Liver disease, frequently caused by hepatitis B virus (HBV) and/or hepatitis C virus (HCV) co-infection, is a significant cause of global health burden. About 130–170 million people are infected with HCV, representing ~3 per cent of the world's population. NS5B is responsible for the synthesis of negative-sense RNA and subsequently of positive-sense RNA that is incorporated into progeny virions. Importantly, the selective pressures that shape non-structural regions of the viral genome are distinct from those targeting structural genomic regions. For instance, highly conserved secondary RNA structures limit NS5B diversity. Immune-mediated selection pressures contribute to NS5B polymorphism, and HLA-restricted epitopes may overlap with sites of drug resistance. Immune- or drug-selected mutations in NS5B dramatically reduce viral replication in vivo, although compensatory mutations may develop. NS5B variability also impacts pathogenesis as a higher mutation rate is associated with elevated ALT levels, and NS5B enzymatic activity positively correlates with ALT levels. As additional non-structural gene inhibitors are developed, characterization of the factors that shape HCV diversity in vivo will be necessary to limit HCV replication and increase the effectiveness of new antiviral agents. The HIV Epidemiologic Research Study (HERS) was established in 1993 to prospectively define the biological, psychological, and social effects of HIV in US women. Serum samples were obtained from women who were HCV RNA positive but HIV negative, HIV/HCV co-infected with CD4 <350, and HIV/HCV co-infected with CD4 ≥350. HCV RNA was extracted using the QIAamp UltraSens Virus Kit and subjected to RT-PCR for the entire NS5B region (~1,798 bases). Next-generation sequencing was used to evaluate intra-patient and interpatient NS5B diversity. NGS data were visualized in Integrated Genome Viewer. Consensus sequences were aligned in ClustalX 2.1 and BEAST v1.8.4 under an uncorrelated log-normal relaxed molecular clock, the general time-reversible model with nucleotide site heterogeneity estimated using a gamma distribution, and a chain length of 100,000,000 with sampling every 10,000th generation.

### A40 Genotypic diversity of HCV in Kosovo with an emphasis on phylogenetic investigation of subtype 4D

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Hepatitis C virus (HCV) infection is a global health problem, affecting up to 3 per cent of the world's population, with the highest prevalence found among intravenous drug users and patients on hemodialysis. In Kosovo, a small developing country at Balkan Peninsula, there is lack of data on the prevalence of HCV genotypes in specific risk groups. The aim of the study was to determine HCV genotypes circulating in Kosovo and to further examine the spread of HCV genotype 4 in the country by employing phylogenetic analysis. A total of 437 HCV RNA positive hemodialysis patients, intravenous drug users and other patients were selected for genotyping by sequencing core region of HCV genome and using NCBI Genotyping tool. For the purpose of investigating the molecular epidemiology and transmission routes of genotype 4 in Kosovo, the HCV NS5b region was also sequenced. Major clusters were identified in a quick neighbor joining tree and HCV control sequences selected employing HCV BLAST search tool. Finally, maximum likelihood phylogenetic trees were constructed by using PhyML 3.0, with automatic substitution model selection Smart Model Selection. Transmission clusters were identified according to approximate likelihood ratio test (aLRT) branch support values obtained. In 383 out of 437 HCV RNA positive patients the HCV core region was successfully sequenced. The following HCV subtypes were determined: 1a (227/383; 59.3 per cent), 4d (95/383; 24.8 per cent), 1b (28/383; 7.3 per cent), 3a (28/383; 7.3 per cent), 2c (4/383; 1.0 per cent), and 2k (1/383; 0.3 per cent). A total of eighty-eight partial NS5b sequences were obtained, mostly from hemodialysis patients. This indicates that subtype 4d is epidemiology distinct from that of subtypes 1a and 3a, since genotype 4d has not been observed among injecting drug users in Kosovo so far. The phylogenetic tree of subtype 4d obtained revealed several clusters, suggesting several introductions of this subtype into dialysis units. In conclusion, 4d is the second most prevalent HCV subtype in Kosovo. Phylogenetic analyses showed several introductions of this subtype to the country and further spread among dialysis units thus demanding an urgent change in infection control practices in order to prevent further transmission of HCV.

### A41 Characterization of NS5 coding region resistance associated substitutions from DAA-naïve GT1 HCV-infected patients in a Portuguese cohort

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Hepatitis C virus (HCV) is considered to be the leading cause of hepatocellular carcinoma (HCC) and other co-morbidities. During recent years, several highly effective regimens of direct-acting antivirals (DAAs) with excellent rates of success became available. However, therapeutic failure may occur in up to 10 per cent of treated individuals, and one of the main causes for this failure is the presence of resistance-associated substitutions (RASs) present before treatment initiation. Our aim was to study the profile and prevalence of baseline RASs in the NS5 coding region of DAA-naïve GT1 HCV infected patients, and then understand the impact of the found RASs in the response to treatment by ascertaining an association between treatment failure and the presence of major NS5 RASs. Plasma RNA from eighty-one GT1 HCV infected patients was extracted using the NucliSens® easyMAG system, followed by an in-house nested RT-PCR of the NS5 coding region. PCR products were purified and subsequently sequenced with the 3130xl ABI PRISM Genetic Analyzer. Sequences were finally aligned and edited using ChromasPro v1.7.6, and analyzed online with hcv.geno2pheno.org. NS5A RASs were present in 28.4 per cent (23/81) of all GT1 infected patients, with GT1a showing the highest prevalence followed by GT1b (17 vs. 11 per cent, respectively). Major NS5A RASs were detected in 23.6 per cent (13/55) of GT1a infected patients (M28V, Q30H/R, L31M, and Y93C/H) and in 15.4 per cent (4/26) of GT1b infected patients (L31M and Y93H). The most commonly detected NS5A RAS was Y93C/H with a prevalence of 9.9 per cent (8/81) in all GT1 infected patients, followed by L31M and Q30H/R with a prevalence of 8.6 per cent (7/81) and 6.2 per cent (5/81), respectively. Furthermore, Y93C/H showed a higher prevalence in GT1b patients than in GT1a, namely 11.5 per cent (3/26) vs. 9.1 per cent (5/55), respectively. NS5B RASs

showed a prevalence of 14.8 per cent (12/81) in all GT1 infected patients, and were only detected in GT1b, being mainly represented by C316N with 38.5 per cent (10/26) of GT1b infected patients. The combined NS5A RASs (Q30H + Y93H), causing high level resistance to all NS5A inhibitors, were detected at baseline in one HIV/HCV GT1a co-infected patient who later failed a treatment with SOF + LDV for 12 weeks. Finally, an isolated Y93H mutation was also detected at baseline in a GT1b mono0-infected patient experiencing recurrence. Overall 38.3 per cent (31/81) of all GT1 HCV infected patients presented NS5 RASs at baseline, in which 58.1 per cent (18/31) were co-infected with HIV/HCV whereas only 38.7 per cent (12/31) of HCV monoinfected patients showed baseline RASs. Moreover, 27.3 per cent (15/55) of GT1a infected patients presented NS5 RASs at baseline, whereas patients infected with GT1b showed the highest prevalence of natural RASs, namely 61.5 per cent (16/26). These data support the usefulness of resistance testing prior to treatment initiation, thus preventing relapses associated to the presence of baseline RASs, as a statistical significant association was found between treatment failure and the presence of major NS5 RASs, namely Y93C/H ($P = 0.04$). However, this reduced sampling can constitute a limiting factor since it may underestimate the statistical analysis, and lead to relatively higher RASs rates when comparing to other previous studies.

### A42 Genetic variability and phylogeography of hepatitis B virus genotype D in Brazil

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Hepatitis B virus (HBV) has been classified into ten genotypes (A–J), some of which are divided into subgenotypes. Genotype D (HBV/D) has a worldwide distribution, and ten subgenotypes (D1–D10) have been described so far. Brazil has received different migratory flows over time. The evolutionary history of HBV/D in Brazil is not well understood and few HBV/D complete genome sequences are available. The aim of this study was (1) to examine the distribution of HBV/D subgenotypes in Brazil, (2) to determine the full-length genomic sequences of HBV/D isolates from different regions, and (3) to investigate the origin and spread of HBV/D subgenotypes in the country. All Brazilian HBV/D sequences with known subgenotype ($n = 215$) were retrieved from GenBank. HBV/D3 was the most prevalent (56 per cent) subgenotype, followed by HBV/D4 (25 per cent), HBV/D2 (17 per cent), and HBV/D1 (2 per cent). Although HBV/D was circulating countrywide, most (57 per cent) isolates were from the South region, which was the only region where all four subgenotypes were found. In addition, forty-five new full-length sequences (one D1, eleven D2, thirty-two D3 and one D4) were determined. To investigate the origin and spread of HBV/D in Brazil, we compiled different datasets of complete genomes for HBV/D1–D4, using Brazilian and worldwide sequences. Phylogeographic analysis, performed using BEAST v.1.8.2, indicated that the most probable origins of HBV/D1 and HBV/D2 were Syria and Eastern Europe, respectively, with times of the most recent common ancestor (tMRCA) in the early nineteenth century for HBV/D1 and the second half of the twentieth for HBV/D2, corroborating historical data on migrations to Brazil. Martinique was found to be the origin of Brazilian HBV/D4, probably reflecting the population of African slaves brought to the Americas. However, the methodology used was not able to determine from where and when HBV/D3 was introduced in Brazil, possibly due to different introduction routes.

### A43 Molecular epidemiology of hepatitis B virus in South Kivu, an eastern province of the Democratic Republic of Congo

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Hepatitis B virus (HBV) is characterized by a wide genomic variability that could play a role in different clinical manifestations and response to therapy. The ten HBV genotypes show a distinctive geographical distribution worldwide and genotypes A, D, and E are the most frequently found in Africa. There are only limited studies on HBV genotype distribution in Democratic Republic of Congo (DRC), all performed in the western part and showing a vast majority of genotype E. We performed a study to determine the genotype distribution of HBV in South Kivu (DRC). Blood screening was performed during 2014–2015 at the Hospital Provincial General de Reference of Bukavu where serum samples of newly detected Ag HBs positive subjects were collected. These samples were sent and analysed at the Cliniques Universitaires Saint Luc, Belgium. We undertook HBV DNA load measurement by Abbott RealTime HBV assay on the m2000 system, genome sequencing using an in-house method targeting the S and P overlapping region, phylogenetic analysis using Geneious 4.0 software, and additional mutational analysis focused on the identification of mutations (P region) associated with antiviral resistance using the online HBVseq tool (Stanford University). Genotype determination was performed in forty-one patients. HBV genotype A was detected in 40/41 (97.6 per cent) and HBV genotype E in 1/41 (2.4 per cent). Only two mutations were observed and concerned the I169T nucleotide substitution, both in genotype A samples. The phylogenetic analysis showed that nearly all South Kivu genotypes A (39/40) are closely related to A1 subgenotype strains found in Rwanda, Haiti, and Martinique while only one single strain attached to the A2 subgenotype cluster was isolated. The only remaining genotype E case was linked to the western African E crescent. HBV genotype A seems to be the most predominant genotype in eastern DRC with the majority belonging to the Afro-Asian subgenotype (A1). This contrasts with the western part of RDC where genotype E is the most frequently found genotype. These results support the hypothesis of an East–West genotypic demarcation. Moreover, the low genetic variability of HBV in South-Kivu is suggestive of strong local endemicity.

### A44 Complete HPV genomes from cervical samples using next-generation sequencing in Luxembourg

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While next-generation sequencing using rolling circle amplification (NGS-RCA) of human papillomavirus (HPV) has been conducted in HIV-HPV co-infected women, we performed a pilot