Genetic variability of hepatitis C virus NS3 protease from chronically infected patients submitted to telaprevir-based therapy

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Background: Hepatitis C virus (HCV) infection is a global health problem. New direct-acting antivirals are being developed to increase the therapeutic successful for chronic hepatitis C. The presence of protease inhibitors (PIs) resistance mutations can influence on therapy efficacy.

Objective: The aim of this work is to analyze the genetic variability of HCV NS3 protease gene from chronically infected patients, before, during, and after combined triple therapy (PI telaprevir, pegylated interferon and ribavirin). Using a high throughput methodology of next-generation sequencing, the identification of major and minor frequent viral variants is allowed.

Methodology: The patients are being selected from Hepatology Service of Hospital Clementino Fraga Filho, Rio de Janeiro, and clinically monitored. Blood samples are being collected at baseline and 4, 8, 12, 24, 48 weeks of treatment, as well 24 weeks after the end. Viral RNA is being extracted from serum, cDNA produced and a nested PCR from HCV NS3 protease are being performed. PCR-products libraries are being produced. An emulsion PCR and Ion sphere particles enrichment are being processed, loaded onto 316™ semiconductor chip and sequenced in Ion PGM™ sequencer. Sequences obtained are being analyzed using bioinformatics tools, specially the CLC Genomics Workbench v.7.5 software, comparing with HCV reference sequences for HCV-1a and 1b. Synonymous (S) and non-synonymous (NS) substitutions are being assessed; including those already described as PIs resistance mutations.

Results and Discussion: Until no w, 80 patients were selected to start triple therapy and are being monitored. For 16 patients, several S and NS substitutions were detected at baseline and 4 weeks-therapy, including those at low-frequency. Nine PI-resistance mutations were identified in 10 patients. However, there was no correspondence between resistance mutations detected at baseline and 4-weeks therapy, suggesting that resistance associated-variants (RAVs) detected at baseline are not good predictors of emerging RAVs under PI-treatment.

Keywords: hepatitis C virus NS3 protease, telaprevir, drug resistance.