DETECTION OF Q80K POLYMORPHISM USING REAL-TIME PCR AND NEXT GENERATION SEQUENCING PRIOR TO SIMEPREVIR THERAPY IN HCV PATIENTS

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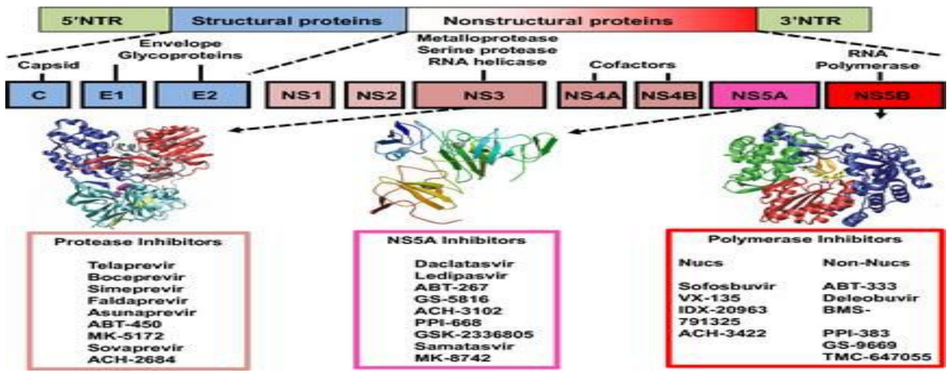
BACKGROUND

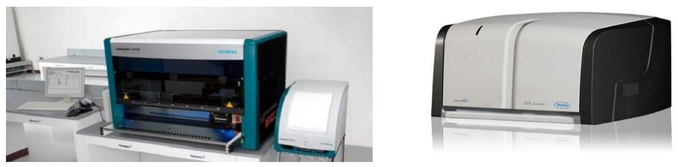
Hepatitis C Virus infection is the leading cause of chronic liver disease and hepatocellular carcinoma in Western Countries.
Therapy improvements have been accomplished in last years: the treatment with direct-acting antiviral agents (DAAs) is associated with a high rate of sustained virological response. Simeprevir is a novel NS3/4 viral protease inhibitor that has been recently approved for the treatment of HCV infection in a multidrug therapeutic approach.
However the presence of natural variants, mainly Q80K polymorphism in HCV genotype 1a, is associated with resistance to simeprevir. Thus, a screening for Q80K mutation is highly recommended prior to drug administration.



The aim of our study was to provide the Q80K prevalence at baseline in a group of 126 patients eligible for DAAs treatment at Sant'Orsola-Malpighi Hospital, Bologna, Italy, using Real Time Polymerase Chain Reaction (RT-PCR) and direct sequencing.

MATERIALS AND METHODS

Between April and July 2015, we enrolled 126 patients attending to Hepatology Unit with presumptive HCV genotype 1a infection, to detect Q80K polymorphism before simeprevir treatment. RNA extraction from plasma samples has been performed with ROBOT EZ1 (Qiagen®), and the extracted nucleic acid has been amplified by Versant® kPCR (Siemens Healthcare Diagnostics) using a Q80K Polymorphism Kit (Clonit srl).
Other tests were performed to explain unamplified samples by RT-PCR, such as genotype identification and detection of viral load.
Finally, selected samples showing no RT-PCR amplification were analyzed with a different protocol: after RNA extraction, the synthesized HCV cDNA was amplified using a home-made nested PCR and subsequently sequenced using GS Junior 454 (Roche®).



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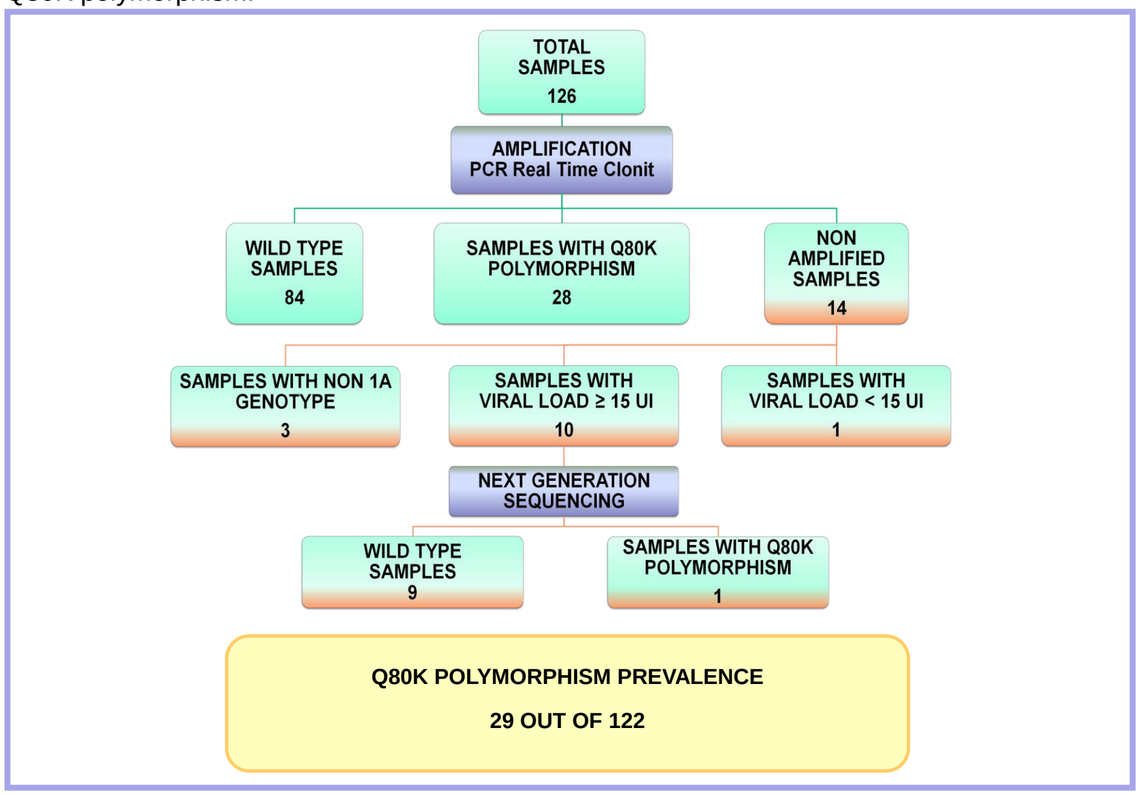
RESULTS

Polymorphism analysis obtained with RT-PCR detected 84 wild type strains (75%) and 28 strains with Q80K mutation (25%).
Fourteen samples showed no RT-PCR amplification and further analysis revealed that one showed an undetectable viral load, while three had a genotype different from 1a. Next Generation Sequencing (NGS) was performed on the remaining 10 samples, previously amplified using a home-made nested PCR: nine were HCV wild type and only one carried the Q80K polymorphism.



CONCLUSIONS

Overall, in our study, Q80K mutation occurred in 29 out of 122 subjects. The polymorphism has a substantially high prevalence in our cohort and this highlights the value of screening HCV patients before subjecting them to the simeprevir therapy. Both methods showed a good performance, and our data could suggest a diagnostic algorithm: a first step based on the RT-PCR, a rapid and completely automatic platform, keeping NGS as a second level test for those samples that show an unexpected result.