Diploma thesis

Evidence of nosocomial transmission of hepatitis C in the region of West Styria

Submitted by

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Graz, 17.09.2014 Gisela Gußmack

Declaration of Originality

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Graz, 17.09.2014

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1 Abstract

1.1 English

Background: There are about 170 million people infected with the hepatitis C virus (HCV) worldwide. Up to 1992, HCV was mainly transmitted by blood and blood products but in about 30% of patients with chronic hepatitis C, the way of transmission is unknown. In the routine diagnostic laboratory, patients living in a region of West Styria were identified to be infected with HCV subgenotype 2a which is found to be rare in Austria.

Objectives: To investigate the exposure to risk factors of HCV infection in a group of patients with HCV subgenotype 2a infection living or having lived for more than 10 years in a well-defined West Styrian region. To identify and discuss the most probable source of transmission in this group of patients.

Materials and Methods: Patients were invited to fill out a questionnaire. Information about the patients' exposure to risk factors especially those related to the health care system was collected. The patients were asked to report on procedures posing a risk of HCV transmission such as colonoscopy, gastroscopy, surgery, and blood transfusion and the location where the procedure(s) had been performed.

Results: Of 23 patients that met the inclusion criteria, 16 patients participated in the study. All participants had been exposed to invasive procedures at health care institutions. The 3 health care settings, where the majority of patients had undergone procedures posing a risk of HCV transmission were identified: dentist A, urologist A, and anesthesia at hospital A. Of the 16 patients, 9 were exposed to invasive procedures at dentist A and 11 patients to invasive procedures at urologist A. 13 patients had at least one event that required general anesthesia at hospital A; however, the time period they had undergone anesthetic procedures was found to be more than 40 years.

Conclusion: The majority of patients were exposed to invasive procedures at multiple health care settings. The number of patients that had one or more cystoscopies at urologist A was remarkable and may be the most probable source of transmission in this group of patients.

1.2 German

Hintergrund: Hepatitis C ist eine weltweit verbreitete Erkrankung, die durch das Hepatitis C Virus hervorgerufen wird. Rund 170 Million Menschen sind von dieser Erkrankung betroffen. Bis 1992 wurde HCV hauptsächlich über Blut sowie Blutprodukte übertragen. In rund 30% aller Patienten, die an chronischer Hepatitis C leiden, ist der Übertragungsweg allerdings unbekannt. In einer Region in der Weststeiermark wurden Patienten gefunden, die mit dem, in Österreich sehr seltenen, HCV Subgenotyp 2a infiziert waren.

Ziele: Ziel dieser Studie war es, die Risikofaktoren zu identifizieren, denen diese Patienten ausgesetzt waren. Mögliche Infektionsquellen werden diskutiert und die wahrscheinlichste beschrieben.

Material und Methoden: Die Patienten wurden eingeladen einen Fragebogen auszufüllen. Informationen über die Exposition der Patienten bezüglich Risikofaktoren für eine HCV Übertragung, besonders innerhalb des Gesundheitswesens, wurden erhoben. Die Patienten wurden gebeten medizinische Eingriffe, die ein Risiko für die HCV Übertragung darstellen wie z.B. Koloskopie, Gastroskopie, Operationen und Bluttransfusionen sowie die dazugehörigen Durchführungsorte anzugeben.

Ergebnisse: 16 der 23 Patienten, welche die Einschlusskriterien erfüllten, nahmen an der Studie teil. Alle Teilnehmer waren invasiven Eingriffen in Gesundheitseinrichtungen ausgesetzt worden. In den folgenden 3 Einrichtungen wurden Eingriffe beim Großteil der Patienten durchgeführt, die ein Risiko für die HCV Übertragung darstellen: Zahnarzt A, Urologe A und Anästhesie im Spital A. Neun der 16 Patienten waren invasiven Eingriffen bei Zahnarzt A ausgesetzt, 11 Patienten Eingriffen bei Urologen A. Bei 13 Patienten war zumindest einmal eine Allgemeinnarkose im Spital A erfolgt, jedoch innerhalb eines Zeitraums, der sich über mehr als 40 Jahre erstreckte.

Schlussfolgerung: Der Großteil der Patienten war invasiven Eingriffen an mehreren Gesundheitseinrichtungen ausgesetzt. Die Anzahl der Patienten, bei denen Zystoskopien durch den Urologen A erfolgt waren, war beachtlich, sodass dieser Risikofaktor als wahrscheinlichste Infektionsquelle erscheint.

2 Introduction

2.1 The hepatitis C virus

Hepatitis C is caused by a major global pathogen, the hepatitis C virus (HCV) and was previously known as non-A, non-B hepatitis, before the agent was identified in the late 1980s (1, 2).There are about 170 million people infected with HCV worldwide (1, 2).

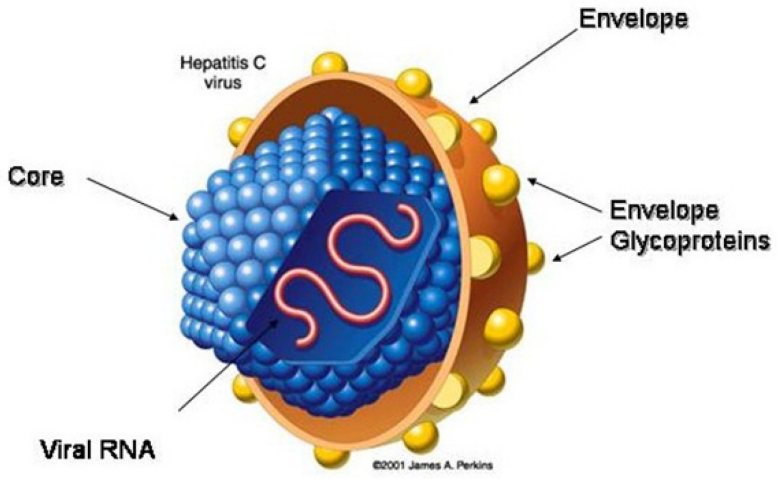

Fig. 1 The hepatitis C virus (3)

The HCV is a positive-sensed, single-stranded enveloped, linear RNA-Virus. HCV is the only member of the genus *hepacivirus* and belongs to the family of *flaviviridae*, although its genome has similarities in organization to that of *flaviviruses* and *pestiviruses* (3). The HCV genome is about 9.6 kB in length and includes a long open reading frame (ORF). The ORF is flanked by 5' and 3' untranslated regions (5'UTR and 3'UTR), that are highly conserved (1, 2). The

5’UTR region contains the internal ribosome entry site (IRES), which initiates viral replication by binding directly to the 40S ribosome subunit without any protein translation factor (2, 4, 5). The ORF encodes a polyprotein that is processed to nonstructural and structural proteins. The structural proteins are the nucleoside protein, the core (C) protein, and two envelope proteins (E1 and E2) (1, 2). Furthermore, there are nonstructural proteins containing enzymes with protease and helicase activity and viral polymerase (NS3, NS4A, NS4B, NS5A, and NS5B) that form the viral replication complex and p7 and NS2 that are important for virion production but are not required for replication (2).

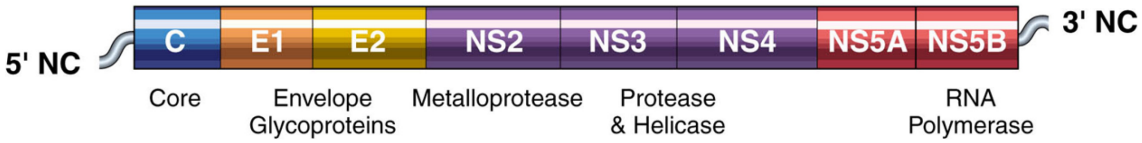

Fig. 2 Organization of HCV genome (kindly provided by Harald H. Kessler).

The two envelope proteins are glycosylated and form a heterodimeric complex (1, 2). They are essential targets for antibodies (1). They comprise hypervariable regions (HVR-1 and HVR-2) that develop fast under antibody selection pressure (1, 2). Often, individuals with HCV infection produce antibodies which react with synthetic peptides representing the HVR-1 sequence of the virus they are infected with (2). It looks like that the appearance of such antibodies modifies the *quasispecies*, a swarm of closely related genetic variants of HCV within one single person (2). The genetic diversity is highest in the segment of the HVR-1 region that encodes for envelope protein E2, and lowest in the 5’UTR and 3’UTR segments of the core gene (Figure 3) (2, 6, 7). Another important cause for the development of *quasispecies* is the lack of proofreading capacity of RNA polymerase (2).

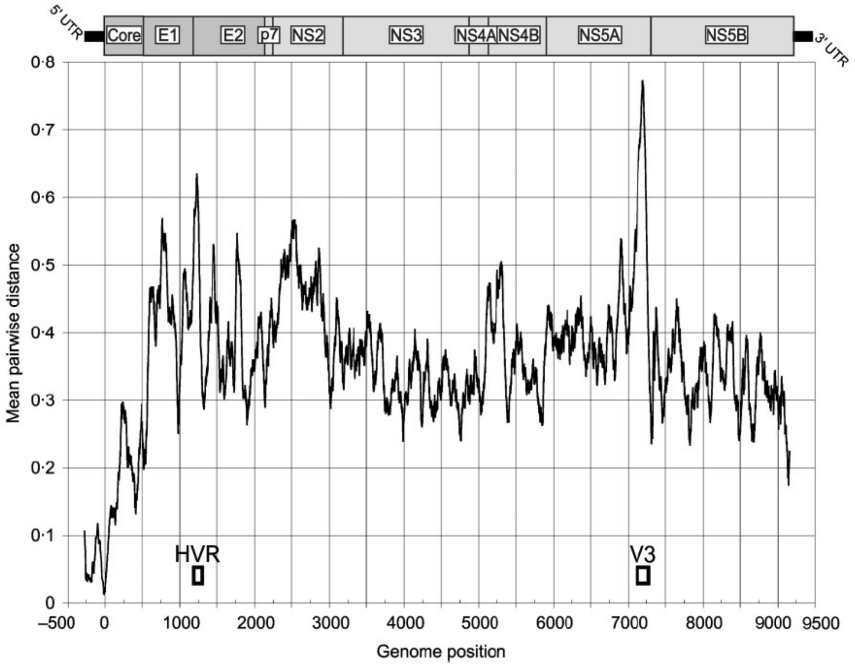

Fig. 3 Genetic diversity of the HCV genome (8).

Replication of HCV usually takes place in hepatocytes, although virus has been detected also in lymphocytes and dendritic cells (1). Viral entry into hepatocyte is a complex, not yet fully understood, process (1, 2). Numerous cellular receptor molecules such as CD81, LDL receptor, DC-SIGN, L-SIGN, macrophage scavenger receptor class B1, tight junction proteins occludin and claudin-1 are likely to play a role in attachment and penetration into the hepatocyte (1, 2). After penetration and up-taking the virus into a cellular endosome, local pH changes may modify the conformation of the envelope proteins, which leads to fusion with the endosomal membrane and release of viral RNA into cytoplasm (2). The cap-independent translation of the viral polyprotein starts with the help of the IRES, followed by processing the polyprotein, leading to production of the viral proteins core, E1 and E2, p7, and five nonstructural proteins (NS3, NS4A, NS4B, NS5A, and NS5B), which form the replicase complex (2). This complex directs the synthesis of a negative-strand copy of the HCV genome that is used as template for the production of multiple copies of positive-strand genomic RNA (2). After enclosing the genomic RNA into new viral particles, the virus gets released (2).

2.2 HCV genotypes and subgenotypes

There is not only heterogeneity in HCV sequences in single patients (*quasispecies*) but also an impressive genomic heterogeneity between different patients (genotype variation) (2). According to phylogenetic evaluation studies, it is estimated that there are at least six major HCV genotypes (2). Depending on the genomic region that has been analyzed, sequences from different genotypes can diverge in up to 50% of nucleotides (2). There is no proof that there are differences in transmission, level of replication or rates of progression between HCV genotypes, even though several studies have been performed (2).

Within one genotype, there are usually several subgenotypes (subtypes) that have about 75-85% of nucleotides within the core/E1 and NS5B region in common (2, 9). In comparison, variants of *quasispecie*s have 91–99% identity in these regions (2, 10).

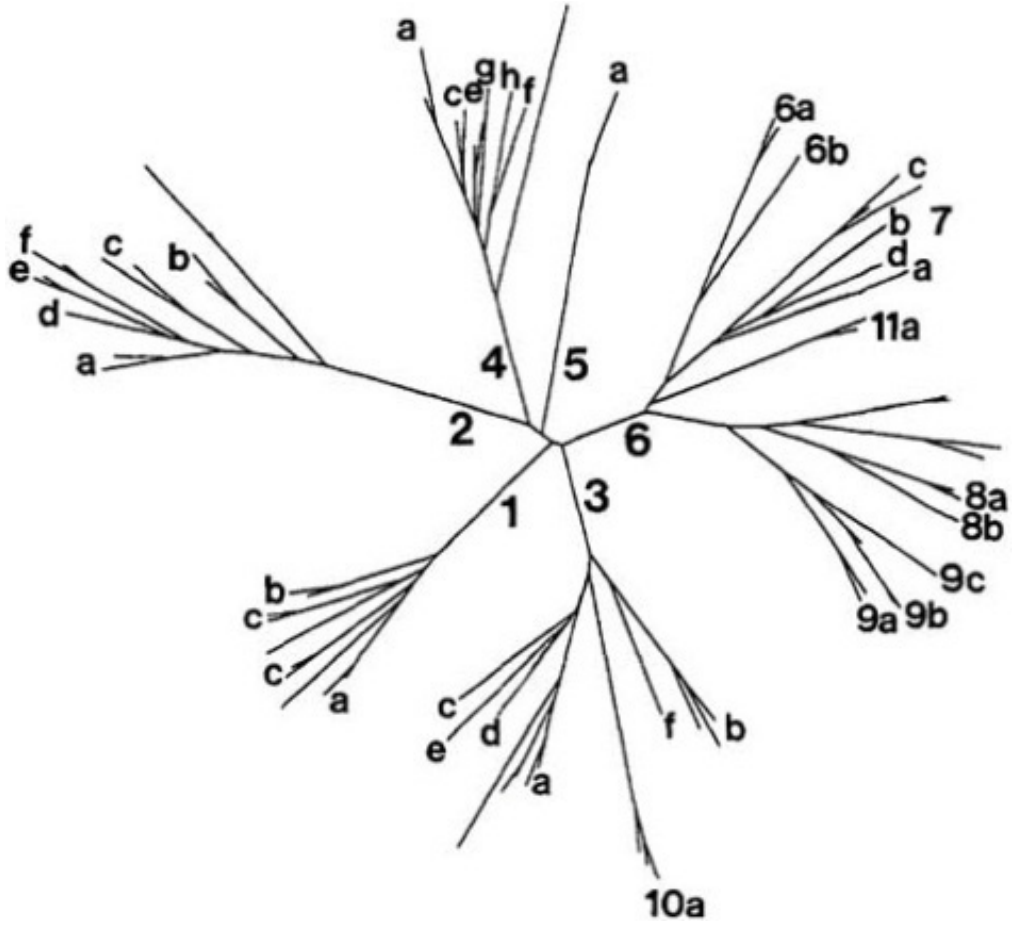

Fig. 4 Phylogenetic tree of HCV NS 5B sequences. Major branches are labeled with type numbers, minor branches with subtypes designations (11).

While some HCV genotypes are distributed all over the world, others are more typical for a certain region (Fig. 5). In general, HCV genotype 1 is the most common type in Europe and Northern America. HCV genotype 3 is endemic all over the world, mainly associated with intravenous drug abuse in Western countries. HCV genotype 4 is predominant in Egypt (1).

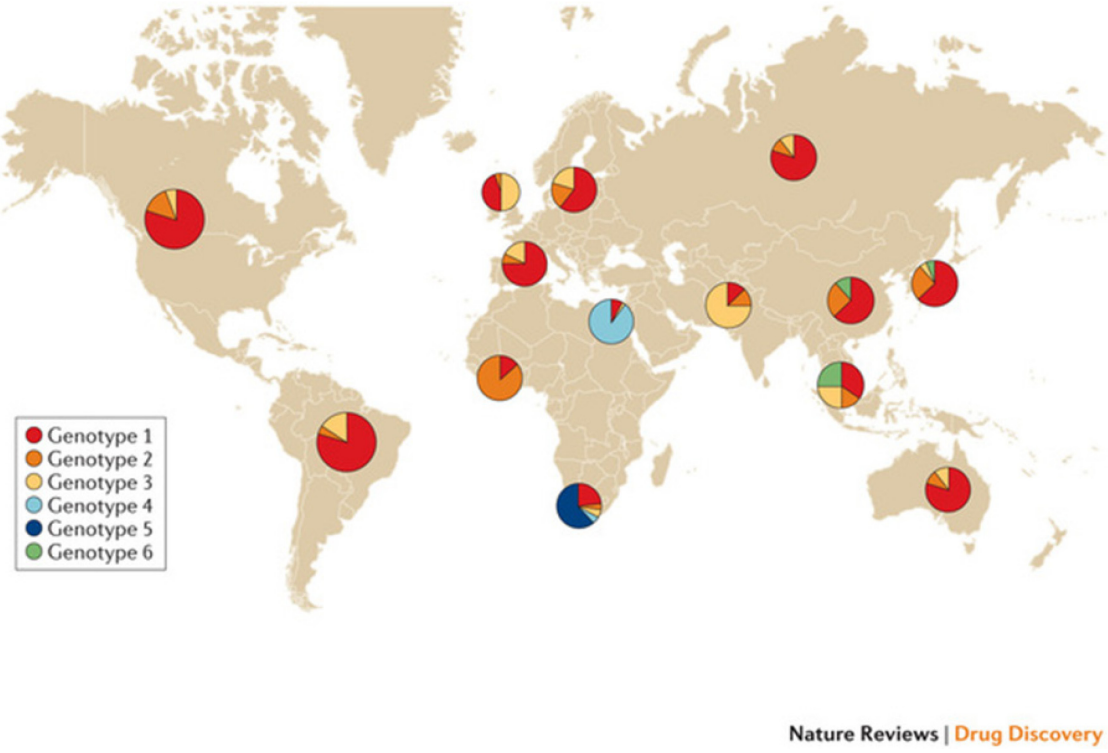

Fig. 5 Worldwide distribution of HCV genotypes (12).

There are few data available regarding HCV genotype distribution in Austria. In an Eastern Austrian study (Vienna and surroundings), HCV genotype 1 was found to be the predominant HCV genotype, infecting almost 75% of individuals with CHC, followed by HCV genotype 3 (approximately 16%), HCV genotype 4 (approximately 5%), and HCV genotype 2 (approximately 3%) (13). In Upper Austria, corresponding numbers were 80% for HCV genotype 1, 12% for HCV genotype 3, 4.5% for HCV genotype 2, and 3% for HCV genotype 4 (14).

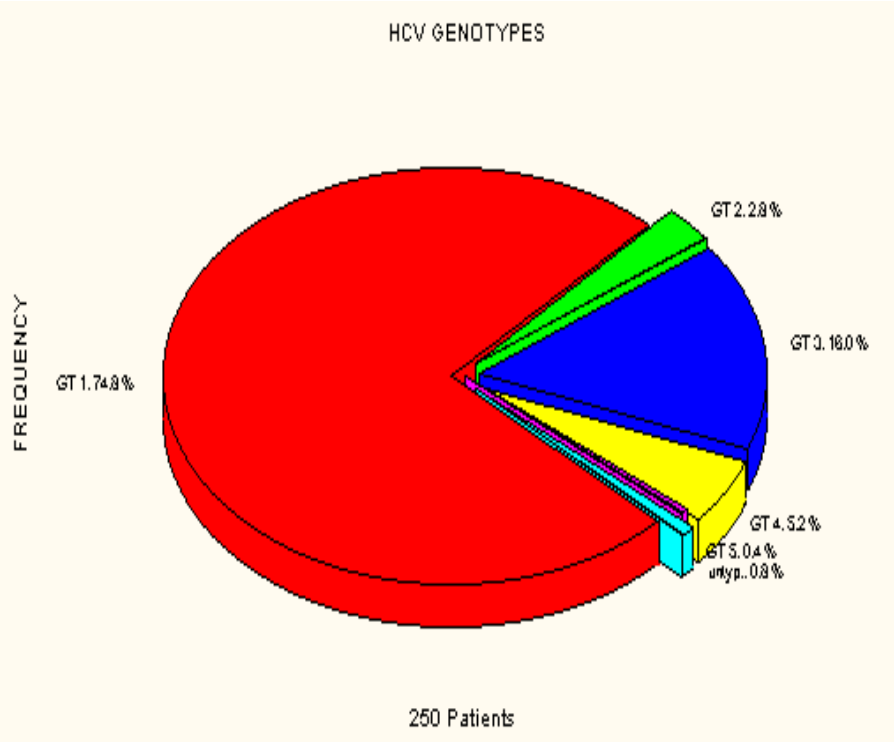

Fig. 6 HCV genotype distribution in Austria (13)

2.3 Transmission of HCV

The HCV can be found in blood, saliva, tears, and several body fluids including cerebrospinal and seminal fluid (2, 15). HCV is most frequently transmitted by blood and blood products when inoculated intravenously (for instance by blood transfusion). Although humans are the only natural host of HCV, chimpanzees have been infected experimentally, for instance by intravenous inoculation of saliva (1, 2, 16).

In developed countries, the transmission is nowadays mainly associated with intravenous drug abuse (1). The way of transmission from mother to child as well as that between sexual partners is possible, though uncommon in comparison to HBV. Another way of HCV transmission is through percutaneous medical procedures (2). In up to 30% of all patients with CHC, the source of the infection is not clearly identified (17).

2.3.1 Nosocomial transmission

Nosocomial transmission still plays an important role in the spreading of HCV. Before the introduction of screening blood products with a sensitive second-generation assay in 1992, patients with hemophilia and recipients of blood transfusion were at high risk of being infected (1).

Several cases of transmission events through medical procedures in nosocomial settings have been reported. Patient-to-provider, patient-to-patient or provider-to-patient transmission was described. The most frequent transmission from patient-to-health care worker is a needle stick with a hollow-bore, injection-style needle contaminated with blood from an HCV infected patient (18). Transmission after human bites and through splashes of blood of infected patients onto health workers' mucous membranes have also been reported, although very rarely (18, 19, 20).

There are numerous case reports of patient-to-patient transmission. HCV spread in an hemodialysis center has been suggested to be caused by environmental contamination, contaminated dialysis machines, inadequate infection control procedures, understaffing of the dialysis unit, and dialyzing infected and noninfected patients in the same area (18). In addition to infection in a hemodialysis setting, there is a variety of medical interventions and procedures that have been associated with HCV transmission such as administration of contaminated immunoglobulin, contamination of a multidose vial, contaminated intravenous administration devices, gynecological procedures, orthopedic procedures, endoscopy, colonoscopy, cardiothoracic surgery, organ transplantation, anesthesiologist's procedures and several more (18, 21, 22, 23). It is suggested that they are mainly due to inadequate infection control procedures or inadequate disinfection of devices or objects (18, 23).

Although provider-to-patient transmission has been less common, individual cases have been reported in the last years, some of them shown in Table 1 (18). As some cases are due to low adherence of hygiene guidelines, other provider-to-patient transmissions are associated with intravenous drug addiction of the provider.

Table 1
Studies investigating provider-to-patient transmission.

Source of infection	Number of infected patients
Cardiac Surgeon (24)	5
Anesthesiology Assistant (25)	5
Anesthesiologist (26)	3
Anesthesiologist (27)	33
Anesthesiologist (28)	275
Anti-D immune globulin (29)	390
Midwife (30)	1

2.4 Clinical manifestations

2.4.1 Acute hepatitis C

Acute HCV infection is usually asymptomatic, with 80% of patients presenting with no or a few symptoms (1, 2, 31). Typical symptoms such as malaise, nausea, and right upper abdominal pain may be present, commonly in a relatively mild form. Symptoms of pruritus, jaundice, pale stool, and dark urine are not frequent (1, 2). Only in every 5th patient, jaundice can be observed (2, 31). The incubation time varies between 2 and 10 weeks (17). Individuals developing typical symptoms of acute hepatitis (such as jaundice) are more likely to clear HCV infection spontaneously, the reactions of the body being associated to a strong immune response (1, 2). Generally, up to 25% of infected persons will clear HCV spontaneously (1).

Within days of exposure, the viral RNA can be detected in blood, while the development of HCV antibodies may require up to several weeks (1, 17). Serum

levels of liver specific enzyme such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and sometimes bilirubin are usually elevated (2).

Acute hepatitis C rarely develops into a fulminant form. Especially, patients additively infected with HAV show an increased probability of fulminant liver disease (2).

2.4.2 Chronic hepatitis C

If the hepatitis C infection persists longer than 6 months, it has become a chronic infection. Up to 30% of patients with CHC develop liver cirrhosis after 20-30 years (17). Co-Factors such as alcohol intake, elder age, or co-infection with HIV or HBV increase the risk significantly (17).

The majority of patients with CHC is asymptomatic or has few unspecific symptoms such as fatigue, tiredness, nausea, loss of appetite, and enlarged liver and spleen (2). The ALT level remains normal in 25% of patients with CHC, although there is HCV RNA detectable in blood (31). If elevated, serum aminotransferase levels may reach up to twice as high as normal levels (1). The majority of patients with CHC that stay asymptomatic in the acute phase of disease are not aware of their infection. They may be diagnosed accidentally after routine screening (for instance, for blood donation or after giving history of drug abuse), or because of an incidental finding of abnormal liver function test (20).

2.4.3 Complications of CHC

Besides the infection's impact on the liver, HCV is strongly associated with a couple of extrahepatic manifestations, such as lymphoproliferative disorders, essential mixed cryoglobulinemia, sporadic porphyria cutanea tarda, and membranoproliferative glomerulonephritis (1, 2). Further, in patients with HCV infection Sjögren's syndrome, autoimmune thyreoiditis, lichen planus, and type 2 diabetes mellitus have been found more often compared to non-infected people (1).

As already mentioned above, liver cirrhosis develops in up to 30% of patients with CHC after 20-30 years of infection (17). Common complications are ascites and variceal bleeding that occur in 80% of patients with cirrhosis within 10 years (1). After 5 more years, 50% of these patients develop liver failure, HIV coinfected patients progress even more rapidly (1).

A severe complication of CHC is the hepatocellular carcinoma (HCC). Every year, 1-5% of patients with cirrhosis are diagnosed with HCC. Sudden worsening of prior symptoms and signs of cirrhosis can be noticed, often associated with right upper quadrant pain (1, 2).

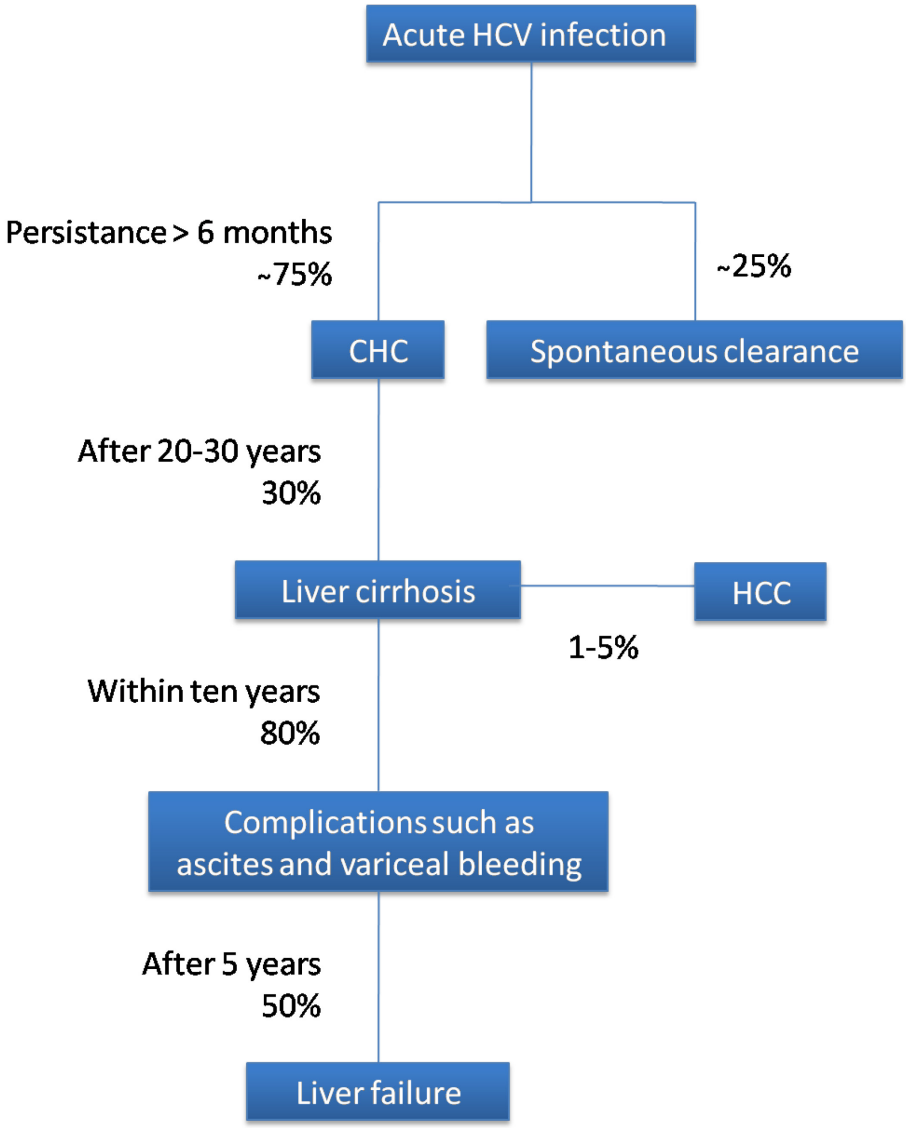

Fig. 7 Natural progress of HCV infection.

2.5 Diagnostics

2.5.1 Indirect detection – serology

In the majority of patients with HCV infection, the disease is initially diagnosed by detection of antibodies to recombinant HCV polypeptides by using an enzyme immunoassay (EIA) (1, 2). The EIA measures antibodies directed against NS3, NS4, core, and NS5 sequences (2, 32). EIAs are very important for screening. Third-generation assays show a high sensitivity of up to 97%. Antibodies can be detected 6 to 8 weeks after transmission (2). In immunocompromised patients, such as patients co-infected with HIV or on hemodialysis, the appearance of antibodies may be delayed or even absent, leading to a false-negative EIA result (1). Six months after exposure, more than 95% of patients have developed antibodies (1). The high sensitivity of EIAs can lead to false-positive results, especially in low prevalence populations (1, 2). Thus confirmatory testing must be performed to confirm a positive EIA result (1, 2). Today, all positive EIA results are confirmed by screening on HCV RNA. In case of a positive EIA test result but undetectable HCV RNA, a recombinant immunoblot assay (RIBA) can be useful to indicate whether the patient resolved a prior infection or the EIA test result was false-positive due to lack of specificity (2). The interpretation of results obtained by different HCV assay is shown in Table 2.

Table 2
Interpretation of results obtained by HCV antibody and nucleic acid testing.

Anti-HCV	HCV RNA	Interpretation
+	+	Acute or chronic HCV infection
+	-	Resolution of HCV infection; false positive serological result*
-	+	Early acute HCV infection; CHC in setting of immunosuppression
-	-	Absence of HCV infection

*An immunoblot assay may reveal unspecific reactivity of the immunoassay; if positive, repetition of HCV RNA testing within 6 -12 months suggested.

2.5.2 Direct detection - HCV RNA testing

Nucleic acid testing is not only important for confirmation after a positive HCV antibody test but also essential for assessing the response to antiviral therapy (1, 33). Presently, reverse-transcription real-time PCR (qPCR) is by far the most commonly used technology for detection and quantitation of HCV RNA. Today, commercially available assays for quantitation of HCV RNA are ultrasensitive and show a broad dynamic range (33).The lower limit of quantitation (LLQ) usually ranges between 10 and 15 IU/ml and the upper limit of quantitation (ULQ) between 1.8×10^7 and 1.0×10^8 IU/ml (34).

2.6 Treatment of HCV infection

The aim of anti-HCV therapy is the eradication of HCV (1). Eradication of HCV can be assumed in case of a sustained virological response (SVR), which means the absence of HCV RNA in blood six months after the end of treatment (1, 2, 35).

With introduction of interferon alpha (IFNa) for anti-HCV therapy in 1991, SVR rates of up to 16% of all patients treated could be achieved. The combination of IFNa with the guanosine analog ribavirin (RBV) and later pegylated IFNa (PegIFNa) with RBV led to a significant increase of SVRs (35, 36).

Studies showed that the HCV genotype influences the response to therapy (Fig.8). Further important factors with an impact on the success of treatment include younger age (<40), absence of cirrhosis, low body weight (BMI <30 kg/m²), Caucasian or Asian ethnic background, and low viral load at baseline (1, 35). In addition, a favorable IL28B polymorphism contributes to a higher chance of SVR in IFN-based treatment regimes (1).

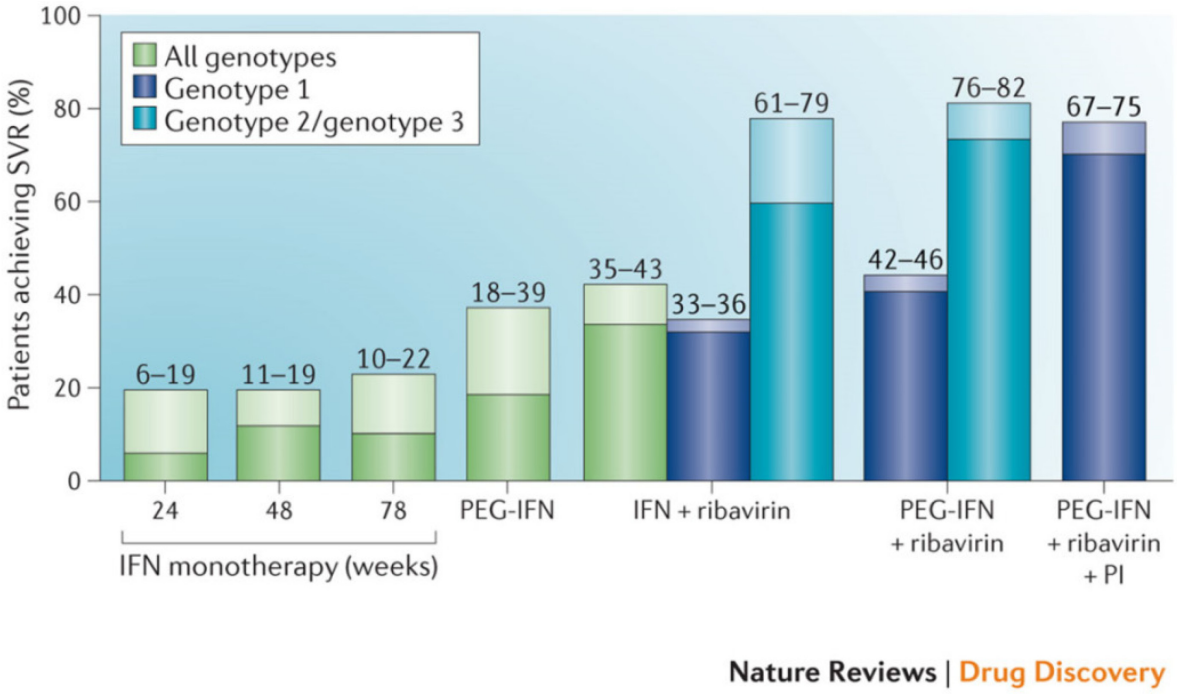

Fig. 8 Improvement of SVR rates in patients with chronic hepatitis C through evolution of anti-HCV therapies (12).

With dual therapy, the response to treatment was not satisfying at all, especially in genotype 1 (Fig. 8). New generation drugs have thus been developed, including NS3/4A protease inhibitors (the "-previrs"), NS5B polymerase inhibitors (the "-buvirs"), NS5A inhibitors (the "-asvirs"), and cyclophilin inhibitors (the "-sporivirs") (Fig. 9). Another host-target agent is mirvirsen, which directs to the liver-specific micro-RNA miR-122, leading to a significant suppression of viremia and showing no major side effects (36). Furthermore, the use of interferon lambda could be an alternative to interferon alpha in the future (36).

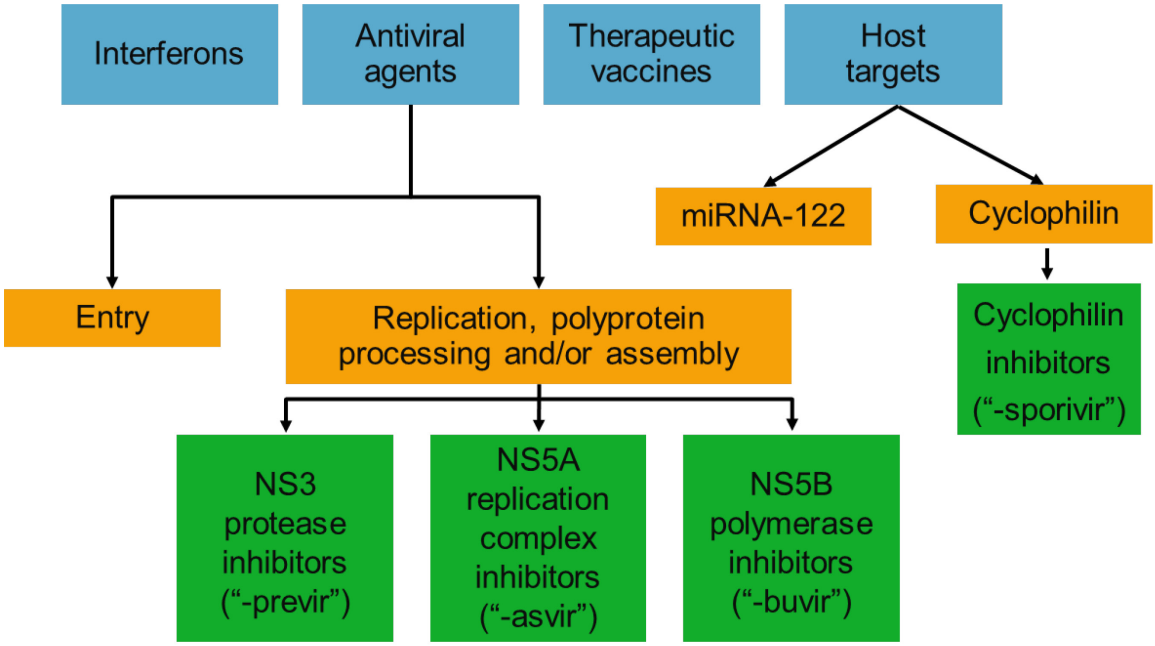

Fig. 9 Different classes of direct acting antivirals (DAAs) for treatment of CHC (kindly provided by Harald H. Kessler).

Currently, triple therapy with PegIFNa, RBV, and one of the first generation protease inhibitors is state-of-the-art (Fig 10) (35, 36). The first new DAAs approved by the US Food and Drug Administration (FDA) and the European Medicine Agency (EMA) in 2011 were telaprevir and boceprevir, inhibitors of the NS3/NS4 protease. These new drugs are only effective in HCV genotype 1

infections. Triple therapy including telaprevir or boceprevir has improved the SVR rate significantly and has also led to a shortening of treatment duration (1, 35, 36).

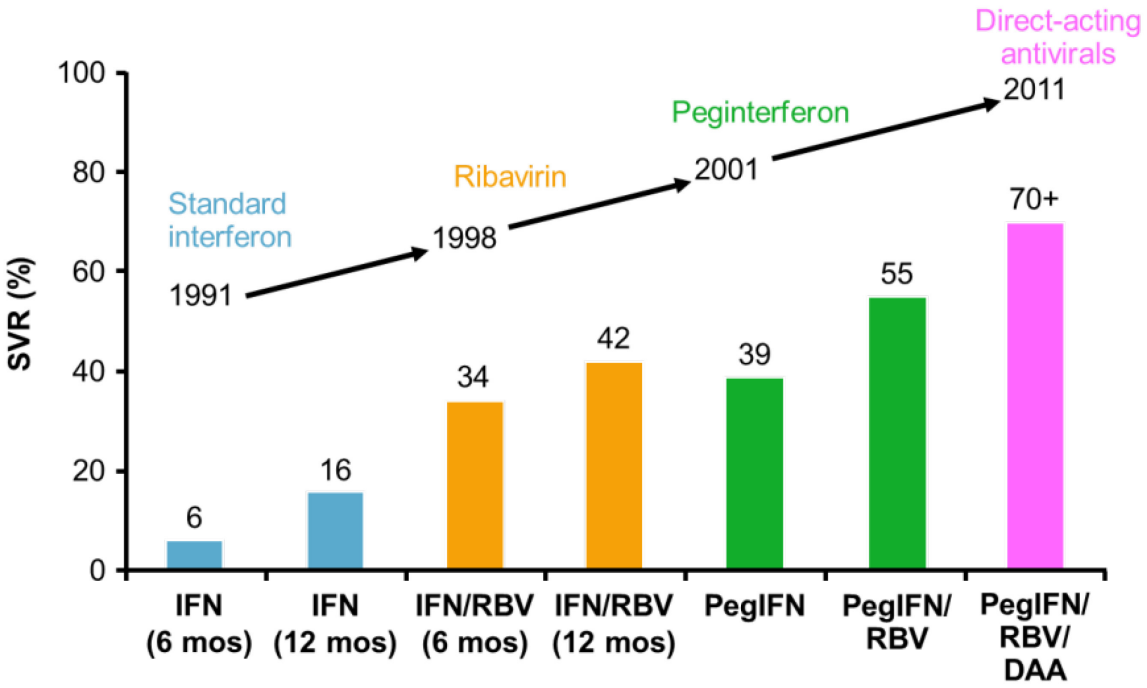

Fig. 10 Evolution of anti-HCV treatment (kindly provided by Harald H. Kessler).

However, side effects are common with anti-HCV therapy. IFN may lead to fatigue, rash, influenza-like symptoms, bone marrow suppression, mood swings, and severe depression (1). Its use is contraindicated in patients with autoimmune disease, with uncontrolled depression and mental illness, after renal and cardiac transplantation, and with decompensated pulmonary disease (1, 36). RBV can lead to hemolytic anemia during treatment (1). It is contraindicated in pregnancy and patients with advanced renal disease (36). Side effects of therapy with first-generation protease inhibitors include rash with pruritus (telaprevir) and (severe) anemia (boceprevir and telaprevir) (Fig. 11).

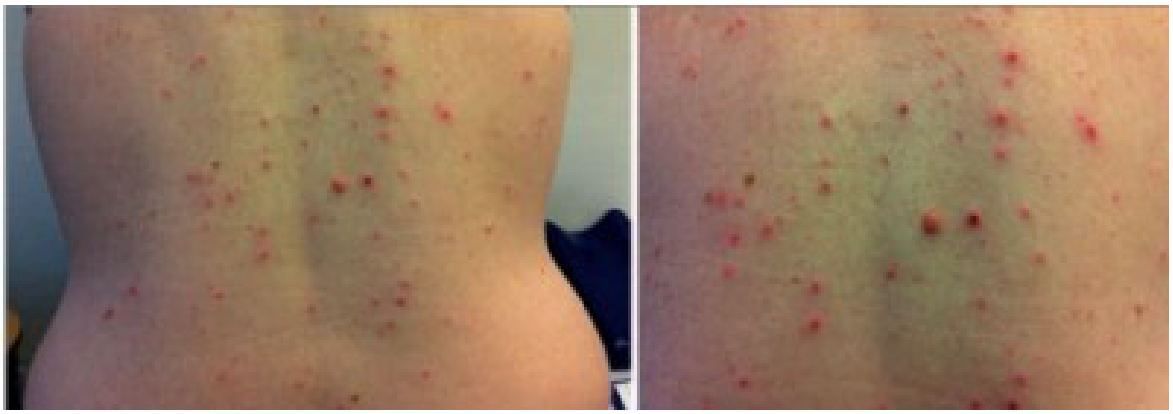

Fig. 11 Patient with rash and severe pruritus following telaprevir-based anti-HCV triple therapy (37).

2.7 Aims of the study

In this study, the exposure to risk factors of HCV infection was investigated in a group of patients with HCV subgenotype 2a infection living or having lived for more than 10 years in a well-defined West Styrian region (district of Voitsberg) with a previously unknown way of transmission. Furthermore, this study aims at identification and description of the most probable source of transmission in this group of patients.

3 Materials and methods

This study was conducted at the Institute of Hygiene, Microbiology, and Environmental Medicine (IHMEM), Medical University of Graz, according to principles of the Declaration of Helsinki. The study protocol and all study procedures were reviewed and approved by the local ethics committee. All participants gave written informed consent.

In a routine laboratory, the Molecular Diagnostics Laboratory, IHMEM, Medical University of Graz, thirty-four patients (28 females and 6 males) with CHC were identified to be infected with HCV isolate HCJ5 (GenBank Accession No. D10075) of HCV subgenotype 2a. Twenty-three patients infected with this isolate having lived or living in the West Styrian district of Voitsberg for at least 10 years were invited to participate in this study. Patients received a letter including an explanation of the purpose of the survey and a questionnaire (see Adnex 1). To identify the possible way of transmission and to find a possible common source of infection, data about medical interventions and other events that could have led to HCV infection were collected.

In the questionnaire, patients were asked when they were diagnosed with HCV for the first time and whether they had contact with any healthcare setting prior to diagnosis. In case of one or more contacts, date(s), hospital(s) including department(s), and the reason(s) for the contact(s) were investigated. Furthermore, the patients were asked if certain medical procedures such as gastroscopy, colonoscopy, blood transfusion, plasma donation or dialysis had been performed. Further possible reasons of infection such as drug abuse, ozone therapy, tattooing, piercing, manicures, and pedicures were also included in the questionnaire. Additionally, patients were asked to name their dentist(s), general practitioner(s), and specialist(s) including gynecologists, urologists, etc. they had contacted.

All data were collected anonymously. Each patient received a code number. After sending the questionnaire by surface mail to the patients, they were contacted by phone and asked whether they wanted to participate or not. In case they agreed, a personal meeting was arranged, the purpose of the survey was explained, and the questionnaire completed.

4 Results

4.1 Socio-demographic data

Of 23 patients invited to participate in this study, 3 patients did not respond to the letter and repeated phone calls, 2 patients did not want to participate due to health problems or unclear reasons, and 2 patients had already died.

Of the 16 patients participating in the study, 14 were female and 2 were male. The participants were born between 1938 and 1967 (Fig.12). The mean age was 66 years. All of the 16 patients included in this study were Austrians. None of the patients had been employed in the healthcare setting, had ever taken any illicit drugs, had a tattoo made, had had a manicure or pedicure, and had been in prison. All of the patients claimed no knowledge of how they had acquired their hepatitis C infection.

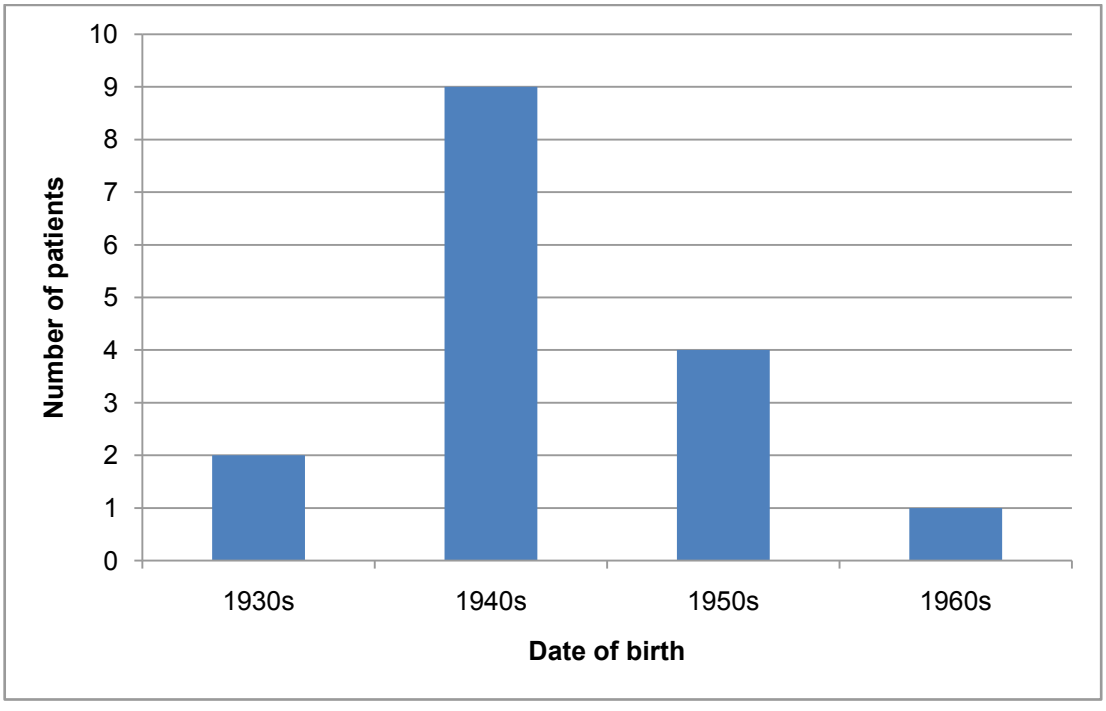

Fig. 12 Age distribution of patients participating in the study.

4.2 Hospital exposure

Table 3 shows a summary of reported hospital exposure with invasive procedure(s) of the study population prior to diagnosis of HCV infection. All of the patients were hospitalized at least once in their lives. Nine patients had exposure to invasive procedure(s) at the division of surgery at hospital A, 8 patients at the division of obstetrics and gynecology at hospital A, and 5 patients at the division of internal medicine of this hospital. Four patients had exposure to the department of surgery at hospital B and 4 patients to the department of obstetrics and gynecology of the identical hospital. Three patients had exposure to the division of surgery of hospital C. Six patients reported a single exposure to alternative hospitals.

Invasive procedures performed at the corresponding divisions of hospital A requiring general anesthesia are shown in Table 4. Thirteen patients had at least one event that required general anesthesia that had been performed between 1953 and 1995.

Table 3
Hospital exposure with invasive procedure(s) including endoscopy prior to HCV diagnosis.

No. patients	Division of surgery/ hospital A	Division of obstetrics and gynecology/ hospital A	Division of internal medicine/ hospital A	Department of surgery/ hospital B	Department of obstetrics and gynecology/ hospital B	Division of surgery/ hospital C	Other divisions
1		X		X			
2		X					
3	X			X			
4		X					X
5	X	X	X				
6	X		X	X		X	X
7		X		X	X		
8	X	X	X				X
9	X	X					
10					X	X	X
11	X						
12	X	X					
13			X				X
14					X	X	X
15	X						
16	X		X		X		
Total	**9**	**8**	**5**	**4**	**4**	**3**	**6**

Table 4
Patients with events that required general anesthesia performed at hospital A.

No. Patients	Division of obstetrics and gynecology/ hospital A	Division of surgery/ hospital A
1	Curettage	
2	Caesarean section	
3		Appendectomy
4	Caesarean section	
5	Tubal occlusion	Appendectomy
6		Appendectomy
7	Curettage (2x)	
8	Hysterectomy	Varicectomy
9	Hysterectomy	Inguinal hernia surgery (2x)
11		Appendectomy
12	Caesarean section (2x)	Appendectomy, tonsillectomy
15		Appendectomy
16		Tonsillectomy, appendectomy

4.3 Exposure to gastroscopy

In Table 5, exposure to gastroscopy prior to diagnosis of HCV infection is summarized. Of the 16 patients, 6 had at least one gastroscopy prior to HCV diagnosis. Three of them had exposure to internal specialist A and 2 patients to internal specialist B. Two patients had (additional) exposure to the division of internal medicine of hospital A where the procedure was performed. One patient reported a single exposure to an alternative internal specialist.

Table 5
Exposure to gastroscopy prior to HCV diagnosis.

No. patients	Internal specialist A	Internal specialist B	Division of internal medicine/ hospital A	Other specialists
1		X		X
5			X	
7	X			
8	X		X	
13		X		
16	X			
Total	**3**	**2**	**2**	**1**

4.4 Exposure to colonoscopy

Exposure to colonoscopy is summarized in Table 6. Of the 16 patients, 8 had at least one colonoscopy prior to HCV diagnosis. Two patients had exposure to the internal specialist A, 2 patients to the internal specialist B, and 2 patients to the division of internal medicine of hospital A. Three patients reported a single exposure to alternative hospitals.

Table 6
Exposure to colonoscopy prior to HCV diagnosis.

No. patients	Internal specialist A	Internal specialist B	Division of internal medicine/ hospital A	Other specialists
1		X		
4				X
5			X	
8	X			
10				X
13		X		
14				X
16	X		X	
Total	**2**	**2**	**2**	**3**

4.5 Exposure to invasive dental procedures

Table 7 summarizes the exposure to invasive dental procedures of the study population prior to diagnosis of HCV infection. All of the patients but one had invasive dental procedures. Nine patients had exposure to dentist A, 4 patients to dentist B, 2 patients to dentist C, 2 patients to dentist D, and 2 patients to dentist E. Two patients had exposure to an outpatient clinics operated by a social insurance company. Eight patients reported an exposure to alternative dentists.

Table 7
Exposure to invasive dental procedures prior HCV diagnosis.

No. patients	Dentist A	Dentist B	Dentist C	Dentist D	Dentist E	Outpatient clinic of social insurance company	Other dentists
1		X					X
3	X			X			
4	X						X
5			X	X			
6		X	X				
7	X				X		X
8	X						
9	X						X
10	X						X
11	X				X		
12	X	X					X
13		X					
14							X
15	X					X	
16						X	X
Total	**9**	**4**	**2**	**2**	**2**	**2**	**8**

4.6 Exposure to invasive urologic procedures

Table 8 summarizes the exposure to invasive urologic procedures of the study population prior to diagnosis of HCV infection. Eleven patients had exposure to urologist A and three had additional exposure to urologist B. All of the 11 patients treated by urologist A had a cystoscopy because of urinary tract infection.

Table 8
Exposure to invasive urologic procedures prior HCV diagnosis.

No. patients	Urologist A	Urologist B
1	X	X
2	X	
4	X	
5	X	
6	X	
7	X	
9	X	
10	X	X
12	X	
13	X	X
15	X	
Total	**11**	**3**

4.7 Exposure to interventions of general practitioners

Table 9 summarizes the reported exposure to the study population through procedures of general practitioners prior to diagnosis of HCV infection. All of the patients had procedures performed at least once in their lives. All of them reported multiple exposures to injections and/or infusions such as childhood vaccinations and analgesics. Five patients had exposure to procedures at general practitioner A, 2 at general practitioner B, 2 at general practitioner C, 2 at general practitioner D, 2 at general practitioner E, and 2 at general practitioner F. Fourteen patients reported additional exposure to alternative general practitioners.

Table 9
Exposure to interventions of general practitioners.

No. patients	General practitioner A	General practitioner B	General practitioner C	General practitioner D	General practitioner E	General practitioner F	Other general practitioners
1				X		x	
2							x
3							x
4	x				X		x
5							x
6							x
7			x				x
8	x						x
9	x						x
10							x
11	x	x	x				x
12					X		x
13				X		x	x
14							x
15	x	x					
16							x
Total	**5**	**2**	**2**	**2**	**2**	**2**	**14**

4.8 Exposure to transfusion of blood and blood products

Patients who received blood and/or blood products prior to HCV diagnosis are shown in Table 10. Of 16 patients, 4 received blood and/or blood products prior HCV diagnosis.

Table 10
Exposure to transfusion of blood and/or blood products.

No. patients	Blood transfusion
2	X
8	X
12	X
16	X
Total	**4**

4.9 Other risk factors

Ozone blood therapy

Two patients (patient 10 and patient 15) reported that they had ozone blood therapies performed by two different general practitioners.

Plasma donation

One patient (patient 12) donated plasma.

Piercing

One patient (patient 16) had a piercing.

4.10 Multiple exposures

The 15 patients with exposure to the 3 most frequently reported health care settings were investigated on multiple nosocomial exposures (Fig. 13). All but 2 of the 15 patients showed exposure to more than one health care setting.

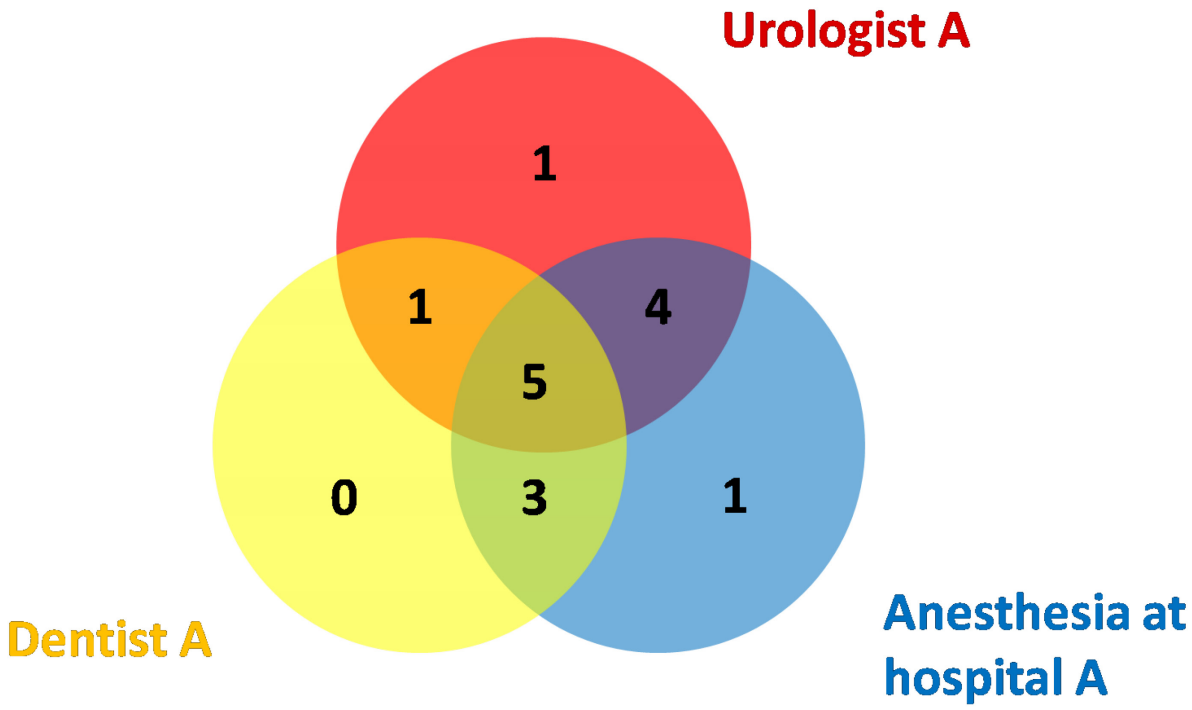

Fig. 13 Amount of patients that had undergone invasive procedures at urologist A, dentist A or general anesthesia at hospital A.

5 Discussion

HCV is a global major pathogen with about 170 million people infected worldwide (1, 2). When persisting longer than 6 months, HCV infection has become chronic. CHC can lead to severe complications such as liver cirrhosis, variceal bleeding, and hepatocellular carcinoma (1). There are at least six major HCV genotypes that can further be divided in subgenotypes (2). Genotype 2 is found in 3 - 4.5% of Austrian CHC patients (13, 14).

In this study, all participants were infected with subgenotype 2a. With regard to a common source of HCV infection, the majority of patients had undergone invasive urological procedure(s), procedure(s) requiring general anesthesia, and/or invasive dental procedure(s) at 3 different health care settings: urologist A, hospital A (division of surgery and division of gynecology and obstetrics), and dentist A. Fifteen of the 16 patients were exposed to at least one of these three health care settings. The remaining patient was exposed to procedures at other health care institutions.

According to recent studies, anesthetic procedures represent a possible mode of transmission for HCV (25, 26, 27). Viral transmission can occur due to contamination of multidose medication vials designed for single-patient use; however, used on multiple patients as recently reported (20). Furthermore, HCV can be transmitted by the re-use of contaminated needles and syringes (21).

In this study, 13 patients had at least one surgery requiring general anesthesia performed at hospital A. Because the time period was found to be more than 40 years, with single, widely varying events, it is assumed that a common source of infection related to anesthetic procedures seems very unlikely. It is suggested that different anesthetic teams performed the invasive procedures throughout the time.

Patient-to-patient transmission during colonoscopy was shown in a case report of Bronowicki et al. in 1997 identifying an insufficient disinfection of the endoscope as the most likely cause of HCV transmission in that case (23). In the present study, 6 participants were exposed to gastroscopy and 8 participants were exposed to colonoscopy prior to diagnosis of HCV infection. Besides improper disinfection, anesthetic procedures performed for endoscopic diagnostics were reported to represent an additional risk factor (22). However, in this study, patients

underwent colonoscopy at different health care settings with a lack of a common staff. Therefore, it seems unlikely that the transmission took place during endoscopy.

Regarding invasive dental procedures, there may be a transmission risk, although there is a need for more studies on this issue (38). In the present study, all participants, but one had exposure to invasive dental procedures prior to HCV diagnosis. Of the 16 participants, 9 had at least one invasive dental procedure at dentist A.

A possible contribution to HCV transmission by general practitioners, who had treated the participants with injections and/or infusions, was also studied here. No common source of infection could be identified in one of the numerous general practitioners. Remarkably, one patient who was found to have hardly any similarities with all other patients was treated by a general practitioner who was infected with the same HCV subgenotype and isolate.

The most interesting finding observed in the present study was that urologist A performed irrigations by cystoscopies in 11 of all 16 patients. Transmission of HCV infection through cystoscopy has not yet been reported in literature. However, transmission cannot be ruled out if the irrigating equipment is reused and unrecognized reflux of fluid occurres. The irrigation system may then become contaminated as described in a study by Molina-Navarro in 1999 (39). It is estimated that during the period of time when urologist A was working, it was usual to re-use fluid and the giving-sets (fluid administration apparatus), as it occurred in the West Midlands. Although that study described only refluxes in men, the possibility of refluxes in women cannot be ruled out, as there were twice as many male patients than female patients participating in that study.

An interesting and notable point in the present study is the ratio of female to male patients – 14 female patients and 2 male patients. If the transmission had taken place during urologic procedures, an important factor might be that female patients often visit a urologist already at a younger age because of recurring urinary tract infections. Men typically see a urologist when they are older, mainly because of prostatic hyperplasia. It is estimated that the transmissions occurred in the 70s, 80s and/or 90s of the last century. Many of the male patients that were treated at that time may have already died. This would explain why the majority of participants were female.

Urologist A was contacted by phone; however, as he is aged over 90, no further reliable data was available. At least he denied experiencing symptoms compatible with hepatitis and did not remember having had elevated aminotransferases at any time.

A general limitation of this study is that the data collected only relied on the memories of the participants. Most patients who develop acute hepatitis C do not have or only have unspecific symptoms making it very difficult to determine the onset of the disease. It is estimated that the transmission had occurred decades of years ago. As a result, it was very difficult to investigate all the possible risk factors properly. Unfortunately, it was not possible to evaluate, if patients had invasive procedures at the same health care setting on the same day that would indicate a patient-to-patient transmission. The majority of patients were only able to remember the year in which they had undergone the procedure(s). Filed data were incomplete and did not go back far enough. Additionally, it has to be mentioned that the participants could have denied uncomfortable questions such as the past use of illicit drugs.

Another limitation is the small number of patients that participated in this study. Of 23 patients, seven patients did not answer the letters or phone calls, had already died, or did not want to participate. As a result, it was only possible to investigate 16 patients that were identified to be infected with this rare HCV subgenotype.

In conclusion, the majority of patients included in this study were exposed to invasive procedures at multiple health care settings. The number of patients that had one or more cystoscopies at urologist A was remarkable and may be the most probable source of transmission in this group of patients.

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Adnex 1

Fragebogen zur Hepatitis C Infektion

Studien-ID-Nr.:

Patient/Patientin:

Geburtsdatum:

Seit wann ist bei Ihnen die Hepatitis C bekannt?

Hatten Sie vor der Diagnose Hepatitis C Krankenhausaufenthalte?

☐ja ☐nein

Wenn ja, nennen Sie bitte das Krankenhaus, den Zeitraum sowie den Grund für Ihren Aufenthalt (z.B. Operation).

Wo?	Wann?	Warum?

Wurden bei Ihnen folgende Untersuchungen oder Behandlungen durchgeführt?

Gastroskopie (Magenspiegelung)?

☐ja ☐nein

Wenn ja:

Wann und wo?_____

Durchführender Arzt?_____

Koloskopie/Rektoskopie (Darmspiegelung)

☐ja ☐nein

Wenn ja:

Wann und wo?_____

Durchführender Arzt?_____

Ozon/Eigenbluttherapie

☐ja ☐nein

Wenn ja:

Wann und wo?_____

Durchführender Arzt?_____

Haben Sie jemals eine Bluttransfusion oder Plasmaspende erhalten?

☐ja ☐nein

Wenn ja:

Wann und wo?_____

Haben Sie jemals Blutplasma gespendet?

☐ja ☐nein

Wenn ja:

Wann und wo?_____

Wurde bei Ihnen jemals eine Dialyse durchgeführt?

☐ja ☐nein

Wenn ja:

Wann und wo?_____

Haben Sie eine Tätowierung/ein Piercing?

☐ja ☐nein

Wenn ja:

Wann haben Sie es sich stechen lassen?_____

Von wem?_____

Gehen oder gingen Sie jemals zur Maniküre oder Pediküre?

☐ja ☐nein

Wenn ja:

Wann und wo?_____

Haben Sie jemals Drogen gespritzt oder geschnupft?

☐ja ☐nein

Wenn ja:

In welchem

Zeitraum?_____

Welche Zahnärzte/welche Zahnärztinnen behandelten Sie bisher? In welchem Zeitraum?

Wer?	Wann?

Welche Hausärzte/welche Hausärztinnen behandelten Sie bisher? In welchem Zeitraum?

Wer?	Wann?

Bei welchen Ärzten/Ärztinnen (z.B. orthopädische Behandlungen) waren Sie außerdem vor ihrer Diagnose der Hepatitis C in Behandlung? In welchem Zeitraum?

Wer?	Wann?

Fallen Ihnen noch weitere mögliche Ursachen ein, die für Ihre Hepatitis C-Erkrankung verantwortlich sein könnten?

☐ja ☐nein

Wenn ja:

Welche?_____

_____

_____

Vielen Dank für Ihre Hilfe!