Modeling of patient virus titers suggests that availability of a hepatitis C vaccine could reduce HCV transmission among injecting drug users

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Abstract

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Supplementary Materials

Figure S1. Illustration of HCV transmission via a shared syringe.

Figure S2. Sensitivity analysis of Eq. 2 (Hill function) as a function of viral load.

Figure S3. P_{trans} generated by Eq. 1 and Eq. 2.

Figure S4. Probability of HCV transmission via a rinsed low dead space syringe during reinfection using Eq. 2.

Figure S5. Cumulative probability of transmission via shared needles over 6 months for the reinfected low titer group based on frequency of sharing using Eq. 2.

Table S1. Critical probabilities of HCV transmission and corresponding viral $\log_{10}$ IU/mL

Table S2. Estimated q distribution calculated from chimpanzee reverse-titration experiments.

Data file S1. Titers obtained for *in vitro* syringe studies

The major route of hepatitis C virus (HCV) transmission in the United States is injection drug use. We hypothesized that if a HCV vaccine was available, vaccination could impact HCV transmission among people who inject drugs by reducing HCV titers after viral exposure without necessarily achieving sterilizing immunity. To investigate this possibility, we developed a mathematical model to determine transmission probabilities relative to the HCV RNA titers of needle/syringe sharing donors. We simulated sharing of two types of syringes fitted with needles that retain either large or small amounts of fluid post-expulsion. Using previously published viral kinetics data from both naïve subjects infected with HCV and reinfected individuals who had previously cleared an HCV infection, we estimated transmission risk between pairs of serodiscordant injecting drug users, taking into account syringe type, rinsing, and sharing frequency. We calculated that the risk of HCV transmission through syringe sharing increased ~10-fold as viral titers ($\log_{10}$ IU/mL) increased ~25-fold. Cumulative analyses showed that, assuming sharing episodes every 7 days, the mean transmission risk over the first 6 months was >90% between two people sharing syringes when one had an HCV RNA titer >5 $\log_{10}$ IU/mL, regardless of infection outcome. For those with pre-existing immunity that rapidly controlled HCV, the cumulative risk decreased to 1–25% depending on HCV titer and syringe type. Our modeling approach demonstrates that even with transient viral replication following exposure during injection drug use, HCV transmission among people sharing syringes could be reduced through vaccination if a HCV vaccine were available.

One Sentence Summary:

A future HCV vaccine could reduce hepatitis C virus transmission among those sharing syringes, even in the presence of post-exposure viral replication in vaccinees.

Introduction

Injection drug use is the leading cause of hepatitis C virus (HCV) transmission in the United States (1) with an estimated 60% of all HCV infections attributed to sharing needles, syringes or other drug paraphernalia (2). As a consequence, people who inject drugs are an important target population for testing the efficacy of HCV vaccines under development and have been recruited to take part in a Phase I/II prophylactic HCV vaccine study (ClinicalTrials.gov Identifier: NCT01436357) designed to induce T cell responses to the HCV non-structural proteins. To date, the only efficacy data for prophylactic HCV vaccines have been obtained from chimpanzees infected with HCV [(reviewed in (3)]. These studies have shown that prophylactic vaccines have successfully induced HCV-specific immune responses and led to reductions in viral replication soon after challenge, indicating that the vaccines effectively primed antibody or T cell responses that could attenuate viral replication (4). However, none of these animal studies achieved sterilizing immunity by completely preventing infection upon viral challenge. Consistent with these findings, data on the spontaneous clearance of natural HCV infections show that pre-existing immune responses do not completely prevent infection and viral replication upon re-exposure in humans or chimpanzees (4, 5). A major problem following HCV infection is viral persistence, which can lead to chronic liver disease. Therefore, prevention of chronic liver disease rather than sterilizing immunity has come to be seen as an acceptable goal for HCV vaccines currently under development. As such, it has been proposed that vaccines would not need to achieve

sterilizing immunity in injecting drug users in order to reduce disease spread. Transmission of low amounts of HCV (10 to 100 RNA copies) in naïve chimpanzees has been shown to result in initiation of HCV infection i.e., HCV RNA amplification in blood (6–9). In contrast, blood samples containing trace amounts of RNA (<10 RNA copies) have been shown to be non-infectious (6, 7, 10); this lack of infectivity may be dependent upon the RNA to infectious virus ratio in any given sample. Reverse titration studies in chimpanzees have shown that not all HCV RNA molecules are infectious and that more than one RNA copy is needed to initiate infection (11).

It is plausible to assume that the viral titer of transmitted blood is related to the probability of transmitting infection, in that small amounts of low-titer blood are less likely to contain infectious virus. We hypothesized that the HCV titer of an infected individual would directly impact the probability of transmitting infectious particles in the small volumes of blood contained within contaminated needles and syringes. Therefore, if titers are sufficiently reduced, vaccines would not need to achieve sterilizing immunity in people who inject drugs in order to reduce disease transmission. However, how low these viral titers would need to be to substantially reduce the chance of transmission from shared syringes with attached needles is unknown.

HCV transmission risk is challenging to study due to difficulties in culturing natural isolates and the limited availability of animal models to study viral replication (12). Nonetheless, several informative studies on HCV survival in injection equipment or transmission through syringe sharing have been completed (13–16). Simulations of injection practices and syringe contamination using HCV cell cultures have shown that although titers decline within the first few days (14, 16), the virus can maintain infectivity for prolonged periods of up to 6 weeks (13, 16) and therefore could potentially be transmitted to an uninfected individual despite extended periods between syringe sharing events. Additionally, the volume of residual blood in shared needles/syringes has been shown to directly impact the amount of virus that could be transferred (15). The amount of residual donor blood could be affected by the type of syringe used. High dead space syringes retain more fluid that cannot be expelled from the syringe (i.e. fluid remaining within the needle and between the syringe hub and the plunger following expulsion of liquid) than do low dead space syringes.

We anticipated that several factors could affect the probability of HCV transmission via syringe sharing (illustrated in fig. S1) including: (1) the proportion of blood transmitted from used syringes relative to syringe type (low or high dead space), (2) syringe rinsing, (3) the amount of HCV RNA in the blood of donors, (4) the relative volumes of blood and drug in the needle and syringe, and (5) the fraction of infectious HCV RNA in blood. How these factors could combine to affect HCV transmission remains unclear. To address this, we experimentally estimated HCV carry-over in two different combinations of syringes with needles, a low dead space syringe with a permanently attached small bore needle and a high dead space syringe with a detachable high bore needle. We developed an RNA titer-based mathematical analysis of HCV transmission between people sharing these two types of needle and syringe combinations. We applied this model to previously published viral kinetics data from HCV-infected subjects comprising naïve individuals (17, 18) and reinfected individuals who had previously cleared an HCV infection (19, 20). We then

assessed the potential of T cell based HCV vaccines (i.e. vaccines that do not induce neutralizing antibodies) to subsequently reduce HCV transmission.

Results

HCV RNA carry-over in low and high dead space syringes

Figure 1 shows our study approach, equations used and sources of HCV data. We assessed HCV RNA carry-over from used needle and syringe combinations *in vitro* (Data file S1), as described in Materials and Methods. Throughout this study references to syringes or the term "syringe sharing" assumes the use of a syringe with an attached needle. We obtained mean RNA titers from HCV-positive human plasma, HCV-positive human plasma after syringe expulsion, and normal (uninfected) human plasma that had been drawn into contaminated syringes with attached needles with or without tap water rinse. These data are presented for low dead space syringes with a permanently attached needle (Fig. 2A) and high dead space syringes with a detachable needle (Fig. 2B). RNA extractions for normal plasma and water samples prior to syringe rinsing were always negative for RNA (Figs. 2A and 2B).

HCV-positive plasma before and after syringe expulsion showed similar titers for both syringe types (5.36 to 6.79 $\log_{10}$ IU/mL) (Figs. 2A and 2B left panels). We observed carry-over of HCV RNA in 100% of low (17/17) and high (15/15) dead space syringe samples when no rinsing was employed. Titers ranged from 2.27 to 4.32 $\log_{10}$ IU/mL and 4.54 to 4.97 $\log_{10}$ IU/mL for low and high dead space syringes, respectively (Figs. 2A and 2B). After rinsing, we observed HCV RNA carry-over in 83% (10/12) and 100% (12/12) of low and high dead space samples, respectively, with carry-over titers ranging from 1.57 to 3.81 $\log_{10}$ IU/mL and 2.51 to 4.39 $\log_{10}$ IU/mL (Figs. 2A and 2B). These data indicate that although water rinsing reduced the amount of HCV RNA carried over in syringe equipment, there was still a risk of HCV transmission.

We used the above values to estimate percentage carry-over of any HCV-positive sample in low (Fig. 2A) and high (Fig. 2B) dead space syringes for any single sharing event. The average percent carry-over without rinsing was 0.6% (ranging from 0.02 to 2.6%) for low dead space syringes and 3.1% (range 1.4 to 7.2%) for high dead space syringes (Figs. 2A and B). When rinsing was applied, these values were reduced to 0.12% (range 0.003 to 0.8%) for low dead space syringes and 0.38% (range 0.03 to 0.77%) for high dead space syringes. When these studies were repeated using HCV-positive plasma diluted 1:100, we obtained similar carry-over values (Figs. 2C to 2D), indicating that HCV RNA carry-over was independent of the starting RNA titer.

Cell culture HCV (HCVcc) carried over in low and high dead space syringes

We wished to assess if the percentage transmission of infectious virus particles was consistent with the carry-over values obtained for HCV RNA. It was possible that infectious HCV may not have been carried over as easily as RNA or that manipulations with needles and syringes could have impacted the infectivity of the sample. In such a case, using RNA carry-over to estimate viral transmission could result in inaccurate predictions. We

performed the same carry-over experiments using a cultured 1b/JFH1 chimeric virus (HCVcc) (Data file S1). The HCVcc virus titers calculated for syringe-treated samples are shown in Figs. 3A and 3B for low and high dead space syringes with attached needles, respectively. Consistent with the results seen for RNA titers in plasma, we saw similar amounts of infectious virus in the starting samples before and after expulsion for both syringe types. We obtained mean viral titers of 4.9 to 5.1 log_{10} focus forming units (ffu)/mL for the HCVcc pre- and post-expulsion samples, respectively (Figs. 3A and 3B, left panels). We also observed carry-over of infectious virus using both syringe types, with or without rinsing, although as seen for the plasma studies, more virus was carried over using high dead space syringes (ranges 4.9 to 5.1 log10 ffu/mL, unrinsed; 2.3 to 2.4 log10 ffu/mL, rinsed) (Fig. 3B) compared to low dead space syringes (ranges 2.4 to 2.5 log10 ffu/mL, unrinsed; 0 to 2 log10 ffu/mL, rinsed) (Fig. 3A). The percentage carry-over for unrinsed and rinsed low dead space syringes was 0.26% (range 0.23% to 0.28%) and 0.04% (range 0.003% to 0.08%), respectively (Fig. 3A). For high dead space syringes, the percentage carry-over was 0.87% with no rinsing and 0.19% with rinsing (range 0.16% to 0.22%) (Fig. 3B). Thus, using cell culture HCV, we found the percentage transmission of infectious virus was consistent with that obtained for RNA in human plasma.

Determining Transmission Probability of HCV

To calculate transmission probabilities, we used the RNA carry-over data obtained from our studies with the human HCV plasma samples. The human plasma samples were the most representative of both the virus type and matrix (plasma is more similar to blood than cell culture media) that would be transferred during syringe sharing events. Additionally, the empirical data obtained with these plasma samples gave higher carry-over and greater variability in carry-over percentages compared to our studies with HCVcc and, therefore, represented the highest risk scenario in terms of virus transmission.

We predicted the probability of transmission (P_{trans}) for individual syringe sharing events as a function of viral load for low and high dead space syringes with needles attached using an 11:1 RNA to infectivity ratio, considering both rinsed and unrinsed syringes (Eq. 1 and Fig. 4). Table 1 shows the source for each of the parameters used in Eq. 1 and the values, where applicable. The 11:1 infectivity ratio (value q) was determined from empirical data generated in chimpanzee reverse titration studies using acute phase plasma prior to seroconversion (table S2) (8, 9, 11, 22). These studies have provided direct comparisons between RNA titers and infectious titers of HCV RNA-positive samples in the absence of neutralizing antibodies. This type of sample would be applicable to individuals immunized with a T cell based vaccine as they would not have circulating antibodies to HCV envelope proteins that might influence viral infectivity.

We calculated that P_{trans} was   90% if an individual had a titer >5 log_{10} IU/mL, regardless of syringe type or use of a rinse step (Table 2). With low dead space syringes P_{trans} increased 10-fold as titers increased from ~3.2 to ~4.7 log_{10} IU/mL (rinsed) and ~2.6 to ~4.1 log_{10} IU/mL (unrinsed) (Fig. 4A). When using high dead space syringes the risk was greater and increased 10-fold as titers increased from ~2.8 to ~4.3 log_{10} IU/mL (rinsed) and ~1.9 to ~3.4 log_{10} IU/mL (unrinsed) (Fig. 4B). If an individual had an HCV titer below ~2.3 log_{10}

IU/mL, then P_{trans} for sharing low dead space syringes was predicted to be <5% (Table 2, table S1). In contrast, if high dead space syringes were to be shared, the viral titer would need to be <1.6 $\log_{10}$ IU/mL in order to reduce P_{trans} to <5%.

Impact of HCV RNA Kinetics on the Probability of Transmission

Our calculations of P_{trans} were based on viral titers in a syringe donor at a single time point. However, titers during acute HCV infection fluctuate until they eventually result in spontaneous clearance or long-term persistence (also called chronicity) (17). We previously reported (17) three *a priori*-defined RNA kinetic patterns during primary acute infection in human subjects: full control (spontaneous clearance of HCV RNA within 6 months post infection; n=52) (Fig. 5A), incomplete control (1 $\log_{10}$ IU/mL decline in HCV RNA following the peak titer followed by persistence; n=44) (Fig. 5B) and persistence (increase or < 1 $\log_{10}$ IU/mL decline in HCV RNA following the peak titer; n=66) (Fig. 5C). Using median RNA titers calculated from subjects classified into the three different kinetic patterns (Figs. 5A to 5C), we estimated P_{trans} for any single syringe-sharing event using a rinsed low dead space syringe with an attached needle. The full control group showed a reduction in P_{trans} to 40% by month three and elimination by month four (Fig. 5A). The incomplete control group showed a reduction in P_{trans} in months 4 to 5 following transient control of HCV replication; however, this was followed by a later increase in P_{trans} upon loss of control and a return of viral titers to higher levels at 6 months (Fig. 5B). In contrast, the persistent infection group maintained transmissibility risk at >90% throughout the first 6 months of the acute phase of infection (Fig. 5C).

Successfully vaccinated individuals would ideally have immune responses that would control HCV replication, resulting in viral titers that were lower and of shorter duration than those seen in naïve subjects, as has been reported for HCV reinfection in people who had previously cleared an infection (5, 19). Upon analysis of serial HCV RNA data obtained within 1 to 90 days after confirmed reinfection in subjects who had previously successfully cleared a known primary infection (19), we observed three different outcomes: rapid clearance (within 4 months)/low titer (Fig. 5D), rapid clearance/high titer (Fig. 5E), and chronic infection (Fig. 5F).

To simulate the potential impact of a T cell based vaccine on HCV transmission between people who inject drugs and share syringes, we analyzed RNA profiles from subjects who were reinfected following spontaneous clearance of HCV. We applied median RNA profiles from each of the three identified groups to our transmission equation to estimate P_{trans} for any single syringe sharing event using rinsed low dead space syringes with an attached needle over a 6-month period. During the first month following exposure, P_{trans} was reduced by more than 90% in the rapid clearance/low titer group (Fig. 5D), compared to the first month of acute infection in naïve individuals (Figs. 5A to 5C), and transmission was eliminated by month 2. Transmission probability was >99% during the first 6 weeks in the rapid clearance/high titer group (Fig. 5E), but was almost zero from 6 weeks onwards (Fig. 5E). In contrast, the chronic infection group maintained transmissibility risk at > 40% throughout the 6 months and showed a risk of transmission at >90% for the majority of the time period analyzed (Fig. 5F).

Cumulative Transmission Probabilities in Potential Vaccinees

The calculations above apply to single syringe sharing events between an infected person and an uninfected syringe recipient. To understand transmission risk based on syringe sharing over time, we calculated the cumulative probability of transmission between an infected and uninfected syringe sharer over 6 months for high (daily) to low (monthly) sharing frequencies using either rinsed or unrinsed, low or high dead space syringes with attached needles for all of the infection profiles shown in Fig. 5. For high titer individuals (>5 $\log_{10}$ IU/mL), HCV transmission probabilities were >99.9% throughout the 6-month time period studied regardless of sharing frequency, syringe type, and irrespective of viral kinetics. This included all acute phase profiles in naïve subjects (Figs. 5A to 5C) and reinfected subjects other than those with a rapidly clearing, low titer profile (Figs. 5E and 5F). When we applied kinetics from the rapidly clearing, low titer reinfected group (Fig. 5D), we found that the frequency of syringe sharing had a significant impact on cumulative transmission, although reduction also depended upon syringe type and rinsing. For example, if rinsed syringe sharing occurred daily, the cumulative probability of transmission was <50% (Figs. 6A and B). Moreover, if sharing frequency decreased to weekly for rinsed syringes, the cumulative transmission probability declined to <~10%, and further still with less frequent sharing (Figs. 6A and B).

Transmission Risk as a Function of Viral Load over Time

Our cumulative analyses demonstrated that transmission risk should be estimated as a function of viral load over time, not just a single event with a single titer. We calculated the area under the curve (AUC) using our plots of viral titer against time (Fig. 5). Applying this to our cumulative analyses revealed that individuals with AUCs of over ~100,000 Titers*Months had a >99% chance of viral transmission to their sharing partner regardless of syringe type or sharing frequency. This value corresponds, for example, to a titer ($\log_{10}$ IU/mL) of >5 for 1 month, 4.5 for over 3 months, or 4.2 for over 6 months. In cases where the individual's viral load and time of infection resulted in AUCs <100,000 Titers*Months (e.g. the individual shown in Fig. 5D), reducing syringe sharing frequency could considerably decrease the likelihood of transmission (Fig. 6).

A Potential Non-Linear Relationship between P_{trans} and Viral Load

In order to consider non-linear changes in P_{trans} as a function of viral load, we also tested a Hill function as an alternative to Eq. 1. The Hill function (Eq. 2) can be formulated as $P_{trans} = (nq)^{\beta} / \left((nq)^{\beta} + p_{50}^{\beta} \right)$, where n and q are the same as in Eq. 1, p_{50} represents the value of nq for which $P_{trans} = 50\%$ (fig. S2A), and β controls the steepness of the change in P_{trans} (fig. S2B). Eq. 2 qualitatively matches Eq. 1 for $\beta = 1.4$ and $p_{50} = 0.46$ for low dead space syringes (fig. S3). Reconsidering our studies of transmission probability over time (Figs. 5 and 6), we found that a difference in β did not lead to qualitative changes in transmission probability over time (fig. S4) or the extent to which lower sharing frequency reduced transmission probability (fig. S5). For example, for rinsed low dead space syringes and rapid clearance with low viral load, we found that in our analysis using Eq. 1 the overall transmission probability was ~30% for daily sharing and <1% for monthly sharing, whereas for an extreme example of a Hill function with $\beta = 0.5$, the transmission probability was

95% for daily sharing and 10% for monthly sharing (fig. S5). These results show that even under very different P_{trans} steepness conditions, low sharing frequency using rinsed low dead space syringes could greatly reduce transmission probability, consistent with Eq. 1.

Discussion

In order to reduce the spread of HCV in people who inject drugs, effective interventions are needed that will reduce the chances of virus transmission (23). Vaccine development or antiviral treatment may be able to augment other efforts that can impact infection incidence, such as harm reduction initiatives (e.g. needle and syringe programs, opioid substitution therapy, and behavioral counseling (24, 25)). However, low HCV titers may still be transiently present in individuals receiving vaccines (4). To assess how low viral titers need to be in order for the spread of the virus to be impacted, we performed simulated syringe sharing experiments using low and high dead space syringes with needles attached and developed an equation for HCV transmission based on viral titers when either of these syringe types is used in combination with the needles tested in our studies. We also examined the effect of water rinsing and sharing frequency on the probability of transmission using these two syringe types.

Our approach used empirical data generated in a set of experiments designed to assess RNA carry-over from an infected syringe donor to an uninfected individual (Figs 2 and 3). Our RNA carry-over data were consistent with prior studies on syringe dead space (15, 26). The average difference in dead space between low and high dead space syringes has been estimated as 10-fold (27). We found a 3 to 6-fold difference in the mean carry-over of viral RNA between these two types of syringes. There is conflicting data in the literature as to the benefit of low vs high dead space syringes in preventing virus transmission despite the lower amount of contaminated blood that can be transmitted from the former [reviewed in (27)]. Our data can partially explain these discrepancies. Using an RNA to infectivity ratio of 11:1, we estimated that syringe sharing could result in >90% transmission probability for any single event if HCV titers were $>5 \log_{10}$ IU/mL (28). This high transmission risk could explain reports showing that needle sharing programs have been less successful at preventing the spread of HCV compared to HIV in people who inject drugs (29), while still acknowledging the general positive impact of needle sharing programs on reducing HCV transmission (28). The inclusion of additional prevention strategies, including opioid substitution therapy along with high coverage of needle and syringe exchange programs, will be essential for universally reducing HCV transmission rates (30). Conversely, we show that if viral titers were to be reduced below $2.3 \log_{10}$ IU/mL, the transmission probability could almost completely be eliminated (<5%) for low dead space syringes. For high dead space syringes, the titer would need to be reduced below $1.6 \log_{10}$ IU/ (63 IU/mL). Thus, if infected individuals test negative for HCV RNA using highly sensitive assays (detection limits <20 IU/mL), transmission risk would be close to zero.

One limitation of our work assessing transmission probability is the use of an RNA titer to infectivity ratio established from reverse titration studies in chimpanzees (table S2), as such a ratio has not been established for human transmission. The RNA to infectivity ratio was also established using plasma obtained during the acute phase of infection prior to

seroconversion (8, 9, 11, 21). Thus, the presence of neutralizing antibodies to HCV in a plasma sample may have an impact on the proportion of transmitted virus that can result in an infection. Such antibodies are known to be present in sera from chronically infected patients (31, 32) and in patients that spontaneously clear infections (5). The presence and effectiveness of neutralizing antibodies is difficult to analyze mathematically, but such a scenario would not apply to vaccinees receiving T cell based vaccines targeting only the non-structural proteins of HCV. If future vaccine clinical trials or infectivity studies establish new infectivity to RNA ratios, such as 1:100 or 1:1000, our approach (Eq. 1) could be adapted using a recalculated parameter q. We also assumed a linear relationship between the viral titer and HCV transmission probability using Eq. 1. If transmission is found to change in a non-linear fashion as a function of viral load, e.g. when neutralizing antibodies are present, our approach from Eq. 1 could be adapted to Eq. 2. However, our predicted data, using Eq. 1, are consistent with studies on occupational exposure that demonstrated a relationship between virus titer and infection in exposed health care workers (33). It was shown that the risk of transmission increased 11-fold when health care workers were exposed to sera with a high viral load (>6 log10 copies/mL) compared with exposure to sera with a lower viral load (4 log10 copies/mL) (33).

For single syringe sharing events, we observed a significant impact on transmission risk from individuals who had pre-existing immune responses to HCV (i.e. those who had previously cleared infections) and rapidly controlled the virus (Fig. 5D) compared to naïve, exposed subjects with no pre-existing immunity (Figs. 5A to 5C) and those with little or no control of their secondary infection (Fig 5F). In all naïve, acute phase subjects the risk of transmission was predicted to be 50% within the first 3 months regardless of outcome (clearance or persistence), whereas transmission was eliminated or reduced to <5% in the reinfected subjects that rapidly cleared the virus within 1 (Fig. 5D) to 2 (Fig. 5E) months following re-exposure. Our mathematical modeling analyses suggest that immunity-inducing HCV vaccination could have a marked long-term effect on HCV spread through a population of injecting drug users and emphasizes the need to reduce titers in HCV-infected people who inject drugs in order to reduce HCV transmission. When analyzing cumulative risk, we found that the frequency of syringe sharing remained a pivotal factor in potential virus spread between syringe sharing partners, even if their HCV titers were reduced. This has implications for the maintenance of harm reduction strategies to address potential risk compensation (i.e., re-engaging in high risk practices) by vaccinated drug users who perceive no risk of reinfection or transmission to others. We considered a defined 6-month period following infection but did not assess the longer-term impact of rapid clearance in potential vaccinees. An individual with high titers (>5 $\log_{10}$ IU/mL) for a short period would be infectious for the first week or month but non-infectious for the remaining period; in contrast, a chronically infected individual would remain infectious for the full 6 months and beyond. Both the long-term vaccination effects of reduced/transient viral load and the transmission probabilities developed here could serve as important concepts in a comprehensive and complex modeling approach (e.g., agent-based) that considers the events of sharing syringes in space and time along with the dynamic and complex interplay of factors at the individual level (e.g., risk behavior), the structural level (e.g., access to clean needles and syringes), and the social level (e.g., injection networks) (34). Such

comprehensive models have the potential to fully investigate the impact of reduced viral titers, reduced periods of infection, and multiple syringe sharing partners on the spread and long-term prevalence of HCV.

Applying our models of HCV transmission via syringe sharing to RNA kinetics data, we developed a basis for understanding transmission relative to viral titers (Eq. 1) and predicted the titers necessary to substantially reduce transmission probability among serodiscordant people who inject drugs. Of note, we show that for accurate consideration of transmission risk due to syringe sharing, probability should be estimated as a function of viral load over time, not just a single event with a single titer. Overall, our data strongly support previous reports that viral load is an important factor in HCV transmission (33), similar to the relative increase in HIV (35) and herpes simplex virus transmission (36) risk via sexual intercourse with increased viral load. Notably, our analyses indicate that vaccination of people who inject drugs would not need to achieve sterilizing immunity to reduce HCV transmission provided that harm reduction strategies also were maintained.

Materials and Methods

Study Design

The objective of this study was to assess whether reduced HCV RNA titers in vaccinees would reduce the spread of the virus within drug user populations where contaminated syringes were shared. Data on transmission of HCV RNA from contaminated low and high dead space syringes with attached needles was generated through *in vitro* studies (3 independent experiments). Each experiment consisted of drawing HCV-positive human plasma or cell culture virus into a clean syringe via the attached needles, expelling the sample, drawing normal (HCV-negative) human plasma into the same syringe and testing the RNA titer in the expelled, contaminated plasma. These data were used to calculate the percentage of RNA transmitted from syringes and used in a model-based mathematical analysis to calculate the probability of RNA being transmitted to an HCV-naïve injecting drug user from an HCV-infected injecting drug user based upon the RNA titer in the infected individual. Previously published HCV RNA kinetic data from the InC3 study were obtained for naive (n=162) and reinfected (n=21) subjects (17–20) and a mathematical analysis was applied to calculate the transmission probability for a single sharing event at each timepoint over a 6-month period based on the median titers observed. We extended these studies to consider the cumulative probability of HCV transmission if the same two serodiscordant injecting drug users shared syringes on a regular basis over a 6-month period (i.e. daily, weekly or monthly) based on the previously published viral kinetics data. Each sharing event was considered to use a newly contaminated syringe with attached needle, not the same syringe, and we assumed no loss of infectivity due to delay between contamination and use by the naïve individual. For the human HCV RNA data, all participants provided written informed consent and protocols were approved by local human subject research review committees.

HCV Patient Data

Data for well-characterized HCV RNA kinetics from naïve and reinfected subjects were obtained from the InC3 Study (17–20). All participants provided written informed consent and protocols were approved by local human subject research review committees. Among 643 participants, we studied two subgroups in detail: those with well-characterized acute infection (17) and those with reinfection (19).

Among 162 participants with well-characterized primary acute infection, i.e. HCV RNA detected within three months of the date of infection and a subsequent HCV RNA test within 120 days of the peak RNA, we previously identified three *a priori*-defined patterns of HCV RNA kinetics: i) spontaneous clearance, ii) partial viral control with persistence (1 $\log_{10}$IU/mL decline in HCV RNA levels following peak) and iii) viral plateau with persistence (increase or <1 $\log_{10}$IU/mL decline in HCV RNA levels following peak). The estimated date of HCV infection was calculated based on a hierarchy using all serological (anti-HCV antibodies), virological (HCV RNA), and clinical data (symptoms and liver function tests) as previously described (17). Briefly, for individuals testing HCV RNA positive and anti-HCV negative, date of infection was estimated as four weeks prior to HCV RNA detection. For individuals with symptomatic acute HCV, date of infection was estimated as six weeks prior to onset of symptoms (jaundice or ALT >400 IU/L). For individuals with a negative anti-HCV test followed by either a positive anti-HCV or positive HCV RNA test, seroconversion was assumed to occur at the mid-point between the last negative and the first positive test. Date of infection in this group was deemed to be six weeks prior to the estimated seroconversion date if the first positive test was an anti-HCV test and four weeks prior to the estimated seroconversion date if the first positive test was only an HCV RNA test. Overall, 32% of participants had spontaneous clearance, 27% had partial viral control with persistence and 41% had viral plateau with persistence (17).

To simulate viral kinetics in vaccinees, i.e. those with pre-existing immunity, we first identified 28 individuals with re-infection based on evidence of spontaneous clearance as previously described (17) (two consecutive undetectable HCV RNA tests at least one month apart) or viral suppression (one undetectable HCV RNA test) after primary infection (HCV seroconversion), followed by reappearance of HCV viremia and a change in HCV viral sequence. We excluded 7 individuals who had no quantifiable viral RNA titer, only a qualitative result, (n=3) or only one data point (n=4). Among the remaining 21 individuals, 16 had one episode, three had two episodes and two had three episodes (total of 28 episodes). Among 27 episodes, 10 episodes (37%) were considered chronic and 17 episodes (63%) were considered self-clearing. The time of re-infection was calculated based on the mid-point between the final undetectable HCV RNA test and the first detectable HCV RNA test indicating HCV reinfection (19).

Viral titer imputation for reinfected subjects

In order to analyze viral kinetics in reinfected subjects, we estimated the viral load before the virus was first detected to in turn estimate the date at which the individual was first re-infected (19). We assumed that in the first two weeks following reinfection, the viral load increased in an exponential manner to that of the first measured data point. If the first

measured data point was before 0.5 months we assumed an exponential increase up to that point. From the time t=0.5 months to the first measured time, we assumed that the viral load was constant. We then used linear interpolation, using a log scale, to obtain a continuous graph of the viral load at any given time during the infection.

We next determined population-level statistics by classifying individuals as belonging to one of three classes: low-titer ($<4\log_{10}$ IU/mL) with rapid clearance (within 4 months) (n=12), high-titer ($>4.0 \log_{10}$ IU/mL) with rapid clearance (n=5), or chronic infection (persistence of virus for more than 6 months) (n=10). Within each group, we used the previously defined linear interpolation function to estimate the viral load of each individual at each time point. We then estimated the viral load of the group as the median of the values. Since the data on different individuals extends to different time points, data for later time points may not have been available for all individuals. In these cases we calculated the median of the viral load values of the remaining individuals.

HCV Samples

RNA studies used HCV genotype 1b human plasma (kindly provided by Mei-Ying Yu, CBER/FDA), which had been used to generate part of a CBER HCV RNA panel (37). Infectious virus studies used 1b/JFH1 chimeric HCVcc (38).

Simulated Syringe Sharing

Two syringe types (insulin and tuberculin) were chosen to represent low and high dead space syringes, commonly used for injecting various types of drugs (39). For low dead space syringes we used 0.5 mL U-100 insulin syringes with fixed 27-gauge 0.5 inch needles (Terumo Medical, MD). For high dead space syringes we used 1 mL tuberculin syringes fitted with Precision Glide 23-gauge 1 inch needles (Becton Dickenson, NJ). To simulate real-life situations where syringes are rinsed in an attempt to reduce transmission prior to sharing, we used tap (chlorinated) water for rinsing between simulated contamination and uptake of negative plasma.

HCV Positive Plasma

HCV positive plasma was diluted 1:10 or 1:100 in normal human plasma (Innovation Research, Novi, MI); 0.5 mL was drawn into a syringe via the needle and expelled. Subsequently, 0.5 mL of normal plasma was drawn into the same syringe and expelled into a clean tube. In rinsing studies, 0.5 mL of tap water was drawn into the syringe and expelled to waste prior to drawing up normal plasma (0.5 mL). Samples were extracted as 100 μL aliquots and assessed for RNA titers by real-time RT-PCR, limit of detection 200 RNA copies/mL (40). Using this real-time RT-PCR assay we previously obtained ratios of 2.7 RNA copies per HCV IU using WHO international standards (40), therefore all titers were divided by 2.7 for expression as IU/mL. Syringe-treated samples negative in the real-time RT-PCR assay were assigned a value of half of the limit of detection (i.e. 100 RNA copies/mL or 37 IU/mL). Percentage carry-over was calculated by dividing the non-log transformed HCV RNA titer in the contaminated, expelled plasma in each individual experiment by the non-log transformed titer of HCV RNA in the positive plasma in the same

experiment and multiplying by 100. Each experiment was repeated 2 to 4 times generating 4 to 5 extracted aliquots per experiment to give a total of 8 to 17 data points per result.

1b/2a Chimeric cell culture HCV

Chimeric cell culture HCV samples were diluted 1:5 in normal plasma and subjected to the same treatment described above except that tap water was passed through a 0.2 μm filter. Titers were assessed using Huh7.5 cells as previously described (38). Briefly, diluted virus samples were inoculated onto Huh7.5 cells, 100μl per well in duplicate. Following incubation at 37°C for three hours inocula were removed and replaced with fresh cell growth medium. Cells were incubated for 3 days at 37°C then fixed and stained as previously described (38). Experiments were performed twice. Pre-carry-over samples were diluted 10-fold and tested in triplicate to assess titers, and carry-over samples were diluted 2-fold and tested in duplicate. Percentage carry-over was calculated as described above using FFU/mL titers in place of RNA titers.

Simulations, Sensitivity, and Statistical Analyses

Assuming infectivity by one infectious unit is independent of infectivity by other infectious units, we used a binomial function (Equation [Eq.] 1 below), reminiscent of a binomial function used by Rottingen et al. (41), to estimate the probability of a syringe sharing event leading to transmission of an infectious dose between an HCV-infected and serodiscordant individual (P_{trans}), where n = the number of transferred HCV RNA units and q = the ratio of infectious dose to HCV RNA units (Tables 1 and S2).

We used published data from 12 chimpanzee reverse titration studies employing multiple HCV genotypes (8, 9, 11, 22) to calculate that the median number of RNA molecules required to result in an infection is ~9 with a range of 3 to 13 (table S2). The transformed infectivity to RNA ratios (q) were best described as a gamma distribution with calculated median and Q1-Q3 as 17% and 7%−33%, respectively (Table s2).

$$p_{trans} = 1 - (1 - q)^n \quad \text{(Eq.1)}$$
$$where \quad n = VL \cdot s \cdot \rho$$

We simulated P_{trans} as a function of syringe type (low or high dead space) and syringe rinsing (with or without a rinse step). We determined that n was based upon viral load (IU/mL) in the syringe donor's blood (*VL)*, percent carry-over from the syringe-needle combination based on empirical RNA data generated in this study (ρ), and the relative volume of donor blood in the mixture *(s)* (outlined in Table 1). Parameter n was determined by multiplying each of these three parameters. We took set viral loads and chose randomly from ranges and frequencies for RNA carry-over (ρ) and relative volume of donor blood in the mixture *(s)*. Parameter *s* accounts for situations where blood is drawn into a syringe prior to injection to ensure that the dissolved drugs are injected into a vein (42). We assumed that *VL* does not diminish between contamination and syringe sharing.

Sensitivity analyses used 1000 Monte Carlo samples (RStudio version 3.2.0, Boston, MA). Statistical comparisons were performed using Mann Whitney test and Graphpad Prism software 5.0. P-values <0.05 were considered statistically significant. Area Under the Curve (AUC) was calculated using the Trapezoidal Rule and implemented in Python (version 2.7).

Cumulative transmission probability

We calculated P_{trans} for each group using the pre-established values for ρ, s and q from the *in vitro* experiments and literature as described above. For the variable representing the viral load, *VL*, we used the median viral load of all individuals in the given group at the specific time of the injection.

We assumed that the first injection took place at $t = 0$ (which was also the time of infection). The next injection took place at time $t = freq_{injection}$, where $freq_{injection}$ represents the frequency with which the individual injected with shared syringes. We assumed that the individual continued to inject (via shared syringes) with a frequency equal to $freq_{injection}$ and used the viral load corresponding to that time to calculate the probability of transmission. The overall probability of *no transmission* occurring is

$p_{no\ trans}^{tot} = \left(1 - p_{trans_{t1}}\right) * \left(1 - p_{trans_{t2}}\right) * \ldots$ and thus the overall probability of transmission is

$p_{trans}^{tot} = 1 - p_{no\ trans}^{tot}$. This approach calculates the likelihood of transmission from one specific viremic individual to another specific non-infected individual with whom they share syringes.

Considering a non-linear relationship between P_{trans} and viral load—A Hill function as an alternative to Eq. 1 can also be used, reminiscent of (43). The Hill function could be formulated as Eq. 2 where p_{50} represents the value of $n \cdot q$ (n and q as described in Table 1) for which $P_{trans} = 50\%$, (fig. S2A) and β controls the steepness of the change in P_{trans} (fig. S2B):

$$P_{trans} = \frac{(nq)^{\beta}}{(P_{50})^{\beta} + (nq)^{\beta}} \quad \text{(Eq.2)}$$

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.
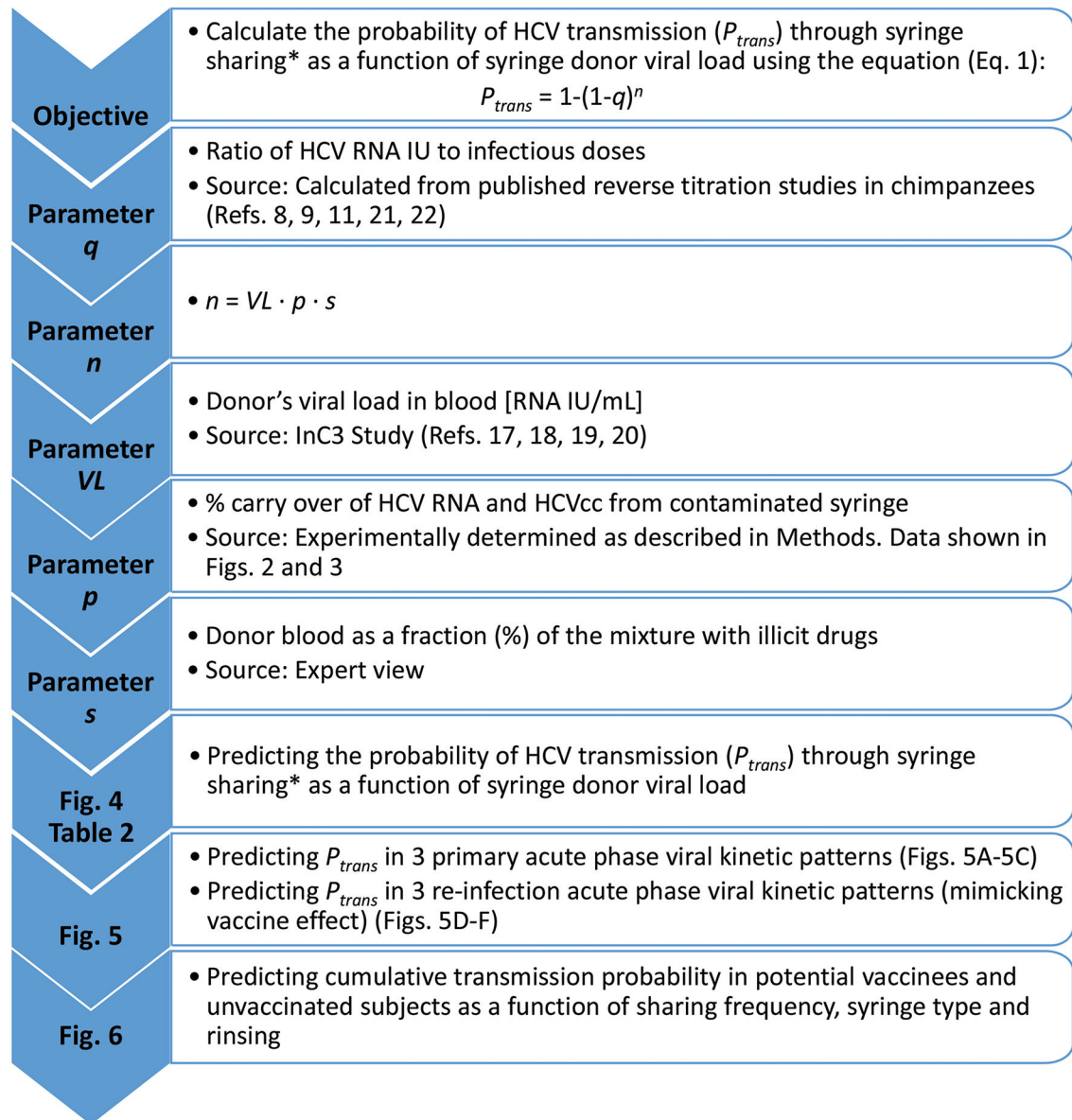
Acknowledgements

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Objective
- Calculate the probability of HCV transmission (P_{trans}) through syringe sharing* as a function of syringe donor viral load using the equation (Eq. 1):
$$P_{trans} = 1-(1-q)^n$$

Parameter q
- Ratio of HCV RNA IU to infectious doses
- Source: Calculated from published reverse titration studies in chimpanzees (Refs. 8, 9, 11, 21, 22)

Parameter n
- $n = VL \cdot p \cdot s$

Parameter VL
- Donor's viral load in blood [RNA IU/mL]
- Source: InC3 Study (Refs. 17, 18, 19, 20)

Parameter p
- % carry over of HCV RNA and HCVcc from contaminated syringe
- Source: Experimentally determined as described in Methods. Data shown in Figs. 2 and 3

Parameter s
- Donor blood as a fraction (%) of the mixture with illicit drugs
- Source: Expert view

Fig. 4 Table 2
- Predicting the probability of HCV transmission (P_{trans}) through syringe sharing* as a function of syringe donor viral load

Fig. 5
- Predicting P_{trans} in 3 primary acute phase viral kinetic patterns (Figs. 5A-5C)
- Predicting P_{trans} in 3 re-infection acute phase viral kinetic patterns (mimicking vaccine effect) (Figs. 5D-F)

Fig. 6
- Predicting cumulative transmission probability in potential vaccinees and unvaccinated subjects as a function of sharing frequency, syringe type and rinsing

* The term "syringe sharing" denotes the sharing of syringes with attached needles

Fig. 1.
Study design, equation parameters and sources of data. The goals of each analysis are shown together with corresponding figures relating to data generated.
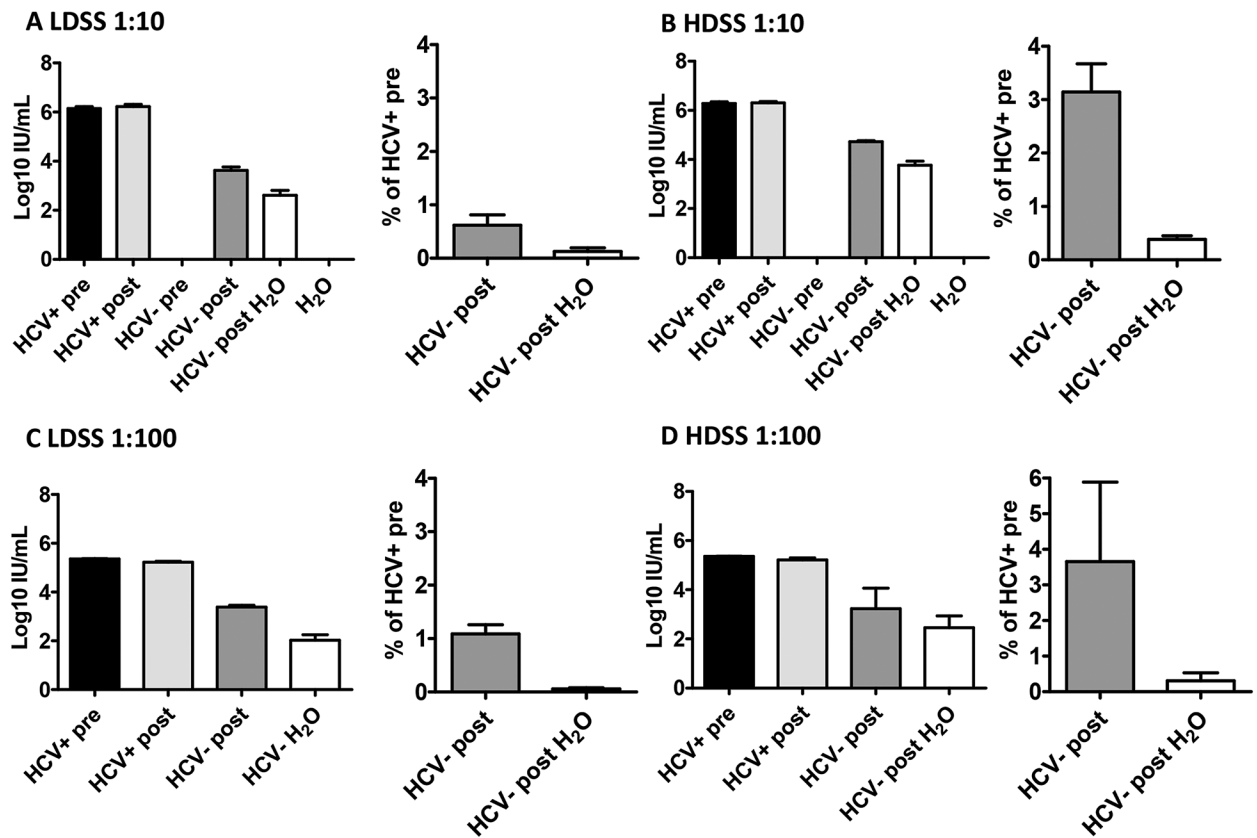
Fig. 2. RNA carry-over of HCV Positive Human Plasma in two different syringe types with attached needles.

Mean RNA titers and percentage carry-over for HCV-positive human plasma samples (diluted 1:10) after being carried over in **(A)** low dead space syringes (LDSS) and **(B)** high dead space syringes (HDSS) (mean of 3 independent experiments). Mean RNA titers and percentage carry-over for human plasma samples (diluted 1:100) after being carried over in (**C**) low dead space syringes and (**D**) high dead space syringes (mean of 3 independent experiments). Bars represent s.e.m. Means were calculated using log-transformed data. HCV + pre= HCV-positive plasma before uptake into a syringe; HCV+ post= HCV-positive plasma after expulsion from the syringe; HCV- pre= HCV-negative plasma before uptake into a syringe; HCV- post= HCV-negative plasma after uptake and expulsion from a contaminated syringe; HCV- post H_2O= HCV-negative plasma after uptake and expulsion from a contaminated syringe rinsed in tap water.

A LDSS

B HDSS
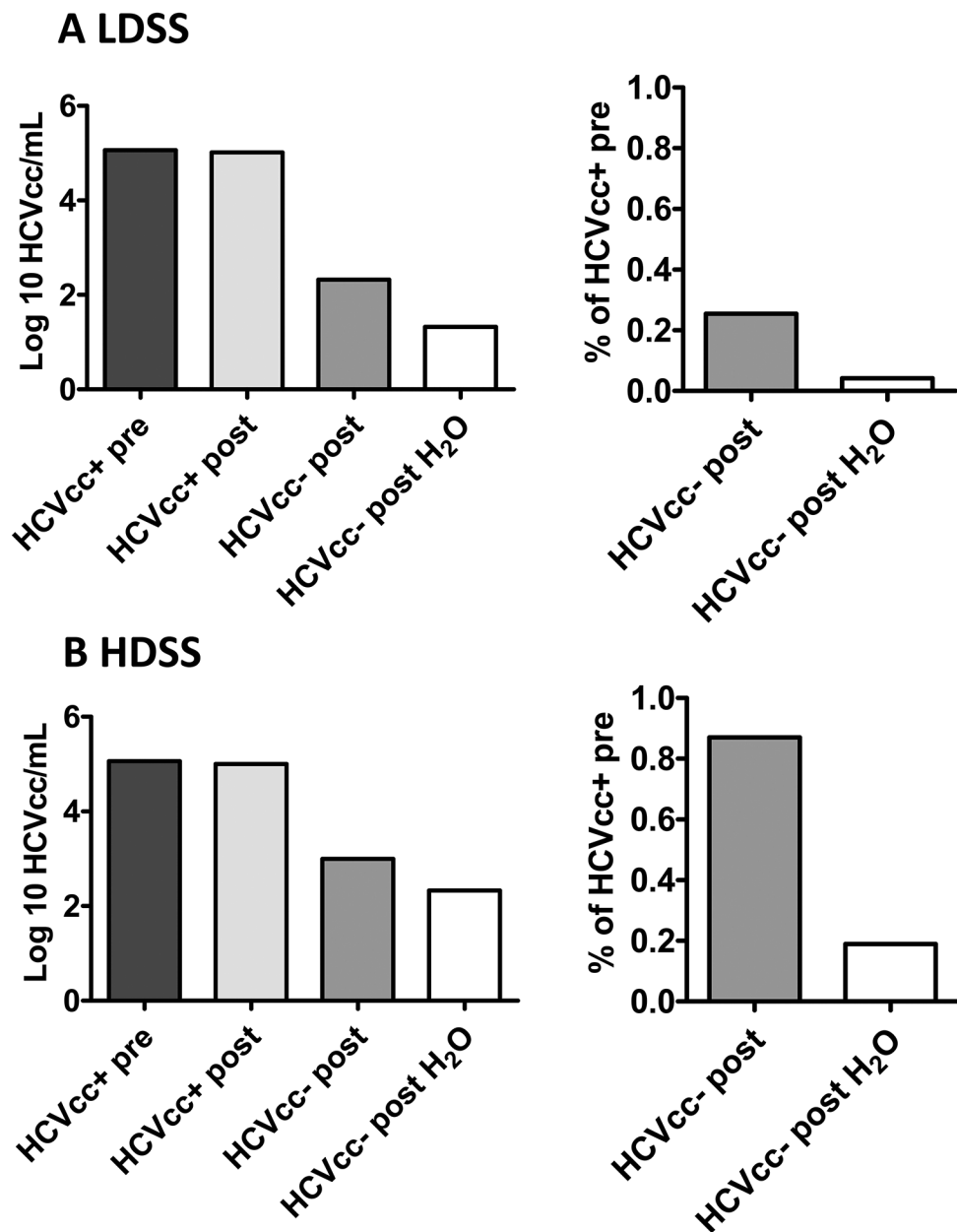
Fig. 3. Carry-over of HCV cell culture virus (HCVcc) positive samples in two different syringe types with attached needles.

Shown are mean titers and percentage carry-over of HCVcc before uptake into (**A**) a low dead space syringe (LDSS) (B) a high dead space syringe (HDSS). HCVcc+ pre= HCVcc-positive sample before uptake into a syringe; HCVcc+ post= HCVcc-positive sample after expulsion from the syringe; HCVcc- post= HCVcc-negative plasma after uptake and expulsion from a contaminated syringe; HCVcc- post H_2O= HCVcc-negative plasma after uptake and expulsion from a contaminated syringe rinsed in tap water. Data represent the mean values of 2 independent experiments. Percentages were calculated as described for Figure 2 using HCVcc titers instead of HCV RNA titers. Means were calculated using log transformed data. Ranges taken from all experiments are reported in the text.
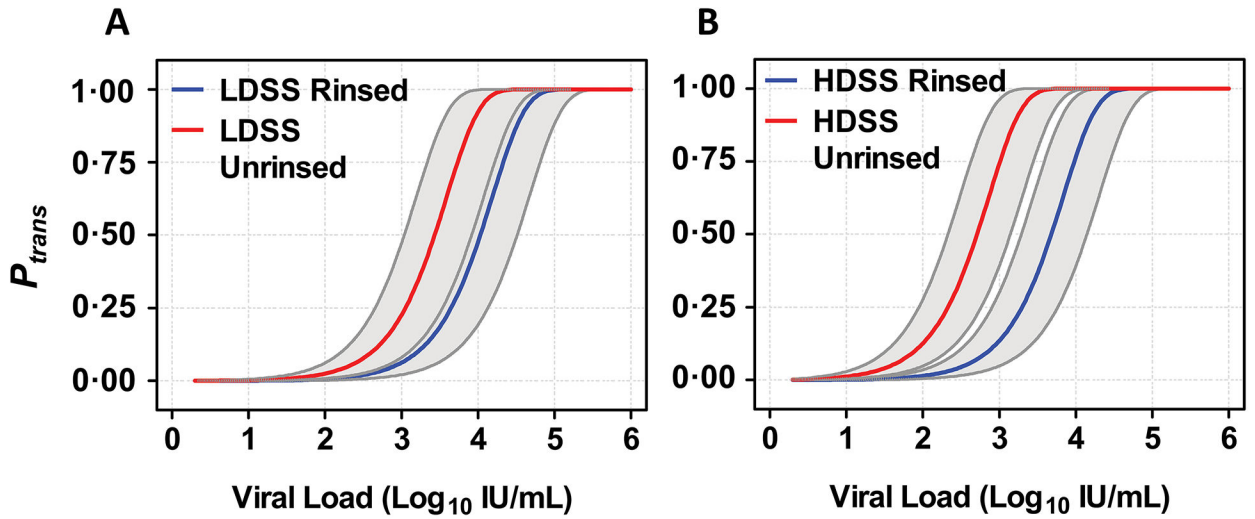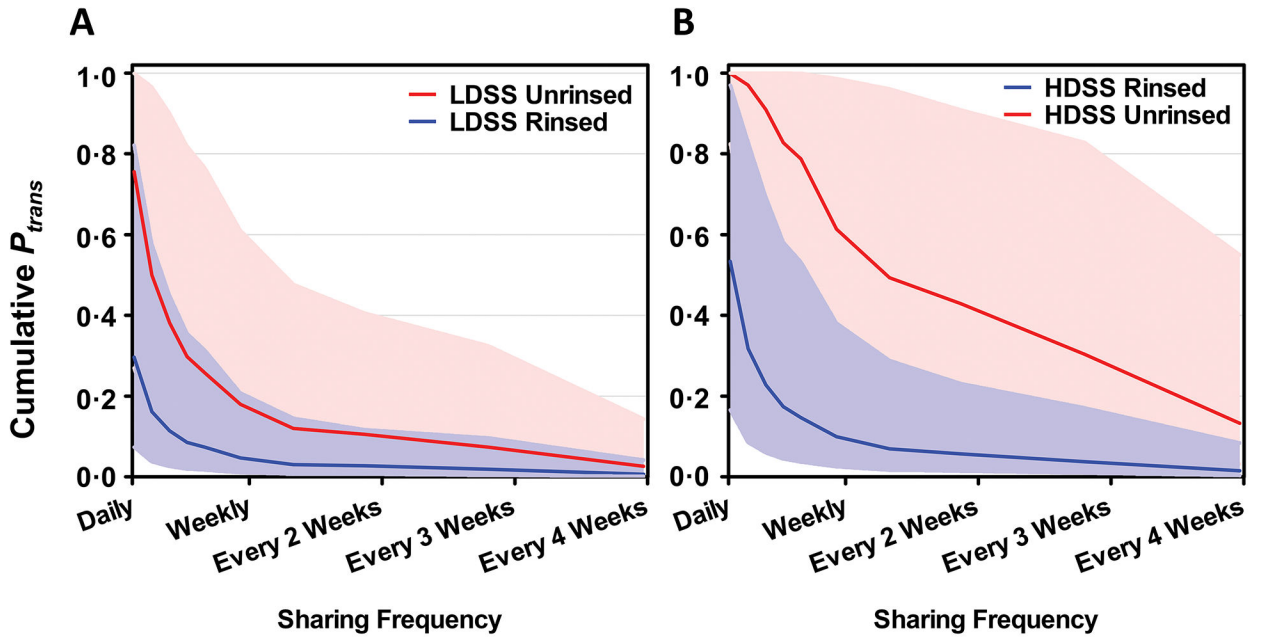
Fig. 4. Probability of HCV Transmission Based on HCV RNA titer.

Probability of transmission (P_{trans}) was calculated using Eq.1, described in Materials and Methods, for increasing viral load as a function of syringe type, with and without rinsing. Set viral loads were taken and combined with randomly chosen ranges and frequencies for RNA carry-over and relative volume of donor blood in the drug mixture (ranges for these parameters are shown in Table 1). **(A)** Calculated values for P_{trans} as a function of viral load using rinsed and unrinsed low dead space syringes (LDSS). **(B)** Calculated values for P_{trans} as a function of viral load using rinsed and unrinsed high dead space syringes (HDSS). Grey bands show the interquartile range based on Monte Carlo sensitivity analyses.
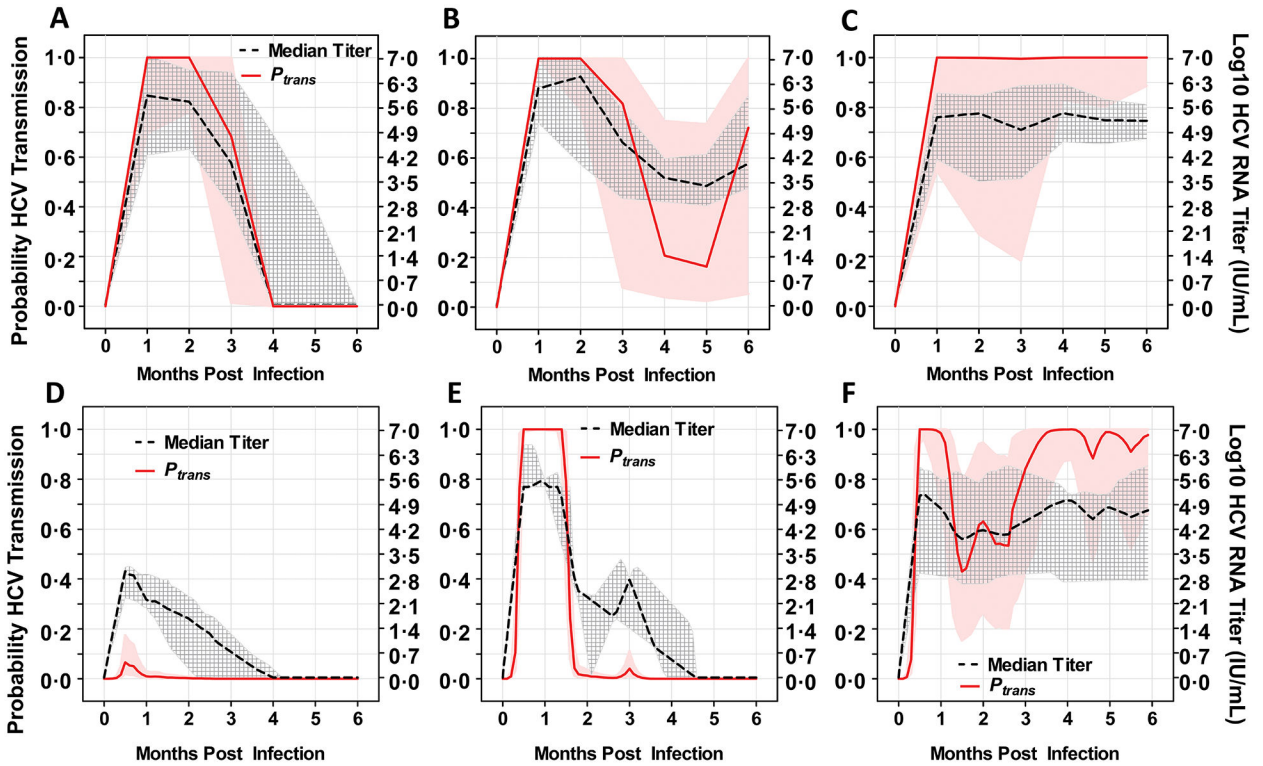
Fig. 5. Probability of HCV transmission via a rinsed LDSS during acute phase infection and reinfection.

Probability of transmission (P_{trans}) (solid red) and syringe donors' median viral titers (dotted black) following different HCV infection outcomes in naïve patients (**A**, **B** and **C**) and patients who had cleared primary infections and experienced reinfection (**D**, **E** and **F**). Data are presented as the median HCV RNA titer for patients grouped by outcome: (**A**) naïve acute phase, self-clearing (**B**) naïve acute phase, incomplete control (**C**) naïve acute phase, persistence (**D**) reinfection acute phase, low titer with rapid clearance (**E**) reinfection acute phase, high titer with rapid clearance (**F**) reinfection acute phase, chronic infection. Gray cross-hatched bands represent the interquartile range of measured data points of all individuals in the group at the given time points. Pink bands show the interquartile range of transmission probability based on Monte Carlo sensitivity analyses.

Fig. 6. Cumulative probability of transmission (P_{trans}) via shared syringes over 6 months for the reinfected low titer group based on frequency of sharing.

This patient group corresponds to that shown in Fig. 5A. (**A**) LDSS, low dead space syringe; (**B**) HDSS, high dead space syringe. Data are shown for rinsed (blue line) or unrinsed (red line) syringes. Bands show the interquartile range of cumulative transmission probability based on Monte Carlo sensitivity analyses. Of note, all viral kinetic profiles for other groups, either following primary infection or re-infection, resulted in a cumulative transmission probability of >99.9% over a 6-month period.

Table 1.
Equation 1 parameters, values and sources.

Transmission probability was calculated based on Equation 1 (Eq. 1). The parameters *VL*, *s*, ρ, and *q* used in Eq. 1 are described together with values and their ranges. Sources are identified; either calculated in this study, taken from previously published data or estimated from experts familiar with practices employed by injecting drug users. IU, international units; HCV, hepatitis C virus; HDSS, high dead space syringe; LDSS, low dead space syringe; IQR, interquartile range (Q1-Q3).

Parameter	Description [units]	Value [Range]	Scenario	Source
n	Number [#] of transferred HCV RNA IUs by contaminated syringe	$VL \cdot \rho \cdot s$		Calculated as described in text and in Fig. 1
VL	Donor's viral load in blood [RNA IU / mL]	Variable [0 to 6 $\log_{10}$ IU/mL]	Experimental	Values input for testing (Fig. 4 and Table 1)
		Time-dependent: 3 primary acute-phase viral kinetic patterns	Self-limiting, transient, chronic outcome (Figs. 5A, B and C)	InC3 Study (17–20)
		Time-dependent: 3 reinfection viral kinetic patterns	Self-limiting (Low and high titer) and chronic outcome (Figs. 5D, E and F)	
s	Donor blood as a fraction of the mixture with illicit drugs [%]	25% [5%−50%]		Expert view
ρ	HCV RNA carry-over	0.6% [0.02 to 2.6%]	LDSS	Estimated in the current study (Fig. 2)
		3.1% [1.4 to 7.2%]	HDSS	
		0.12% [0.003 to 0.8%]	LDSS + rinse	
		0.38% [0.03 to 0.77%]	HDSS + rinse	
q	Ratio of infectious doses to HCV RNA IU	17% [IQR 7–33%]		Table S2 based on published studies (8, 9, 11, 21, 22)

Table 2:

Probability (mean ± standard deviation) of HCV transmission as a function of viral load.

To estimate the probability of HCV transmission (Eq. 1) means were calculated using a Monte Carlo procedure. For parameter ρ (Eq. 1), samples were generated using the truncated normal distribution with the standard deviation equal to ¼ of the experimentally determined range. For parameter s, values were chosen from a uniform distribution between 0.05 and 0.5, and q values were chosen from a gamma distribution with calculated median and Q1-Q3 as 17% and 7%−33%, respectively, (table S2). HDSS, high dead space syringe; LDSS, low dead space syringe.

HCV titer [$\log_{10}$ IU/mL]	Mean HCV transmission probability (± SD)			
	LDSS		HDSS	
	Unrinsed	Rinsed	Unrinsed	Rinsed
1.0	0.57(±1.25)	0.16(±0.30)	2.58(±4.16)	0.30(±0.55)
1.5	1.78(±3.53)	0.51(±0.96)	7.58(±10.36)	0.97(±1.72)
2.0	5.43(±8.60)	1.67(±3.00)	19.97(±20.84)	3.09(±5.10)
2.5	14.12(±17.34)	4.89(±7.85)	40.81(±30.08)	8.66(±12.00)
3.0	31.00(±28.03)	12.95(±16.67)	65.67(±31.68)	21.18(±22.10)
3.5	54.04(±33.41)	28.73(±27.39)	84.54(±25.09)	42.25(±30.94)
4.0	75.50(±30.68)	50.95(±33.81)	94.26(±16.41)	66.45(±32.01)
4.5	88.90(±23.29)	72.52(±31.79)	98.04(±9.78)	84.66(±25.26)
5.0	95.31(±15.79)	87.22(±24.31)	99.37(±5.73)	94.12(±16.89)
5.5	98.13(±10.09)	94.79(±16.41)	99.78(±3.29)	97.89(±10.19)
6.0	99.30(±6.03)	97.91(±11.36)	99.94(±1.25)	99.31(±6.11)