Overall vertical transmission of HCV, transmission net of clearance, and timing of transmission.

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Summary:

Taking account of infections that would have cleared spontaneously before detection, the rate of HCV vertical transmission is 7.2% (95%CrI 5.6-8.9) in mono-infected women, but transmission "net" of clearance is 3.1% (1.8-4.4) at 3 years, and 2.4% (1.1-4.1) at 5.

Short title: HCV vertical transmission and clearance

Abstract

Background: It is widely accepted that the risk of HCV vertical transmission (VT) is 5-6% in mono-infected women, and that 25-40% of HCV infection clears spontaneously within 5 years. However, there is no consensus on how VT rates should be estimated, and there is a lack of information on VT rates "net" of clearance.

Methods: We re-analysed data on 1749 children in 3 prospective cohorts to obtain coherent estimates of overall VT rate and VT rates "net" of clearance at different ages. Clearance rates were used to impute the proportion of uninfected children who had been infected and then cleared before testing negative. The proportion of transmission early in utero, late in utero and at delivery was estimated from data on the proportion of HCV RNA positive within three days of birth, and differences between elective caesarean and non-elective caesarean deliveries.

Findings: Overall VT rates were 7.2% (95% credible interval 5.6-8.9) in mothers who were HIV negative and 12.1% (8.6-16.8) in HIV-co-infected women. The corresponding rates net of clearance at 5 years were 2.4% (1.1-4.1) and 4.1% (1.7-7.3). We estimated that 24.8% (12.1-40.8) of infections occur early in utero, 66.0% (42.5-83.3) later in utero, and 9.3% (0.5-30.6) during delivery.

Conclusion: Overall VT rates are about 24% higher than previously assumed, but the risk of infection persisting beyond age 5 years is about 38% lower. The results can inform design of trials of to prevent or treat pediatric HCV infection, and strategies to manage children exposed in utero.

Keywords
Hepatitis C virus; HCV; vertical transmission; spontaneous clearance; net transmission

With the discovery of direct acting antivirals (DAAs) to treat hepatitis C virus (HCV), attention is turning to interventions either in pregnancy or in infancy to prevent or treat vertically acquired infection. The WHO's target of HCV elimination by 2030 [1] has added further urgency to this issue. According to a 2014 meta-analysis vertical transmission (VT) occurs in 5.8% of infants of HCV-RNA positive mothers who are not HIV-co-infected, and 10.8% if mothers also have HIV [2]. A proportion of vertically infected infants clear spontaneously by age 5 years: 20%-40% is cited in reviews and guidelines [3, 4], but a recent analysis reported 66% clearance, with rates initially high then declining over the first 3 years [5].

This pattern of clearance means that the VT rates reported in the literature depend on the age at which infection status is ascertained and also on the timing of diagnostic tests. The lack of standardization in testing schedules and in methods for calculating transmission rates has long been a cause for concern [6, 7]. Some studies have included all children meeting the definition of infection even if they subsequently clear, while others do not; some report outcomes at 18 months. Each strategy will produce a different estimate of the VT rate.

A second problem is that some infections may clear before they are detected and confirmed. An infant whose first RNA test is at 3 months and is negative would be counted as uninfected in a prospective study, but they may have been infected and then cleared before 3 months. If the first negative RNA test was at 6 months, an initial infection would have had longer in which to clear, and the probability that the child had originally been infected would be correspondingly greater.

The likelihood that unobserved infection and clearance is occurring alongside the variation in how detected infections are counted introduces a profound lack of clarity about how to interpret the reported VT rates.

This paper aims to give a coherent account of the underlying VT rate and the VT rate net of clearance at different ages. This is needed to inform strategies for prevention, diagnosis, and treatment of vertically-acquired infection, and to plan trials of preventive and therapeutic interventions.

We use data on individual mother-child pairs from three published European cohorts to estimate, for the first time, both the overall rate of confirmed VT and the VT rates net of clearance at ages up to 5 years. The overall (underlying) VT rate is estimated by correcting for infections that may have cleared before they were detected. VT rates net of clearance are then estimated by applying clearance rates, estimated previously from the same data [5], to the overall VT rate.

Our analysis also looks at the impact of mother's HCV-RNA viral load, mother's HIV coinfection, and mode of delivery. We investigate the timing and mechanism of infection, by estimating the proportion of infection that occurs early in utero, later in utero and during delivery. This may help inform the optimal timing of preventive treatment in pregnancy.

METHODS

Data sources

Three prospective studies following infants born to HCV antibody positive mothers were included: European Pediatric HCV Network (EPHN) [4, 8-10]; the British Paediatric Surveillance Unit (BPSU) study, which included 3 hospitals in Dublin, Irish Republic and centres across the UK [11]; and the ALHICE study (Alpes-Maritimes, Languedoc, Haute Garonne Infection C chez l'Enfant) [12]. The selection of these studies has been described previously [5], along with details of their pediatric testing schedules. The Faculty of Health

Sciences Research Ethics Committee, University of Bristol, approved these analyses of historic data.

Definitions

Infants were regarded as *Infected* if they were ever anti-HCV positive after 18 months and/or had at least two positive RNA tests at any age. Those who did not meet the Infected definition were considered *Uninfected* if they tested RNA negative at any age after 6 weeks or if their final anti-HCV test was negative. Remaining children were considered *Indeterminate*. Note that "infected" is to be interpreted as "ever-infected" because infected infants can subsequently clear infection, and that "uninfected" infants may have been infected and cleared. Supporting details are given in Supplementary Materials.

Ages at which tests are performed play a key role in the estimation of the probability that each Indeterminate infant was infected, and that each uninfected infant had been infected then cleared. We define *Age at last anti-HCV positive under 18 months:* the later the last positive anti-HCV test, the more likely the infant is to be infected. We also define *Age at last RNA negative under 6 weeks:* the later this is the less likely the infant is to have been infected. *Age at first anti-HCV negative test or the first negative RNA test over 6 weeks, whichever is earliest* is the age when the infant is first known to be uninfected: the later this is the more likely the infant is to have been infected and cleared.

Statistical methods

Our objective was to estimate the risk of vertical infection, the impact of risk factors (mother's HIV and HCV-RNA viral load), and the proportions of infection transmitted Early in Utero (EiU), Late in Utero (LiU) and at delivery. The proportion transmitted EiU is informed directly

by the proportion HCV RNA positive in the first 3 days. Assuming that children delivered by elective caesarean cannot acquire infection during delivery, the difference between overall transmission rates in ECS and non-ECS modes of delivery informs the proportion of non-EiU transmission that is LiU as opposed to occurring during delivery, among those not delivered by ECS.

Infection at each stage, EiU, LiU or during delivery, is conditional on not being infected at an earlier stage. Data is available on risk factors (Study: EPHN, BPSU, ALHICE; mother's HIV status; and mother's HCV viral load measured as near as possible to delivery: Low, High (>600 copies/ml)). Risk factors impact on risk of transmission in each of the three routes as they would in a standard logistic regression, but it is assumed that the odds ratios are the same for each route. We assumed that the log odds ratio associated with higher viral load could depend on HIV status. This interaction was constrained so that the log risk attaching to mothers' positive HIV status and high HCV viral load combined had to be no less than the log risk of either factor alone, but could not be more than both added together. Standard interaction and main effect models were investigated as sensitivity analyses. All models controlled for study effects.

Mother-child pairs lacking data either on mode of delivery or mother's HIV-status, and cases where the mother was known to be HCV RNA negative were excluded. Mother's HCV RNA infection status was unknown in 67% of the remaining records, and where RNA status was known to be positive, HCV viral load was unknown in 43% (Table 1). We included data with missing HCV RNA on the assumption that the proportions of mothers with low viral load, or no detectable RNA, were exactly the same as in mothers in the EPHN study with the same HIV status and mode of delivery whose HCV RNA status was known. Robustness of conclusions to these assumptions was assessed in sensitivity analyses assuming that the odds of both no HCV

RNA and of low viral load were both either 1.6 times higher or 1.6 times lower, which we considered implausibly extreme.

In outline, the statistical analysis estimates the probability that each child of indeterminate status is infected, taking into account their risk group, the age when they were last anti-HCV positive, and the age at the last HCV-RNA negative if this was under 6 weeks. Similarly, the probability that each uninfected child was originally infected and then cleared is calculated, based again on risk group, and on the age when they were first ascertained as uninfected. These probability calculations are shown in Supplementary Table S1. The estimated probabilities of infection in each indeterminate and uninfected child are then summed and added to the number of children with confirmed infection to estimate a notional transmission rate. Uninfected children who were originally infected but then cleared are thus "restored" to the underlying overall VT rate. Then, the net VT rates at selected ages are estimated by applying the clearance rate to the overall VT rate.

The statistical analysis was carried out using Bayesian Markov Chain Monte Carlo estimation. Details of the statistical methods are given in the Supplementary Materials.

RESULTS

The proportions infected, indeterminate and uninfected and the risk factor distributions are shown in Table 1.

Numbers of ever-infected children

Figure 1 panel A1 shows the probability that uninfected children were anti-HCV positive by age; B1 the probability that infected children were RNA negative under 6 weeks of age; C1 the probability that an infected child had not cleared by age. These functions had been estimated

from the three cohorts in advance, and are used together with the information in panels A2, B2, C2 to estimate the probability that individual uninfected and indeterminate children are infected. Panel A2 is a histogram showing age at last positive anti-HCV among children with indeterminate status; B2 shows age at last RNA negative under 6 weeks in uninfected and indeterminate children; C2 shows age at first RNA or anti-HCV negative among uninfected children. The mean age when uninfected children of mono-infected women were first known to be uninfected was 5.2 months in, and 4.4 months in HIV co-infected women

Table 2 illustrates the results of imputing the probability of infection in each indeterminate and uninfected child. In addition to the 96 observed infections, there were a further estimated 10.2 infections among the 223 children with indeterminate status, and a further 9.0 unobserved infections among the 1430 nominally uninfected infants, representing 8.6% and 7.8% respectively of the total 115.2 infections. In the entire combined cohort of 1749, the nominal VT rate is 6.6% (6.2 – 7.1) (Table 2). This is in a study population that includes 67% mothers who were anti-HCV positive but with unknown HCV-RNA status, a proportion of whom – probably around 30% - would have been RNA negative and would not have transmitted.

Risk factors and timing of transmission

Analysis of risk factors (Table 3) suggests no important differences between studies, and strong effects of both maternal HIV status and maternal HCV-RNA viral load. Also shown are the absolute risks of transmission at each stage: early in utero, late in utero and at delivery, in the HIV negative low HCV-RNA viral load group. The proportion of transmission by each route (Table 4) indicates that in non-ECS deliveries, 24.8%, 66.0% and 9.3% of transmissions occur early in utero, late in utero and at delivery. Among ECS deliveries we estimated 27.5% early and 72.5% late in utero. However, relatively few infected children, only 25, were tested in the first 3

days, of whom 9 (36%) tested positive, contributing to the wide credible intervals in estimated proportion of infection transmitted at delivery.

The overall VT rates by maternal HIV status, HCV viral load and mode of delivery are shown in Table 5, and the average *net* VT rates at ages from 3 months to 5 years are plotted in Figure 2 separately for children of mono-infected and HIV-co-infected mothers. In these groups overall transmission risks are 7.2% and 12.1% respectively, falling to VT rates net of clearance at 5 years of 2.4% (1.1-4.1) and 4.1% (1.7-7.3).

Sensitivity analyses

Sensitivity analyses (Table 6) suggest that the overall VT rates and the proportion of infection by each route are relatively insensitive to how or whether the impact of HCV RNA on transmission depends on HIV status, and to assumptions about the distribution of HCV RNA (high or low viral load, or negative) in data where this information was missing. Goodness of fit statistics fail to distinguish between the alternative models (a difference of less than 3 is not regarded as meaningful), and none of the variations in modelling assumptions raise or lower key estimates by more than 5%, well within the statistical uncertainty of the preferred model.

DISCUSSION

HCV vertical transmission rates reported in the literature are based on infection status assessed at different ages, with no consensus on how to take account of spontaneous clearance. We have therefore developed an approach that estimates how many uninfected children may have been infected and cleared before their infection was detected and confirmed, based on a previously estimated clearance rate [5], and which then calculates VT rates net of clearance at ages from birth to age 5 years.

When comparing results to previous literature, it is useful to consider VT rates in HIV uninfected and HIV co-infected mothers separately. The most recent meta-analysis of VT rates [2] reports 5.8% VT in HIV negative women. If we now apply 25%-40% clearance rates [13] (average 32.5%) to this, we would predict that 3.9% of infants born to HCV-RNA positive mothers remain infected at 5 years. These figures can be compared to our estimated 7.2% overall transmission in mono-infected women and 2.4% net transmission at age 5 years. Thus, according to our analysis, the extent of VT is 24% higher than the accepted estimate, while the extent of chronic infection remaining at 5 years is 38% lower. Credible intervals should of course be taken into account (Figure 3).

As a "reality check" we may note that the meta-analysis VT rate of 5.8% [2] is 81% of our estimate of 7.2%. If we refer this to the time-to-clearance curve [5], we find that this would represent a VT rate net of clearance at just under 6.8 months, which accords closely with the average age at which uninfected children were first known to be uninfected, 5.2 months. A similar exercise in HIV-co-infected women would show that the meta-analytic estimate of 10.8% represents a VT rate net of clearance at 3.6 months given our 12.1% overall VT rate: this compares to the 4.4 month average age at which children of HIV infected mothers were first known to be uninfected.

The analysis has a number of limitations. Much of the data was collected at a time when PCR tests were less accurate: various estimates of sensitivity and specificity of the tests used during this period have been made [5, 14, 15], but, like most investigators, we have taken test results at face value for the sake of simplicity. This may have impacted on the classification of children as infected, uninfected and indeterminate, on the assumed time to loss of anti-HCV in uninfected infants, time to clearance, and time to positive RNA in infected infants.

Our estimate of the VT rate in HIV co-infected women, 12.1%, may be of little contemporary relevance. The majority of co-infected women would have been treated with the less potent anti-retroviral drugs available up to 2003. More recent European cohort studies including HIV/HCV co-infected women with a high coverage of antiretroviral therapy suggest substantially lower HCV VT rates, in the range 2.8%-5.9% [16-18].

A further important drawback is the extent of missing data on mother's viral load and HCV RNA status. Although sensitivity analyses reveal that results are robust against large changes in the assumed proportions RNA negative or with low viral load, this lack of data has prevented us from investigating whether HIV and HCV-RNA status might impact transmission differently in utero or at delivery, or on clearance rates themselves. These questions do not appear to have been investigated previously, but can be researched within the framework we have introduced.

Finally, one can question whether the timing and frequency of tests in our cohorts was sufficient for accurate estimation of VT and clearance rates. In conventional analyses less frequent testing will impact on the numbers counted as infected or uninfected. By contrast, in our analyses less frequent testing will translate into greater statistical uncertainty in estimated time to clearance, which is then reflected in the credible intervals on overall and net VT rates. In theory, our methods should estimate the same clearance and VT rates that would be observed if children were tested every day, regardless of testing intervals. How close it comes to this ideal depends on sample size, with larger numbers needed if testing is less frequent. It is therefore relevant to note that although there was insufficient testing in the first three days, the intensity of subsequent testing was comparable to what would be expected in a well-conducted study today: the median age at the first HCV RNA test in the entire cohort was 4 days in the 88% who were ever-tested;

the median times between successive tests after that (whether antibody or HCV RNA) were 2.8, 3.1, and 3.8 months respectively.

A major contribution of this paper is that it introduces methodology for simultaneously estimating overall VT rates and rates net of clearance. The novel element is the imputation of previously cleared infections among uninfected children, based on the age at which they were first known to be uninfected. This extends similar methodology used to impute the number of infections among indeterminates, both in the present paper and in earlier studies of HCV [11] and HIV, before PCR testing became widely available [19-21].

The second contribution is the findings on VT net of clearance, which may help inform the design of trials of treatments in pregnancy to prevent vertical transmission, in spite of the shortcomings in the data. A recent phase I trial has been completed [22] and further trials are under way [23, 24]. Currently, the recommended care of children exposed in utero is to delay diagnosis until 18 months and then refer anti-HCV positives for RNA confirmatory testing at 3 years prior to treatment [3]. This strategy may not be viable where there is substantial loss to follow-up, as has been reported in infants born to HCV-infected women in the US [25-28]. Our results may therefore also be relevant to evaluate alternative diagnostic and pediatric treatment strategies if and when treatments are licensed for use in children under 3 years.

Notes

Author contributions AEA conceived and carried out the analyses with the assistance of FG. AEA wrote the first and subsequent drafts of the paper. KS carried out the literature search and review. AEA, AJ, IJC, and DMG were co-investigators on the HCVAVERT project, and AJ was the principal investigator. EC was a researcher on the HCVAVERT project. LP, EM-B, DMG and KB were senior or principal investigators on the 3 contributing studies: EPHN (European

Pediatric HCV Network); ALHICE (Alpes-Maritimes, Languedoc, Haute Garonne Infection C chez l'Enfant); BPSU (British Pediatric Surveillance Unit). GI provided clinical input on hepatology and management of pediatric HCV. Curation of the original data files available to the project was the responsibility of LP and CT (EPHN), DMG and KB (BPSU), and EM-B (ALHICE). Subsequent data processing was by FG and AEA. All authors critically reviewed and revised drafts as necessary and approved the final version for submission.

Acknowledgements The authors express their gratitude to all those who have contributed to the EPHN, BPSU and ALHICE studies.

Disclaimer The funding sources did not have any influence on study design, data collection, analysis and interpretation of the data, writing of the report, or the decision to submit for publication.

Financial support. This work was supported by the Medical Research Council (UKRI) [MR/R019746/1], through the Joint Global Health Trials scheme. Work at GOSICH was supported by the National Institute of Health Research Biomedical Research Centre, Great Ormond Street Hospital.

Potential conflict of interest CT has received honoraria for presentations from ViiV Healthcare (paid to author); reports grants or contracts unrelated to this work (paid to institution) from European Commission, Child Health CIO, Public Health England, Penta Foundation, and ViiV Healthcare via Penta Foundation; and has participated on the Infectious Disease in Pregnancy Screening Programme Advisory Board. IJC reports a Medical Research Council Global Health Trials development grant paid to institution and a Gilead HCV Elimination competitive grant paid to institution, both unrelated to this work. EC reports a contract or grant from ViiV Healthcare paid to institution via the Penta Foundation. All other authors have no potential conflicts.

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Table 1. Infection status and risk factor distribution in the three cohorts, after removal of records with missing HIV, missing mode of delivery, and RNA-negative mothers. NK: not known. ECS: Elective caesarean section. RNA+: HCV RNA positive. Mean and 95% CrI.

		EPHN		BPSU		ALHICE		TOTAL	
		n	%	n	%	n	%	n	%
TOTAL		1256	100	342	100	151	100	1749	100
Infection status	Infected	69	5.5	15	4.4	12	7.9	96	5.5
	Indeterminate	121	9.6	102	29.8	0	0.0	223	12.8
	Uninfected	1066	84.9	225	65.8	139	92.1	1430	81.8
Mother's HIV	No	1053	83.8	321	93.1	105	69.5	1479	84.6
	Yes	203	16.2	21	6.9	46	30.5	270	15.4
Mother's HCV Viral Load	Low	167	13.3	0	0.0	94	62.3	261	14.9
	High	29	2.3	0	0.0	39	25.8	68	3.9
	NK but RNA+	240	19.1	0	0.0	4	2.6	244	14.0
	RNA NK	820	65.3	342	100	14	9.3	1176	67.2
Mode of Delivery	ECS	373	29.7	26	3.3	35	23.2	434	24.8
	Non-ECS	883	70.3	316	96.7	116	76.8	1315	75.2

Table 2. Observed and unobserved infections, nominal overall vertical transmission rates.

	Infected	**Indeterminate**	**Uninfected**	**Total**
Totals	96	223	1430	1749
Observed infections	96	-	-	115.2 (108.7-124.2)
Unobserved infections	-	10.2 (7.1 – 13.8)	9.0 (4.3-17.1)	
VT rate %	-	4.7 (3.2-6.3)	0.6 (0.3-1.2)	6.6 (6.2-7.1)

Table 3. Risk of vertical transmission by route, and odds ratios for study and risk group, median and 95% CrI.

	Median	2.5%	97.5%
Risk of transmission, %, by route			
Early in utero	1.38	0.64	2.59
Late in utero	3.88	2.24	5.87
Delivery	0.38	0.03	1.97
Odds ratios: study			
EPHN	1 (ref)	-	-
BPSU	1.23	0.64	2.20
ALHICE	0.98	0.47	1.87
Odds ratios: risk group			
HIV-, Low VL	1 (ref)	-	-
HIV-, High VL	2.66	1.19	6.12
HIV+, Low VL	1.75	1.08	3.12
HIV+, High VL	3.43	1.69	8.03

Table 4. Percent of vertical infection by stage, mean and 95% CrI.

	Elective Caesarean	**Non- Elective Caesarean**
Early in utero	27.5 (13.3-45.8)	24.8 (12.1-40.8)
Late in utero	72.5 (54.2-86.7)	66.0 (42.5-83.3)
At delivery	-	9.3 (0.5 – 30.6)

Table 5. Overall VT rates, by subgroup.

Mode of delivery	HCV Viral load	HIV -ve			HIV +ve		
		mean	2.5%	97.5%	mean	2.5%	97.5%
ECS	**Low**	5.6	3.7	7.6	9.6	5.6	14.8
	High	14.2	7.1	23.5	17.5	10.2	27.7
Non-ECS	**Low**	6.1	4.2	8.2	10.6	6.2	16.2
	High	15.3	8.1	24.6	19.1	11.4	29.8
Weighted average		7.2	5.6	8.9	12.1	8.6	16.8

Table 6. Sensitivity analyses. Comparison of preferred model and alternatives. The preferred model is constrained interaction and assumes that 68.9% of the HIV -ve with unknown HCV RNA status are RNA positive, and 90.7% of the HIV +ve. Goodness of fit is posterior mean deviance.

	Goodness of fit	Overall VT, % HIV-	Overall VT, % HIV+	Transmission by stage, %	
Preferred model					
Constrained interaction	741.6	7.2	12.1	24.8	9.3
Statistical uncertainty in preferred model					
Lower (2.5%) credible limit	-	5.6	8.6	12.1	0.5
Upper (97.5%) credible limit	-	8.9	16.8	40.8	30.6
Model choice:					
Main effect model	742.4	7.2	11.8	24.6	9.3
Simple interaction model	743.0	7.2	11.8	24.6	9.2
Proportion Low HCV viral load and proportion RNA -ve					
Both odds lower by a factor of 1.6	742.2	7.4	12.2	24.6	9.1
Both odds higher by a factor of 1.6	740.9	7.0	12.1	24.8	9.4

FIGURE LEGENDS

Figure 1. Panel **A1**. Assumed proportion of uninfected children remaining anti-HCV positive at each age up to 18 months. **B1** Assumed proportion of infected children who are not initially RNA positive remaining RNA negative by age up to 6 weeks. **C1** proportion of infection not yet cleared, by age up to 5 years. **A2** proportion of indeterminates with last anti-HIV +ve at each age. **B2** proportion of uninfected and indeterminate with last RNA negative at each age (< 6 weeks). **C2** proportion of uninfected children with the first test indicating they were uninfected at each age.

Figure 2. Overall vertical transmission (horizontal lines) and vertical transmission net of clearance at different ages: by mother's HIV and weighted average of HIV- and HIV+.

Figure 3. Left bar: VT rate of 5.8% (95%CrI: 4.2%-7.8%) in HCV mono-infected women [2], and spontaneous clearance 32.5% (25%-40%) [29, 30]. Right bar: this study with VT rate of 7.2% (5.6-8.9) with 65.1% clearance (50.1%-81.6%). Blue segments: infection that clears within 5 years. Red segments: infection remaining after 5 years.

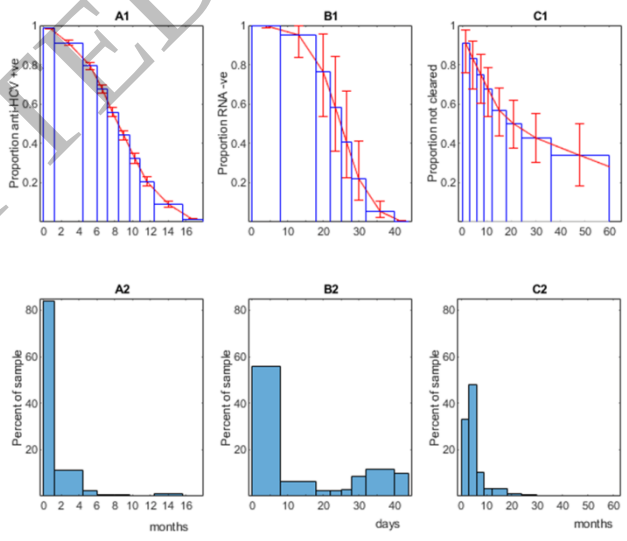

Figure 1
40x33 mm (5.7 x DPI)

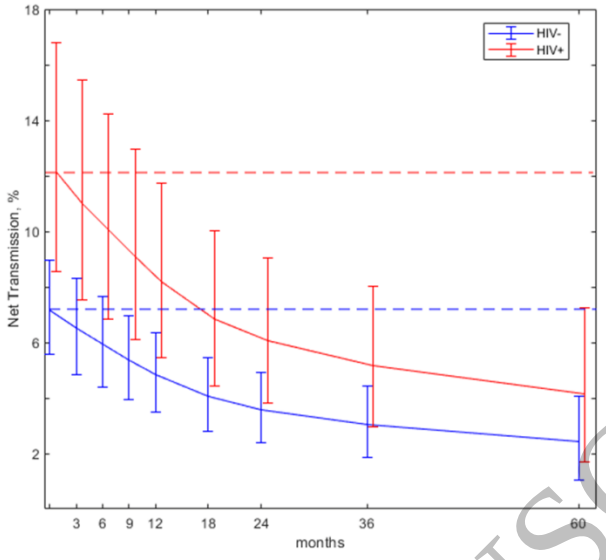

Figure 2
36x33 mm (5.7 x DPI)

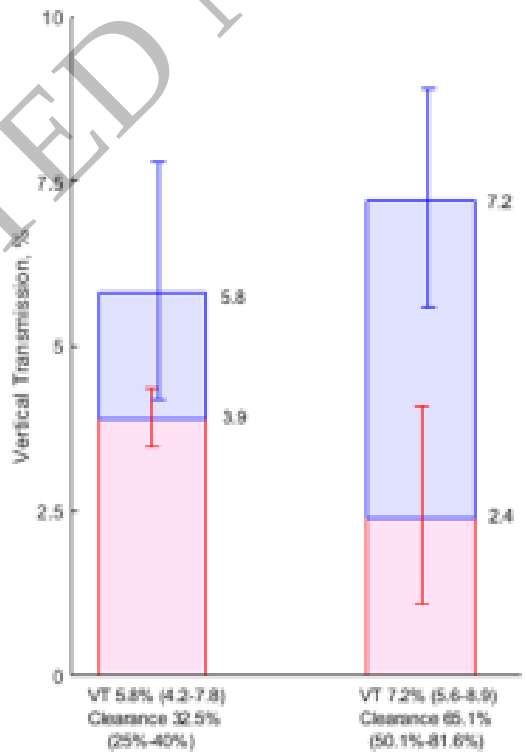

Figure 3
22x31 mm (5.7 x DPI)