SUPPLEMENTARY MATERIAL

MATERIAL AND METHODS

Reverse Transcription-PCR

Briefly, 5µL of all extracted HCV-RNA was mixed with 10µL 5xBuffer, 2.5µL DMSO, 20pmol of downstream primer and of upstream primer, 0.5µL Transcriptor One-step enzymes, to a final volume of 50µL. Reverse transcription was performed at 60°C for 40min followed by the PCR reaction (GeneAmp 2700 PCR system, Applied Biosystems, Foster City, CA, USA). After denaturing for 7min at 94°C, 35 cycles of 10sec at 94°C, 30sec at 53°C, and 30sec at 68°C were performed, with a final 7min step at 68°C, and maintained at 4°C after the PCR reaction.

REFERENCES

Lower response rates for HCV-genotype 1a compared to HCV-genotype 1b to Direct-acting antiviral (DAA) agents (NS3 protease, NS5A and non-nucleoside NS5B-polymerase inhibitors) [1-12].

Moreover, at least for HCV-genotype 1, both the frequency and the pattern of resistance to different DAA classes are subtype-specific [13, 14]

Subtype-specific differences in genetic barrier to resistance [15-19].

Current assays have not been designed to confidently identify subtypes and mixed infections [20-23].
Programmes used: Clustalothe genetic distances are computed according to the Kimura-80 model (K80) with a gamma parameter of 0.42. The data analysis system consists in four R modules, scripts use the packages Biostrings and ape.[24-28].

Injecting drug use is still the major driving force of the uncontrolled HCV epidemic throughout the World [29-31].

Since even regimens containing potent agents, such as nucleos(t)ide NS5B polymerase inhibitors, have been shown to select subtype-dependent resistance [4, 5, 32, 33]

Inter-genotypic recombinants [34, 35].

SUPPLEMENTARY REFERENCES


SUPPLEMENTARY FIGURE LEGENDS.

Supplementary Figure 1 (S1). UPGMA phylogenetic tree of the NS5B segment of 339 nucleotides from the Reference Sequences used to classify HCV isolates into the 67 subtypes accepted in 2014 (Smith et al 2014; Hepatology **59**:318-327). Bootstrap confidence levels are included on the branches (blue numbers). Genotype 1 references are colored in dark green, G2 in dark orange, G3 in blue, G4 in pink, G5 in light green, G6 in yellow.

Supplementary Figure 2 (S2). Nucleotide alignment of the 67 subtypes from a region of 500 nucleotides on NS5B. Primers for RT-PCR are colored in dark orange and Heminested primers in yellows.