International Journal of Infectious Diseases 41 (2015) 1–2

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Case Report

Diagnosis of hepatitis C virus genotype 2k/1b needs NS5B sequencing

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ARTICLE INFO

Article history:
Received 28 September 2015
Received in revised form 12 October 2015
Accepted 12 October 2015

Corresponding Editor: Eskild Petersen, Aarhus, Denmark

Keywords:
Hepatitis C virus
HCV 2K/1b
Belgium

SUMMARY

Hepatitis C virus (HCV) is probably the most common cause of liver cirrhosis and hepatocellular carcinoma worldwide. The correct identification of HCV genotype has important clinical implications as a marker of responsiveness to antiviral therapy and serves as a guideline for the duration of treatment. The VERSANT HCV Genotype 2.0 Assay failed to detect HCV genotype 2k/1b. HCV genotype 2k/1b detection requires NS5B sequencing.

1. Introduction

Hepatitis C virus (HCV), a single-stranded enveloped RNA virus, is probably the most common cause of liver cirrhosis and hepatocellular carcinoma worldwide and is one of the major global public health problems. HCV is highly heterogeneous, with seven confirmed major genotypes and 67 confirmed subtypes. Each genotype displays a different geographic distribution.[1] Since different HCV genotypes react differently on available antiviral therapies, the correct identification of HCV genotype serves as a marker of responsiveness and an indicator for duration of treatment.

A few cases of HCV genotype 2k/1b have been reported in Western Europe.[2–5] However, to our knowledge, no cases of HCV genotype 2k/1b have been reported in Belgium thus far.

2. Case series

Four patients in AZ Sint-Jan Bruges, Belgium were identified retrospectively as having HCV recombinant genotype 2k/1b between January 2012 and December 2014. The sequencing strategy was performed using the HCV NS5B sequencing method (Murphy et al., 2007), which has recently replaced the VERSANT HCV Genotype 2.0 Assay (Siemens Medical Solutions Diagnostics, Tarrytown, NY, USA) used in our laboratory.[6]

2.1. Patient 1

In May 2012, a 33-year-old male chronic intravenous (IV) drug user (heroin and 'krokodil') from Georgia was admitted to our hospital with pancytopenia, B-symptoms, haematemesis, and hepatosplenomegaly. Laboratory results revealed a significant bone marrow suppression: thrombocytopenia (87×10^6/l), anaemia (3.25×10^{12}/l), leukopenia (2.4×10^9/l), an elevated erythrocyte sedimentation rate (120 mm/h), and a mild elevated lactate dehydrogenase (LDH; 711 U/l); there were no other abnormalities. Broad serological screening for possible causal agents was negative: hepatitis B surface antigen (HBsAg), HIV (HIV Ag/antibody (Ab)), hepatitis A virus (HAV) immunoglobulin M (IgM), cytomegalovirus IgM, *Toxoplasma gondii* IgM, Epstein–Barr virus (viral capsid antigen) IgM, *Brucella spp*, *Coxiella burnetti*, Leishmania, and Histoplasma antibodies. Serological HCV testing was positive. HCV RNA was detected (viral load (VL) 4.3×10^6 IU/ml) and initially diagnosed as genotype 2a/2c by the VERSANT HCV Genotype 2.0 Assay, although NS5B sequencing revealed HCV genotype 2k/1b.

A contrast-enhanced abdominal computed tomography (CT) scan revealed hepatosplenomegaly. CT examination of the head and thorax showed no adenopathy, and neither did a CT of the abdomen. Further investigation revealed disseminated tuberculosis (TB) with a positive intradermal tuberculosis test and 'foreign body' hepatic granulomatosis possibly due to his past IV drug use. Tuberculostatics were started and clinical symptoms regressed. His HCV infection remained untreated considering the more urgent need for four-drug TB therapy (rifampicin, isoniazid, ethambutol, and pyrazinamide) and possible drug interactions. Three months later, during a follow-up visit, he presented with general malaise, fatigue, anorexia, and headache. A manifest regression of his hepatosplenomegaly and adenopathy was observed. Laboratory workup showed a normal blood cell count and decreased inflammatory parameters. The HCV infection remained untreated and the TB four-drug regimen was continued. In December 2012, he became homeless and was lost to follow-up.

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http://dx.doi.org/10.1016/j.ijid.2015.10.010

2.2. Patient 2

In September 2012, a 32-year-old Russian (Chechen) man was admitted with a provisional diagnosis of HCV genotype 2a/2c hepatitis (HCV antibody (HCAb)-positive). He had a pre-existing chronic hepatitis B virus (HBV) infection (HBsAg, hepatitis B surface antibodies (HBsAb), hepatitis B core antibody (HBcAb), and hepatitis B envelope antibody (HBeAb) positive, but hepatitis B envelope antigen (HBeAg) negative). The patient had a history of recurrent sexually transmitted diseases and *Helicobacter pylori*-positive gastritis. HCV RNA was detected (17.0×10^6 IU/ml). NS5B sequencing confirmed HCV genotype 2k/1b, originally misclassified as 2a/2c. For his HCV infection, he received treatment with peginterferon (PEG-IFN)/ribavirin (RBV) therapy, with an initial decrease in the viraemia, but no sustained virological response (SVR) after 24 weeks. Based on his HCV genotype 2k/1b and in parallel with the chronic active HBV infection, a longer treatment protocol of 48 weeks was necessary. In July 2013, his HCV RNA levels remained undetectable, indicating successful clearance of the virus. One year later, an increase in his HCV VL (28.0×10^6 IU/ml) and a stable HBV VL (26 IU/ml) was observed.

2.3. Patient 3

In March 2013, a 38-year-old Georgian woman was admitted with respiratory bound upper-right abdominal quadrant pain of many years duration. She mentioned a past HBV infection (HBsAb and HBcAb positive, due to a needle accident). Clinical examination was normal. Laboratory workup showed a mild elevation of liver enzymes (alanine aminotransferase (ALT) 72 U/l and aspartate aminotransferase (AST) 49 U/l) along with an active HCV infection with HCAb positivity and detectable HCV RNA (VL 2.4×10^6 IU/ml). NS5B sequencing revealed HCV genotype 2k/1b. PEG-IFN treatment was started (intended duration 48 weeks). After 32 weeks of therapy, the treatment was stopped due to progressive and intolerable neurological complaints, including muscular weakness, general malaise, fatigue, and a depressive mood disorder. During follow-up visits, laboratory parameters returned to the physiological range and the HCV viraemia remained undetectable, indicating successful clearance of the virus.

2.4. Patient 4

In December 2014, a fourth case of HCV 2k/1b was diagnosed in a 34-year-old Chechen man. An HCV VL of 16.9×10^6 IU/ml was observed, as well as a negative HBV serology. He refused antiviral therapy and a diagnostic liver biopsy, stating that he wanted to be treated and followed in his homeland. Once discharged from prison, the patient was lost to follow-up without prescription treatment.

3. Discussion

The recombinant HCV genotype 2k/1b was first described in Saint Petersburg in 2002 in IV drug users.[7] Since its discovery, only a few cases of HCV genotype 2k/1b have been reported in Western Europe.[2–5] In all four cases, the patients originated from the Caucasus, where HCV genotype 2k/1b is endemic. A sequencing strategy led to the detection of HCV genotype 2k/1b. However, clinical laboratories often use the VERSANT HCV Genotype 2.0 Assay for routine HCV genotyping. This molecular second-generation line probe assay (LiPA) may potentially misclassify these HCV strains as HCV genotype 2a/2c.[8,9] Based on these findings, the number of patients infected with HCV 2k/1b may be underestimated.

It is widely recognized that patients infected with genotype 2 in particular are more likely to achieve a faster SVR than patients infected with genotype 1. Seventy-five to eighty percent of genotype 2 patients achieve SVR after 24 weeks of antiviral treatment (PEG-IFN plus RBV) versus only 50% of genotype 1 patients after 48 weeks treatment. Furthermore, in general 70% (genotype 1a) to 80% (genotype 1b) of patients achieve a SVR when protease inhibitors are included in the therapy.[10]

Due to the limited information on susceptibility of the recombinant 2k/1b HCV genotype to antiviral treatment, no recommendations exists on the duration of therapy or optimal therapy.[11] Studies show discrepant results regarding IFN treatment of HCV 2k/1b and SVR. One study has shown a higher susceptibility of the 2k/1b genotype to PEG-IFN/RBV treatment than another.[4,10] In 2014, Hedskog et al. showed lower SVR rates among patients infected with recombinant HCV forms on direct-acting antiviral (DAA) therapy.[11] HCV 2k/1b patients could therefore be considered for longer treatment durations or DAA therapy.[4,9,10] Regarding our cases, a longer PEG-IFN-based treatment, 48 weeks instead of the expected 24-week protocol for genotype 2a/2c, was needed for two patients to obtain SVR. A new DAA, sofosbuvir, has recently been approved in Europe for the treatment of chronic HCV patients (all genotypes) and has shown high rates of SVR when given with RBV.[12] Based on the actual strict reimbursement criteria in Belgium, none of these 2k/1b patients would have been eligible for treatment with sofosbuvir at the moment of diagnosis and genotyping (infections with recombinant HCV strains are not mentioned within these criteria). The detection of these cases stresses the importance of determining the HCV genotype by NS5B sequencing. Exact HCV genotyping and subtyping facilitates treatment options and serves as a guide for clinical decision-making.

Funding: None.

Conflict of interest: The authors have no conflicts of interest.

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