REVIEW

Minimum infective dose of HIV for parenteral dosimetry

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Summary: The probability of HIV-1 transmission in a small blood exposure such as a needlestick injury or an unsafe medical injection has been estimated indirectly. Now that several comparable laboratory simulations have provided data on inoculum volumes for such exposures, the epidemiological evidence supporting these estimates can be validated and qualified using dosimetry. This review of data on infective titre, viral load and injection inoculum volume compares three approaches to HIV dosimetry. Agreement across the three approaches indicates that unsafe medical injections are several times more likely to transmit HIV-1 than needlestick accidents, and that the risk remains substantial if injection equipment is wiped, rinsed or flushed prior to re-use. The 50% infective dose of HIV in blood exposures ranges from one virion (two copies RNA) in primary infection with CCR5 co-receptor using strains of HIV-1 to 65,000 copies HIV-1 RNA in blood from an asymptomatic source patient. The median transmission risks for unsafe intravenous or intramuscular injections using equipment cleaned but not sterilized after use on a symptomatic pre-AIDS patient are 1.8% (95% confidence interval [CI] 0.1–3.2%) and 0.8% (95% CI 0.1–1.4%), respectively.

Keywords: HIV, nosocomial, iatrogenic, parenteral, Africa

INTRODUCTION

HIV-contaminated blood has been recovered from syringes used for medical injections in Cameroon and in the USA.[1,2] This evidence that unsafe medical injections contribute to the spread of HIV has been contested on the grounds that only a small fraction of contaminated syringes have the potential to transmit HIV.[3] In 14 of 32 HIV-positive syringe flushes from the syringe assay in Cameroon, for example, only one copy of HIV RNA was detected.

Estimates of the transmission efficiency of HIV in injections, from records on health worker occupational HIV exposures, studies of injection drug users and retrospective analysis of nosocomial outbreaks, range from 0.3% to 7%.[4,5] Estimates of the relative importance of these small blood exposures to the AIDS pandemic in Africa accordingly vary across orders of magnitude.[6,7] These estimates are undermined by (1) variability in the circumstances in occupational needlestick injuries, (2) investigators' rejection of self-reported needle-sharing rates among injection drug users (presuming substantial under-reporting because they expected more inefficient HIV transmission per injection than was observed) and (3) the inclusion of transfusions among the invasive procedures not specifically identified in the nosocomial outbreak investigations. Nosocomial HIV transmission to at least 628 Romanian children who received no transfusions, discovered in 1991–1992, is thought to be somehow contingent on exceptional circumstances.[8] The World Health Organization (WHO) has modelled the probability of transmitting HIV in an unsafe medical injection as 1.2%, a compromise averaging two competing estimates based on needlestick injury data.[6] The present review applies an alternative approach to estimating the HIV transmission risk in an unsafe medical injection, validating and qualifying the needlestick injury analogy with dosimetry.

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METHODS

This review describes injection risks by assigning likely inoculum volumes to specific types of needlestick injuries for which transmission probabilities can be derived from a case-control study of transmission outcomes in health workers. The corresponding transmission probability is estimated again using the observed tissue culture infective titre ($TCID_{50}$/mL) in patients with HIV. A third estimate is obtained by calculating the transmission probability associated with a given inoculum volume from the viral load in patients at specific clinical stages of disease.

Although for transfusion risk assessments, an infective dose of HIV-1 is estimated to be 10 virions (or approximately 20 copies HIV RNA), this is assumed to be an overly conservative estimate in comparison with the 50% tissue culture infective dose ($TCID_{50}$).[9] The $TCID_{50}$ is a relative measure of infectivity. The 50% infective dose in animal models is given in units of $TCID_{50}$ and not RNA copies/mL because the ratio of viable HIV-1 particles to defective virus particles *in vivo* is estimated to range from 1:1 to 1:100, and some estimate 90–99% of HIV RNA is defective.[10,11]

A systematic search for studies reporting both the infective titre of HIV in the blood of patients at various clinical stages of infection and viral load relied on the reference lists of the papers identified first for additional sources. Papers reporting the infective titre for cell-free isolates of HIV-1 are excluded

as unrepresentative, because infection with HIV is cell-mediated *in vivo*.[12] Sample sizes under five are also excluded. In the systematic search for studies reporting inoculum volumes in needlestick injury simulations, our literature search again relied almost entirely on the reference list of the first paper identified,[13] and the reference lists of each paper thus identified, as well as all sources listed in Google Scholar as citing one of these papers.

Laboratory data on the survival of HIV in syringes and on exposed surfaces are reviewed to translate the results into practical estimates of the risk of HIV transmission in unsafe intramuscular or intravenous medical injections. The Centers for Disease Control and Prevention has pointed out that these are high-titre experiments.[14] However, the observed time to elimination of half of the virus present in the initial sample (more than 24 hours in a rinsing pan) is largely independent of the concentration of the standard.[15]

RESULTS

Infective titre

Translating the $TCID_{50}$ into a measure of host infectivity involves comparison with animal models that differ in susceptibility to HIV. For the chimpanzee, the ID_{50} ranges from 4 to 300 $TCID_{50}$ for various isolates of HIV; however, chimpanzees are relatively resistant to HIV.[16–20] The chimpanzee's 50% infective dose is much lower for chimpanzee-adapted virus (titrated from chimpanzee to chimpanzee), ranging from 2 to 5 $TCID_{50}$.[21] In pig-tailed macaques, the 50% infective dose of SHIV-IIIB ranges from 0.1 to 1.0 $TCID_{50}$ in an intravenous challenge.[22] These data suggest that for host-adapted HIV, a 50% infective dose falls within the range of 0.1–5 $TCID_{50}$. Centring this estimate on 1.0 $TCID_{50}$ reflects the assumption that HIV-1 is presently better adapted to human hosts than a strain of HIV-1 titrated through several chimpanzees is adapted to chimpanzees.

From data presented in Table 1, the probability of seroconversion for small volume blood exposures can be modelled linearly with a y intercept at zero in one of the two ways: using the patient's viral load or the infective titre in $TCID_{50}$/mL. In African AIDS patients median viral load is 500,000 copies/mL.[23] The viral load typical of African patients with symptomatic HIV-1 infection is 300,000 copies/mL.[24] The viral load in the asymptomatic stage in African patients is approximately 10,000 copies/mL.[25] In acute infection, viral load is typically two log over the chronic stage or 1,000,000 copies/mL.[26]

Table 1 **$TCID_{50}$ per mL and viral load in treatment-naïve HIV-1 patient plasma**

Clinical stage of HIV-1 infection	No.	$TCID_{50}$ per mL (range)	Copies RNA per $TCID_{50}$ (range)
Acute patient plasma (CCR5 strains only)[27]	15	59,150 (1600–170,000)	2 (1–4)
Acute patient plasma (non-CCR5/dual strains)[28]	17	7413 (316–1,258,925)	Not reported
Asymptomatic patient plasma[29]	29	27 (2–275)	6239 (477–20,007)
Symptomatic patient plasma[29]	8	115 (7–417)	7463 (933–22,404)
AIDS patient plasma[29]	11	67 (9–398)	17,493 (584–117,803)

CCR5 – exclusive CCR5 co-receptor using strains, CXCR4 – CCR5 and CXCR4 co-receptor using strains

The great infectivity of sera from patients with acute infection is important, as patients with acute HIV infection are likely to receive injections for fever, particularly where a laboratory test to exclude malaria cannot be performed. The elevated infectivity of sera from symptomatic patients who have not progressed to AIDS likely reflects CCR5 co-receptor usage in all strains in this sample of patients. The exclusive usage of CCR5 co-receptors is associated with enhanced HIV transmission efficiency and reduced pathogenicity, because macrophages and dendritic cells are almost exclusively permissive to CCR5 co-receptor using variants, whereas CXCR4 co-receptor using variants that evolve later in infection are transferred more efficiently from dendritic cells to autologous CD4+ T-cells and are associated with more rapid progression to AIDS.[30] Elevated reverse transcriptase activity and greatly elevated integrated HIV-1 DNA titre have been observed in the culture of HIV-1 strains using CCR5 receptors, in the presence of relatively few viral particles.[31] Thus in early stages of infection, the infective titre is probably a more reliable indicator of transmission risk than patient viral load.

Needlestick injuries

A health worker's risk of acquiring HIV in a needlestick injury involving an HIV-positive patient is known to be slight at $<0.4\%$.[4] The risk of transmission in an unsafe medical injection is often assumed to be similar. However, most cases of HIV transmission to a health worker involve procedures such as phlebotomy that are performed with a large gauge needle, visibly contaminated with blood, whereas most needlestick injuries are superficial scratches in which the hole of the needle fails to penetrate the skin. Only one case-control study differentiates among outcomes of needlestick injuries with or without such risk factors.[32]

In this case-control study (27 cases, 488 controls), the risk of transmission associated with a venous procedure is 1.3%, for a deep injury 2.3%, and for an injury with an 18-Gauge needle 3.8%, compared with a 0.3% transmission risk in needlestick injuries without these risk factors.[5] From these data and the inoculum volumes from needlestick simulations, a probability of infection per μL inoculum volume can be derived, with a good fit ($P = 0.01$, $r^2 = 0.91$) given in Equation 1:

$$P = 1.3 \times \text{volume}(\mu\text{L}) + 0.27 \quad (1)$$

Notably most source patients in those needlestick accidents leading to seroconversion had progressed to AIDS. This risk factor increases the probability of HIV transmission by a factor of 1.9. Symptomatic HIV infection that has not progressed to AIDS is the referent. No information is available in the case-control study for needlestick accidents involving patients with acute HIV infection.

Needlestick simulations

Ten needlestick injury simulations using diverse syringe types and sizes were identified by search, and nine comparable to injection risks are summarized in Table 2. Important differences in methodology include the media into which the inoculum is delivered (fluid media may overestimate risk due to capillary action), whether the syringe was flushed with blood and

Table 2 **Needlestick injury and injection simulation inoculum volumes (μL)**

Media	Barrier	Depth	Inoculum	Diameter/gauge	No.	Mean	Range or 95% CI	Ref.
Wet cotton	Plastic film	–	Insertion	0.80 mm	8	0.21	0.01–0.75	34
				0.63 mm	8	0.06	0.01–0.17	
		<5 mm	Injected	0.45 mm	2	5.99	4.53–5.44	
None	NA	–	Residual in syringe and needle	20 Gauge	10	183	85–281	35
				22 Gauge	10	138	85–190	
				23 Gauge	10	100	37–163	
				26 Gauge	10	34	26–42	
				27 Gauge	10	7.8	5.8–9.8	
Agarose gel	None	2 mm	Insertion	Suture	–	0.133	–	13
		5 mm		Suture	–	0.683	–	
Paper prefilters (22 mm)	Glove	5 mm	Insertion	18 Gauge	–	3.4	2.8–4.0	36
				20 Gauge	–	2.1	1.9–2.3	
				25 Gauge	–	0.6	0.4–0.8	
				0.27 Suture	–	2.0	1.7–2.3	
				0.23 Suture	–	1.2	1.1–1.3	
		10 mm	Insertion	18 Gauge	–	4.8	4.0–5.6	
				20 Gauge	–	2.7	2.4–3.0	
				25 Gauge	–	0.8	0.7–0.9	
				0.27 Suture	–	3.1	2.4–3.7	
				0.23 Suture	–	1.6	1.5–1.7	
		20 mm	Insertion	18 Gauge	–	7.6	6.2–9.0	
				20 Gauge	–	4.1	3.1–5.1	
Buffer	Parafilm	<16 mm	Insertion	0.5 mm	20	0.034	0.004–0.26	37
–	None	–	Expelled 2x	0.5 mm	20	34	18–67	
None	Latex	2.4 mm	Insertion	–	–	0.064	–	34
Gower's solution	Parafilm	–	Insertion	22 Gauge	20	1.40	0.00–6.13	38
	None	–	Expelled 1x	22 Gauge	20	1.29	0.01–4.24	
Jellified medium	None	3 mm	Insertion	22 Gauge	15	0.07	0.03–0.10	39
		15 mm		22 Gauge	15	0.24	0.16–0.32	
		6 mm		16 Gauge	15	0.47	0.13–0.61	
		6 mm		25 Gauge	15	0.05	0.04–0.06	
	Glove	6 mm		22 Gauge	15	0.12	0.06–0.18	
Paper prefilters	None	5 mm	Insertion	18 Gauge	–	2.0	–	40
				20 Gauge	–	1.0	–	
				22 Gauge	–	0.5	–	
				25 Gauge	–	0.5	–	
				0.18 Suture	–	0.5	–	
				0.27 Suture	–	1.0	–	
		10 mm	Insertion	18 Gauge	–	3.0	–	
				20 Gauge	–	1.5	–	
				22 Gauge	–	1.0	–	
				25 Gauge	–	0.6	–	
				0.18 Suture	–	0.6	–	
				0.27 Suture	–	2.0	–	
		20 mm	Insertion	18 Gauge	–	5.5	–	
				20 Gauge	–	2.5	–	
				22 Gauge	–	2.0	–	
				25 Gauge	–	0.5	–	
Pig's foot	Glove	–	Insertion	18 Gauge	–	0.5	–	
				0.27 Suture	–	<0.01	–	

CI = confidence interval

whether all contamination retained in the syringe and needle was recovered or passive transfer was measured. The excluded study of surgical risk applied blood to a glove surface and then introduced contamination to underlying fresh pig skin using a needle.[33]

For venous needlestick injuries, a 22-gauge phlebotomy needle is referent, while for non-venous (i.e. intramuscular) injections a 25-gauge syringe is typically used. The average inoculum volume in a needlestick simulation using an 18 gauge syringe is 2.87 μL (95% confidence interval [CI] 1.01–4.72), for a 22-gauge syringe it is 0.66 μL (95% CI 0.03–1.28) and for a 25-gauge syringe it is 0.3 μL (95% CI 0.05–0.55).

The median inoculum in an injection simulation is 5.99 μL for a 22-gauge syringe (0.5 mm diameter), inserted less than 5 mm. This is a factor of 9.1 greater than the inoculum volume when a 22-gauge syringe is inserted but the plunger of the syringe is not depressed. A depth of 1 cm is assumed for both unsafe injections and deep needlestick injuries, and this increases the inoculum volume by a factor of 1.4 over the referent, insertions of only 5 mm.

For intravenous injections, an inoculum volume of $0.7 \times 9.1 \times 1.4\ \mu L = 8.9\ \mu L$ is obtained. For comparison the inoculum volume in a deep needlestick injury with a 22-gauge phlebotomy needle is $0.7 \times 1.4\ \mu L = 1.0\ \mu L$. Much greater volumes (average 32 μL) have been recovered from syringes used by injection drug users that were not visibly contaminated with blood.[41] The practice of flushing the syringe with blood one or two times to recover any residual drug probably accounts for this difference, and would not occur in a medical injection.

Intramuscular injections involve far less blood than intravenous needlestick injuries. Applying the same multiplicative risk for deep insertion and for injection, the inoculum volume in an intramuscular injection would be $0.3 \times 9.1 \times 1.4\ \mu L = 3.8\ \mu L$. Much smaller inoculum volumes have been recovered from syringes used to perform non-intravenous medical injections (the most common are intramuscular injections). A syringe assay performed on autoclaved injection equipment in Tanzania found only 1.5–90 nL blood on 34.1% of syringes used in laboratories and sexually transmitted disease clinics and on 7.2% of those used in wards and outpatient departments.[42] In a US syringe assay, no more than 4.6 nL blood was recovered from any syringe used for non-intravenous medical injections on HIV-positive patients.[2] Unfortunately, neither assay measured the total volume of infectious contamination retained in these syringes. This risk factor is indicated in the latter study, which attributed the detection of HIV RNA in some syringe flushes to the presence of interstitial fluid.

Effects of cleaning

HIV does not replicate outside the body and drying rapidly reduces infective virus by one $\log_{10}$ ($TCID_{50}$/mL). Only half of viable HIV in blood is lost over 24 hours in wet conditions, however.[43,15] The use of rinsing pans to prepare equipment for reuse has been observed even in South Africa, where such pans are also used for washing hands while soaking medical instruments.[44] HIV is stable for temperatures up to 44°C, declining by a factor of 10 between 48 and 52°C. The use of boiled water allowed to cool, as opposed to boiling water, in rinsing pans would not necessarily inactivate virus.

In an assay of syringes used to simulate injection drug use, after one day of storage the detectable HIV was reduced by 75–90%.[45] In a similar experiment, small volumes of blood (2 μL) retained amplifiable virus for only one day.[46] Considering that 0.5% of virus remains at five days, loss of infectivity is evidently log-linear, and curve fitting suggests that only 25% of virus remains infective after two hours, but 20% still remains after five hours.

The impact of wiping a needle before reuse is mechanically equivalent to passing a needle through one glove layer, a protective measure evaluated in several needlestick simulations. In the closely comparable experiments using paper prefilters to collect the inoculum (correspondence across syringe sizes and penetration depths $P < 0.0000001$), gloving did not reduce inoculum volume at all (relative risk [RR] 0.68 for no glove).[36,40] In other comparable simulations, gloving is protective (volume reduced by 50–85%).[36,39,40]

Expelling the contents of the syringe once or twice is equivalent to rinsing needle and syringe without disassembling first, and removes only 1–25% of residual contamination.[35,37,38] However, in an injection experiment in which a 1 mL syringe was flushed with blood and then rinsed once before reuse, the inoculum volume was reduced by 74%.[34] A similar result was obtained by flushing syringes twice with bleach and clean water after drawing in only a minimal visible amount of blood, to simulate the practice of registering to confirm needle placement in a vein.[47] Flushing a syringe once with water has been shown to eliminate virus that could replicate in culture in 70% of syringes in another assay, while flushing twice removed replicative virus in 95% of syringes, with similar results when rinsing syringes with 1:10 diluted bleach.[48]

Estimates

Table 3 presents the estimated transmission probabilities corresponding to specific inoculum volumes that represent two types of medical injections, for four categories of source patients. A 90% loss of infective virus is assumed regardless of which cleaning method is used. In the absence of any cleaning method, much more frequent nosocomial transmission seems likely, as has been observed in Romania.[49]

These findings validate the use of case-control data on risk factors for HIV infection in needlestick injuries to approximate the risk to patients from unsafe medical injections, and show that the stage of infection in the source patient is critically important.

DISCUSSION

Needlestick injury simulations with diverse methods have found similar inoculum volumes under similar circumstances. Few experiments have simulated injections, and in these simulations inoculum volumes range from 1.3 to 34 μL. The estimated transmission probabilities supported by dosimetry using these simulation data are not more precise than earlier estimates, but they consistently indicate that unsafe intravenous injections are far more likely to transmit HIV than needlestick injuries, even if injection equipment is cleaned before re-use.

For these estimates to describe the risk to injection drug users who share needles, earlier investigators' assumption that injection drug users under-report how frequently they share needles must be rejected.[50,51] A more recent experiment with anonymous survey methods revealed no tendency for drug users to under-report needle sharing, although self-reports of other HIV risk behaviours such as unprotected sex are influenced by social desirability bias.[52] Another factor that may bias estimates of HIV transmission efficiency in injections from epidemiological data on injection drug users is informed selective

Table 3 **Transmission probabilities for intravenous and intramuscular injections**

Stage	Injection type	Inoculum (μL)	Case control (%)	Infective titre (%)	Viral load (%)
Acute	Intravenous	8.9	–	100	100
Acute	Intramuscular	3.8	–	100	100
Asymptomatic	Intravenous	8.9	–	1.2	0.1
Asymptomatic	Intramuscular	3.8	–	0.5	0.0
Symptomatic	Intravenous	8.9	1.2	5.1	1.8
Symptomatic	Intramuscular	3.8	0.5	2.2	0.8
AIDS	Intravenous	8.9	2.3	3.0	1.3
AIDS	Intramuscular	3.8	1.0	1.3	0.5

needle sharing, as opposed to random mixing. Selective sharing has been reported by injection drug users, although the dyads in which most needle sharing occurs can be partnerships of convenience or brief friendships and may not reflect a strategy to avoid HIV exposure.[53,54]

The source patient's clinical stage of HIV disease largely determines the risk from the smallest of blood exposures. Time from seroconversion is usually not determined for HIV patients in developing countries. For this reason, the stage of disease that predominates in African hospital settings remains an unknown. Asymptomatic patients may be largely crowded out of in-patient settings where the AIDS burden on hospitals is severe, while terminally ill patients may elect for home care, but there is no evidence to show this.

The evolution of HIV-1 away from exclusive CCR5 co-receptor usage towards more promiscuous co-receptor usage complicates transmission dynamics. CCR5 co-receptor usage does not differ between B and non-B HIV-1 clades or among ethnic groups.[55,56] However, environmentally triggered cytokine production favouring the expression of CCR5 co-receptors on CD4+ T-cell surfaces is suggested in African populations.[57] Thus, our use of data on serial dilutions using culture media not enhanced in CCR5 expression may underestimate the probability of HIV transmission to African patients in small blood exposures.

All HIV transmission involves CCR5 co-receptors, but in approximately half of patients HIV strains evolve to also use CXCR4 or other co-receptors, at which point viral load increases but infective titre equivalent per virion decreases.[56,58–60] The duration of the increased risk posed by infection with strains using CCR5 co-receptors may extend until immune activation increases the expression of CXCR4 co-receptors on T-cell surfaces.[61] But within this timeframe the degree of increased risk varies widely. In Western sera from patients with multi-drug resistant HIV-1, a highly elevated infective titre (100,000 $TCID_{50}$/mL) has been observed 47 weeks beyond initial presentation, and up to 4.8 years later.[62] In contrast, the low infective titre average reported for asymptomatic patient sera in Table 1 is from a sample of patients that includes eight individuals with exclusive CCR5 co-receptor using strains, and the difference in infective titre between these and other strains in the sample is non-significant.[29] Multi-drug resistance is not independently associated with elevated infective titre.[62]

Assuming a symptomatic pre-AIDS source patient, the median transmission risks for unsafe intravenous and intramuscular injections using equipment cleaned but not sterilized between use and reuse are 1.8% (95% CI 0.1–3.2%) and 0.8% (95% CI 0.1–1.4%), respectively. The estimates from patient infective titre may be more accurate and are significantly greater than the transmission risk assumed in the WHO's 2004 model of the global burden of disease from unsafe medical injections.[6] The latter estimates suggest that 22% or more of incident HIV infections in sub-Saharan Africa in 2007 resulted from unsafe medical injections, although overlap with heterosexual transmission can be assumed.[7]

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