Development and Validation of a Pharmacogenetic Test for Correct Management of HCV Patients Treated with Pegylated-Interferon (PegINF) plus Ribavirin (RBV)

E. Bugin[1,2], K. Bortolozzo[1], E. Ragazzi[2]

[1] AB ANALITICA Advanced Biomedicine s.r.l, Padua, Italy
[2] Dept. of Pharmaceutical Sciences, University of Padua, Italy

Hepatitis C virus (HCV) infection is a major health problem with more than 170 million infected individuals worldwide. Approximately 30% of the infections are cleared spontaneously, whereas 40% of the patients develop a chronic infection that can lead to cirrhosis, hepatocellular carcinoma and end-stage liver disease. The current standard of care is a combination treatment with Pegylated Interferon (PEG-INF) and Ribavirin (RBV), but its efficacy and tolerability are limited. Many efforts were made to identify the factors that influence host resistance to HCV infection and treatment outcome. Several Genome-Wide Association Studies have independently demonstrated that *IL28B* (Interleukin 28B) gene sequence variations are strongly associated with spontaneous clearance of the virus, early viral kinetics and with PEG-INF/RBV therapy response in different populations. The rs12979860 CC variant upstream of *IL28B* gene is associated with a twofold higher SVR (Sustained Virological Response) rate in Europeans and a threefold higher rate in African-Americans undergoing Peg-INF/RBV treatment, in comparison to non-CC genotype. Other studies reported an association of some functional variants of the ITPA (Inosine Triphosphatase) protein with the protection from RBV-induced anemia.
The aim of this study was to develop an innovative system to genotype the most significant genetic polymorphisms used for predicting the treatment response and the risk of adverse reactions in patients infected with HCV. Four polymorphisms were investigated in this study: rs12979860 and rs8099917 in the *IL28B* gene and rs7270101 and rs1127354 in the *ITPA* gene.
Human genomic DNA was extracted from peripheral blood using a commercial kit. The genotyping of the genetic variants was performed by combining a multiplex PCR with a Reverse Line Blot hybridization assay (mPCR/RLB). The target sequences were amplified with a PCR-SSP (*Sequence Specific Primer PCR*), using biotinylated primers. Sequence alignment by ClustalW was used to identify single base variations. The amplification products were analyzed by direct sequencing to evaluate the specificity of the PCR-SSP method. For each investigated SNP, two ASO (Allele Specific Oligonucleotide) probes, recognizing the different alleles, were designed. The mPCR/RLB protocol was set up with samples of known genotype.
In order to evaluate the performance of the method, serum samples obtained by 100 HCV infected patients were selected on the basis of HCV genotype. Genotyping results were compared with those obtained both by sequencing and by Real Time PCR. The test was performed with both manual and automated blotting analysis methods. An external validation with 40 samples was carried out to assess the reproducibility of the test.
All tested samples were accurately genotyped showing a 100% analytical specificity. This underlines the reliability of the assay. The test provided a result within a DNA concentration of 1 to 500 ng/reaction.
The RLB is a convenient way to identify up to 8 targets in 20 individual specimens simultaneously. It is more flexible and less costly than DNA microarrays while providing the same specificity and sensitivity. The system developed in this study analyzes the most important host genetic factors influencing the efficacy of the Peg-INF/RBV therapy and determining the risk of RBV-induced anemia. This test is the only assay on the market that analyzes *ITPA* and *IL28B* polymorphisms together. Therefore, it represents the most complete approach for determining the genetic constitution in regard to *IL28B* and *ITPA* for personalized management of patients with Hepatitis C.