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Validation of hepatitis C virus RNA detection using capillary blood by fingerprick (GenXpert system)– Hepatitis C fingerprick study

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Abstract (239 words)

To achieve the ambitious goals of the WHO to eliminate hepatitis C virus (HCV) infection as a public health threat by 2030, innovative approaches are needed to improve the uptake for screening and treatment in people who inject drugs (PWID). Important barriers to care are difficult venous access and the two-step approach in current point-of care tests, using an HCV antibody screening test followed by a confirmatory HCV RNA test. In this study we aimed to validate the new GenXpert instrument to diagnose HCV RNA by fingerprick. This prospective study was conducted in a cohort of PWID in 6 alcohol/drug clinic sites and 1 outreach project in Belgium between January 2018 and March 2019. Plasma and capillary whole blood samples were collected by venepuncture and fingerprick, respectively. Sensitivity and specificity of the GenXpert system were compared to the gold standard Artus HCV RNA kit. Of 153 participants enrolled, 147 (96.1%) had results of both the GenXpert system and Artus HCV RNA kit available. HCV RNA was detected in 35/147 (23.8%) by the Artus HCV RNA kit and in 36/147 (24.8%) by the GenXpert. Median quantitative HCV RNA viral load on fingerprick was 28,700 IU/ml (IQR 4,070-65,875) vs. 1,900,000IU/mL (IQR 416,466-2,265,510) on plasma. The GenXpert instrument had a sensitivity of 100% (95% CI 90-100%) and a specificity

of 99.1% (95.1-99.9%). The overall diagnostic accuracy was 99.3% (96.3%-99.9%).This study validates the excellent performance of the GenXpert instrument to assess HCV RNA in capillary whole blood by fingerprick in a PWID cohort.

Introduction

In 2015, it was estimated that 71.1 (62.5-79.4) million people were chronically infected with hepatitis C virus (HCV) infection globally.[1] Of these, 6.1 million people reported to have used drugs in the previous year.[2,3] Recent research has proven that HCV infection is curable, even in people who actively use drugs, due to the availability of safe and effective direct acting antiviral (DAA) therapy.[4-6] Nevertheless, people who inject drugs (PWID) are confronted with multiple barriers to diagnosis, linkage to care and treatment.[7] In prior studies, barriers identified were the difficult venous access for venepuncture, or the need for multiple visits when using point of care tests. These often have a two-step approach, as HCV antibodies are first tested, followed by HCV RNA.[8,9]

Recently, the GenXpert (GX) Instrument was approved in the European Union for detecting HCV in plasma derived from venous blood.[10,11] This innovative technology made it possible to provide on-site diagnosis of chronic HCV infection within hours. Nevertheless, the validated technology still required a venepuncture. In 2017, Grebely et al. described the successful use of capillary blood samples derived by fingerprick to measure HCV RNA by the GX instrument.[12] Sensitivity of the Xpert HCV Viral Load assay for HCV RNA detection in samples collected by fingerprick was 95·5% (95% CI 84·5–99·4) and specificity was 98·1% (95% CI 93·4–99·8). In this study, we aimed to validate the results of the GX instrument in a cohort of PWID receiving opiate agonist therapy (OAT).

Methods

Study design and participants

This is a prospective, multicenter interventional study. Participants were recruited from 7 different sites (6 alcohol and drugs clinic sites, 1 outreach project by mobile van) across Belgium between January 2018 and March 2019. Inclusion criteria were age ≥ 18 years and receiving OAT. During the outreach week, only patients receiving OAT, and who had a positive rapid HCV Ab test were invited to participate. All participants provided written informed consent. The study (17/027U) was approved by the Ethics Committee of

Ziekenhuis Oost-Limburg on 23/11/2017. The study was executed according to the rules of Good Clinical Practice.[13]

Study procedure

Eligible participants were recruited when attending when attending one of the participating alcohol and drug clinic sites, or during one week of clinical outreach with a mobile van. All participants underwent both blood collection through a fingerprick alongside a standard venous blood sample. As this study was a validation of the GX instrument, the same methodology was used as in the study of Grebely et al.[12]

A capillary whole-blood sample was collected from participants via a fingerprick (MiniCollect Safety Lancet; Greiner Bio-One, Monroe, Frickenhausen, Germany) using procedures recommended by WHO and collected into a 100 µL minivette collection tube (Minivette POCT 100 µL; Sarstedt, Nümbrech, Germany).[14] Immediately after collection, 100 µL of capillary whole blood was placed directly into the Xpert HCV Viral Load cartridge (GXHCV-VL-CE-10; Cepheid, Sunnyvale, CA, USA; lower limit of quantification of 10 IU/mL) followed by the addition of 1 mL buffer (Cepheid, Sunnyvale, CA, USA) without mixing, for on-site HCV RNA testing. The cartridge was then loaded into the GX instrument (< 15 minutes), and capillary whole-blood sample volumes of less than 100 µL were recorded. Xpert HCV Viral Load testing of capillary whole blood was done on an on-site GX R2 6-colour, four module machine (GXIV-4-L System; Cepheid, Sunnyvale, CA, USA) operated by a trained member of the clinical research team as per manufacturer's instructions. Data were analysed with GX Dx software (version 4.6a). The time taken to obtain a result from Xpert HCV Viral Load testing is 108 min. Participants were not provided with the result of their Xpert HCV test results, because the Xpert HCV Viral load test was not approved for clinical use in Belgium.

Blood samples were immediately stored in a fridge (2-6°C), and transported to the laboratory where they were processed (< 6 hours) by the standard of care. Five mL venous blood collected in a K2EDTA (edetic acid) spray-coated collection tube was centrifuged for 20 min at 1500 × g and plasma was collected and aliquoted into 1 mL fractions. HCV viral load was measured using an Artus HCV RNA kit, with RNA extraction on the QIAsymphony of QIAGEN and amplification on the RotorGene of Qiagen. These results were communicated to the participants. All participants were also asked to complete a short questionnaire on the preferred methodology of testing (Table 3).

Statistical analysis

All analyses were performed using SPSS version 24 and Medcalc diagnostic test evaluation. Sensitivity and specificity of the Xpert HCV RNA point-of-care test were analyzed using both detectable and quantifiable thresholds (limit of quantification >10 IU/mL) compared with Artus HCV RNA kit as the gold standard (limit of quantification >21 IU/mL). Assuming a prevalence of chronic HCV of 30% and a sensitivity and specificity of 100%, 150 samples would provide a 95% CI of 23–38% for the prevalence estimate and a 95% CI of 92·1–100·0% for the estimates of sensitivity and 96·5–100·0% for specificity.[12]

Results

An overview of the included patients is provided in Figure 1. In total, 203 clients were addressed to participate in this study, of which 194 at the centre for alcohol and drug abuse, and 9 during screening days in a mobile van. As there was no financial incentive available, and screening was already systematically performed at the centre for alcohol and drug abuse, 50 clients (24.7%) were unwilling to participate. Six of the 153 participants had incomplete results: in two patients, the venepuncture failed with regards to failed phlebotomy due to absence of venous access. In an additional four patients, the fingerprick assay could not be performed; three were due to the lack of capillary blood in the fingers because of cold or callus formation, and in one an error occurred due to motion in the mobile van. In total, results of 147 participants were available for both the GX instrument and the Artus HCV RNA kit. Baseline characteristics of these participants are provided in Table 1. All participants had used drugs, and the majority had ever injected drugs (95.2%). Quantitative RNA results were available in 34 participants, whereas genotyping was only performed in 28. The latter due to the fact that this was not always automatically performed by the laboratory, or due to limited sample volume. The far majority of patients had genotype 1a (40.0%)or 3a (31.4%). Only one participant was also infected with HIV, but at the time of sampling, this patient did not use antiretroviral therapy. As such antiretroviral therapy could not interfere with the test results of the GX instrument.

Results of the detection of HCV RNA for both the Xpert viral load assay on fingerprick and the Artus HCV RNA kit on venous blood samples are provided in Table 2. These results indicate a sensitivity of 100% (95% CI 90-100%) and a specificity of 99.1% (95.1-99.9%). As such, the positive predictive value of the GX instrument was 97.2% (83.3-99.6%) and the negative predictive value was 100% (95% CI could not be calculated. The overall diagnostic accuracy was 99.3% (96.3%-99.9%). As shown by the Bland–Altman plot analysis (Figure 2), HCV RNA concentrations detected by the Xpert HCV Viral Load assay of finger prick were a mean of 0.76 (SD 0.82) log10 IU/mL lower than those measured by the Artus HCV RNA kit on venous blood samples. The limits of

agreement indicate that 95% of the differences between Xpert HCV Viral Load assay and the Abbott RealTime Viral Load assay are between –2.37 and 0.84 log10 IU/mL.

Finally, the results of the preference of testing are provided in Table 3. Both venous sampling and fingerprick testing were considered to be very acceptable by the majority of patients that participated (78.9% and 93.8%, respectively) on a Likert scale of 1 to 5 with 1 being very unacceptable and 5 being very acceptable. Nevertheless, almost all PWID (>95%) preferred fingerprick testing to venous sampling. The preferred time-to-result was almost evenly distributed between 1-2 hours. However, only 15% of PWID admitted that they would wait 120 minutes at the centre for their result. The others preferred to be contacted by telephone the same day. The majority of participants (70.7%) preferred the fingerprick tests because of the fast time-to-result.

Discussion

This study confirms the findings of an excellent sensitivity and specificity of the HCV Xpert HCV Viral Load test for HCV detection in capillary blood collected by fingerprick testing compared to the Artus HCV RNA kit on venous blood samples in people attending drug health clinics in Belgium as was published earlier by Grebely et al.[12] As this approach simplifies the diagnosis of chronic HCV infection, it can be used as a one-step approach, without the need of venous sampling, simplifying the diagnosis of HCV infection in PWID.

To our knowledge, this is only the third study to evaluate the on-site point-of care fingerprick capillary whole blood collection test for HCV RNA detection in a clinical setting.[12,15] It is the first one to be done in a cohort of PWID outside Australia. As outlined by Grebely et al. these data were necessary to further evaluate the performance of this assay in a validation cohort of PWID.[12] Furthermore, it adds to the existing evidence that on-site testing can be performed in PWID. This is crucial as it has already been shown that integrated care can improve the uptake for screening and treatment.[16-18] In comparison to the studies performed in Australia, we included slightly more females (23.1% vs. 12.7-15.2%) and our participants were younger (median age 39 vs. 44 years). Furthermore, we included more people with a history of injecting drug use (95.2% vs. 65.3-71.7%), although the rate of active injecting drug use was only slightly higher (52.4% vs. 44.6-45.7%). As such, we could state that we have reached our target group of individuals with a high-risk of HCV infection.

There was only one discrepant result during the study, where one active injecting drug user had a positive result on the fingerprick assay, which was not confirmed by the clinical laboratory. However, as lab values

were collected using standardized clinical testing forms, these were always performed using the two step methodology where first HCV antibodies (Ab) were tested, and when positive, HCV RNA was tested. In this patient, HCV Ab were negative, which could mean the patient was either recently infected (acute infection), or that the result was false positive. Furthermore, quantitative RNA levels were below 10IU/mL in this patient on the GX system. At a follow-up blood test 2 months later HCV Ab were still negative.

The bias observed when comparing the quantification results by Bland-Altman plot was around -0.76 log10 IU/mL. These results are different from the ones that have been observed during the clinical trials with the CE IVD version.[15] In our study, the manual pipetting of diluent and sample could have increased the variability of the quantitative RNA levels which could explain the bias of these quantification results. As such the comparison of quantification might have been difficult due to the off-label approach. Importantly, this bias did not influence the actual positive predictive value, as all patients positive for HCV RNA on the Artus HCV RNA kit were also positive for HCV RNA on the fingerprick assay.

In three patients, sampling by fingerprick was not possible due to cold extremities and a thick intima layer of the skin. This sampling error was slightly higher than in the study in PWID in Australia, (1.9% vs 0.9%).[12] In one case, an error occurred due to motion in the mobile van. This was also described previously as a possible complication of mobile testing.[12] In the outreach setting, still a two-step approach was used, using first HCV Ab rapid fingerprick testing (Oraquick®). These tests provide a rapid diagnosis of HCV Ab (<20 minutes), with a high diagnostic accuracy (sensitivity 98%/specificity 99%).[19] This provided the opportunity to screen more patients at one screening event, as the long processing time (108 minutes) and limited capacity (4 loading docks) would have limited our screening uptake. With a shorter processing time and/or larger capacities this step could be eliminated to also diagnose acute infections.

The preference of participants for fingerprick testing was consistent with earlier findings.[20] Importantly, nearly half of the participants admitted that they have difficult venous access and the fingerprick was less painful. As such, this innovative method could help remove the barriers to diagnosis for PWID due to poor venous access or stigmatization by health care providers because of difficult phlebotomy.[21,22]

This study had several limitations. This study was performed with the HCV Xpert viral load assay which still had a time to result of 108 min in contrast to the final assay with a time to result of 60 min. As we used an assay with four modules**,** this relatively long time-to-result limited the capacity per screening event. Furthermore, as this study was performed in an observational cohort, those more engaged to care might be

more easily contacted, and as such, this study population might not be representative for the whole population of PWID. Furthermore, a lot of PWID were already aware of their HCV status before entering this study, as 94% of the PWID were previously tested by venepuncture. As the study was executed during a time period when a fibrosis stage of > F2 was still required for the reimbursement of DAA therapy, the lack of a financial incentive, the lack of a need for HCV testing, and the frustration of not being able to access therapy could explain the relatively high refusal rate of 25% to participate in the study.[23]

Conclusions

This study reports an excellent performance of the GX instrument on fingerprick capillary blood compared to the Artus HCV RNA kit on venous blood, with a sensitivity of 100% (95% CI 90-100%) and a specificity of 99.1% (95.1-99.9%). These findings validate the earlier reports of the usefulness of rapid HCV RNA testing in PWID in a separate cohort.

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Figures

Figure legends

Figure 1. Inclusion profile of the study.

Figure 2. Bland-Altman plot of differences. Xpert HCV Viral Load assay for HCV RNA detection in plasma samples compared with the Artus HCV RNA kit on venous blood in plasma; n=41, bias –0.76, 95% limits of agreement –2.37 to 0.84.

Tables

Table 1. Baseline characteristics of participants with both test results available.

	Participants (n=147) (n, %)
Age (years)	Median 39 (IQR 33-48)
Gender	
Male	113/147 (76.9%)

Female	34/147 (23.1%)
Ever injected drugs	
Yes	140/147 (95.2%)
No	4/147 (2.7%)
No answer	3/147 (2.0%)
Active injecting drug use (< six months)	
Yes	77/143 (52.4%)
No	66/143 (44.9%)
Infected with HIV	1/147 (0.7%)
Currently treated with antiretroviral therapy	0/1 (0%)
Ever treated for hepatitis C infection	24/147 (16.3%)
HCV RNA detected	35/147 (23.8%)
Quantitative RNA on fingerprick sample (IU/ml)	Median 28,700 (IQR 4,070-65,875)
Quantitative RNA on venous sample (IU/ml)	Median 1,900,000 (IQR 416,166-2,265,510)
Genotype	
1a	14/35 (40.0%)
1b	2/35 (5.7%)
3a	11/35 (31.4%)
4	1/35 (2.9%)
Not available	7/35 (20.0%)

IQR = interquartile range, HIV = human immunodeficiency virus

Table 2. Sensitivity and specificity of the Xpert viral load assay (GX instrument) on fingerprick samples compared to the Artus HCV RNA kit on venous blood samples.

		Artus HCV RNA kit		
		Detected	Undetected	Total
GX instrument	Detected	35	1	36
	Undetected	0	111	111
	Total	35	112	147

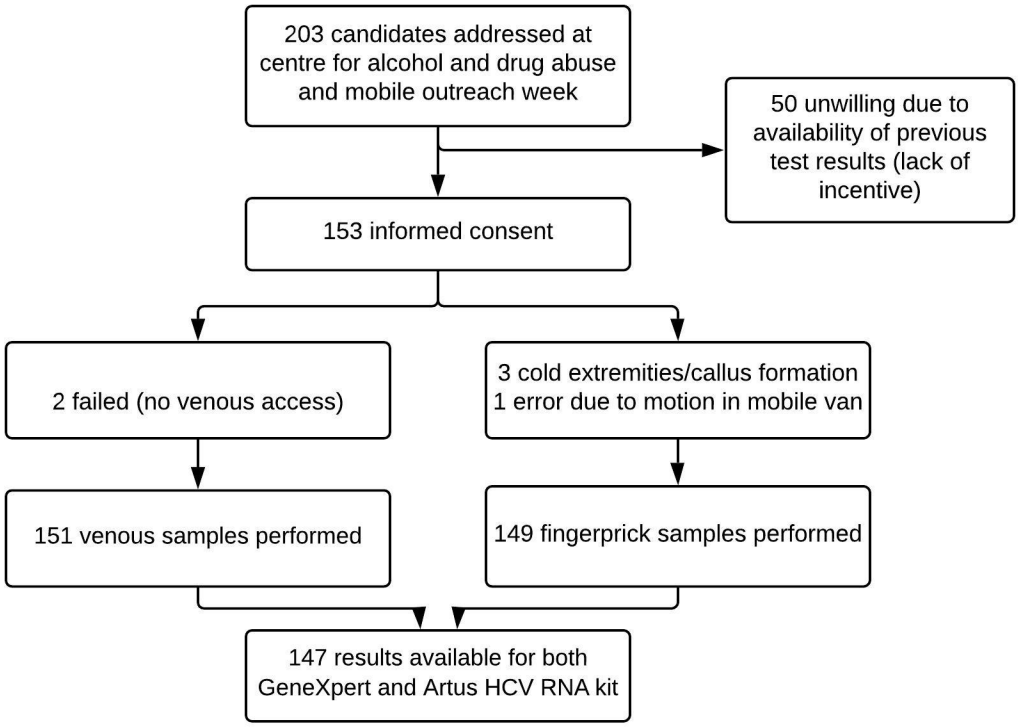
Xpert HCV Viral Load assay (GX instrument) lower limit of detection 10IU/mL.

Artus HCV RNA kit lower limit of detection 21IU/mL.

Table 3. Results of preference of testing methods by participants.

	Participants (n=147) (n, %)
Previously tested by venepuncture	138/147 (93.9%)
Previously tested by fingerprick	4/147 (2.7%)
Would you like to know the result the same day?	
Yes	110/146 (74.8%)
No	1/146 (0.7%)
Does not matter	35/146 (23.8%)
How long would you be willing to wait for your result?	
I would not wait	34/144 (23.1%)
30 min	8/144 (5.4%)
45 min	12/144 (8.2%)
60 min	3/144 (2.0%)
90 min	65/144 (44.2%)
120 min	22/144 (15.0%)
How acceptable is it for you to be tested by a venepuncture?	Median 5 (IQR 5-5)
How acceptable is it for you to be tested by a fingerprick?	Median 5 (IQR 5-5)
Which test would you prefer?	

Venous sample (result within 2 weeks)	0/145 (0%)
Venous sample (result within 120 min)	0/145 (0%)
Venous sample (result within 60 min)	5/145 (3.5%)
Fingerprick sample (result within 2 weeks)	2/145 (1.4%)
Fingerprick sample (result within 120 min)	63/145 (43.5%)
Fingerprick sample (result within 60 min)	65/145 (44.8%)
Either fingerprick or venous sample (result within 120min)	9/145 (6.2%)
Either fingerprick or venous sample (result within 60min)	1/145 (0.7%)
Why do you prefer the fingerprick sample? (multiple answers possible)	
It is fast	104/145 (70.7%)
It is reliable	56/145 (38.1%)
It is less painful	72/145 (49.0%)
There are difficulties to perform venous samples	71/145 (48.3%)



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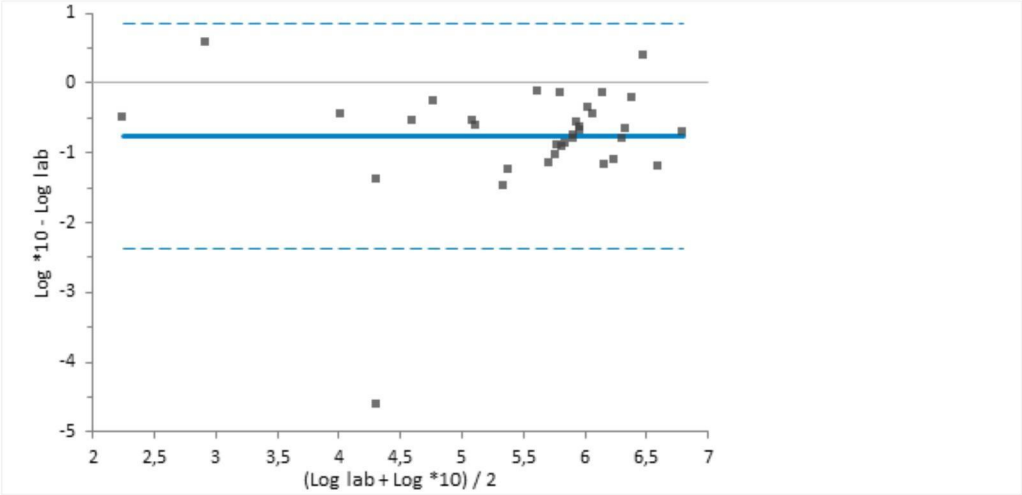

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