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Hepatitis C virus transmission in a Dutch haemodialysis unit: detailed outbreak investigation using NS5A gene sequencing

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SUMMARY

Background: Haemodialysis is a risk factor for hepatitis C virus (HCV) transmission. Two patients receiving haemodialysis in a Dutch dialysis unit in The Hague were found to seroconvert to HCV in December 2016 after the yearly routine control for blood-borne viruses. Following the presumed time of infection, three chronically infected HCV patients were identified as possible index cases.
Aim: To confirm inter-patient transmission and to identify the source.
Methods: Molecular investigation and review of medical records were performed.
Findings: Both of the incident cases and one of the three possible index cases were demonstrated to be infected with HCV genotype 2b based on 5′UTR sequencing. Epidemiological relatedness between these viruses was further investigated by sequencing of the NS5A region. Phylogenetic analysis clearly identified the incident cases and the index case to represent a cluster distinct from unrelated controls with HCV genotype 2b. Detailed review of the medical records identified two possible incidents that might have resulted in the HCV transmission cases: contamination of the venous pressure-sensing port due to high venous pressures or incomplete compliance with infection control precautions of the unit staff during handling of two incidents, that occurred at the same time in a single haemodialysis session with the index patient as well as both incident cases present.
Conclusion: This study demonstrates that detailed incident recording in combination with state-of-the-art molecular investigations such as sequencing of the NS5A region resulted in unravelling a set of two HCV transmissions that occurred at a haemodialysis unit.

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Introduction

In February 2017, the National Inspection for Healthcare was notified of two cases of hepatitis C virus (HCV) transmission in a Dutch haemodialysis unit in The Hague. HCV infection is a significant health problem. In 2015, an estimated 71 million people globally were chronically infected with HCV [1]. Chronic infection can lead to cirrhosis, end-stage liver disease, and hepatocellular carcinoma [2,3]. Each year, ~399,000 people die from HCV complications, mostly from liver cirrhosis and hepatocellular carcinoma [1].

HCV transmission occurs mainly via blood-to-blood contact [4,5]. Less frequent routes are sexual transmission, perinatal transmission, acquisition from mucous membrane exposure, body fluids, and colonoscopy [4–6]. Cases of HCV transmission in healthcare settings have been reported but the number of reported transmissions is still limited, probably because acute HCV infections are not always detected due to the asymptomatic nature of the infection [7–9]. However, HCV transmission in haemodialysis units has been reported several times [10–13]. The relatively frequent identification of HCV transmission in haemodialysis patients may be due to these patients being tested for blood-borne virus infections every six to 12 months, according to the guidelines [14]. Most reported transmissions are associated with infection control-related events including inadequate compliance with standard infection control precautions by healthcare workers (poor hand hygiene and glove use), inadequate cleaning and disinfection of environment and haemodialysis machines, and use of multi-dose vials [15–20].

Genomic sequencing is a very useful technique to investigate classification, epidemiology, evolution, resistance, outbreaks, and transmission of micro-organisms. When transmission of HCV was suspected in a Dutch haemodialysis unit, sequencing of different HCV regions and strains was used to confirm transmission and to determine the source. This report describes in detail the outbreak investigation, including the sequencing results and the possible routes of transmission.

Methods

Outbreak investigation

To investigate whether HCV transmission had occurred at the dialysis unit or outside, an outbreak management team (OMT) was convened comprising the nephrology staff, the quality officer of the renal department, the infection prevention officer, medical microbiologists, and the community health services.

The OMT investigated the following topics: possible incident cases; external risk factors; time of infection; possible index cases; possible routes and risk factors of transmission at the dialysis unit; relatedness between HCV strains. Relatedness between HCV strains was investigated by amplification and sequencing of the 5′UTR and NS5A region.

Extraction, amplification and sequencing

Extraction, polymerase chain reaction (PCR) amplification and sequencing of genomic RNA was performed from sera of index, incident cases and 10 unrelated controls. The unrelated controls consisted of clinical samples collected from HCV genotype 2b-infected individuals attending the Haaglanden Medical Centre and were called unrelated controls 1–10 (UC1–10).

Total nucleic acid was extracted from 200 μL serum using the MagnaPure96 system (Roche Diagnostics, Pleasanton, CA, USA) with the DNA and Viral NA Large Volume Kit and Viral NA Universal LV 3.1 run protocol, and eluted in 50 μL of elution buffer for subsequent use in amplification reactions.

Samples were analysed using Sanger population sequencing. Independent amplification and sequencing of part of the 5′-UTR and NS5A regions was performed using dedicated DeepChek® Assays (ABL SA Group, Luxembourg). Sequencing was performed on an ABI 3730 sequencer. Fasta files of the 5′UTR results were used for HCV typing in the Los Alamos HCV typing tool. The NS5A sequencing results were used for phylogenetic analyses.

Phylogenetic analyses

NS5A nucleotide sequences from the index and incident cases were compared pairwise using the progressive pairwise aligner of Geneious 9.1.6. Phylogenetic analysis was performed using the Tamura–Nei model as part of Mega 6.0.2 sequence analysis software, and was based on the NS5A sequences from the index, incident cases and a panel of epidemiologically unrelated controls. These controls included nine genotype 2b nucleotide sequences obtained from GenBank (Table I) and 10 nucleotide sequences retrieved by sequencing clinical HCV strains (described earlier). The results were processed into a phylogenetic neighbour-joining tree. The tree was resampled with 1000 bootstrap replications to test the robustness of the data.

Results

Setting

In December 2016 HCV transmission was suspected in a Dutch haemodialysis unit of a secondary teaching hospital in The Hague. The hospital operates from three different locations and in 2017 had 35,043 admissions, 18,453 day-care visits, and 87,012 emergency visits. The dialysis centre consists of two units (unit A and B), which operate from two of the three locations. The two units consist of 44 dialysis stations in total and provide haemodialysis and peritoneal dialysis to 130 and 30 patients per year, respectively.

Table I
Nucleotide sequences obtained from GenBank

HCV subtype 2b gene for polyprotein[a] isolated from patient no.	Accession no.
11	AB661380.1
24	AB661387.1
54	AB661392.1
55	AB661393.1
58	AB661395.1
86	AB661399.1
113	AB661402.1
103	AB661423.1
106	AB661425.1

[a] The polyprotein is post-translationally processed in 10 protein products including the NS5A product.

Transmission was suspected in patients visiting unit A after the annual routine testing for blood-borne viruses. Two patients tested newly positive for HCV antibodies (ADVIA Centaur XP; Siemens Healthcare) on December 9th, 2016. All other patients that attended unit A, and all patients of unit B, tested HCV antibody negative. In addition, patients who had transferred to another haemodialysis centre or had undergone renal transplantation, were traced, screened for HCV antibodies and found to be negative.

To identify possible secondary cases, rescreening for HCV antibodies was performed after three months. This additional testing revealed no secondary cases.

Risk factors

For the transmission cases, the community health services investigated risk factors outside the hospital, such as tattoos, sexual behaviour, dialysis overseas, use of drugs, acupuncture, and pedicure. All these risk factors for transmission were excluded. Furthermore, both patients had no relatives or friends known to be infected with HCV. The only risk factor shared between the patients was dialysis.

Time of infection

To determine the time of infection, stored samples from the two possible incident cases were retrospectively tested for HCV RNA (Cobas® AmpliPrep/Cobas® TaqMan HCV Test, v2.0; Roche) and HCV antibodies (Table II). Both incident cases tested negative on June 7th, 2016. However, on August 11th, 2016 a positive HCV RNA PCR was reported for incident case 1, with a viral load of 371,000 IU/mL. The HCV antibody test was non-reactive at that stage, but was positive on December 6th, 2016. In January 2017 the patient died of a non-HCV-related disease (pneumonia). Incident case 2 tested positive for HCV RNA (3320 IU/mL) and HCV antibodies on December 6th, 2016. There were no blood samples available between June 7th and December 6th. The period from exposure to seroconversion lasts eight to nine weeks on average. Because antibodies were already detectable on December 6th, 2016, the time of infection would have been at least eight to nine weeks earlier.

In both possible incident cases the viral load became undetectable (Table II) subsequently, suggesting full recovery from the infection. However, follow-up HCV PCR testing of incident case 2 became positive again, indicating progression to chronic disease (HCV RNA positive >6 months). Incident case 1 died in January 2017 of a non-HCV-related disease (pneumonia). Only one subsequent PCR result was available after the undetectable viral load, which was positive with a low viral load of 30 IU/mL. The viral loads in both cases demonstrated a pattern of intermittent viraemia, which has previously been observed in acute HCV infection [21].

The laboratory results showed that incident case 1 had become infected between June 7th and August 11th, 2016, and incident case 2 between June 7th and December 6th, 2016. Following the presumed time of infection, three chronically infected HCV patients were mentioned by the nephrology staff as possible index cases. Two of these patients received haemodialysis at unit A. The third possible index case received haemodialysis at unit B, but temporarily received haemodialysis at unit A from June 10th until June 14th, 2016, because of admission to the hospital of unit A.

Molecular investigation

To confirm inter-patient transmission with a reasonable certainty and to identify the source, genotyping based on 5′UTR sequencing was performed. Both of the incident cases and one of the three possible index cases were infected with HCV genotype 2b. The other two possible index cases harboured genotype 4a and 1a respectively, and, based on that result, were excluded from further investigation. The possible HCV genotype 2b-harbouring index case was known with high viral loads. HCV RNA was 2.93×10^6 IU/mL on December 6th, 2016.

The 5′UTR sequences did not allow discrimination of the putative transmission cluster from unrelated HCV genotype 2b 5′UTR sequences. Therefore, a more discriminatory HCV gene region, NS5A, was selected and sequenced. NS5A nucleotide sequences of the index case and incident cases were compared. Pairwise nucleotide identity between the index case and incident case 1 was 95.4% and 97.4% between the index case and incident case 2.

Phylogenetic analysis

To investigate relatedness between HCV strains, a neighbour-joining tree was constructed based on NS5A

Table II
Laboratory results of the incident cases

Incident case	Jun 7th, 2016	Aug 11th, 2016	Dec 6th, 2016[a]	Dec 15th, 2016	Dec 27th, 2016	Jan 2017	Mar 7th, 2017	Sep 5th, 2017
Case 1								
HCV antibody	Non-reactive	Non-reactive	Reactive	Reactive		†		
HCV RNA PCR	Not detected	371,000 IU/mL	Not detected	30 IU/mL		†		
Case 2								
HCV antibody	Non-reactive		Reactive		Reactive		Reactive	Reactive
HCV PCR	Not detected		3320 IU/mL		Not detected		1290 IU/mL	115,000 IU/mL

HCV, hepatitis C virus; PCR, polymerase chain reaction.
[a] Yearly routine control.
† Death.

nucleotide sequences from both incident cases, the index case, 10 unrelated controls and nine HCV genotype 2b sequences obtained from GenBank. Phylogenetic analysis clearly identified the incident cases and the index case to represent a distinct cluster in relation to all other sequences included (Figure 1).

Route of infection

There was no epidemiological link between the index case and the two incident cases outside the dialysis unit. Therefore, it was strongly suspected that transmission happened at some stage at the dialysis unit. Various audits were performed to evaluate the infection prevention and control procedures at the unit. No shortcomings were observed during these audits. Universal procedures were followed, including proper hand hygiene, glove usage, vascular access care, environmental and machine surface cleaning, and chemical and heat disinfection of the machine after each dialysis. No multi-dose medication vials or material were shared between patients.

Medical records were reviewed for incidents that were reported to have happened during haemodialysis and to identify the days of dialysis and the specific machines that were used for the index and incident cases. Review of the medical records revealed that the index case and both incident cases received haemodialysis at the same time on June 11th and June 14th, 2016 (Figure 2). The index case used machine 22F on June 11th; the two incident cases used the same machine, but almost one month later, on July 5th and July 7th, 2016. On June 11th, 2016 an incident was recorded for the index case: clotting of the extracorporeal system occurred, resulting from the use of heparin-free haemodialysis. This resulted in high venous pressures and possible contamination of the venous pressure-sensing port. Incident case 1 was known with high venous pressures, which could cause a risk for inter-patient transmission from the index case to incident case 1 via a contaminated venous pressure-sensing port. It is not known whether incident case 2 suffered from high venous pressures.

To investigate whether contamination of the venous pressure-sensing port with HCV could potentially explain the transmission, this device was removed and flushed with sterile water, after which PCR was performed on the eluted water. The HCV RNA PCR result on the eluted water was negative.

On June 11th, 2016 a second incident was reported for incident case 1 because of bleeding of the arteriovenous fistula after dialysis. In addition, incident case 2 needed help post dialysis with pressing the fistula. The incidents occurred at almost the same time. Reconstruction of these incidents with renal staff members revealed that a member who had handled the two incidents reported for the index case and incident

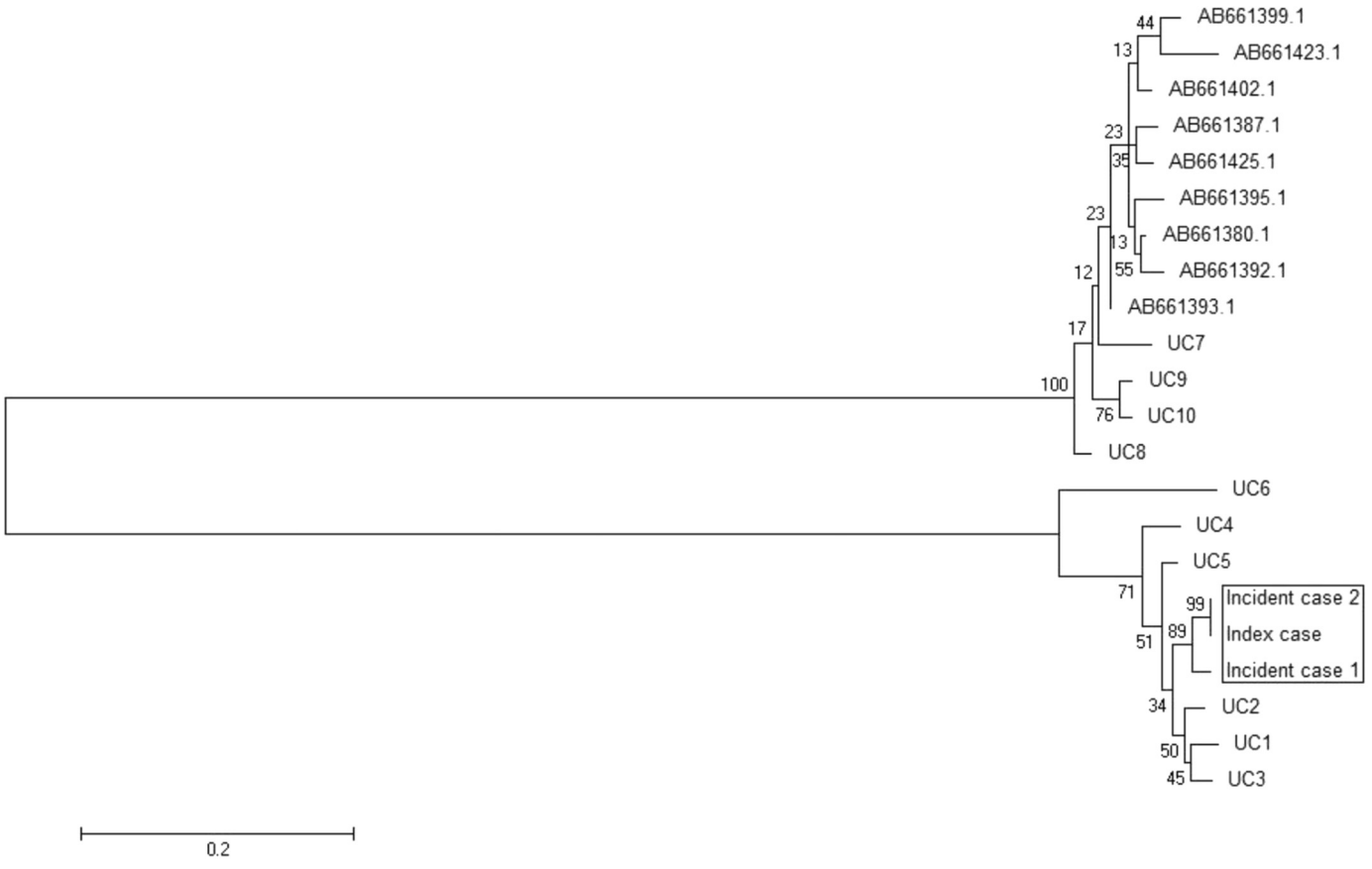

Figure 1. Neighbour-joining phylogenetic tree based on hepatitis C virus (HCV) genotype 2b NS5A nucleotide sequences, showing the relation between the index case and the incident cases (highlighted cluster) and the unrelated controls included in this study. Unrelated controls included 10 nucleotide sequences retrieved from clinical strains (UC1–10) and nine nucleotide sequences obtained from GenBank. The value on each branch is the occurrence of the branching order in 1000 bootstrapped trees. The scale bar represents 0.2% differences in nucleotide sequences.

Unit A	10.06.16	11.06.16	12.06.16	14.06.16	16.06.16	18.06.16	21.06.16	23.06.16	25.06.16	28.06.16	30.06.16	02.07.16	05.07.16	07.07.16
Incident case 1		A											22F	
Incident case 2		B												22F
Index case		C 22F												

Figure 2. Timeline with days of dialysis (in grey), use of the same machine (22F) and events or incidents that took place for the index and incident cases. (A) Incident: bleeding of arteriovenous fistula post dialysis. (B) Event: help post dialysis with pressing fistula. (C) Incident: clotting of extracorporeal system.

case 1, respectively, also helped pressing the fistula of incident case 2. It cannot be excluded that, due to urgency and incident stress, incomplete compliance — for example by not changing gloves — may have occurred.

Discussion

Two cases of HCV transmission from a single index occurred at the haemodialysis unit of a Dutch secondary care hospital. The first indication of epidemiological linkage between the viral strains present in one of the potential index patients and both incident cases was obtained from sequencing part of the 5′UTR region that revealed a shared HCV genotype 2b. Globally, the prevalence of HCV genotype 2b is only 1%, and this genotype is also known to represent a minority of HCV infections in The Netherlands [22].

Analysis of HCV genotype 2b intra-genotype variation between NS5A genes of the incident and index cases in relation to unrelated controls was performed to generate further evidence for genotypic relatedness between the potential transmission cluster cases in comparison to unrelated controls. A phylogenetic tree was generated based on NS5A nucleotide sequences. High level of identity between the HCV strains, shown as a distinct cluster in the phylogenetic tree with unrelated genotype 2b HCV controls, indicates that the genotype 2b HCV strains derived from the incident cases and the presumed index case are closely related.

The small nucleotide differences observed in the NS5A sequences between the HCV strains of the index and incident cases can most likely be explained by the bottleneck effect that is part of viral transmission [23,24]. HCV has an extremely high mutation rate, caused by the high replication rate and the lack of proofreading activity, resulting within an infected individual in a swarm of related genomic variants, called the viral quasi-species. It is thought that quasi-species within an individual may allow the virus to evade the immune response or to escape treatment. Even though a swarm of viral quasi-species is present in an index patient, a single or a few founder viruses are responsible for establishing the HCV infection in the recipient: the transmission bottleneck effect. Population-based sequencing of the viral quasi-species from various related infected individuals reveals small nucleotide differences between the sequences, as also observed in the genotype 2b transmission cluster presented here.

In an attempt to identify possible causes and routes for the observed transmission, medical records were reviewed and infection control inspections were performed. Two possible routes of transmission were identified: contamination of the venous pressure-sensing port and incomplete compliance with standard infection control precautions, probably provoked by two incidents that occurred at the same time in a single haemodialysis session in which the index patient as well as both of the incident cases were present.

Different studies have reported the risk of blood contamination of haemodialysis machines beyond pressure-sensing ports [25–27]. Pressure-sensing ports are devices inside the machine that convert pressure into an electronic signal that can be displayed. They serve an important role in monitoring of blood pressures within the arterial and venous circuit. High venous pressures can cause flooding of blood into the pressure tubing set, leading to contamination of the venous pressure-sensing port, which is not accessible to routine disinfection. The two incident cases used the same machine as the index case; however, the period in between was almost a month. When contamination of the venous pressure-sensing port had occurred, transmission could only have taken place when the virus on the port remained infectious. HCV can survive outside the body. The duration of continued infectivity has been demonstrated to be dependent on environmental factors such as temperature, humidity as well as the virus titre. Studies that assessed environmental stability of HCV demonstrated that viral infectivity in a liquid environment remained for up to five months at lower temperatures (4°C) [28–30]. However, in a dry environment at room temperature, the viral infectivity was lost within a few hours to days. This makes it unlikely that contamination of the pressure-sensing port has been the shared source of equipment that caused the transmission. In addition, the HCV RNA PCR result of the eluate was negative. A positive result would demonstrate contamination of the venous pressure-sensing port, whereas a negative result cannot rule out contamination, but this makes it unlikely that this route would have caused the transmission.

The second possible transmission route is lack of compliance by one or more healthcare workers with hospital hygiene procedures, even though no shortcomings were observed during inspections performed as part of the investigation. On June 11th, 2016 two significant incidents occurred at almost the same time that disturbed the normal work process and possibly caused temporal stress to the unit staff. First, clotting of the extracorporeal system occurred with the index case. This was followed by bleeding of the arteriovenous fistula of incident case 1. In addition, incident case 2 needed help post dialysis with pressing the fistula. These incidents were handled by shared staff present at the haemodialysis unit. Although the exact route of transmission could not be determined, reconstruction of the two incidents suggests that transmission via healthcare workers' hands — for example by not changing gloves — might be the most plausible cause.

Following the results of the outbreak investigation, the renal staff entered several corrective actions including: education and training of the healthcare workers, detailing strict adherence to all infection control policies and procedures at any time; increased periodic auditing; implementation of a daily meeting at the end of the day, where incidents or other breaches in the normal work process are evaluated for training purposes.

In conclusion, the present study demonstrates the superiority of genotypic identification and sequencing to unravel a viral outbreak. Sequencing of the NS5A region appeared to be an appropriate target to investigate relatedness between HCV strains. So far, haemodialysis patients with HCV, who are not eligible for renal transplantation, are not treated, despite the generally high viral load in chronically infected HCV individuals. With the availability of extremely efficacious direct-acting antivirals that have few adverse effects, this policy should be reconsidered in order to decrease the HCV viral load in infected individuals and thereby reduce the risk of HCV transmission.

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Conflict of interest statement
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