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# From IEDs to AIDS? Detection of HIV in human corpses by rapid screening tests after suspected intentional transmission in terrorist attacks

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## ABSTRACT

**Objectives** We evaluated the feasibility of intentional transmission of HIV by means of suicide bombing and rape as a terrorist tactic in asymmetric conflicts by evaluating the recognised optimum conditions for biological warfare. We also estimated the suitability of a fourth-generation rapid test for HIV detection in the blood of dead terrorists killed in the completion of their mission.

**Methods** The feasibility of deliberate transmission of HIV for terroristic ends was evaluated on the basis of published experience from passive biological warfare research. In addition, blood from four recently deceased HIV-positive patients and four HIV-negative control corpses, stored at 4°C in a mortuary, was analysed at 12, 24, 36 and 48 h postmortem by rapid serological testing.

**Results** The feasibility of HIV infection for terroristic purposes was established. The fourth-generation HIV rapid test we evaluated identified all HIV-positive samples and was negative for all HIV-negative samples.

**Conclusions** Rapid HIV testing from the remains of dead terrorists in the deployed military environment is possible. Samples should be acquired quickly, basic sample preparation is advisable and consequent decisions concerning postexposure prophylaxis should take into account the diagnostic gap in early infections.

### Educational points

- The feasibility of intentional transmission of HIV by means of suicide bombing and rape as a terrorist tactic in asymmetric conflicts in terms of the recognised optimum conditions for biological warfare was confirmed.
- Rapid HIV testing from remains of dead terrorists in theatre is possible.
- Rapid acquisition of sample material from the corpses of dead index persons should be aimed for.
- Delays in HIV testing should never delay application of postexposure prophylaxis in case of potential transmission events.

## INTRODUCTION

Modern warfare frequently involves asymmetric conflicts such as those recently encountered in Iraq and Afghanistan. Irregular opposing forces tend to launch non-conventional attacks including suicide bombing, use of improvised explosive devices (IEDs) or similar operations with the primary goal of spreading terror.

If the suicide bomber is infected with parenterally communicable viruses such as hepatitis B virus (HBV), hepatitis C virus (HCV) or HIV, the blast that dismembers the assassin can disseminate infective tissue fragments. Even in the case of non-severe injuries to the victims of the blast, transmission of virus may occur through breaking the intact skin barrier or contaminating even intact mucous membranes, such as by implantation of bone fragments from the assassin.

Sexual assault is a war crime according to UN resolution 1820 (S/RES/1820 (2008)); it also provides a feasible way as part of armed conflicts to deliberately spread parenterally and sexually communicable diseases such as HIV infection for terroristic purposes.[1]

Irrespective of the means of transmission, it may not be possible to reliably capture terrorist attackers alive and in the case of suicide bombers, only fragments of the attacker's corpse may be available for diagnostic purposes as a result of the force of the explosion.

A similar diagnostic problem may arise if military medical personnel are inoculated with blood or tissue, for example, as a result of needle-stick injuries or scalpel cuts, from an injured patient who dies before blood samples can be acquired in the deployed medical setting. Such situations are not infrequent in the authors' own experience in Afghanistan.

Broad evaluation studies of commercially available, field-compatible, rapid diagnostic test kits for HIV testing in theatre with material from human corpses are largely lacking. Consequently, at present the distribution of HIV postexposure prophylaxis (PEP) to victims of accidental events or deliberate transmission attempts or accidents is based solely on suspicion, if the index person—that is, the source of infection transmission—dies before samples can be acquired.

We evaluated the feasibility of deliberate transmission of HIV for terroristic ends and describe the evaluation of a rapid HIV test using material from human corpses to estimate its suitability for diagnostic purposes on the remains of terrorist attackers under field conditions.

## METHODS

### Analysis of the feasibility of HIV transmission for terroristic purposes by means of suicide bombing and rape

Optimum conditions for the success of biological warfare have been defined on the basis of experience from passive biological warfare research dealing with counter-measures in cases of attacks

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with biological weapons.[2 3] These conditions include infectious agent-related features—a consistent given effect (death or disease), high infectiousness and contagiosity in low doses, a short and predictable incubation time, difficulty in identifying the agent in the target population, suitability for mass production, storage and weaponisation, stability during dissemination and low persistence after delivery. Aside from agent-related features are features of the target population—little or no acquired immunity and little or no access to immunisation or treatment and also aggressor-related features—the aggressors' ability to protect or treat their own forces and population against the agent. The deliberate transmission of HIV by suicide bombing and conflict-associated rape was assessed for its feasibility in biological warfare in terms of the considerations.

## HIV testing using corpse fluids

### Samples and sample preparation

Stored blood samples were used from a previous study.[4] They were acquired from recently deceased human corpses of four known HIV-positive patients and four HCV-positive, HIV-negative patients that served as negative controls in this study at the Institute for Forensic Medicine, University Hospital Eppendorf, Hamburg, Germany. The first samples were taken 12 h after death, which was regularly possible if patients had died at the University Hospital Eppendorf and were sent to the mortuary in time. If patients had died outside the hospital and there was delay in sending them to the mortuary, so that the 12-h period could not be achieved, the first sample was acquired directly on admission to the mortuary. Subsequent samples were taken at 24, 36, and 48 h postmortem, respectively. For organisational reasons, the 24-h sample of one HIV-positive patient and the 48-h samples of two controls could not be taken and were therefore excluded from the analysis.

Before their arrival at the Institute for Forensic Medicine, the corpses were stored at room temperature (20–25°C), usually for less than 12 h. After arrival at the mortuary, they were stored under standardised conditions at 4°C prior to acquisition of samples.

The sampling procedure comprised disinfection of the sampling site, percutaneous acquisition of 20 ml whole blood through sterile TSK Supra hollow needles (2.00×100, TSK Laboratory, Tilburg, The Netherlands) from peripheral large vessels such as the femoral or subclavian artery and/or vein, and, in rare instances, from the heart if sampling from peripheral vessels failed, and transfer of the blood into labelled, empty 10 ml test tubes (Harre Co., Hannover, Germany).[5] The samples were centrifuged twice at 1000 *g* for 10 min each to separate corpuscles and nuggets of fat, resulting in material similar to haemolysed serum. Prior to testing, the samples were stored at −20°C.

### Rapid HIV testing

Determine® HIV-1/2 Ag/Ab Combo rapid tests (Alere, Stockport, UK) with a sensitivity of 100% and a specificity of at least 99.49% (manufacturer's validation) are currently used on deployed military operations by the Medical Services of the German Armed Forces and were used for HIV screening with the specimens described above at the Institute for Medical Microbiology, Virology and Hygiene, University Hospital Eppendorf, Hamburg, Germany. The fourth-generation tests, screening for both p24 antigen and antibodies against HIV, show better sensitivity for antibody responses than 'antibody-only' based third-generation rapid tests in case of established HIV infections.[6] The sensitivity for early infections without antibody response is poor, however, with no more than 0%–2% antigen detections.[6–8] The manufacturer does not provide data on the test's reliability with postmortem samples. The sample material was used as described by the manufacturer for serum.

### Assessment of HIV viral load

From all analysed HIV-positive corpses, determination of viral load was attempted by quantitative PCR using the Cobas Amplicor HIV-1 Monitor test, V.2.0, without prior RNA extraction directly from the sample materials.[4]

## RESULTS

## Analysis of the feasibility of HIV transmission for terroristic purposes by means of suicide bombing and rape

### Agent-related features

HIV infections lead to chronic disease and, in the long term, to AIDS and death, with the rare exception of persons with coreceptor mutations.[9] While HBV infections can be prevented by vaccination and acute HCV infections have an excellent prognosis after interferon-α therapy,[10] there is currently no cure for HIV infections. Although the incubation time is not short, it is predictable. If the terrorist's goal is long-term spread of terror rather than immediate military success, the onset of a severe, chronic, communicable disease may be more effective from the terrorist's point of view than immediate or rapid death of the victim.

The consequences for those affected are considerable. In addition to lifelong therapy, people infected with HIV often suffer from social stigmatisation,[11] from the effects of the infection on relationships, sexual life and general vitality and from employment-related disadvantages. In most armed forces, HIV infection is an exclusion criterion for military service in order to reduce the risk of infection of comrades in combat. The loss of hard to replace remunerative and often specialised employment in the army can put financial, psychological and emotional stress on even the most established relationships. In addition, each episode of sexual intercourse will be accompanied by the silent fear of infecting the partner. The risk of infection can be reduced with antiretroviral therapy by >90%,[12] with the use of condoms by 80%[13] and with a pre-exposure prophylaxis (PrEP) of the sexual partner by 40%–90%.[14 15] According to the report of the representative for military affairs of the German legislature in 2011, around 80% of marriages of German soldiers end in divorce, a risk that might be further increased by acquisition of HIV while on operations. By depriving affected soldiers of their livelihoods and by devastating their private lives and relationships, a terrorist attack that includes the deliberate transmission of HIV aims directly at the core element of society: the family.

The risk of infection is >90% if considerable amounts of infectious blood are transmitted, as is known from incidents involving contaminated blood products,[16 17] particularly if the viral load is high, as it typically is for the first stages of infection. Accordingly, a terrorist may launch an attack in the early weeks following the self-infection. Although the risk of sexual transmission is considerably lower,[18] genital injury due to forcible sexual intercourse will increase the risk of transmission in the situation of rape. Reliable data to address the directly attributable risks are unsurprisingly lacking, but a relevant risk has to be expected from theoretical considerations.

Due to the non-specific nature of the symptoms of a primary HIV infection[19] compared with the overt blast injuries after suicide attacks, transmission has a good chance of remaining unnoticed despite well-defined criteria for deliberate outbreak events,[20–22] which will facilitate spread among the intimate

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contacts of the affected. Further transmission is not unlikely since soldiers are usually young and in a stage of life with the highest degree of sexual activity. In contrast, screening for sexually transmitted diseases including HIV is likely to be done after sexual assaults, so that further HIV transmission is only likely in situations where a rape is wilfully ignored.

Mass production, storage and weaponisation are not necessary for the terroristic use of HIV. Infected individuals who might serve as a source of the virus are distributed around the world, making the virus easily accessible by the taking of blood samples from them for potential self-inoculation by the prospective attacker.

The mode of transmission by blast-induced fragmentation of the assassin's body may be critical because the high temperatures associated with the blast might inactivate a large proportion of the virus. However, this is not certain as the duration of the heat is short, and studies of this aspect are completely lacking. If the virus load is high enough, individual virus particles may remain in a replicative stage, particularly in less intensely heated body fragments such as bone or cartilage fragments. Rape-associated spread might be more effective in this regard, as the virus is incorporated directly. In contrast, as an enveloped virus, HIV does not remain infective for a long time outside the vital environment.[23]

### Population-related features

The target population is generally susceptible as no vaccination is available and protective mutations in coreceptors are rare.[24] The combined use of tenovovir–emtricitabine has recently been licensed as PrEP, but only validated for sexual transmission.[15 25–27] It remains unclear whether potential PrEP effects might be overpowered by transmission of high virus loads. Lifelong therapy of the infection is possible, but all attempts at a cure have failed so far, with the exception of a transplantation of stem cells with a homozygous δ-32-mutation of the gene coding for the CCR5 receptor,[28] which is not a realistic option due to the severe risks associated with such a procedure.

### Aggressor-related features

The fact that there is no cure for the aggressor is not relevant in the case of a suicide attack because the assassin's death is an intended part of the operation. On the contrary, it may actually be easier for a terrorist commander to persuade potential suicide assassins to fulfil their mission if they are infected with a disease that is associated with a high degree of social stigmatisation and a poor prognosis without expensive medication. In contrast, HIV infection is more problematic for a common rapist because, once infected, there is at present no way back (Table 1).

To summarise, both suicide bombing and rape would be effective for intentional HIV transmission for terroristic purposes, but suicide bombing-associated infections have the best chance of remaining undetected and potentially leading to further spread of the virus.

## Rapid HIV testing with the Determine HIV-1/2 Ag/Ab combo rapid tests

All the analysed samples from known HIV patients with sampling time points ranging from 12 to 48 h postmortem tested antibody-positive and antigen-negative for HIV using the Determine tests. All control samples remained negative for both HIV-specific antibodies and p24 antigen (Table 2).

## Assessment of viral load

Although viral RNA or DNA can generally be reliably detected postmortem, the results of the quantitative PCR in the four deceased patients tested in this study were widely dispersed in

**Table 1** Estimation of the efficacy of attempts to deliberately spread HIV by suicide bombing or rape as biological warfare tactics in asymmetric conflicts

| Optimal conditions for biological warfare | Deliberate HIV transmission by suicide bombing | Deliberate HIV transmission by rape |
|---|---|---|
| **Agent-related features** | | |
| Consistently produced given effect (death or disease) | Fulfilled | Fulfilled |
| High infectiousness and contagiosity in low doses | Not fulfilled, but irrelevant because of the biological foreign body implantation | Not fulfilled, but high risk of transmission due to direct genital inoculation and expected genital bleeding |
| Short and predictable incubation time | Fulfilled (not short, but predictable) | Fulfilled (not short, but predictable) |
| Difficulty in identifying the agent in the target population | Fulfilled (no routine screening is performed) | Not fulfilled |
| Suitability for mass production, storage and weaponisation | Not fulfilled (but also not necessary for terroristic purposes as infected patients may serve as a continuous source and the assassin uses his/her own body as a weapon) | Not fulfilled (but also not necessary for terroristic purposes as infected patients may serve as a continuous source and the assassin uses his/her own body as a weapon) |
| Stability during dissemination | Unclear (no relevant studies) | Fulfilled |
| Low persistence after delivery | Fulfilled | Fulfilled |
| **Target population-related features** | | |
| Little or no acquired immunity | Fulfilled | Fulfilled |
| Little or no access to immunisation or treatment | Partly fulfilled (postexposure prophylaxis may be efficient within a short time frame, otherwise lifelong treatment but no cure is possible) | Partly fulfilled (postexposure prophylaxis may be efficient within a short time frame, otherwise lifelong treatment but no cure is possible) |
| **Aggressor-related features** | | |
| Ability to protect or treat own forces and population against the agent | Not fulfilled (but also not necessary, if the attacker's death is intended) | Not fulfilled (but also not necessary, if the attacker's death is intended) |

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**Table 2** Results from measured specimens of the four analysed HIV-positive patients and four HIV-negative controls at 12 h (at admission), 24, 36 and 48 h postmortem

| Hours postmortem | Determine antigen band | Determine antibody band | RNA copy number in PCR |
|---|---|---|---|
| Patient 1 (HIV +) | | | |
| 12 h | Negative | Positive | 3500 |
| 24 h | Negative | Positive | 4600 |
| 36 h | Negative | Positive | 4300 |
| 48 h | Negative | Positive | 5100 |
| Patient 2 (HIV +) | | | |
| 12 h | Negative | Positive | 50 |
| 24 h | Negative | Positive | 620 |
| 36 h | Negative | Positive | 300 |
| 48 h | Negative | Positive | 360 |
| Patient 3 (HIV +) | | | |
| Admission | Negative | Positive | 160 000 |
| 24 h | Negative | Positive | 200 000 |
| 36 h | Negative | Positive | 6800 |
| 48 h | Negative | Positive | 9800 |
| Patient 4 (HIV +) | | | |
| 12 h | Negative | Positive | 1400 |
| 24 h | – | – | – |
| 36 h | Negative | Positive | Inhibited sample |
| 48 h | Negative | Positive | Inhibited sample |
| Control 1 (HIV –) | | | |
| Admission | Negative | Negative | – |
| 24 h | Negative | Negative | – |
| 36 h | Negative | Negative | – |
| 48 h | – | – | – |
| Control 2 (HIV –) | | | |
| 12 h | Negative | Negative | – |
| 24 h | Negative | Negative | – |
| 36 h | Negative | Negative | – |
| 48 h | Negative | Negative | – |
| Control 3 (HIV –) | | | |
| 12 h | Negative | Negative | – |
| 24 h | Negative | Negative | – |
| 36 h | Negative | Negative | – |
| 48 h | Negative | Negative | – |
| Control 4 (HIV –) | | | |
| 12 h | Negative | Negative | – |
| 24 h | Negative | Negative | – |
| 36 h | Negative | Negative | – |
| 48 h | – | – | – |

Dashes indicate that the analysis was not performed due to organisational reasons.

different directions. The 36- and 48-h samples of one HIV-positive patient could not be used for PCR analysis due to heavy inhibition. In two of the other three cases, the RNA level remained stable (cases 1 and 2), while in case 3 a decrease of more than 90% at 36 h postmortem was observed (Table 2).

## DISCUSSION

Intentional HIV transmission has previously been used as a weapon of war[29] and has been best documented for transmissions by sexual assault, such as in the Democratic Republic of the Congo.[29–31] Transmission of viruses through suicide bombing has been described for HBV in papers from Israeli and British authors[32–34] and biological foreign body implantation (of teeth) from suicide bombers has been witnessed by the German Armed Forces Medical Services (Maj Anja Schlegel, personal communication). Deliberate transmission of HIV by this mode has however not been described so far although it is not unlikely.

Although there is discussion as to whether deliberate transmission of HIV fulfils the legal definition of bioterrorism,[35 36] it comes very close to the optimum conditions for effective biological warfare[2 3] from the point of view of a terrorist with the primary goal of spreading fear.

After an attempt at deliberate transmission, PEP should be administered as quickly as possible,[37] preferably within the first 2–4 h after exposure, because the time between the first contact of HIV with its target cell and its internalisation into the cell is as low as 30 min to a few hours.[38] The severe consequences of an HIV infection in comparison with the manageable side effects of antiretroviral drugs should encourage a generous administration, with the option of stopping the treatment if the index individual is tested HIV-negative.

In the field setting, such HIV testing has to be based on simple, undemanding procedures, such as the Determine HIV-1/2 Ag/Ab Combo rapid test that is in use by the German Armed Forces. Our data suggest that the Determine HIV-1/2 Ag/Ab Combo test might be suitable for rapid HIV testing on the blood of a dead index person in theatre. The observed band pattern with positive antibody bands but negative p24 antigen bands is not surprising for the chronically infected patients analysed because the antigen is disguised in immune complexes unless these are dissolved by prior acid treatment. Correct results were demonstrated for analyses up to 48 h after death.

In contrast, quantitative PCR was shown to be prone to sample inhibition, presumably because of progressive haemolysis. However, if sample inhibition is excluded by inhibition control PCRs, transmissibility from both living index persons and corpses is unlikely where the HIV viral load is undetectable, that is, where someone has been stable on antiretroviral treatment.

Undeniably, interpretation of the diagnostic study results is limited by several factors. One major limitation is the low number of samples, which is difficult to avoid because of the scarcity of the required samples; a multicentre approach might lead to more reliable data. In addition, standardised sample preparation, including centrifugation, will not be possible under field conditions. Furthermore, the corpses used were stored at 4°C after arrival in the mortuary; such optimal preservation, which was confirmed by low protein degradation over the assessed 48 h (data not shown), is very different from field conditions. Studies in which human corpses are allowed to decompose at room temperature for several days to the necessary samples to be acquired are unlikely to be done for ethical reasons. All of these factors will contribute to considerably worse sample quality in theatre, potentially leading to worse results.

Consequently, rapid acquisition of sample material from the corpses of dead index persons should be aimed for, as the sample quality will worsen with increasing time and sample acquisition will become increasingly difficult if the—potentially dismembered—corpse exsanguinates. As the rescue of the injured will have priority in theatre, there may be considerable delay until there is time to attend to sample acquisition from dead terrorists. Although centrifugation of blood from corpses in theatre using a hand-operated centrifuge is theoretically possible, such a procedure is highly unlikely in combat. If no centrifuge is available, sedimentation as a minimum should be the aim in order to prevent the introduction of debris material into the test system. Aspiration of a fat film on top of the sample should also be avoided.

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## CONCLUSIONS

Determine HIV-1/2 Ag/Ab Combo rapid tests can in principle be used for rapid HIV screening from corpses under field conditions. The risk of a false-negative test result may be diminished by using freshly acquired samples. However, a negative test from corpse materials never completely excludes HIV infection, particularly in the very early stages of infection in which the sensitivity of the test is low.

In addition, the Determine HIV-1/2 Ag/Ab Combo rapid tests can be used with samples from dead enemy fighters and with recently deceased patients if needle-stick injuries or similar biological contamination of medical personnel occurs during attempts to save the patients' lives as theoretically anyone may be infected with HIV, irrespective of age, sex or social status. HIV testing should be done in accordance with the respective national laws of the investigator's country; discussion of legal aspects would be beyond the scope of this work.

Delays in HIV testing should never delay application of PEP in case of potential transmission events but should only inform decisions about stopping it. A decision on the potential cessation of antiretroviral PEP must always be made by the responsible physician with regard to all aspects that might affect the likelihood of deliberate or accidental transmission.

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