Evaluation of a new real time PCR method for the determination of HCV NS3/4A Q80K polymorphism

Authors: Dario Russo[2], Antonella Marzorati[2], Alessio Colombo[1], Dimitra Mougiou[3], Orfanou Eleni[3], Georgia Gioula[3]

1. Fleming Research Service Lab , Via Bernardo Quaranta 57, 20139 Milano (MI)
2. Clonit srl, Via Bernardo Quaranta 57, 20139 Milano (MI)
3. Laboratory of Hepatology, B' Clinic of Internal Medicine, Aristotle University of Thessaloniki, Greece

Objectives: Hepatitis C Virus (HCV) infection constitutes a world health problem. The vast majority of this infection leads to chronic liver disease, hepatocellular carcinoma, and end-stage liver diseases. The standard treatment for HCV genotype 1 chronic infection has been a combination of pegylated interferon (PEG-IFN) and ribavirin (RBV) for 48 weeks or longer. Simeprevir (TCM435, Olysio™; Janssen Therapeutics) is one of the second-generation HCV NS3/4A protease inhibitors that allow significant improvement of SVR rates for HCV genotype 1 infected patients.
Simeprevir, when used in combination with pegylated interferon and ribavirin, has shown a limited effect in HCV genotype 1a samples that have the naturally occurring Q80K polymorphism. The aim of this study was to detect the Q80K polymorphism in HCV genotype 1a positive samples using a new real time PCR method.

Methods: Plasma samples from 52 HCV-positive patients infected with HCV genotype 1a were randomly selected from the Laboratory of Hepatology, B' Clinic of Internal Medicine, Medical School, Aristotlle University of Thessaloniki and analyzed.
RNA extraction was performed using QIAamp Viral RNA Mini kit (QIAGEN) on the QIAcube instrument. Viral genotyping was determined using the VERSANT HCV Genotype 2.0 Assay (LiPA) (Siemens Healthcare Diagnostics).

Real time PCR was performed using the new Q80K polymorphism kit (Clonit srl) on the VERSANT kPCR AD, Life Technologies 7500 Fast, and Rotor-Gene Q instruments. All samples were also analyzed by NS3/4A sequencing using Life Technologies 3500 genetic analyzer system at Fleming Research labs – Milan

Results: According to our results, 7 out of 52 (14%) tested clinical samples harbored the Q80K polymorphism; the remaining 44 (86%) harbored the wild type variant.
During the evaluation, one sample could not be analyzed using the real time PCR method. Sequence analysis showed that a different amino acid substitution (Q80L) was present, which was not recognized by the assay

Conclusion: Clinical trial data showing the decreased rate of sustained virologic response in the presence of HCV genotype 1a virus with the Q80K polymorphism and the prevalence of this polymorphism shown in our study, we conclude that it is important to screen for this mutation to discriminate patients eligible for treatment with Simeprevir.
This new real time PCR based assay correlates perfectly with sequencing; moreover the number of clinical samples harboring Q80 polymorphisms observed in this study is consistent with previous reports. It is also an easy method to implement and can be an effective tool to determine HCV NS3/4A Q80K polymorphisms.