses a screening option for index persons who have died after mass-casualty incidents in high-prevalence settings in the field?

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ABSTRACT
Introduction We tested a commercially available rapid hepatitis C virus (HCV) test assay for its potential use for analyses of corpses as a screening option for index persons who have died after mass-casualty incidents in high-prevalence settings in the field.

Materials and methods 50 blood samples were drawn from 16 recently deceased confirmed HCV-positive patients whose corpses were stored at 4°C in the mortuary and were analysed at admission and up to 48 h post mortem by rapid serological testing using the ImmunoFlow HCV test (Core Diagnostics, Birmingham, UK) in comparison with automated serological assays and PCR. Samples from 50 HCV-negative corpses were also analysed.

Results The blood of only four of the 16 HCV-positive corpses reacted clearly with the ImmunoFlow HCV test, while in five cases the result was only weakly reactive and three cases showed very weak reactivity. Four of the infected corpses showed initially negative results, three of which became very weakly reactive 48 h post mortem. 49 out of 50 samples (98%) from HCV-negative corpses tested negative.

Discussion The rapid test system we investigated showed insufficient sensitivity regarding the identification of HCV positivity. Automated serological testing or PCR should be preferred if it is realistically available in the deployed military setting.

INTRODUCTION
Medical assistance in mass-casualty incidents, for example, in resource-limited areas of the world with high hepatitis C virus (HCV) prevalence, may lead to direct, unprotected contact with blood from dying injured individuals. In the case of contact of blood with mucous membranes or injured skin, there is considerable risk of HCV transmission. The early stages of HCV infection are associated with excellent prognosis if treated with interferon-α therapy. Knowledge of the positive HCV status of a corpse from which infectious material has been inoculated will increase awareness of the need for HCV follow-up for timely identification of potential infection by serology, liver enzyme assessment and quantitative PCR to exclude spontaneous viral clearance.

HCV is prevalent in virtually all conflict areas of the world, such as in Afghanistan. In the course of 6 years from 2007 to 2012, at the laboratory of the German field camp in Mazar-e Sharif, Afghanistan, HCV antibodies were detected in 1/110 (<1%) blood samples from Afghan government officials, in 2/23 (10%) samples from Afghan civilians who were employed by the German Armed Forces and in 3/145 (2%) samples of deployed German soldiers (unpublished data).

METHODS
HCV testing using corpse fluids Samples and sample preparation
Stored blood samples from a previous study were used in this analysis. They were derived from recently deceased human corpses of 16 known
HCV-positive and of 50 HCV-negative deceased individuals who were donors of corneal and tissue samples. All samples were acquired at the Institute for Forensic Medicine, University Hospital Eppendorf, Hamburg, Germany. The first samples from the HCV-positive patients were taken on their admission to the mortuary and 12 h post mortem, which was routinely possible if the patients had died at the University Hospital Eppendorf and were sent to the mortuary in time. If HCV patients had died outside the hospital and there was delay in sending them to the mortuary, the 12-h sampling could not be done. Subsequent samples were taken at 24, 36 and 48 h post mortem, respectively. Overall, 50 samples from the 16 HCV-positive corpses were tested that were drawn on admission (n=5), 12 h post mortem (n=7), 16.5 h post mortem (n=1), 18 h post mortem (n=1), 24 h post mortem (n=13), 36 h post mortem (n=11) and 48 h post mortem (n=12). Samples of 50 HCV-negative patients were acquired without delay after their admission to the mortuary. Subsequent sampling was covered by the ethical clearance for this study for infectious corpses only. Accordingly, only one sample could be obtained from HCV-negative corpses.

Before their arrival at the Institute for Forensic Medicine, the corpses were stored at room temperature (20–25°C). After arrival at the mortuary, they were stored under standardised conditions at 4°C prior to acquisition of subsequent samples.

After disinfection of the sampling site, 20 mL whole blood was acquired percutaneously through sterile TSK Supra hollow needles (2.00x100, TSK Laboratory, Tilburg, The Netherlands) from peripheral large vessels, that is, femoral or subclavian artery and/or vein, and, in rare instances, from the heart if sampling from peripheral vessels failed. The material was subsequently transferred into labelled, empty 10 mL test tubes (Harre Co., Hannover, Germany).4 5

The more or less haemolysed samples were centrifuged twice at 1000 g for 10 min each to separate corpuscles and particles of fat, resulting in a material similar to haemolysed serum. Prior to testing the samples were stored at −20°C.

**Rapid HCV testing**

ImmunoFlow HCV tests (Core Diagnostics, Birmingham, UK) with a sensitivity of 100% and a specificity of 99% for serum (manufacturer’s data), which are certified for diagnostic use in Europe, are currently under evaluation for potential use in the deployed operational theatre by the Medical Services of the German Armed Forces and were used for HCV screening with the specimens described above at the Institute for Medical Microbiology, Virology and Hygiene, University Hospital Eppendorf, Hamburg, Germany. In an evaluation study by the US Armed Forces, the test was shown to have a sensitivity of 96.4% and a specificity of 97.9% for plasma. However, there was deterioration of sensitivity to 26.2%–34.5% and of specificity to 89.2%–98.8% if whole blood subject to unfavourable storage and temperature conditions was analysed.6 Data on the reliability of the ImmunoFlow HCV test with samples from corpses are not available. The sample material was used as described for serum by the manufacturer.

Samples were considered reactive if they showed a reaction equal to or stronger than the control reaction. A less visible band than the control was considered weakly reactive, and a hardly visible band was defined as a very weak reactivity. If no reaction could be seen, the samples were considered negative.

**Confirmation testing and estimation of sample quality**

As the diagnostic gold standard, all 50 samples from HCV-positive patients and the 50 HCV-negative samples were tested with the HCV V3.0 kit on the AXSYM system (Abbott, Wieshadden-Delkenheim, Germany). The results are depicted as signal/cut-off (s/co) units with a reference value of <1.00.

In case of positive HCV results by automated serological testing, determination of HCV load was attempted by the quantitative Cobas Taqman PCR (Roche Diagnostics Deutschland GmbH, Mannheim, Germany) with a detection limit of 15 IU/mL directly from the sample materials without prior RNA extraction.

To estimate the degree of protein degradation in the samples of the HCV-positive patients, whole-serum protein values (reference value 64–83 g/L) and albumin (reference value 35–50 g/L) were determined (Dimension Vista, Siemens, Berlin and Munich, Germany). For organisational reasons, these analyses could not be performed for all samples from all study patients. HCV-negative corpses were not included in this analysis.

**Statistics**

Results for groups of samples from HCV-positive corpses with clearly positive bands, weak bands or very weak bands, and without visible bands in the rapid antibody testing-based ImmunoFlow HCV tests were compared with the s/co unit values of the automated AXSYM system. The distribution of the measured results across the four rapid test categories was displayed using boxplots as a graphical variant of an ANOVA analysis.

**RESULTS**

**Rapid HCV testing with the ImmunoFlow HCV test**

Altogether 12 analysed samples from 4/16 deceased patients (25%) known to be HCV-positive and with sampling time points ranging from admission to 48 h post mortem clearly tested positive for HCV antibodies using the ImmunoFlow rapid test. In five corpses (11 samples), the test was only weakly reactive; one of these patients became clearly positive 24 and 48 h post mortem. In three of the HCV-positive corpses (seven samples) the rapid test was very weakly reactive; only one of these samples became weakly reactive 36 h post mortem. The other four HCV-positive corpses (14 samples) tested negative, both initially and at consecutive analyses up to 36 h post mortem (Figure 1). However, at 48 h post mortem three out of four of these samples switched from a negative to a very weakly reactive test result. Of note, for three out of the four negatively tested HCV-positive corpses, the infection had been known for 1.5, 6 and 9 years, respectively, excluding weak antibody reactivity due to early seroconversion. The corresponding data were not available for the fourth negatively tested corpse. Among the samples from the HCV-negative corpses, 49 out of 50 tested negative for HCV antibodies and one sample was weakly reactive.

The control bands of the rapid HCV test appeared in every analysis. Accordingly, false negative results would indeed have been given as negative and not merely as non-interpretable.

**Automated serological testing and PCR-based assessment of viral load**

Automated HCV antibody testing on the AXSYM system was positive for all HCV-positive corpses, and negative for all HCV-negative corpses. For the HCV-positive corpses, the mean test values (s/co) ranged from 3.75 to 125 with a median of 27.5. The quantitative results remained stable within the 48-h analysis period, with most results being scattered within a ±13% range (median) and 2.9% and 43.5% of the respective mean values being the lowest and highest deviations. The mean value (s/co) of the HCV-negative corpses was 0.1 with an SD of
96.3% and a median of 0.08. Of note, the negative AXSYM result of the sample scoring falsely positive by rapid testing was confirmed as negative using the ADVIA Centaur HCV assay (Siemens, Munich, Germany) with a test value (s/co) of 0.1 (<0.8).

Positive PCR results were obtained from 10 out of 16 (62.5%) HCV-positive corpses; all HCV-negative samples remained negative. Switches from positive to negative and vice versa depending on time post mortem were not observed with PCR. Mean values of quantitative PCR were between 1900 and 27 500 000 IU/mL for the positively tested samples; the SDs of the measured results at the different postmortem time points ranged from 34.8% to 91.1% of the respective mean values, with a median of 63.8%.

Comparison of rapid testing and AXSYM-based testing
For the measured s/co units of the AXSYM system, there was no difference between the groups of HCV-positive corpses with negative rapid tests and with very weak bands in the rapid tests with mean s/co values of 20.7 (±11.7) and 12.0 (±11.4) (p>0.13), respectively. In contrast, the groups with weak bands (s/co 45.3±27.9) and clearly positive rapid tests (s/co 79.3±39.5) differed from each other and from all other groups (p<0.05). The cut-off for reliable positive results in rapid testing was an s/co value >40 of the AXSYM system (Figure 2).

Analysis of protein degradation between admission and 48 h post mortem
In 12 out of 15 HCV-positive corpses (80%), the initially observed absolute values were considerably decreased in comparison with reference values from living patients, indicating protein degradation before the corpses were transferred to the mortuary. For whole-serum protein, mean values ranged from 27 to 90 g/L (reference 64–83) with a median of 42 g/L, and for albumin they ranged from 9.2 to 39.5 g/L (reference 35–50) with a median of 16.1 g/L. However, the whole-serum protein as well as albumin remained stable during the storage of the corpses at 4°C in the mortuary in the interval between admission and 48 h post mortem. For whole-serum protein, the SDs of the measured results at the different post-mortem time points ranged from 2.6% to 30.7% of the respective mean values with a median of 8.5%; for albumin, the SD ranged from 0% to 18.0% of the mean values with a median of 6.3%. These data reflect the stable results in antigen/antibody-based (and thus ultimately protein-based) HCV testing on the AXSYM system.

DISCUSSION
In mass-casualty care, such as in humanitarian aid missions, contact with blood and its incorporation via mucous membranes or needle-stick injuries is likely. The risk of HCV acquisition from an infected index person in case of needle-stick injuries is about 3% and may be higher in case of high viral load.7–9 The yearly number of professional HCV infections worldwide is estimated to be 16 000.10 There is considerable risk of HCV transmission in countries with high endemicity. Humanitarian aid missions in conflict areas with a high HCV burden are associated with an increased risk. In asymmetric military conflicts, there is the additional jeopardy of infection due to ballistic...
transmission via bone fragment implantation during suicide bombing.\textsuperscript{3} For example, in Afghanistan, HCV seroprevalence of 36.6\% among injecting drug users\textsuperscript{11} provides an easily accessible source for the self-inoculation of potential suicide assassins with fresh, replicative virus-containing blood.

Post-exposure measures after accidental inoculation of replicative virus are currently not available for HCV infection. However, the course of the disease can be significantly affected by therapeutic measures, particularly if the infection is detected at an early stage. If treatment is started at an early stage of infection, interferon-\(\alpha\) sole therapy can lead to resolution rates up to 90\% or even higher.\textsuperscript{1} In chronic infections, favourable outcomes are still common, but the therapeutic schemes are more complex\textsuperscript{1} and therapeutic success is at least partially dependent on the virus subtype\textsuperscript{12} and the H-2B alleles of the infected individual.\textsuperscript{13}

Early identification of an HCV infection largely depends on the awareness that a potential transmission event might have occurred. The long interval of several months between the transmission event and final seroconversion\textsuperscript{14} makes it difficult to recall every potentially relevant incident.

Compliance with post-exposure screening procedures after needle-stick injuries and related accidents is generally low, as was recently clearly shown for a German University Hospital,\textsuperscript{15} especially regarding the important follow-up screenings. Failure to consider the possibility that a particular index patient might be infected is the most typical reason for this non-compliance.\textsuperscript{15}

It is therefore desirable to have reliable HCV antibody test results from the index patient to increase the awareness of the risk to the injured in case of positive test results. In resource-limited settings, such screenings have to be based upon rapid screening tests. Rapid HCV antibody tests were shown to have varying sensitivities and specificities, both showing a wide range from 70\% to 100\% in various studies,\textsuperscript{6} 16–22 with a further decline in reliability when pre-analytic conditions were poor.\textsuperscript{6} 23

In detail, the results seem to depend on the test system\textsuperscript{6} and the test setting.\textsuperscript{21} Particularly in high-risk settings such as Uganda and India, rapid HCV testing shows unreliable results in comparison with traditional enzyme immunoassays and PCR.\textsuperscript{17} 24 Rapid HCV testing of blood donors in rural Cambodia and Vietnam showed a sensitivity as low as 86.6\%, an unacceptably poor result for blood donor screening.\textsuperscript{25} Both poor pre-analytic conditions\textsuperscript{6} 23 and HIV positivity\textsuperscript{16} 26 can lead to declines in the sensitivity of rapid HCV test systems. Specificity may be poor as well, as demonstrated by a Pakistani study showing 2.35\% falsely positive anti-HCV test results from blood with an immunochromatographic device.\textsuperscript{27}

The best results from rapid tests are usually achieved in standardised evaluation studies, leading to sensitivities and specificities >98\%,\textsuperscript{22} 28 29 even for rapid multiplexed immunoassays (MBio Diagnostics, Boulder, Colorado, USA),\textsuperscript{30} and for various sample materials such as venous blood, finger-stick blood, serum, plasma or oral fluid (OraSure Technologies, Inc., Bethlehem, Pennsylvania, USA).\textsuperscript{31} The OraQuick HCV test (OraSure Technologies, Inc.) in particular, suitable for blood and oral fluid, scored well in various evaluation studies.\textsuperscript{6} 18 31–33 In contrast, the rapid chromatographic Toyo anti-HCV test (Toyo Diagnostics UK, London, UK) showed a poor specificity of 88\% in spite of a sensitivity of 99\% for serum and EDTA blood.\textsuperscript{34} The sensitivity of the BioRapid HCV test (biorapid GmbH, Umrich, Germany) on donor plasma was only 84\%.\textsuperscript{6} The Chembio finger-stick blood, Chembio oral fluid (Chembio Diagnostics, Inc., Medford, New York, USA) and MedMira (MedMira Inc., Halifax, Canada) finger-stick blood tests achieved sensitivities of 87.1\%, 85.4\% and 80.0\% and specificities of 99.0\%, 100\% and 100\% with serum, respectively.\textsuperscript{20} In another study, Chembio and MedMira rapid tests showed sensitivities of 96.2\%–98\% and 86.8\%–88.3\% and specificities >99.5\% with serum, respectively, while OraSure tests showed sensitivities and specificities of 97.8\%–99.3\% and >99.5\%, respectively.\textsuperscript{26} Another study suggested sensitivity of 98\% and specificity of 100\% with serum for the MedMira rapid technique.\textsuperscript{35} Sensitivity and specificity of the SM-HCV rapid tests (SERO-med Laborspezialitäten GmbH, Dollnstein, Germany) with serum were 98\% and 100\%, respectively.\textsuperscript{36} The rapid HCV diagnostic kit by J. Mitra Co. (New Delhi, India) exhibited sensitivity of 87.5\% and specificity of 100\% with serum.\textsuperscript{37} It is evident that the reliability of various HCV rapid test systems is variable.

If the index patient dies before blood samples can be acquired, for example, in the course of a mass-casualty event, corpse blood has to be analysed. Because no data on the reliability of rapid HCV screenings from corpse blood are available, we tested the ImmunoFlow HCV test that is under evaluation by the German Armed Forces Medical Service with blood of HCV-positive and HCV-negative human corpses.

We demonstrated that rapid HCV antibody testing is hampered by poor sensitivity, since only four of the HCV-positive corpses were clearly reactive, and five showed a weakly positive result. Low s/co values in automated testing were significantly associated with failure of rapid testing. The relevance of a very weak reactivity obtained in three of the HCV infected corpses is unknown since it was also found in 3/4 HCV-infected patients who tested negative until the final sample was drawn 48 h post mortem. Thus, these very weak positive results might have been due to unspecific binding of components of the haemolysed blood to the matrix. Rapid sample acquisition might reduce the risk of such non-specific results, but with a negative test result HCV infection of the index person cannot be excluded and the exposed persons should be encouraged to be re-tested to rule out seroconversion. As expected, because of the lack of antigen component in the rapid test we assessed, there was no statistical difference between samples with high or low HCV copy numbers in PCR regarding their rapid test results (data not shown). Accordingly, high infectivity can be associated with negative rapid test results.

It is a major limitation of this study that no subsequent samples could be taken from the HCV-negative samples because there was no ethical clearance for this. The question whether progressive decay might have led to a higher rate of false reactivity remains unanswered. However, even in a mass-casualty setting, sample acquisition from deceased index patients should be possible within several hours, corresponding to the estimated average transport time of the HCV-negative corpses to the mortuary in our study.

It is theoretically possible that HCV rapid test kits that scored better with whole blood samples after unfavourable transport and temperature conditions\textsuperscript{6} might have led to better results with corpse blood as well. However, the limited quantities of sample did not allow for broad evaluation studies, so only the test system that is under evaluation by the German Armed Forces Medical Service was assessed to identify potential diagnostic pitfalls of corpse blood analyses by HCV antibody rapid tests.

Automated serological testing scored best concerning reliable discrimination of HCV-positive and HCV-negative corpses as described earlier,\textsuperscript{4} but it is unlikely to be available in the field or in resource-limited areas. The same is true for testing for HCV RNA by PCR, which has previously been shown to be possible even up to 48 h post mortem.\textsuperscript{3} Of the 16 HCV-positive corpses,
were shown to be viraeic throughout follow-up, while six had negative PCR results due either to low viral replication or to cured infection or inhibitory effects of the haemolysed blood.

The observed stability of protein content in the corposse during 48 h post mortem may be attributed to the storage of the bodies at 4°C. If corpses are kept in a tropical environment, protein degradation might be much faster. Basic sample preparation is advisable. If no centrifugation is possible, at least sedimentation of serum samples should be aimed for.

Finally, the interpretation should consider the time-window of serological HCV screenings and the associated risk of infections from antibody-negative HCV-infected index persons if early infection of the dead index persons seems likely. Regarding seroconversion sensitivity, the ImmunoFlow HCV test was 0.4–5 days less sensitive than 7/9 recently compared automated and classical HCV antibody tests in a recent analysis by a commercial assessment service (kindly provided by mölab GmbH, Langenfeld, Germany). Field-compatible HCV antigen tests might be considered in cases of suspected early infection, but their suitability for diagnostic use with corpse material is still to be demonstrated.

CONCLUSIONS

Rapid HCV antibody testing of samples from corpses under field conditions based on the test system investigated here is associated with poor sensitivity and with specificity that depends on time post mortem. Accordingly, results should be interpreted with care, particularly considering the postmortem time point of sample acquisition and the time-window in cases of an early HCV infection of the index patient. Automated serological testing should be preferred if it is realistically available in the circumstances.

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Contributors

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Competing interests

None.

Ethics approval

Ethical clearance was obtained from the Ethics Committee of the Medical Association of Hamburg, Germany (WF-024/09). The Ethics Committee granted permission in the absence of explicit consent by the deceased. Written consent was obtained from the relatives of the deceased.

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