JOURNAL OF VIRAL HEPATITIS
JVH

Journal of Viral Hepatitis, 2015, **22** (Suppl. S3), 19–49 doi:10.1111/jvh.12441

Poster Abstracts

HEPATITIS C VIRUS

Diagnosis

P1

Can simple easily reproducible biochemical tests replace the costly elastography in diagnosis of liver cirrhosis in chronic hepatitis C patients?

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BACKGROUND: In the era of novel antiviral drugs for treatment of chronic viral hepatitis C (HCV), the diagnosis of liver cirrhosis guides both the choice of the drug regimen, duration of treatment and subsequent follow up. Transient elastography (TE) (fibroscan) is a recent, non invasive and reliable method for the diagnosis of cirrhosis in patients with chronic HCV. We aimed at demonstrating the agreement of some of the indirect serum markers of liver fibrosis with TE score in staging of liver fibrosis in chronic HCV patients.

MATERIALS AND METHODS: One hundred and fifty-nine chronic HCV Egyptian patients were evaluated for antiviral treatment using TE. In all patients, real-time PCR for HCV RNA, liver and renal biochemical tests, PT, INR and CBC were done just before TE (no longer than one month). The stage of liver fibrosis was considered F0 if TE score was 0–5, F1 if 5.1–7, F2 if 7.1–10, F3 if 10.1–17 and F4 if it was 17.1–75. Liver fibrosis was considered non significant (NSF) if TE score corresponds to F0 or F1. Liver fibrosis was considered significant (SF) if TE score corresponds to F2 or F3. Liver cirrhosis was diagnosed if it corresponded to F4. The indirect serum markers of liver fibrosis examined were AST/Platelet Ratio Index (APRI), Fibrosis 4 score (FIB4) and fibrosis index (FI). APRI was calculated as (AST/upper limit of normal range)/platelet count (10^9/L) × 100 (1). Liver fibrosis was considered significant if APRI score was ≥0.7 and cirrhosis was considered if the score was ≥1 (2). FIB4 score was calculated using the following formula: (Age × AST)/(Platelets × (sqr(ALT)). If FIB4 score <1.45 = F0–F1. If FIB4 score ≥1.45 and ≤3.25 = F2. If FIB4 score >3.25 = F3–F4 (3). FI was calculated using the following formula: 8.28 − [(0.01 × Platelets (10^9/L) − [1.08 * (10 * serum albumin (g/L))]]. If FI <2.1, no or minimal fibrosis. FI ≥2.1 shows significant fibrosis. FI ≥3.3 predicts cirrhosis (4). The Kappa Statistic was used to assess the method agreement. If Kappa was <0 = less than chance agreement. Kappa 0.01–0.20 = slight agreement. Kappa 0.21–0.40 = fair agreement. Kappa 0.41–0.60 = moderate agreement. Kappa 0.61–0.80 = substantial agreement. Kappa 0.81–0.99 = almost perfect agreement (5).

RESULTS: APRI score agreed with TE score in diagnosis of NSF in 75.8% of cases, in SF in 22.9% and in diagnosis of LC in 84.7% of cases. Kappa was 0.41 denoting moderate agreement. FIB4 agreed with TE score in diagnosis of NSF in 50% of cases, in SF in 52.2% and in diagnosis of LC in 71.2% of cases. Kappa was 0.38 denoting fair agreement. FI agreed with TE score in diagnosis of NSF in 82.1% of cases, in SF in 37.2% and in diagnosis of LC in 47.2% of cases. Kappa was 0.287 denoting fair agreement.

CONCLUSIONS: APRI has a better agreement with TE score in staging of liver fibrosis in chronic HCV Egyptian patients than both FIB4 and FI.

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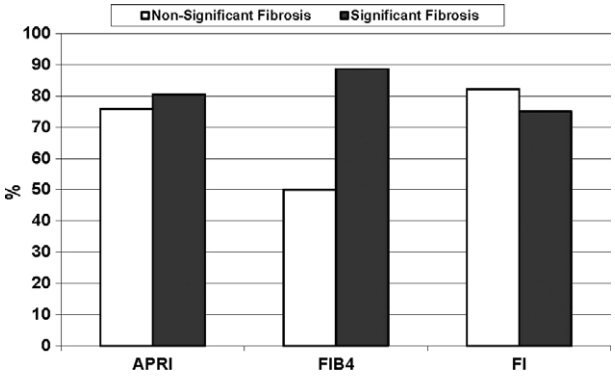

Figure 1 Agreement of APRI, FIB4 and FI with TE score in staging of liver fibrosis. Both significant fibrosis and cirrhosis were grouped as significant fibrosis in this figure.

P2

Agreement of indirect serum markers of liver fibrosis with liver biopsy results in chronic hepatitis C patients

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BACKGROUND: Assessment of liver fibrosis in chronic hepatitis C virus (HCV) infection is the key for decision making. Although liver biopsy (LB) has been considered the gold standard, it is invasive and is subjected to sampling errors and observer variability. We aimed at demonstrating the agreement of some of the indirect serum markers of liver fibrosis with Metavir score in staging of liver fibrosis in chronic HCV patients.

MATERIALS AND METHODS: Two hundred and twenty two chronic HCV Egyptian patients (128 males, 94 females) were evaluated for antiviral treatment. Ultrasonography guided LB was done in 63 patients and liver fibrosis was staged according to Metavir score. Liver fibrosis was considered non significant (NSF) if Metavir score was <F2, significant (SF) if Metavir score was ≥F2 and liver cirrhosis (LC) if it was F4. In all patients, real-time PCR for HCV RNA, liver and renal biochemical tests, PT, INR and CBC were done before LB (no longer than 1 month). The indirect serum markers of liver fibrosis examined were AST/Platelet Ratio Index (APRI), Fibrosis 4 score (FIB4) and fibrosis index (FI). APRI was calculated as (AST/upper limit of normal range)/platelet count (109/L) × 100 (1). Liver fibrosis was considered significant if APRI score was ≥0.7 and cirrhosis was considered if the score was ≥1 (2). FIB4 score was calculated using the following formula: (Age × AST)/(Platelets × (sqr(ALT)) (2). If FIB4 score <1.45 = F0-F1. If FIB4 score ≥1.45 and ≤3.25 = F2. If FIB4 score >3.25 = F3-F4 (3). FI was calculated using the following formula: 8.28 – [(0.01 × Platelets (10^9/L) – [1.08 * (10 * serum albumin (g/L))]]. If FI <2.1, no or minimal fibrosis. FI ≥2.1 shows significant fibrosis. FI ≥3.3 predicts cirrhosis (4). The Kappa Statistic was used to assess the method agreement. If Kappa was <0 = less than chance agreement. Kappa 0.01–0.20 = slight agreement. Kappa 0.21–0.40 = fair agreement. Kappa 0.41–0.60 = moderate agreement. Kappa 0.61–0.80 = substantial agreement. Kappa 0.81–0.99 = almost perfect agreement (5).

RESULTS: APRI score agreed with Metavir score in diagnosis of NSF in 65.5% of cases, in SF in 17.9% and in diagnosis of LC in 75% of cases. Kappa was 0.158 denoting poor agreement. FIB4 agreed with Metavir score in diagnosis of NSF in 48.3% of cases, in SF in 57.1% and in diagnosis of LC in 40% of cases. Kappa was 0.205 denoting poor agreement. FI agreed with Metavir score in diagnosis of NSF in 74.1% of cases, in SF in 59.3% and in diagnosis of LC in 75% of cases. Kappa was 0.410 denoting moderate agreement.

CONCLUSIONS: FI has a better agreement with Metavir score in staging of liver fibrosis in chronic HCV Egyptian patients than both APRI and FIB4.

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P3

Noninvasive assessment of liver fibrosis in hepatitis C: acoustic radiation force impulse imaging (shear wave elastography) and liver fibrosis biomarkers versus liver biopsy

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BACKGROUND: Egypt has the highest prevalence of hepatitis C virus (HCV) infection, averaging 15–25%. Precise estimation of the degree of liver fibrosis is important for determining the prognosis and treatment. Liver biopsy is considered the gold standard for assessing fibrosis grade. Recently many non-invasive techniques for assessing liver fibrosis have been developed, including imaging techniques like transient elastography, shear wave elastography and biomarkers like FIB-4 score and AST/platelet ratio (APRI).

PURPOSE OF THE STUDY: To determine the efficacy of shear wave elastography and biomarkers of liver fibrosis (FIB-4 score and APRI) in the non-invasive assessment of liver fibrosis versus liver biopsy and to compare between them in the assessment of the stages of hepatic fibrosis.

METHODS: One hundred and eighteen Egyptian patients with chronic hepatitis C with mean age 44.6 ± 12.8 years, 86 male and 32 female were included in the study. For each patient complete medical history was taken and physical examination was done. Abdominal ultrasonography, liver function tests, CBC and liver biopsy were also done. Calculation of FIB-4 score and APRI and shear wave elastography examination were done for all the study cases.

SUMMARY OF RESULTS: The shear wave cut off value were 4.8, 6.3 and 9.4 for F ≥2, F ≥3 and F=4 stage of fibrosis, with sensitivity of 81.5% and specificity of 73.1%, AUC 0.78 (0.67:0.89), sensitivity of 93.8% and specificity of 86.5%, AUC 0.92 (0.83:1) and sensitivity of 91.3% and specificity of 98.1%, AUC 0.98 (0.96:1) respectively. FIB-4 score had sensitivity of 40.7% and specificity of 76.9%, AUC 0.57 (0.44:0.7) for F ≥2, sensitivity of 56.3% and specificity of 65.4%, AUC 0.55 (0.39:0.72) for F ≥3 and sensitivity of 52.2% and specificity of 84.6%, AUC 0.7 (0.57:0.83) for F=4 respectively. While APRI had sensitivity of 63% and specificity of 51.9%, AUC 0.52 (0.39:0.65) for F ≥2, sensitivity of 56.3% and specificity of 90.4%, AUC 0.75 (0.6:0.89) for F ≥3 and sensitivity of 69.6% and specificity of 69.2%, AUC 0.71 (0.57:0.84) for F=4 respectively.

CONCLUSION: Shear wave elastography provides more accurate correlation with liver fibrosis stage compared with FIB-4 score and APRI as a non-invasive technique for assessment of liver fibrosis especially in significant stages (F3 and F4).

Natural history and epidemiology

P4

Non-invasive liver fibrosis scores: do they also predict antiviral treatment response, decompensation, hepatocellular carcinoma and significant liver related adverse events?

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BACKGROUND: Non-invasive liver fibrosis scores (NIF) are increasingly applied as an alternative to liver biopsy (LB).

Table 1 - Comparison of predictive accuracy of different non-invasive markers for treatment response, decompensation, HCC and SLRE

	Treatment response*	Decompensation*	HCC*	SLRE*
APRI	0.519 (0.440–0.599), 0.041	0.538 (0.063–0.783), 0.120	0.800 (0.660–0.940), 0.071	0.535 (0.299–0.771), 0.119
FIB 4	0.583 (0.504–0.662), 0.040	0.854 (0.738–0.956), 0.041	0.884 (0.827–0.942), 0.029	0.847 (0.765–0.929), 0.039
LOK SCORE	0.518 (0.438–0.598), 0.041	0.803 (0.858–0.993), 0.127	0.529 (0.288–0.769), 0.123	0.801 (0.552–0.999), 0.129
GUCI	0.521 (0.442–0.601), 0.041	0.604 (0.062–0.775), 0.144	0.843 (0.717–0.970), 0.065	0.600 (0.319–0.882), 0.139
FIBROALPHA	0.551 (0.472–0.631), 0.041	0.644 (0.362–0.999), 0.146	0.615 (0.396–0.835), 0.112	0.644 (0.360–0.929), 0.144
Mod APRI	0.587 (0.508–0.666), 0.040	0.615 (0.101–0.784), 0.132	0.521(0.367–0.927), 0.117	0.608 (0.349–0.867), 0.129
KING SCORE	0.575 (0.509–0.664), 0.041	0.641 (0.016–0.898), 0.150	0.905 (0.858–0.954), 0.025	0.635 (0.342–0.928), 0.149
AAR	0.533 (0.495–0.654), 0.041	0.671 (0.405–0.999), 0.137	0.543(0.365–0.926), 0.034	0.671(0.403–0.939), 0.132
FIBROSIS INDEX	0.555 (0.453–0.613), 0.040	0.767(0.529–0.873), 0.079	0.881 (0.776–0.986), 0.054	0.764 (0.610–0.917), 0.081
FCI	0.531 (0.517–0.673), 0.041	0.646 (0.603–0.998), 0.143	0.604(0.365–0.926), 0.022	0.644 (0.365–0.923), 0.141
GPI	0.500 (0.451–0.611), 0.041	0.500 (0.297–0.800), 0.146	0.496 (0.266–0.725), 0.117	0.500 (0.214–0.786), 0.122
FIBROQ	0.595 (0.476–0.634), 0.040	0.881 (0.730–0.950), 0.076	0.807 (0.723–0.891), 0.043	0.877 (0.727–0.998), 0.065
Advanced grade (Grade 3 and 4)#	0.527(0.446–0.607), 0.041	0.408 (0.175–0.641), 0.119	0.788 (0.614–0.961), 0.089	0.406 (0.176–0.644), 0.112
Advanced Fibrosis (Stages F3 and F4 fibrosis)#	0.538(0.458–0.618), 0.041	0.525(0.232–0.819), 0.150	0.804 (0.631–0.977), 0.088	0.521(0.222–0.810), 0.148
Cirrhosis (Stage F4 fibrosis)#	0.485(0.405–0.0.564), 0.041	0.480 (0.205–0.756), 0.141	0.558 (0.308–0.809), 0.128	0.484 (0.202–0.751), 0.039

*AUROC (95% Confidence interval).
Standard error # on liver biopsy.

However, the role of NIF in predicting antiviral treatment (AVT) response and post treatment significant liver related events (SLRE) in chronic hepatitis C (CHC) is not well established.

AIMS: To compare 12 simple NIF, derived from routine blood investigations, for predicting response to AVT and SLRE. To identify independent predictors of treatment non-response from routine baseline blood investigations.

METHODS: One thousand six hundred and five patients underwent LB (Scheuer classification) and received AVT (pegylated interferon and ribavirin). Twelve simple NIF [AST-platelet count ratio index (APRI), Fibrosis-4 (FIB-4) score, Lok score, GUCI score, Fibroalpha score, modified APRI, King score, AST-ALT ratio (AAR), Fibrosis Index (FI), Fibro score, Fibrosis cirrhosis index (FCI) and Globulin platelet index (GPI)] were calculated from the baseline blood tests prior to AVT. AUROCs were calculated for each of these NIF for predicting non-response to AVT and development of SLRE (defined as development of any event requiring intervention; decompensation and hepatocellular carcinoma (HCC)) on follow-up. From the baseline pretreatment blood tests, we also attempted to identify independent predictors of non-response to AVT.

RESULTS: Mean age 41.9 ± 9.7 years (85% males), predominantly genotype 4 (65%) and genotype 1 (11%). After AVT, there were 1089 (67.8%) responders, 482 (30%) non responders and 34 (2.1%) relapsers. LB results showed stage-0 fibrosis in 1.9%, stage-1 in 32.9%, stage-2 in 39.5%, stage-3 in 19%, and stage-4 (cirrhosis) in 6.6% of the patients. After median follow-up of 6580.5 patient-years; 52 (3.2%) had decompensation (bleed-9, ascites-39, jaundice-22, hepatic encephalopathy-7, spontaneous bacterial peritonitis-10, hepatorenal syndrome-4), 11 (0.7%) had HCC and 62 (3.9%) had SLRE. The predictive accuracy of NIF and LB for non-response to AVT was low. FIB-4, FibroQ and King score showed high accuracy for predicting adverse events. For predicting decompensation, HCC and SLRE, FibroQ (0.88), King score (0.90) and FibroQ (0.87) had highest AUROC respectively. On MVA; age (AOR = 1.036, CI = 1.007–1.065, p = 0.01), ALT (AOR = 0.990, CI = 0.984–0.996, p = 0.002), gamma-glutamyl transpeptidase (AOR = 1.012, CI = 1.007–1.017, $p < 0.001$) and platelet count (OR = 0.989, CI = 0.984–0.995, $p < 0.001$) predicted non-response to AVT. We derived a study score [(0.072 + 0.035 (age, years) – 0.01 (ALT, IU/ml) + 0.01 (GGT, IU/ml) – 0.01 (platelet count, 109/dL)], which at a cut-off of 0.32 had a predictive accuracy of 0.748 (95% CI = 0.708–0.789) for non-response to AVT.

CONCLUSIONS: Predictive accuracy of known NIF for non-response to treatment was low. However age, GGT, ALT and platelet count were independent predictors of non-response to antiviral therapy. Some NIFs have high accuracy for predicting development of decompensation, HCC and SLRE. Application of these simple scores can improve assessment of long term liver prognosis after antiviral treatment for CHC.

P5

Watch and wait – chronic hepatitis C patients before the era of interferon-free therapy in Germany

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In 2013 the first interferon-free treatment options for chronic Hepatitis C (CHC) became available for GT2 and 3, introducing a new era of highly effective and well tolerated oral treatment options for CHC. The data from the cross-sectional study CURRENT-C highlight the epidemiological characteristics of patients with CHC in Germany. The data were collected during the time period immediately before approval of interferon-free treatment options for HCV Genotype 1. One thousand four hundred and seventy one patients from 40 German centers specializing in viral hepatitis treatment were included in the analysis. Patients were enrolled from June through November 2014 and were not actively receiving treatment. The mean age was 52.4 years with 41.2% of the patients being female. Suspected route of infection in male patients was most frequently drug use (46.2%), in female patients blood transfusion and blood products (22.8%). The route of infection was frequently unknown with 28.2% of male and 43.1% of female patients. Compared to male, female patients were older (55.6 vs. 50.1 years) and longer diagnosed (18 vs. 15 years). Native language of the patients was German in 72.2% followed by Russian (14.2%) and Polish (2.9%). HCV genotype (GT) 1 was found in 73.5% (1a 28.5%, 1b 37.6%, 1a+b 0.6%, subtype not specified 6.9%), GT2 in 3.4%, GT3 in 18.2%, GT4 in 4.2%, GT5 or 6 in 0.4%. Liver cirrhosis was diagnosed in 15.7% of the patients (17.1% male, 13.7% female). 43.2% of the patients had already received HCV treatment, most frequently dual therapy with pegIFN + RBV (75.8%) or triple therapy with telaprevir or boceprevir (20.4%). Compared to treatment naïve patients pretreated HCV patients were older (55.1 vs. 50.3 years) and had liver cirrhosis as clinical diagnosis more frequently (22.2% vs. 10.8%). Patients scheduled for HCV treatment within the next 3 months had higher rates of pretreatment (49.4% vs. 37.0%), and

liver cirrhosis (21.4% vs. 10.0%). Facing epidemiological data of Hüppe et al. (2008) (1), Klass et al. stated in 2012 in a comparable setting that the German HCV population progressed in age, disease severity and parameters indicating poor treatment response (2). The current data seem to carry forward this trend towards more difficult to treat patients, indicating the urgent need for new treatment options being now widely available.

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P6

Prevalence and clinical outcome of post transfusion hepatitis C in young and adults

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BACKGROUND: The prevalence of chronic hepatitis C in Sweden is estimated to 0.5%. Before 1991 blood transfusion was a common route of transmission. The primary aim of this study was to estimate the anti-HCV prevalence in subjects receiving blood transfusions in Stockholm county during that period. The secondary aim was to study the effect of age at transfusion on liver disease and treatment outcome.

METHODS: This is a single centre retrospective analysis of subjects found to be anti-HCV tested positive in Stockholm county during a national screening campaign in 2008–2010. Subjects were informed through media and encouraged to perform anti-HCV testing by their general physician if they had received blood transfusions during the period 1965–91. All anti-HCV positive subjects were also HCV RNA tested and those positive were referred to Karolinska University Hospital for further investigation. Inclusion criterion for this study was blood transfusion as the most likely mode of transmission. Subjects with ongoing or a history of drug abuse were excluded. Data on age at transfusion, age at diagnosis, HCV genotype, IL28B genotype, viral load, fibrosis score, liver histology and antiviral treatment was recorded.

RESULTS: One hundred and thirty-four of 7473 (1.8%) tested individuals were anti-HCV positive and of those 102 were HCV RNA positive resulting in a prevalence of chronic hepatitis C infection of 1.4%. In 99 patients data was retrieved showing that 71% were female, 45% of the patients were older than 61 years at diagnosis. Obstetric or gynecological intervention were the most common causes of transfusion. The rate of advanced liver damage was 18% (n = 56). Patients younger than 19 years of age at transfusion (group 1, 23% of the cohort) were significantly more often started on antiviral treatment compared to adult patients (group 2), 65% vs 29% $p < 0.001$. No significant correlation was found between treatment outcome and gender, age at transfusion or IL28B genotype.

CONCLUSION: This look-back study found an anti-HCV prevalence of 1.8% which is higher than in other regions in Sweden. In this study, patients infected during childhood were more likely to receive antiviral treatment than individuals with post transfusion hepatitis C contracted later in life. Additional data on the hepatitis C epidemic in Sweden are needed regarding prevalence and age distribution. If treatment coverage is higher in younger individuals this population should be targeted for general screening.

P7

Viral load dynamics in patients with chronic hepatitis C

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BACKGROUND: In chronic hepatitis C, biannual monitoring is based on surrogate markers such as liver function tests, alpha-feto protein (AFP) or viral load (1,2). HCV viral load in itself is not considered a reliable marker of disease progression. However, it is an important parameter computed into scores for calculating the chance of sustained virologic response (SVR), i.e., GenoFibroTest or Prometheus Index. Therefore, a different viral load at different time points could influence the calculated probability of SVR and the decision to start treatment in this category of patients.

MATERIALS AND METHODS: We performed a study to determine the dynamics of laboratory results, particularly viral load, in genotype 1 chronic hepatitis C patients with FibroScan ≤F3, normal AFP levels and normal abdominal ultrasound.

RESULTS: The study included 34 patients (21 treatment-naïve and 13 with prior treatment failure), with a mean age of 46.3 years (M:F ratio 1:0.55). The mean interval since diagnosis was 4.2 years; the mean FibroScan score was 5.8 kPa. The mean time span between the two laboratory tests was 18 days. In this interval, viral load varied with an average absolute log difference of 0.20 log (min: 0.009 log in 3 days; max: 1.13 log in 11 days elapsed between the two lab tests).

CONCLUSIONS: Clinicians need to be aware that, in chronic hepatitis C patients, a wide variation in HCV viral load can be identified in time, possibly associated with the rate of quasispecies generation. This factor should be taken into account when calculating the chance of SVR and making treatment decisions.

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Treatment and late-stage clinical trials

P8

A single tablet regimen of ledipasvir/sofosbuvir for 12 weeks in HCV genotype 1 or 4 infected patients with HIV-1 co-infection: the Phase 3 ION-4 study

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BACKGROUND AND AIMS: We evaluated the safety and efficacy of the IFN-free, RBV free, single tablet regimen of ledipasvir/sofosbuvir (LDV/SOF) in HCV genotype 1 or 4 patients co-infected with HIV-1 in the Phase 3 ION-4 study.

METHODS: HCV treatment naïve and experienced HIV co-infected patients on stable, approved antiretroviral (ARV) regimens received LDV/SOF (90 mg/400 mg) once daily for 12 weeks. Patients with compensated cirrhosis were eligible. Permitted concomitant ARVs included tenofovir/emtricitabine (TDF/FTC) with raltegravir (RAL), efavirenz (EFV) or ripilvirine (RPV). Safety evaluations included enhanced renal toxicity monitoring, CD4 count and HIV-1 RNA levels. The primary endpoint was SVR12.

RESULTS: Three hundred and thirty-five patients with GT1a (75%), GT1b (23%) and GT4 (2%) were enrolled in the study; 82% were male, 34% were black, mean age was 52 (range 26–72), mean baseline HCV RNA was 6.7 log10 IU/mL (range 4.1–7.8), median baseline CD4 count was 662 cells/uL (range 106–2069), 20% had cirrhosis, 24% were IL28B CC genotype and 55% had not responded to prior HCV treatment. Most patients were taking EFV (48%) or RAL (44%). Table 1 shows SVR4 by ARV regimen. Overall, SVR24 was 96% (321/335); two patients had on-treatment virologic failure due to non-compliance and 10 had virologic relapse after discontinuing treatment. Overall, SVR24 (96%) among non-cirrhotic (F0-F3) patients was similar to SVR4 (94%) among cirrhotic (F4) patients. No patient had confirmed HIV virologic rebound (HIV-1 RNA $\geq$ 400 copies/mL). No patients discontinued study drug due to an AE. AEs occurring in $\geq$10% of patients were headache (25%), fatigue (21%) and diarrhea (11%). No significant lab abnormalities were observed.

CONCLUSIONS: The IFN-free, RBV-free, single tablet regimen of LDV/SOF administered once daily for 12 weeks is highly effective and well tolerated in treatment-naïve and experienced, genotype 1 or 4 HCV-infected patients with HIV-1 coinfection, including those with cirrhosis. Complete SVR24 data will be presented.

Table 1 - SVR4 by ARV regimen

Virologic response	TDF+FTC+ EFV (N = 160)	TDF+FTC+ RAL (N = 146)	TDF+FTC+ RPV (N = 29)	Overall (N = 335)
SVR24, n (%)	151 (94)	142 (97)	28 (97)	321 (96)
On-treatment failure, n (%)	1 (<1)	0	1 (3)	2 (<1)
Relapse, n (%)	8 (5)	2 (1)	0	10 (3)
Other, n (%)	0	2 (1)	0	2 (<1)

P9

Real-world effectiveness of ledipasvir/sofosbuvir 8 weeks chronic hepatitis C treatment

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BACKGROUND AND AIMS: Ledipasvir/sofosbuvir (LDV/SOF) single tablet regimen (STR) is approved for the treatment of chronic hepatitis C (CHC) patients. The ION-3 study showed that 8 weeks of LDV/SOF treatment was non-inferior to 12 weeks in previously untreated GT1 patients without cirrhosis with no benefit for the addition of Ribavirin. According to the label 8 weeks may be considered in this population. The aim of the present analysis is to characterise the population receiving 8 weeks LDV/SOF and to describe outcomes in clinical practice.

METHODS: The first CHC patients treated with 8 weeks LDV/SOF in a single centre in Germany, and for whom sustained virological response after 12 weeks of follow-up (SVR12) will be available in June, were included in the analysis. Baseline characteristics, prior treatment history, safety and effectiveness were investigated. The analysis was performed using descriptive statistics.

RESULTS: Forty-six patients met the inclusion criteria for this analysis. These patients initiated 8 weeks of treatment with LDV/SOF between 24/11/2014 to 27/01/2015. No patient had ribavirin added to the STR. The mean (SD) age was 50.9 (12.4) years and 56.5% were males. The genotype distribution was 52%, 44% and 4% for GT1a, GT1b and GT4, respectively. At entry, 98% of patients had no

cirrhosis, one patient had compensated disease. The METAVIR stage distribution of non-cirrhotic patients at baseline was 39.1%, 32.6%, 19.6% and 8.7% for F0, F1, F2 and F3, respectively. Median (range) HCV RNA at baseline was 5.86 (Q1–Q3 5.38–6.22; Min–Max 3.74–6.67) log10 IU/ml, no patient had HCV RNA ≥6 million IU/mL. No patient was HIV co-infected and one patient was HBV co-infected. Overall, 98% of the patients were treatment-naïve. One patient had relapsed after previous IFN/RBV therapy. At baseline, co-morbidities were reported in 93% of patients, with depression (16%) and arterial hypertension (16%) being most common. Up to date, no discontinuations or relevant Adverse Drug Reactions have been observed. Complete results regarding SVR12, adverse events and discontinuations will be available at the time of presentation.

CONCLUSION: Eight weeks LDV/SOF is predominantly prescribed according to the SPC for treatment-naïve non-cirrhotic CHC patients with HCV RNA <6 million IU/mL at baseline. Preliminary results indicate that LDV/SOF is a safe, well tolerated treatment option with no adverse drug reactions or discontinuations reported so far.

P10

Efficacy and safety of a 12-week simeprevir plus peginterferon/ribavirin regimen in treatment-naïve HCV genotype 4-infected patients with mild-to-moderate fibrosis

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PURPOSE OF THE STUDY: Benefits of shortening peg-interferon-based HCV therapy include reducing the burden of treatment-associated side effects. We evaluated virologic response and safety of a shortened 12-week treatment course with simeprevir plus peg-interferon/ribavirin (SMV+PR) in treatment-naïve chronic HCV genotype 4-infected patients with mild-to-moderate fibrosis (METAVIR F0-F2).

METHOD: This Phase III, open-label study recruited patients from Europe and Saudi Arabia. Genotype 4-infected patients who achieved HCV-RNA <25 IU/mL at Week 2 (detectable/undetectable in IL28B CC, or undetectable in IL28B CT or TT), and undetectable HCV-RNA at Weeks 4 and 8 (Roche COBAS® Taqman®; lower limit of quantification: 25 IU/mL, limit of detection: 15 IU/mL) were eligible to stop all therapy at Week 12. All other patients were assigned to continue PR to Week 24. This analysis was performed when all patients in the 12-week group reached the SVR4 timepoint.

SUMMARY OF THE RESULTS: Overall, 34 of 67 (51%) enrolled patients met the response-guided criteria and were eligible for the shortened 12-week SMV+PR treatment regimen. Of these 34 patients, 68% were male, 77% white, 85%/15% had F0-F1/F2 fibrosis, 41%/38% had G4a/d subtype, and 59% were IL28B non-CC. Race, gender or genotype 4 subtype did not seem to affect eligibility to shorten therapy to 12 weeks; respectively 93%/36%/50% of IL28B CC/CT/TT patients, 54% of F0-1 and 42% of F2 patients were eligible to shorten therapy. All patients eligible to stop therapy at Week 12 achieved SVR4 (34/34). SVR4 by subgroup were: G4a/d: 100% (14/14)/100% (13/13); IL28B non-CC: 100% (20/20). The 12-week SMV+PR regimen was generally well tolerated over the entire treatment phase (Table 1).

Table 1 - Safety profile of patients eligible to receive SMV+PR therapy shortened to 12 weeks.

N (%)	SMV+PR N = 34
Any AE	31 (91)
Any SAE	0 (0)
Worst Grade 3 AE	6 (18)
Worst Grade 4 AE	0 (0)
Treatment-related AE	25 (74)
AE at least possibly related to SMV	10 (29)
AE at least possibly related to RBV	17 (50)
AE at least possibly related to PegIFN	20 (59)
Discontinuations of SMV, PegIFN and/or RBV	0 (0)

Six patients experienced a worst Grade 3 AE (neutropenia [n = 5], neutrophil count decrease [n = 1]). In total, the most common (>15%) AEs were pruritus (26%), neutropenia (26%), fatigue (18%) and decreased appetite (18%).

CONCLUSION: 51% of HCV genotype 4-infected patients met the criteria for stopping SMV+PR treatment at Week 12; all 34 patients achieved SVR4 (SVR4 rate = 100%), including patients with various G4 subtypes and with IL28B non-CC. The AE profile was comparable to that in other trials of SMV+PR.

Practical management strategies

P11

Genotype 3 HCV infection, lower CD4 cell count and higher liver stiffness are related with bone mineral reduction in HIV/HCV co-infected patients (MASTER cohort)

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BACKGROUND: In HIV/HCV co-infected patients, the bone mineral density (BMD) abnormality and risk of fracture are higher than in HIV or HCV mono-infected subjects (1). To study the variables related with low BMD in HIV/HCV co-infected patients, a retrospective analysis of MASTER cohort was implemented.

METHODS: All sequential HIV and HCV Ab+ patients, enrolled in the observational Italian MASTER database, who performed at least one DEXA scan between 2010 and 2013, based on national and international recommendation, were enrolled. Baseline was defined when DEXA scan was performed. Socio-demographics variables, clinic and laboratory data in patients with abnormal DEXA scan (T score <−1) or with normal DEXA scan are compared and analyzed. Osteopenia and osteoporosis were defined following the WHO classification (2). Elastometry was used to define liver fibrosis. Multivariate statistic analysis was performed to detect variables related with abnormal DEXA scan. Analysis was adjusted for the following covariates: age, sex, HCV genotype, presence of SVR after peg-IFN plus ribavirin, liver fibrosis stage, CD4 cell count, HIV viral load, time to HCV exposure, tenofovir use, use of boosted protease inhibitor (PI), calcium, phosphate and 25OH vitamin D plasma level, presence of proteinuria, menopause in women.

RESULTS: The number of HIV/HCV co-infected patients enrolled was 86. In 34/86 (39.5%) the DEXA scan was abnormal: in 27/34 (79.4%) osteopenia was detected and in 7/34 (20.6%) osteoporosis was diagnosed. The median age was 53 and 54 years (IQR 51–54 in booth groups) in patients with abnormal or normal examination respectively; 82 patients were on cART. All subjects assumed tenofovir and 68% a boosted PI. HIV-RNA was undetectable in 93%. CD4+ cell count tended to be lower when DEXA was abnormal (433 cell/mm^3 vs 514 cell/mm^3 p0.07). The exposure to HCV infection was 9.5 years (IQR 7–23) in patients with abnormal and 13 years (10–14) in patients with normal DEXA (p 0.02). Presence of SVR and menopause, among women, were not related with abnormal or normal DEXA. At multivariate analysis, variables related with abnormal DEXA were HCV genotype 3 infection [AOR3,3(1.0–10.9)0.04];T CD4+ cell count <500 cell/mm^3 [AOR 3.6 (95%IC1.1–11.7)0.03] and a liver stiffness >7.5 Kpa [AOR 3.5(1.0–11.6)0.03].

CONCLUSION: In HIV/HCV co-infected patients, included in the MASTER cohort, who performed a DEXA scan, following the international and national guidelines, 40% had an abnormal examination. Genotype 3, lower CD4+ cell count and higher liver stiffness were related with abnormal DEXA despite having achieved SVR.

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P12

Quacks are quick regarding management of viral infections (HBV, HCV) mainly in rural (desert) areas of Pakistan

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BACKGROUND: Hepatitis is a global problem therefore increasing numbers day by day, such silent killer diseases spreading in rural (desert) community of Pakistan widely due to using of unsterilized syringes, and unsterilized instruments. Other causes of viral infection are by using of razor, tattoo marking, and homosexuality/hetero sexuality.

PURPOSE OF STUDY: Our aim was to make the community aware, especially rural (desert) areas, about viral hepatitis and its causing factors and preventive measures and other related problems and object to collect data of hepatitis positive cases mainly treated by, quacks in rural (desert) community by mixture formula drug. Mixture formula contained Noshadhir, Kalmishoro, Ironstone, Salt, Tatri, Sodhaphoro, Lemon cast, Jokhan, Irque Badyan, Ferry Qiune like things mixing in a bottle used for these positive viral (HBV,HCV) infections and abnormal LFT.

RESULTS: Around 95 cases were included with history of abnormal LFT and Billirubin and viral positive (HBV & HCV). Males: 63 (66.3%), Female: 15 (15.7%), Children: 17 (17.8%); Male: 63 = HBV+ve 6 (6.3%), HCV+ve 16 (16.8%), Abnormal LFT = 41 (43.1%); Female: 15 = HBV+ve 3 (3.1%), HCV+ve 5 (5.2%), Abnormal LFT = 7 (7.3%); Children: 17 = HBV+ve2 (2.1%), HCV+ve6 (6.3%), Abnormal LFT = 9 (9.4%); Total: PCR investigated cases: 43(45.2%), Total: Interferon + Ribavirin taken cases: 33 (34.7%); Total: withdrawal/defaulter of treatment: 11 (11.5%), Total: complete course 18 (18.9%); Total: cases viruses –ve3 (3.1%), Total: relapse 15 (15.7%); Total: cases on lamividine/adevir 5 (5.15%), 1 –ve, 4 cases still on & off treatment. Mixture formula taken cases: 69 (72.6%); Male: 43 (45.2%), Female: 12 (12.6%), Children: 16 (16.8%); LFT Found low/normal: 63 (66.3%), Relieve complains: 65 (68.4%), Death: 5 (5.1%)

CONCLUSION: Quacks are quick regarding management of viral infection with mixture formula therapy. Though this is unethical and carcinogenic such kind of practice needs special attention and further work against viral infection, though the world is going to searching new medicines and we are still facing such issue by quacks, there is a continued need for awareness about hepatitis cause, prevention and curative campaigns.

Treatment monitoring and predictors of therapeutic response

P13

Sustained virologic response rates in HCV genotype 1-infected patients treated for 8 weeks with ombitasvir/paritaprevir/r and dasabuvir with ribavirin

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PURPOSE OF THE STUDY: For some patients with HCV genotype (GT) 1, an 8-week treatment duration may be sufficient to eradicate infection with regimens containing multiple direct-acting antivirals (DAAs). We assessed the sustained virologic response (SVR) rates in HCV GT1-infected patients who received 8 weeks of the 3 DAA (3D) regimen of ombitasvir, paritaprevir (identified by AbbVie and Enanta; boosted with ritonavir) and dasabuvir with ribavirin.

METHODS: This *post-hoc* analysis of the open-label AVIATOR phase 2 study examined baseline patient characteristics associated with higher SVR rates. All patients treated with 3D + RBV for 8 weeks were included in the analysis. Wilcoxon rank-sum tests were used to compare baseline characteristics of patients achieving SVR or not.

	8 Week 3D + RBV HCV GT1a SVR24 n/N (%)	8 Week 3D + RBV HCV GT1b SVR24 n/N (%)
Overall	47/56 (84)	23/24 (96)
Baseline viral load, IU/mL		
≤10 M	31/37 (84)	19/19 (100)
≤8 M	30/34 (88)	16/16 (100)
≤6 M	29/32 (91)	13/13 (100)
≤5 M	25/27 (93)	12/12 (100)
≤4 M	20/22 (91)	12/12 (100)
≤3 M	13/15 (87)	9/9 (100)
≤2 M	11/11 (100)	8/8 (100)
≤1 M	9/9 (100)	5/5 (100)

SUMMARY OF THE RESULTS: Eighty treatment-naïve patients without cirrhosis received 8 weeks of 3D + RBV, including 56 with GT1a infection, 46 males, 9 blacks, 56 with IL28B non-CC genotype, and a mean baseline viral load of 6.85 $\log_{10}$ IU/mL. Overall, 70/80 (88%) achieved SVR at post-treatment week 24 (SVR24), with all failures due to relapse. Baseline viral load was significantly higher in patients not achieving SVR24 than in those achieving SVR24 (7.08 vs 6.80 $\log_{10}$ IU/mL, $p < 0.05$). Among GT1b-infected patients, the SVR24 rate was 23/24 (96%); the single GT1b patient that did not achieve SVR24 had F3 fibrosis and a baseline viral load of 11.2 million IU/mL. Among GT1a-infected patients, the SVR24 rate was 47/56 (84%); all 11 patients with a baseline viral load ≤2 million IU/mL achieved SVR24. No differences were identified in mean baseline age, BMI, albumin, or platelet count comparing patients that did or did not achieve SVR. All relapsers were male and 6 had F3 fibrosis. Mean baseline interferon γ-inducible protein-10 (IP-10) was higher in relapsers than those that achieved SVR24 (678 vs 437 ng/L, $p < 0.01$).

CONCLUSIONS: Patients infected with HCV GT1b achieved high SVR24 rates when treated with 3D + RBV for 8 weeks. Higher baseline viral load appears to play a role in increased relapse rates among GT1a-infected patients, supporting the label-recommended 12-week treatment duration for these patients.

P14

ITPA gene variants as markers of ribavirin-induced anemia and white blood cells reduction in Egyptian HCV patients

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BACKGROUND: The non-functional ITPArs6051702 gene polymorphism was associated with ribavirin (RBV)-induced reduction in hemoglobin in some populations (1,2). We explored for the first time the relationship between this non-functional variant in addition to the known functional rs1127354 and rs7270101 ones, and the reduction in hematological parameters: hemoglobin, white blood cells (WBCs) and platelets in Egyptian hepatitis patients treated with RBV.

MATERIALS AND METHODS: Hundred and twenty-three patients treated with pegylated-interferon (peg-IFN) alpha and RBV have been enrolled. DNA was extracted from buffy coat using Qiagen DNA extraction kits; allelic discrimination was performed for ITPArs6051702, rs1127354 and rs7270101 polymorphisms through real time PCR. Evalu-

ated clinical features were delta hemoglobin, WBCs and platelets reductions at 1, 2, 4, 8 and 12 weeks of therapy.

RESULTS: We found that the genotypes AC/CC for rs6051702 at week 4 and CA/AA for rs1127354 at week 8 were associated with lower hemoglobin reduction ($p = 0.012$ and $p = 0.019$, respectively). Association was found between the presence of at least one variant allele of rs1127354 and less WBCs reduction at week 1 and week 4 ($p = 0.038$ and 0.020 respectively). Less WBCs reduction at week 1 in AA/AC genotypes of rs7270101 ($p = 0.025$) has been observed. Multivariate linear regression analysis has been done to detect factors independently associated with hemoglobin reduction at weeks 4, 8 and 12. RBV dose ($p = 0.017$) and ITPArs6051702 ($p = 0.028$) were independently associated with hemoglobin reduction at week 4. CA/AA group for rs1127354 ($p = 0.005$), peg-IFN dose ($p = 0.033$) and sex ($p = 0.036$) were independent predictors at week 8. Peg-IFN type ($p = 0.001$) was the independent predictor at week 12.

CONCLUSIONS: Genotyping of ITPA variants rs6051702 and rs1127354 could be performed to predict anemia before the start of treatment in Egyptian patients.

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P15

Does Metformin improve sustained virological response in patients with chronic hepatitis C?

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BACKGROUND: Evidence indicates that insulin resistance and type 2 diabetes mellitus result in poor sustained viral response (SVR) in patients with chronic hepatitis C (CHC). Metformin is an oral hypoglycemic agent which improves insulin resistance and diabetes control.

AIM: To determine if the addition of metformin to standard antiviral treatment improves SVR in CHC patients.

METHODS: We conducted a prospective pilot study in 43 patients treated for chronic hepatitis C in our department since January 2013. Patients were screened for type 2 diabetes and insulin resistance. All patients received pegylated interferon and ribavirin. Patients with diabetes or insulin resistance received either 500 mg of metformin three times daily for the treatment period. The primary end point was SVR, and secondary end points were viral clearance at weeks 12, and 48, and safety of metformin.

RESULTS: Type 2 diabetes was present in 16 patients (37.2%) and insulin resistance in eight patients (18.6%). The global SVR was 67.4% and was better than that reported in medical literature. The SVR rate in the metformin group was 60% versus 75% in controls which were not significantly different. The triple therapy was well tolerated, but diarrhea was more often seen in metformin group (10%).

CONCLUSION: Adding metformin to peginterferon and ribavirin was safe and improved global sustained virological response. However, further larger randomized controlled trials are needed to confirm these findings.

P16

Serum biomarkers for monitoring pegylated interferon-α plus ribavirin therapy response in chronic hepatitis C patients

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BACKGROUND: The present study aimed to evaluate immunologic aspects induced by treatment of patients chronically infected with hepatitis C virus (HCV) with pegylated interferon alpha (PEG IFN-α) plus ribavirin, correlating serum cytokines/chemokines levels measured in different therapy points and patterns of treatment response.

MATERIALS AND METHODS: The study included 54 therapy-naïve HCV genotype 1 infected patients, treated with PEG IFN-α plus ribavirin for 48 weeks, with the following patterns of response to antiviral therapy: sustained virological response (SVR), non-response (NR), relapse (REL). Patients infected with HCV of other genotypes different from 1, coinfected with HIV and/or HBV, with other hepatic comorbidities, in use of imunossupressants, with history of alcohol abuse and with history of previous antiviral treatment of hepatitis C were excluded. Serum chemokine/cytokine quantification (CCL2, CCL5, CXCL8, CXCL9, CXCL10 and IFN-α) was performed at baseline ($n = 54$: 24 SVR, 16 NR and 14 REL), 12nd ($n = 44$: 17 SVR, 16 NR and 11 REL) and 48th ($n = 36$: 18 SVR, 6 NR and 12 REL) weeks of treatment, and chemokine/cytokine responses were compared among patients with SVR ($n = 24$), NR ($n = 16$) and REL ($n = 14$) to treatment. A control group (CG, $n = 19$) consisted of healthy non-infected blood donors.

RESULTS: At baseline: significant higher levels of CCL2, CCL5, CXCL8 CXCL9, CXCL10 and IFN-α were observed in HCV infected patients (compared to CG); SVR group showed significant lower levels of CXCL8 compared to NR group; IFN-α levels were statistically significant lower in SVR group, in comparison to NR and REL patients; SVR and REL groups

showed similar CCL2 levels; CCL2 and CXCL9 levels were higher in SVR group, in comparison to NR patients. At 12th therapy week: SVR group showed statistically significant uprising levels of CXCL8 and IFN-α, compared to NR patients; CCL5 levels were higher in REL versus NR group.

CONCLUSIONS: Patients chronically infected with HCV present higher serum levels of chemokines/cytokines, due to HCV pro-inflammatory/regulatory character (1,2,3). Serum levels of CXCL8 and IFN-α could be used as surrogate markers to predict response to combined PEG IFN-α plus ribavirin therapy in naïve HCV genotype 1 infected patients.

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Direct-acting antiviral combinations

P17

Daclatasvir plus sofosbuvir with or without ribavirin in patients with HCV genotype 3 infection: interim analysis of a French multicenter compassionate use program

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BACKGROUND: Treatment options for HCV genotype 3 (GT3) patients are limited. The combination daclatasvir (DCV) and sofosbuvir (SOF) for 12 weeks is associated with high SVR rate in genotype 3 non cirrhotic patients (96% SVR12) and a lower response in cirrhotic (63% SVR12). GT3 cirrhotic remain a difficult to treat population and may benefit from the addition of ribavirin (RBV) or extended treatment duration. This analysis reports interim results from a French multicenter compassionate use program of DCV+SOF±RBV in patients with HCV genotype 3 chronic infection.

MATERIALS AND METHODS: The ATU has been managing over 4000 HCV patients from 221 French centers. Patients received DCV+SOF QD for 12 or 24 weeks, with RBV added at the physician's discretion.

RESULTS: Six hundred and one HCV genotype 3 patients with severe fibrosis (F3) or cirrhosis (F4), or HCV extrahepatic manifestations or post-liver transplant HCV recurrence or indication for liver or kidney transplantation enrolled in the program. Most patients were male (75%), HCV mono-infected (83%), cirrhotic (77%), and treatment experienced (73%). The median age was 54.3 years (27–83). 64% and 15% planned to receive DCV+SOF for 24 weeks with or without RBV, respectively, 4% and 17% planned to receive DCV+SOF for 12 weeks with or without RBV, respectively. Baseline median HCV RNA level was 6.07 (1.20–7.62) log10 IU/mL, platelets count 118.5×10^9/L (31–387) and albumin was 39.0 g/L (13–56). Treatment discontinuations were related to adverse event in one patient, death in two patients and patient's decision in 1 patient. Table 1 below shows the interim results in the first 106 patients without liver transplant who reached the week 4 post-treatment visit according to treatment schedule, fibrosis stage and patient status. Efficacy and safety data will be updated in larger population.

CONCLUSIONS: This preliminary analysis is consistent with previous findings and demonstrates that12 weeks of DCV+SOF in GT3 patients results in a high SVR4 in non cirrhotic patient population. Cirrhotic patients appeared to benefit from the extended treatment duration to 24 weeks.

Table 1 - Efficacy of DCV+SOF±RBV regimens in GT3 patient

	12 weeks		24 weeks	
	Cirrhotic	Non cirrhotic	Cirrhotic	Non cirrhotic
	DCV+ SOF±RBV	DCV+ SOF±RBV	DCV+ SOF±RBV	DCV+ SOF±RBV
SVR4	22/29 (76%)	11/12 (92%)	52/59 (88%)	5/6 (83%)

P18

All-oral 12-week treatment with daclatasvir and sofosbuvir in treatment-experienced patients infected with HCV genotype 3: a subanalysis of the ALLY-3 phase 3 study

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BACKGROUND: The phase 3 ALLY-3 study evaluated the all-oral, ribavirin (RBV)-free combination of daclatasvir (DCV; pangenotypic NS5A inhibitor) and sofosbuvir (SOF; NS5B polymerase inhibitor) in patients with GT3 infection. After 12 weeks of treatment, sustained virologic response at post-treatment Week 12 (SVR12) was achieved by 90% and 86% of treatment-naïve and -experienced patients, respectively.

MATERIALS AND METHODS: Treatment-naïve (N = 101) and -experienced (N = 51) patients received open-label DCV 60 mg + SOF 400 mg once daily for 12 weeks. This subanalysis provides further details of efficacy and safety outcomes in the experienced cohort.

RESULTS: Treatment-experienced patients were predominantly male (63%), white (88%) and non-cirrhotic (67%); 75% had baseline HCV RNA ≥800 K IU/mL, and 61% had non-CC *IL28B* genotypes. Patients had previously received IFN-based (n = 42), SOF-containing (n = 7, including 7 treated with SOF/RBV and 1 who was retreated with SOF/peg/RBV) and alisporivir-containing (n = 2) regimens. Prior responses included relapse (n = 31), null response (n = 7), partial response (n = 2), and other forms of nonresponse or IFN intolerance (n = 11). All patients completed 12 weeks of study treatment. SVR12 was achieved in 44 patients (86%); all prior null and partial responders, and IFN-intolerant patients achieved SVR12. SVR12 rates were higher in patients without cirrhosis and in those with *IL28B* CC genotype (Table 1). Treatment failure (relapse) was experienced by 7 patients, including 5 prior IFN/RBV recipients (prior response: relapsers, n = 4; HCV RNA never undetectable, n = 1) and 2 patients who relapsed after prior treatment with SOF/RBV. Of the two prior SOF relapsers, one had cirrhosis with grade 1 steatosis, and one had Fibrotest F3 with grade 2 steatosis, and a baseline NS5A-Y93 resistance-associated variant. There were no serious AEs or AEs leading to discontinuation. Grade 3/4 AEs (a single report of arthralgia) and grade 3/4 lab abnormalities (platelets, n = 1; lipase, n = 1) were uncommon. The most frequent AEs (any grade) were fatigue (26%), headache (20%), nausea (14%) and arthralgia (12%). Safety parameters were similar in those with or without cirrhosis.

CONCLUSIONS: This all-oral, 12-week combination of DCV+SOF achieved high SVR12 rates in GT3 patients previously treated with all oral DAA or IFN-containing regimens. DCV+SOF was well tolerated.

Table 1 - SVR12 rates by patient subgroup

Parameter	SVR12, % (n/N)
All treatment-experienced	86 (44/51)
Prior IFN-based regimen	88 (37/42)
Prior sofosbuvir-containing regimen	71 (5/7)
Prior alisporivir-containing regimen	100 (2/2)
Patients without cirrhosis	94 (32/34)
Patients with cirrhosis	69 (9/13)
Fibrotest F0–3	91 (39/43)
Fibrotest F4	63 (5/8)
NS5A-Y93 baseline resistance-associated variant present	71 (5/7)
NS5A-Y93 baseline resistance-associated variant absent	88 (38/43)
IL28B CC	95 (19/20)
IL28B non-CC	81 (25/31)

P19

Daclatasvir, sofosbuvir, and ribavirin combination for HCV patients with advanced cirrhosis or post-transplant recurrence: phase 3 ALLY-1 study

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BACKGROUND: The pangenotypic combination of daclatasvir (DCV) and sofosbuvir (SOF) achieves high rates of SVR in patients with chronic HCV infection. DCV+SOF has

favorable safety and drug interaction profiles and a high resistance barrier. These attributes support the ALLY-1 study of DCV+SOF with ribavirin (RBV) in patients with advanced cirrhosis or post-liver transplant HCV recurrence, who have a high unmet therapeutic need.

MATERIALS AND METHODS: This open-label study enrolled treatment-naïve or experienced adults with HCV infection of any genotype (GT) in 2 cohorts: (1) advanced cirrhosis, (2) post-liver transplant recurrence. Patients received 12 weeks of treatment with once-daily DCV 60 mg + once-daily SOF 400 mg and RBV (initially 600 mg/day, adjusted for hemoglobin and creatinine clearance). In the cirrhosis cohort, patients transplanted during treatment could receive 12 weeks of extended treatment immediately post-transplant, regardless of treatment duration before transplant. The primary endpoint was HCV RNA LLOQ (25 IU/mL) at post-treatment Week 12 (SVR12) in patients with GT1 in each cohort.

RESULTS: The cirrhosis (N = 60) and post-transplant (N = 53) cohorts were, respectively, 40% and 42% treatment-naïve and 75% and 77% GT1. The Child-Pugh class in the cirrhosis cohort was 20% A, 53% B, and 27% C. MELD score ranged from 8 to 27. No post-transplant patients had cholestatic recurrence or hepatic decompensation. Overall, 83% of patients in the cirrhosis cohort achieved SVR12, with higher SVR12 rates in patients with Child-Pugh class A or B disease than in those with class C (Table 1). In the post-transplant cohort, 94% achieved SVR12. Twelve of the 13 patients without SVR12 relapsed post-treatment. SVR12 rates were comparable regardless of prior treatment experience or baseline demographic characteristics. Four cirrhotic patients received a liver transplant during treatment; 3 of 4 extended treatment post-transplant and all 4 achieved SVR12. The most common AEs (any grade) were headache, fatigue, anemia, diarrhea, and nausea. There were no treatment-related serious AEs. One post-transplant patient discontinued all therapy after 31 days due to headache but achieved SVR12.

CONCLUSIONS: DCV+SOF+RBV for 12 weeks was safe and well tolerated in both cohorts. SVR12 rates were >90% in patients with Child-Pugh class A or B cirrhosis but lower in Child-Pugh class C. SVR12 was achieved by 94% of liver transplant recipients with HCV recurrence.

Table 1 - SVR12 rates by HCV genotype

SVR12, % (n/N)	Advanced cirrhosis cohort	Post-transplant cohort
All patients	83 (50/60)[a,b]	94 (50/53)
GT 1	**82 (37/45)**[c]	**95 (39/41)**[c]
95% CI	68, 92	84, 99
GT 1a	76 (26/34)	97 (30/31)
GT 1b	100 (11/11)	90 (9/10)
GT 1, Child-Pugh A	91 (10/11)	
GT 1, Child-Pugh B	92 (22/24)	
GT 1, Child-Pugh C	50 (5/10)	
GT 3	83 (5/6)[a]	91 (10/11)
GT 2, 4, or 6	89 (8/9)[b]	100 (1/1)

[a]Includes one patient (GT3) who had SVR12 documented after database lock.
[b]Includes one patient (GT4) who discontinued after 3 weeks for liver transplantation and achieved SVR12 off stud.
[c]Primary endpoints.

P20

Daclatasvir plus sofosbuvir for treatment of HCV genotypes 1–4 in HIV-HCV coinfection: the ALLY-2 study

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BACKGROUND: Daclatasvir (DCV; NS5A inhibitor) and sofosbuvir (SOF; nucleotide NS5B inhibitor) are potent, pangenotypic, well-tolerated, once-daily, oral HCV antivirals with limited pharmacokinetic interactions with other agents. DCV+SOF has demonstrated high rates of sustained virologic response (SVR) in HCV monoinfection. Efficacy and safety of DCV+SOF in HIV-HCV coinfection was assessed in a phase 3 study.

MATERIALS AND METHODS: ALLY-2 was a randomized, open-label study in HCV treatment-naïve (n = 151) or -experienced (n = 52) adults coinfected with HIV and HCV (any genotype). Naïve patients were randomized 2:1 to receive 12 or 8 weeks of SOF 400 mg + DCV 60 mg (dose adjusted for concomitant combination antiretroviral therapy (cART): 30 mg with ritonavir-boosted protease inhibitors [PI], 90 mg with nonnucleoside reverse transcriptase inhibitors [NNRTI] except rilpivirine). Experienced patients received DCV+SOF for 12 weeks. Primary endpoint was SVR at post-treatment week 12 (SVR12) in treatment-naïve GT-1 patients who received 12 weeks of DCV+SOF.

RESULTS: Patients were 87% male, 62% white, 34% black, and 14% cirrhotic, had a median age of 52 years, and were infected with HCV GT-1 (83%), GT-2 (9%), GT-3 (6%), or GT-4 (2%). Median baseline (BL) HCV RNA was 6.7 $\log_{10}$ IU/mL and median BL CD4 count was 565 cells/μL. Nearly all patients (98%) were on cART: 50% PI based, 25% NNRTI based, and 25% other regimens (primarily integrase

Table 1 - SVR12 rates by patient subgroups

SVR12, % (n/N)	12 week naïve	12 week exp'd	8 week naïve	SV12, % (n/N)	12 week naïve	12 week exp'd	8 week naïve
All patients	97 (98/101)	98 (51/52)	76 (38/50)	Male	98 (90/92)	98 (42/43)	79 (33/42)
[95% CI]	[89.8, 99.2]	[88.0, 99.9]	[59.7, 87.6]	Female	89 (8/9)	100 (9/9)	63 (5/8)
GT 1	**96 (80/83)**[a]	98 (43/44)	76 (31/41)	Age 65 year	97 (93/96)	98 (48/49)	77 (36/47)
GT 1a	96 (68/71)	97 (32/33)	80 (29/35)	Age ≥65 year	100 (5/5)	100 (3/3)	67 (2/3)
GT 1b	100 (12/12)	100 (11/11)	50 (3/6)	White race	96 (63/66)	100 (31/31)	71 (20/28)
GT 2	100 (11/11)	100 (2/2)	83 (5/6)	Black race	100 (30/30)	95 (19/20)	79 (15/19)
GT 3	100 (6/6)	100 (4/4)	67 (2/3)	*IL28B* CC	100 (28/28)	100 (13/13)	69 (9/13)
GT 4	100 (1/1)	100 (2/2)	-	*IL28B* Non-CC	96 (70/73)	97 (38/39)	78 (29/37)
BL HCV RNA <6 million IU/mL	97 (56/58)	100 (33/33)	79 (27/34)	PI cART	98 (46/47)	96 (22/23)	72 (21/29)
				NNRTI cART	100 (28/28)	100 (12/12)	80 (8/10)
BL HCV RNA ≥6 million IU/mL	98 (42/43)	95 (18/19)	69 (11/16)	Other cART	92 (23/25)	100 (16/16)	78 (7/9)
				BL CD4 200 c/mm^3	100 (4/4)	-	100 (1/1)
No cirrhosis	98 (88/90)	100 (34/34)	77 (34/44)	BL CD4 200–499 c/mm^3	98 (41/42)	100 (12/12)	71 (15/21)
Cirrhosis	89 (8/9)	93 (14/15)	60 (3/5)	BL CD4 ≥500 c/mm^3	96 (53/55)	100 (39/39)	79 (22/28)

BL, baseline; c, cells; cART, combination antiretroviral therapy; CI, confidence interval; GT, genotype; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor
[a]Primary endpoint.

inhibitors). A total of 98% of patients completed study treatment. Among GT-1 patients, SVR12 was achieved by 96% of naïve and 98% of experienced patients after 12 weeks of DCV+SOF and by 76% of naïve patients after 8 weeks; SVR12 rates for non-GT-1 patients in these groups were 100%, 100%, and 78%, respectively. SVR12 was similar in patients with or without cirrhosis and across other demographic and disease subgroups (Table 1). There were no HCV virologic breakthroughs, and HIV control was not compromised throughout the study period. Post-treatment HCV relapse occurred in 1–2% of patients in the 12-week treatment groups and 20% in the 8-week group. There were no treatment-related serious AEs or discontinuations for AEs.

CONCLUSIONS: Treatment of HIV-HCV coinfected patients with DCV+SOF once daily for 12 weeks resulted in an overall 97% SVR12, and was well tolerated. DCV+SOF was effective in cirrhotics, in other demographic and disease categories, and across a broad range of cART regimens without compromising HIV virologic control.

Molecular biology and characterisation

P21

Recovery of HCV-like viruses from naïve MDBK cell line inoculated with rabbit and hare DNA

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PURPOSE OF THE STUDY: Hepatitis C virus (HCV) contains an ssRNA+ genome which, upon virus entry and uncoating, functions as mRNAs and thus can be directly translated into proteins by host cell machinery (1,2). We recently described the presence of endogenous HCV homolog fragments in wild/domestic rabbits and hare genomes and their capacity to replicate in Mardin-Darby Bovine Kidney (MDBK) cell cultures (3). To understand if these small endogenous fragments were able to produce infectious entire virus particles in this same cell line was the purpose of the study.

METHOD: DNA extracts from liver homogenates of a domestic rabbit and a hare were subjected to RNAse treatment and directly inoculated in naïve MDBK cells. Their capacity to generate entire HCV-like virus particles and infectivity were evaluated by immunogold electron microscopy (IEM) with monoclonal antibodies for the NS5 and E2 HCV specific proteins, quantitative Real Time-RT-PCR (qRT-PCR) and matrix-assisted laser desorption/ionization time-of-flight/time-of-flight (MALDI-TOF/TOF-MS/MS) mass spectrometry. A phylogenetic analysis of a final consensus constructed sequence was performed for each tested sample.

SUMMARY OF RESULTS: Specific immunostaining was observed in cell suspensions of passages of inoculated MDBK cell flasks using mouse monoclonal antibodies anti-HCV NS5 (P4 and P7) and anti-HCV E2 (P4) proteins, by IEM. HCV RNA titers were measured by qRT-PCR of inoculated cells at passages P1 to P7, with ranges of 3.75–5.83 log RNA copies/ml and 4.36–5.91 log RNA copies/ml detected from rabbit and hare DNA samples respectively. By MALDI-TOF/TOF-MS/MS a total of 2338 peptide sequences, such as F, Core, E1, E2, p7, NS2, NS3, NS4A, NS4B, NS5A and NS5B HCV were identified with significant protein scores (P.S.) (P.S. >64 are significant, $p < 0.05$). Of these, 1694 and 644 were from the rabbit and hare DNA samples respectively.

The phylogenetic analysis of a constructed consensus sequences of the HCV-like viruses from rabbit (RHCV) and hare (HHCV), using the NJ and the ML methods, revealed that RHCV is more closely related to HCV-1a/HCV-1b genotypes and HHCV to HCV-1b genotype.

CONCLUSION: RHCV and HHCV HCV-like particles are produced in the MDBK cell line, suggesting that the small fragments present in the genomic DNA of the rabbit and hare can, after internalizing the MDBK cells, initiate replication and together with the cell machinery generate novel HCV-like viruses.

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P22

An outbreak of acute HCV infection with genotype 1a in a Haemodialysis Unit in Kocaeli, Turkey

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OBJECTIVE: To analyse an outbreak of acute HCV infection in a private Haemodialysis Unit. The genotype of chronic HCV infection is over 90% genotype 1 (distribution of subgroup analysis approximately 75% 1b, 25% 1a) in Turkey.

MATERIALS AND METHODS: There were 125 patients treated in a private Haemodialysis Unit. They have known 12 HCV infections before the outbreak. A separate machine was used for these patients. Then began new HCV infection cases from July 2013 that not infected before. A total of 26 new HCV infections in serial were identified in approximately 5 months from the same unit. The diagnosis was based on ALT elevations, anti-HCV detection and HCV-RNA detection. Other virological tools including HCV genotype determination and NS 3 gene phylogenetic tree analysis of the acute hepatitis C epidemic were also used to tailor the epidemic nature. HCV genotype/subtypes were identified by phylogenetic analysis of NS3 sequences (codon 27–181 of protease domain). Nucleotide sequences were compared consensus HCV sequences from Strain H77, D90208, HPCPLYPRE, HPCCGAA, HPCJCG, HPCHUMR, HPCCGS and AY051292. Phylogenetic comparisons were performed using neighbour-joining method with the CLC Sequence Viewer 6.9.1 (CLC bio A/S, Denmark). All the personnel who worked there were tested for HCV infection. We took over 100 samples from different machines, solutions that used in the unit.

RESULTS: We identified 26 (10 female and 16 male) with acute hepatitis C infection between July and November 2013. The mean HCV RNA were 7217 432 IU/mL. All of them were known anti-HCV negative before this epidemic. The known HCV positive patients of the center were all genotype 1b. But in this epidemic we found another responsible strain. Phylogenic analysis identified one distinct HCV group and genotype 1a. The index case-patient is not known yet. No multidose medication vials or material was shared between patients. The infected patients had used different dialysis machines. The working personnel were found all negative for HCV infection.

CONCLUSION: During this outbreak, HCV transmission was mainly with a new patient with acute nonsymptomatic infection to another patient via healthcare workers' hands. We could not find another possible source in the unit.

P23

Bioinformatic analysis of codon usage and phylogenetic relationships of different genotypes of hepatitis C virus

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BACKGROUND: Hepatitis C viral infection has six major genotypes. The purpose of this study was to investigate phylogenetically different genotypes of hepatitis C virus and amino acid codon usage in the structure of the virus proteins to discover new methods of treatment regimes.

MATERIALS AND METHODS: Codon usage of the six genotypes of HCV nucleotide sequence was investigated through the online application available on the website of Gene Infinity. Also, phylogenetic analysis and evolutionary relationship of HCV genotypes were analyzed using software MEGA 4.

RESULTS: In the first group genotypes 1 and 5 (74.02%) and in the second group genotypes 2 and 6 (72.43%) have the most similarity on codon usage. Unlike the results of similarity of codon usage, phylogenetic analysis study showed the most closely resembles and correlation between genotype 1 and 4.

CONCLUSION: Genotypes 1 and 4 have the remarkable similarity of genome sequences and proteins, but in terms of preferred codons for genes expression have the greatest difference. More and additionally studies are needed to confirm the results and select the best approach for treatment of these genotypes based of preferred codons.

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Other

P24

A new role for CD4+CD25+ regulatory T cells in promoting development of fibrosis in HCV nonstructural 3/4A transgenic mice

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BACKGROUND: CD+CD25+ regulatory T cells (Tregs) are considered to affect the outcomes of hepatitis C virus (HCV) infection by suppressing HCV-specific T cell responses. However, the role of Tregs in HCV infection, particularly in inflammation and fibrogenesis is still unknown. We here aimed to study the role of CD4+CD25+ Tregs in the development of fibrosis in HCV NS3/4A complex expressing transgenic mice (NS3/4A-Tg).

MATERIALS AND METHODS: Tregs were depleted in NS3/4A-Tg mice by administration of Treg-specific antibodies for 4 weeks, while control group received isotype antibodies. Two weeks later, mice were treated either with olive oil or CCl4 (1 mL/kg) for 2 weeks to induce experimental liver fibrosis. Liver damage, hepatic stellate cells (HSCs), inflammation (particularly M1 and M2 macrophages) and Th1/Th2/Th17 responses were examined using immunohistological staining and quantitative gene expression.

RESULTS: Hepatic expression of a functional NS3/4A protease did not induce spontaneous fibrosis. During CCl4-induced liver fibrosis, depletion of Tregs resulted in decreased collagen-I expression indicating reduced fibrogenesis in NS3/4A-Tg mice. Treg-depletion significantly inhibited hepatic stellate cells (HSCs) proliferation (desmin) and activation (α-SMA) suggesting positive correlation between Tregs and HSCs. Suppressive YM1-positive M2 macrophages and MMP-13 expression were drastically increased in absence of Tregs, whereas IL-1β, TNFα and IL-6 expression was significantly reduced suggesting reduced intrahepatic inflammation. Finally, Treg-depletion skewed the response towards Th2-phenotype, and reduced Th1 and pro-inflammatory Th17 responses in NS3/4A-Tg mice.

CONCLUSION: Tregs not only suppress HCV-specific T cells but also contribute to development of hepatic fibrosis, which widens their role during HCV infection.

P25

Profile of chemokines, circulating microparticles and biological systems approach in patients with chronic HCV infection before and during triple therapy

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INTRODUCTION AND AIMS: To characterize clinical and laboratory aspects, chemokines profile and circulating microparticles in patients with chronic HCV infection before and during triple therapy.

PATIENTS AND METHODS: Twenty patients infected with HCV genotype 1 referred to HCV treatment with triple therapy (telaprevir or boceprevir and PR) were consecutively included. The MCP-1/CCL2, RANTES/CCL5, IL-8/CXCL8, MIG/CXCL9 and IP10/CXCL10 chemokines (BD™

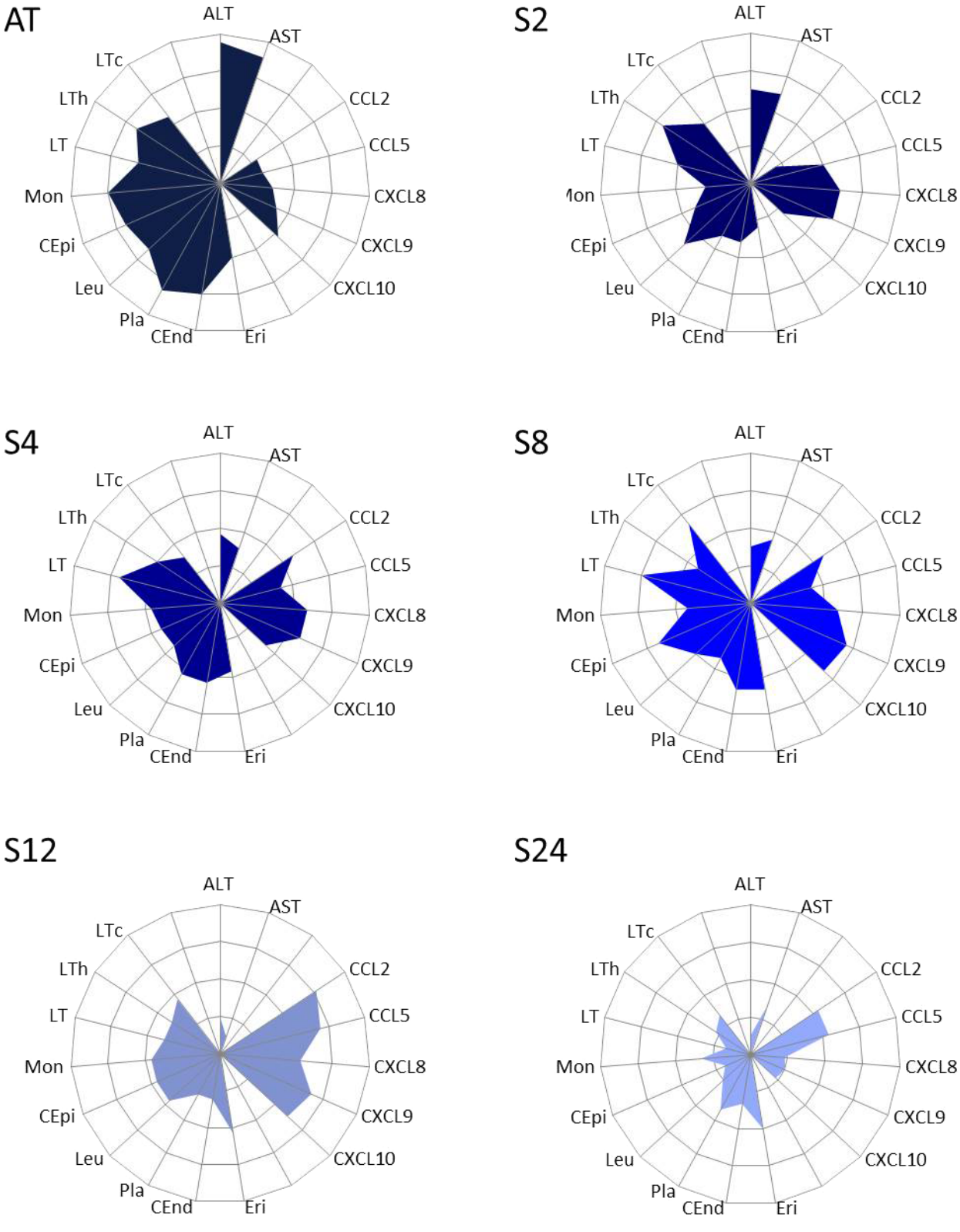

Figure 1 – Analysis of the overall pattern of liver enzymes, chemokines and circulating microparticles of patients with chronic HCV infection before virus (BT) and during treatment (DT) at weeks 02, 04, 08, 12, 24 and 48. Data are presented in radar charts that highlight the minimum, 25%, 50% – median, and maximum, 75% of the population evaluated

Cytometric BeadArray – CBA) and microparticles (MPs) were quantified in plasma by flow cytometry adapted from protocols (Bode and Hickerson Bode, 2000; couperet al., 2010) by comparing the data before treatment (BT) and during treatment (AT) at weeks 2 (n = 20), 4 (n = 20), 8 (n = 19), 12 (n = 18), 24 (n = 16) and 48 (n = 12). Statistical analysis considered significant p value < 0.05 (GraphPadPrism software, San Diego, E.UA., version 5.00).
RESULTS: 13/20 (70%) and 7/20 (35%) were male and female, respectively, mean age 58.5 ± 5.7 years. 14/20 (75%) had HCV G1b and 6/20 (30%) G1a. The circulating levels of chemokines were analyzed: there was an increase of CCL2 at week 12 AT compared to BT. CXCL8 increased at week 12 AT compared to BT. CXCL9 and CXCL10 decreased at week 24 compared to week 12 AT. The MPs originated from lymphocytes TCD3 + decreased at weeks 12, 24 and 48 AT compared to BT and decreased at weeks 24 and 48 compared to week 4 AT. The MPs originated from lymphocytes TCD4 + decreased at the weeks 12 and 24 compared to BT and decreased at week 12 compared to week 4 AT. MPs originated from monocytes decreased at weeks 2, 12 and 24 AT compared to BT and increased at week 48 compared to week 24. The MPs originated from neutrophil decreased at the weeks 2 and 24 AT compared to BT decreased at week 24 compared to week 8 AT and increased at week 8 compared to week 2 AT. After treatment, there was a progressive decrease in liver enzymes and microparticles, which was accompanied by increase of chemokines with a peak at week 12 of treatment. At week 24 post-treatment, there was a reduction in most of the biomarkers compared with the frequency shown before treatment, except for CCL2 and CCL5 which had a frequency similar to that found before the treatment.
CONCLUSIONS: The concentration of liver enzymes is inversely proportional to the treatment continuation, demonstrating a reduction of the liver damage. Circulating chemokines CCL2, CXCL8 were associated with the recruitment of cell types involved in the defense mechanisms against viral infection. In contrast, the chemokines CXCL9 and CXCL10 exhibit reduction throughout treatment. The reduction of MPs during the treatment suggests that it promotes a favorable environment for more effective immune response against the HCV virus. The scenario observed suggests a immunomodulatory profile during treatment until the end of treatment. Financial support for this study was provided by: FIOCRUZ, CNPq and FAPEMIG.

P26

Gene expression profiling of human oxidative stress and antioxidant genes in patients with hepatitis C virus induced liver fibrosis

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BACKGROUND: Hepatitis C virus (HCV) is a main cause of chronic liver disease that affects ~170 million people worldwide (1). In the local Pakistani population, the prevalence of chronic hepatitis C (CHC) is about 6–10% (2). Sixty to eighty percent of the patients develop chronic infection leading to liver fibrosis, cirrhosis and eventually hepatocellular carcinoma (HCC) (3). The progression of disease involves several cellular-level modifications in the host such as cellular infiltration, apoptosis, remodeling and the interferon pathways (4). Oxidative stress is a major contributor in the progression of HCV-induced liver pathogenesis but little is known of the changes in liver gene expression during the early stages of liver fibrosis associated with chronic HCV infection (5). In the present study, we report expression profiling of oxidative stress genes in patients with HCV induced liver fibrosis by using real-time polymerase chain reaction (RT-PCR).
MATERIALS AND METHODS: A total of 50 treatment naïve CHC positive patients and 4 normal samples were selected for expression profiling. The biopsy staging and grading was performed using METAVIR classification. Total RNA was extracted from liver tissues followed by cDNA synthesis and real-time PCR. Expression profiling was performed using oxidative stress and antioxidant pathways arrays. The relative gene expression levels were measured using ΔΔCt method. A fold change of >1.5 and <0.75 along with adjusted p value < 0.05 were considered to be statistically significant. Adjusted p values were calculated using R language. The expression levels of differentially expressed genes were also correlated with the biochemical parameters and treatment outcome of CHC patients via statistical methods.
RESULTS: Expression profiling of a total of 84genes from the oxidative stress and antioxidant pathways was performed. Twenty-eight differentially expressed genes (upregulated:17, downregulated:11) were identified between fibrosis stage (F0,F1,F2) and grade (A1,A2,A3) of CHC patients compare to normal samples (Fig. 1). Gene ontology (GO) enrichment analysis showed that differentially expressed genes clustered into all three GO categories. Furthermore, it was also found that differentially expressed genes correlated with fibrosis stages and biochemical parameters of CHC patients.
CONCLUSIONS: The findings of present study highlights the potential of differentially expressed genes to be used as potential biomarkers and therapeutic targets in CHC patients.

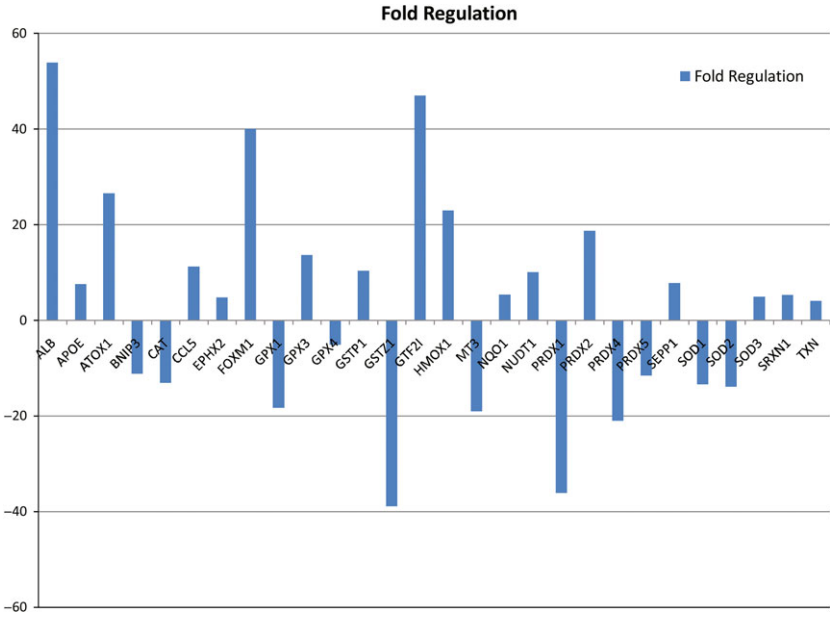

Figure 1 – Expression profiling of differentially expressed genes at fibrosis stages compare to normal samples. The data is represented in fold change.

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P27

High expression of indoleamine 2,3-dioxygenase in cirrhotic livers of HCV patients

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BACKGROUND: HCV is an emerging pathogen that has infected more than 170 million people worldwide. The heme containing enzyme indoleamine 2, 3-dioxygenase (IDO) degrades the amino acid tryptophan into its metabolites, inducing immunosuppression. According to earlier studies, high IDO immunoreactivity is correlated with frequency of liver metastases and poor prognosis of patients. We hypothesized that induction of IDO may suppress T-cell reactivity to viral antigen in chronic HCV infection.

METHODS: Immunohistochemistry was performed on 33 liver biopsies of HCV patients taken from tertiary healthcare hospitals of Rawalpindi and Islamabad exhibiting chronic symptoms and resulting slides were evaluated for IDO expression and scored and graded for liver fibrosis and inflammatory changes.

RESULTS: Our results suggested a significant correlation of IDO expression with increased staging and grading of liver biopsies of HCV positive patients with 72% subjects exhibiting over expression of IDO. Analysis of relationship with clinicopathological parameters revealed a significant correlation of IDO expression with degree of liver scarring on the Knodell and Metavir scoring and grading systems. Results showed that IDO positive staining was present not only in cirrhotic cells, but also in surrounding normal liver cells.

CONCLUSIONS: This evidence suggests an important role of IDO expression in chronic HCV infection and its correlation with liver inflammation and scarring.

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HEPATITIS B VIRUS

Diagnosis and monitoring

P28

The use of terminal restriction fragment length polymorphism for the evaluation of antiviral resistant hepatitis B virus subpopulations in patients with chronic hepatitis B

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BACKGROUND: Antiviral therapies with nucleot(s)ide analogues(NA) is crucial in the treatment of chronic hepatitis B (CHB) as it substantially protects patients from the complications of the disease. However emergence of antiviral resistance in response to long term NA therapy is an important obstacle in the treatment of CHB. In the clinical meaning, resistance is associated with the presence of virological breakthrough which is defined as >1 log increase in the lowest viral load achieved during NA therapy. However, in fact clinical resistance takes place long after (months) the development of genotypic resistance which is associated with the occurrence of resistance mutations in the patients Hepatitis B Virus (HBV) pool. In this respect, determining genotypic resistance is crucial for timely management of the NA therapy. Considering the selective pressure of the NA therapy, the populations of resistant viruses grow over time and become dominant in the patient's HBV pool. In this context to determine antiviral resistant virus populations as soon as they emerge in the viral pool, a genotypic monitoring method with high sensitivity and specificity is of great importance.

MATERIALS AND METHODS: In this research study, to address the above mentioned issue, we investigated the sensitivity and specificity of the Terminal Restriction Fragment Length Polymorphism (T-RFLP) method in detecting HBV subpopulations carrying antiviral resistance mutations. For this aim, differentiation of mutant strains from wild type strains were demonstrated by PCR-RFLP method. With using recombinant plasmids containing mutant and wild type HBV genomes, we constructed artificial quasispecies populations in order to determine the sensitivity of PCR-T-RFLP method in detecting antiviral resistant HBV

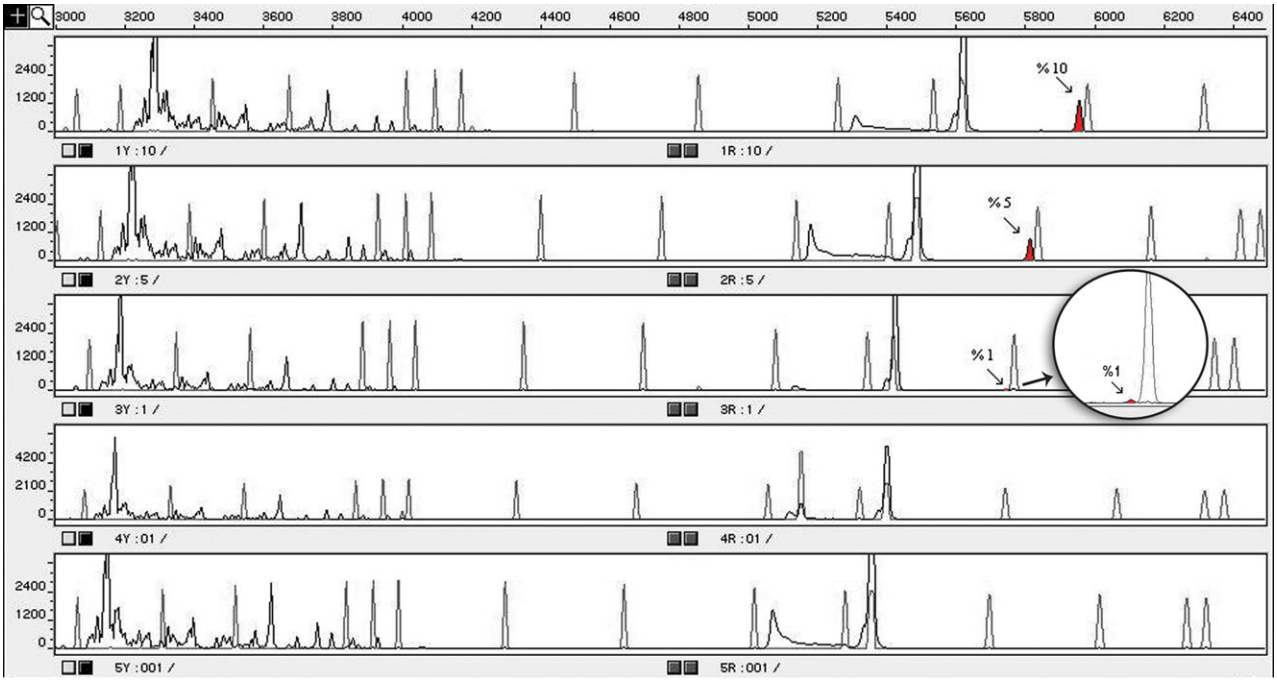

Figure 1 – 2 PCR-T-RFLP assay with quasispecies HBV populations that contain mutant (L180M) HBV subpopulations at various ratios. Five electropherograms represent the T-RFLP assay results of quasispecies HBV populations of which mutant (L180M) to wild type ratios were adjusted to 10%, 5%, 1%, 0.1% and 0.01% from top to the bottom respectively. Peaks (red color) that identify the mutant (L180M) HBV subpopulations representing the 10%, 5% and 1% of the total HBV population were indicated with arrows

subpopulations. Finally by comparing with the DNA sequencing method, we demonstrated the specificity of T-RFLP method in genotyping HBV populations.

RESULTS: We showed that T-RFLP is able to detect HBV subpopulations representing as low as 1% of the whole viral population. Additionally T-RFLP showed 100% concordance with the DNA sequencing method in genotyping HBV populations.

CONCLUSION: Considering the other genotyping methods used in evaluating HBV populations, T-RFLP showed high sensitivity and specificity profiles in detecting antiviral resistant HBV subpopulations. Therefore T-RFLP method can be easily employed in genotypic evaluation of patients' HBV populations during the course of NA therapy.

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Inactive hepatitis B virus carrier and HBeAg negative chronic hepatitis B patient's serum cytokeratin 18 and quantitative HBsAg levels

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BACKGROUND: In long term periods, inactive hepatitis B virus (HBV) carriers, who characterized by HBeAg negativity, normal aminotransferase levels, low serum HBV DNA are expected to have a good prognosis. In HBeAg negative chronic HBV, which is defined as HBeAg negativity, variable aminotransferase levels, low but fluctuating viral load, cirrhosis and hepatocellular cancer risk has increased. Therefore, it is important to differentiate HBeAg negative chronic HBV patients and inactive HBV carriers. Hepatic apoptosis has a major role in pathogenesis of chronic liver disease. Cytokeratin 18 (CK18) is released through blood stream during hepatic apoptosis by the hepatic caspase activity. In recent years, there are new data about that quantitative HBsAg (qHBsAg) may be used for viral monitorization and differentiation of phases of HBV. In our study, we aimed to evaluate serum levels of qHBsAg and CK18 in inactive HBV carriers and HBeAg negative chronic HBV patients and their diagnostic accuracy.

MATERIALS AND METHODS: In healthy controls (n = 25), inactive HBsAg carriers (n = 30) and HBeAg negative chronic HBV patients (n = 30), serum CK18 levels (PEVIVA, Sundbyberg, Sweden) were measured. Also, serum qHBsAg (Architect HBsAg Reagent kit, Sligo, Ireland) levels were measured in patient group (n = 60).

RESULTS: The difference of serum levels of CK18 didn't reach statistical significance between three groups. qHBsAg ($p = 0.11$) level was significantly lower in inactive HBsAg carriers than HBeAg negative chronic HBV patients.

CONCLUSIONS: In conclusion, qHBsAg may be a useful test for differential diagnosis of inactive HBsAg carriers and HBeAg negative chronic HBV patients. CK18 was found efficient for this and for differentiating healthy controls from HBV patients in this study. Therefore, further studies of larger patient scales are necessary in order to generalize this.

Natural history and epidemiology

P30

HBV-linked chronic inflammation and hepatocellular carcinomas in Arctic natives might be triggered by elevated body burden dioxin

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According to the US CDC, the rate of hepatitis B lesions has been high among Alaska Natives, and the annual incidence of hepatocellular carcinoma (HCC) among Eskimo males was five times that of white males in the United States (1). Within 1400 Alaska Natives the Hepatitis B virus (HBV) carriers, the relative risk factor of developing HCC was 148 compared to the general population (2). And yet, the trigger factors are still unclear, although a certain level of environmental concern was raised. To this point, unexpectedly significant airborne transfer of coplanar dioxins (TCDD) from middle to high attitudes has been registered (3). As TCDD is extremely hydrophobic, it increasingly accumulates through the marine food chain thus making Arctic people exposed to larger concentrations of TCDD because their traditional diet includes sea mammal fat. Resulted from that, Arctic Inuit adults have 10–25 times higher body burden of TCDD compared to control samples from Southern Quebec (4). According to current concept of Xenobiotical Virology (5), this level is within the range of TCDD concentrations able to transcriptionally up-regulate HBV, which possesses multiple "dioxin-responsive elements" in the virus promoter regulatory regions. Thus the HCC attributable to HBV infection in the Arctic inhabitants may now need to be revised in light of the fact that new non-viral associations have been discovered. Namely, a molecular mechanism of trans-activation by body burden TCDD of DRE-containing HBV gene is established. So, in addition to using vaccine against HBV, effective medicines targeting the host cell Ah receptor and viral DRE can be utilized to treat Arctic Native carriers of HBV who used to consume TCDD-contaminated marine food.

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P31

Non-responders to hepatitis B vaccine among Egyptian healthcare workers: a pilot study

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BACKGROUND: Hepatitis B virus (HBV) is a widely spreading worldwide virus. HBV can cause acute hepatitis, which in some cases leads to acute liver failure. Approximately 5% become chronic carriers and are at risk of developing liver cirrhosis and hepatocellular cancer. Transmission of HBV can be prevented by vaccination. After immunization, a serum titer of antibodies to hepatitis B surface antigen (anti-HBs) of ≥10 mIU/mL has been shown to be effective in preventing disease and is the generally accepted level for determining that a vaccine response has occurred. Health care personnel are at risk of acquiring HBV infection, and many authorities recommend vaccination. After a standard 3-dose vaccination regime at 0, 1, and 6 months, the rate of response on the basis of an anti-HBs titer of ≥10 mIU/mL is 90–95%. A portion of vaccinated individuals do not develop anti-HBs titers of ≥10 mIU/mL after 3 or more doses and are considered to be non responders.

AIM OF THE STUDY: The study was carried at Mansoura University Children Hospital where health care workers were subjected to hepatitis B vaccination by intramuscular injection. Before vaccination, complete questionnaires were obtained from each participating and viral markers for hepatitis B and hepatitis C were determined. After complete vaccination at 0, 1 and 6 months antibody titers for anti S antigen for hepatitis B virus was measured to determine the presence of protective antibodies titre >10 mIU/ml before vaccination and 1 month after each dose.

RESULTS: The total health care workers subjected to vaccination program were 302 workers they were mainly female nurses (295) and 7 males. The conversion rates for protection antibodies titers was 97.8% while 22.2% failed to show any seroconversion. The analysis of data showed that the presence of immunosuppression condition such as presence of diabetes mellitus and corticosteroids therapy was significantly associated with failure of seroconversion ($p = 0.01$). Moreover, subjects with hepatitis C viremia had significant failure to develop antibodies titer.

CONCLUSIONS: The vaccination program toward hepatitis B virus is an important issue for health care workers. There is a higher failure rate for seroconversion after routine hepatitis B vaccination in our study than that reported worldwide. Several factors adversely affect the antibody response to hepatitis B surface antigen which includes immunosuppression conditions such as diabetes mellitus and corticosteroids. The interesting finding in our study that presence of chronic hepatitis C infection even affect the development of protective antibody titers. Further studies should be carried out on large scale basis for the immunogenic make up for failure of vaccination.

Practical management strategies

P32

Safety and immunogenicity of 2 intramuscular double and high doses hepatitis B vaccine regimen in hemodialysis patients: a randomized controlled trial

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PURPOSE OF THE STUDY: To explore whether two-dose schedule of 20 μg or 60 μg recombinant hepatitis B vaccine (HepB) could better seroconvert among hemodialysis patients, so as to determine the optimum strategy of HepB for hemodialysis patients.

METHOD: Two hundred hemodialysis patients, who were serologically negative for hepatitis B surface antigen (HBsAg), hepatitis B surface antibody (anti-HBs) and in the absence of previous hepatitis B vaccination history, were centrally randomized into two groups, receiving 2 intramuscular injections of the double dose (20 μg) or high dose (60 μg) at weeks 0 and 4. Blood samples were obtained from each selected participant at weeks 24 to evaluate the production of anti-HBs antibodies using enzyme-linked immunosorbent assay (ELISA).

SUMMARY OF RESULTS: One hundred and four patients (54 males and 50 females, 44.02 ± 12.43 years old, 1.89 ± 2.60 years of hemodialysis duration) received 20 μg HepB, and 96 patients (49 males and 47 females, 44.74 ± 13.20 years old, 1.76 ± 1.89 years of hemodialysis duration) received 60 μg HepB. No differences in baseline characteristics (gender, age, marital status, degree of education, duration of hemodialysis, and basic disease, etc) across the study groups were observed ($p > 0.05$). Fifteen patients did not receive the vaccine and were not included in the analysis. Overall, 96 patients in the 20 μg group and 89 patients in the 60 μg group were vaccinated and included in the analyses. At weeks 24, the seroconversion rate of anti-HBs (anti-HBs concentrations ≥10 mIU/ml) and anti-HBs concentrations in the 20 μg group (51.04%, 113.67 mIU/mL) were both significantly lower than 60 μg

group (78.65%, 227.12 mIU/mL) ($\chi^2 = 15.34$, $p < 0.001$; $Z = -4.72$, $p < 0.001$). And the high-level response (anti-HBs concentrations≥100 mIU/ml) (17.72%) in the 20 μg group was also significantly lower than 60 μg group (33.71%) ($\chi^2 = 6.238$, $p = 0.013$). Meanwhile, none of the adverse events (solicited local reactions and systemic reactions) were reported in both groups.

CONCLUSION: In hemodialysis patients, 2 doses of 60 μg recombinant hepatitis B vaccine induced significantly higher seroconversion rate and concentrations of anti-HBs than 20 μg vaccine.

Molecular biology and characterisation

P33

Comparison of detection rate and mutational pattern of drug-resistant mutations between a large cohort of genotype B and genotype C HBV-infected patients in North China

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BACKGROUND: Hepatitis B virus (HBV) genotype C and B (HBV/C and HBV/B) are prevalent genotypes in China, while the association of them with drug resistance has not been well revealed. The study aimed to clarify whether HBV drug-resistant mutations were affected by the two different genotypes.

MATERIALS AND METHODS: A total of 13,847 nucleos(t)ide analog-experienced patients with chronic HBV infection from North China were enrolled. The patients were sampled for HBV resistance testing at visiting Beijing 302 Hospital from July 2007 to March 2013. HBV genotypes and resistant mutations were determined by direct sequencing of HBV Pol/S (nt 54-1277) region, and cloning sequencing was performed if necessary. Well-known primary mutations were taken into account. Briefly, lamivudine-resistant mutation includes rtM204V and rtM204I. Adefovir-resistant mutation includes rtA181V and rtN236T. Entecavir-resistant mutation includes rtM204V/I plus rt184 or rt202 or rt250 substitutions. Coexistence of rtM204V/I conferring resistance to nucleoside analogue lamivudine and rtA181V/rtN236T conferring resistance to nucleotide analogue adefovir is defined as multidrug-resistant mutation.

RESULTS: HBV genotype was 14.3% for HBV/B, 84.9% for HBV/C, and 0.8% for HBV/D across the study population. There was no significant difference in general drug usage patterns between HBV/B and HBV/C patient groups. Lamivudine-resistant mutations was more frequently detected in HBV/C patients compared to HBV/B ones (31.67% vs. 25.26%, $p < 0.01$). Adefovir- and entecavir-resistant mutation incidence was similarly but with different dominant mutational pattern between the two genotypes. Compared with HBV/C patients, HBV/C patients had a higher incidence of rtA181V (5.29% vs. 1.36%, $p < 0.01$) and a lower incidence of rtN236T (2.70% vs. 6.54%, $p < 0.01$) for adefovir-resistant mutation; and a higher incidence of rtM204+rtT184/S202 (3.66% vs. 2.16%, $p < 0.01$) and a lower incidence of rtM204+rtM250 (0.67% vs. 1.46%, $p < 0.01$) for entecavir-resistant mutation. Noticeably, HBV/C patients had a higher incidence of multidrug-resistant mutation than HBV/B patients (0.83% vs. 0.35%, $p < 0.05$). The dominant mutational pattern was rtM204V/I+rtA181V (HBV/C 0.72% vs. HBV/B 0.30%, $p < 0.05$). Seven patients with triple-drug-resistant strains (lamivudion-, adefovir- and entecavir-resistant mutations collocated in the same viral gene) were all infected with HBV/C. None of HBV/D patients was detected with multidrug-resistant mutation.

CONCLUSION: HBV/C-infected patients have a higher risk to develop lamivudine-resistant and multidrug-resistant mutations compared to HBV/B-infected patients.

P34

The rtI233V of hepatitis B virus may serve as a compensatory mutation associated with adefovir resistance

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BACKGROUND: Adefovir dipivoxil (ADV) is still widely used in China for treating chronic hepatitis B, either in single or in combination with nucleoside analog. The study aimed to clarify whether hepatitis B virus (HBV) rtI233V substitution affects ADV resistance.

MATERIALS AND METHODS: A total of 18 419 patients with chronic HBV infection from Beijing 302 Hospital were investigated. HBV complete reverse-transcriptase region of the polymerase was screened by direct sequencing and verified by clonal sequencing if necessary. Replication-competent wild-type and mutant HBV genomic amplicons were transfected into HepG2 cells for phenotypic analysis of viral replication capacity and drug susceptibility.

RESULTS: HBV rtI233V substitution was detected in 38/5344 (0.71%) ADV-treated patients and in 8/13 075 patients without receiving ADV ($p < 0.001$). By contrast, signature ADV-resistant mutations rtA181V and/or rtN236T were detected in 1311 patients, representing 7.12% (1311/18 419) of the study population and 24.53% (1311/5344) of the patients who were receiving ADV at the time of resistance testing. Direct sequencing showed that rtI233V substitution emerged alone in 33 patients, in conjunction with ADV-resistant mutation (rtA181V, rtN236T) in nine patients, and in conjunction with lamivudine-resistant mutation (rtM204I, rtM204V) in

four patients who had experienced LAM prior to ADV treatment. Eight patients with rtI233V ± rtA181V/rtN236T had virologic breakthrough in clinical course of ADV treatment. Phenotypic analysis showed that rtI233V mutants from representative patient 1 and patient 2 exhibited 1.57-fold and 1.51-fold decreased susceptibility to ADV respectively compared to wild-type virus; by contrast, rtN236T and rtI233V+N236T mutants from patient 1 had 6.82-fold and 5.28-fold decreased susceptibility to ADV. The rtI233V, rtN236T and rtI233V+N236T mutants had 97.5%, 30.2% and 69.7% of replication capacity compared to wild-type virus in the absence of antivirals and all remained susceptible to lamivudine, entecavir and tenofovir. Viral replication capacity correspondingly decreased after eliminating rtI233V from the rtI233V+N236T mutant and restored after introducing rtI233V into the rtN236T mutant. In clinical practice, switching-to or combining with entecavir rescue therapy suppressed HBV DNA to undetectable level for rtI233V-related ADV-refractory patients.

CONCLUSION: The rtI233V substitution usually emerged in ADV-treated patients with little impact on ADV susceptibility, but it effectively restored replication capacity of rtN236T mutant, suggesting that rtI233V may partly serve as a compensatory mutation associated with ADV resistance.

P35

Characterization of novel hepatitis B virus (HBV) preS/S-gene mutations from a patient with occult HBV infection

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BACKGROUND: Occult HBV infection (OBI) can be caused by viral preS/S gene mutations. The study aimed to characterize novel HBV preS/S-gene mutants from an OBI patient with hepatocellular carcinoma.

MATERIALS AND METHODS: PreS/S gene mutations of four sequential serums were determined by sequencing. Viral replication and expression characteristics were analyzed.

RESULTS: Twenty-three kinds of preS/S-gene mutant strains that harbored single or combined mutations were cloned from four sequential samples, including 14 novel mutants as follows: (1) sI/T126V; (2) sI/T126V+sG145R; (3) sQ129N+sG145R; (4) PreS1 nt3014-3198 deletion; (5) PreS1 nt3046-3177 deletion; (6) PreS1 nt3046-3177 deletion+s115-116"INGTST" insertion; (7) PreS1 nt3046-3177 deletion+s115-116"INGTST" insertion+sG145R; (8) PreS1 nt3115-3123 deletion+sQ129N; (9) PreS1 nt3115-3123 deletion+s126-127"RPCMNCTI" insertion; (10) s115-116"INGTST" insertion; (11) s115-116"INGTST" insertion+sG145R; (12) s126-127"RPCMNCTI" insertion; (13) s112-123 "KSTGLCK" insertion+sQ129N; (14) PreS2 initiation codon M→I+s131-133TSM→NST mutation. The proportion of two viral strains increased in a stepwise manner in viral pool, i.e., the one with preS1 nt3046-3177 deletion (3.7%, 13.0%, 14.8% and 21.1%), and the one with preS2 initiation codon M→I+s131-133TSM→NST mutation (0%, 26.1%, 59.3% and 73.7%). Phenotypic analysis showed that 9 major mutant strains had either comparable or reduced HBV replication capacity, but all had significant decreased HBsAg level (decreased by 51.2–99.1%) compared with wild-type strain. Western blotting showed that sQ129N or s131-133TSM→NST mutation with an additional N-glycosylated site significantly reduced binding of anti-HBs to HBsAg. Compared with wild-type strain, the replication capacity and surface antigen promoter *II* activity of preS1 nt3046-3177 deletion strain decreased by 43.3% and 97.2%, respectively.

CONCLUSION: OBI presentation of this HBV-infected HCC patient was caused by complicated multiple S-gene mutations. HBsAg reduction mechanism varied among different mutant types. Multiple PreS/S-gene mutations might co-play a role in disease progression.

P36

Annual Viral Hepatitis Surveillance in Kenya: outcome of a year survey in 2014

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BACKGROUND: Viral hepatitis accounts for majority of liver-related diseases worldwide. Hepatitis B and hepatitis C are the two major strains causing the most morbidity and mortality. In Kenya, the status of both infections in the general population is unclear. Current data on the prevalence of viral infections have been based on highly selected groups in limited localities which may not be representative of the situation in the country. To gather data to support national prevention and control strategies of viral hepatitis, we are carrying out population surveys on diverse groups in various parts of the country. Here we present our preliminary data on several groups we screened during the year 2014.

METHODS: Blood samples were obtained from outpatient attendees presenting with fever and jaundice, commercial female sex workers, blood donors, liver clinic attendees, hepatitis B voluntary testing participants during viral hepatitis campaigns. Populations were drawn from Nairobi, Mombasa, Kisumu, Nakuru and Eldoret. Samples were screened for hepatitis B and C using rapid testing kits and ELISA and samples found positive amplified and sequenced.

RESULTS: A total of 15,637 samples were screened for hepatitis B (HBsAg) and 11,580 screened for hepatitis C. These were outpatient clinic attendees 505, sex workers 100, liver clinic attendees 395, blood donors 10,580, and voluntary testing participants, 4057. The prevalence of HBV was 4.7% among outpatient clinic attendees, 3% among commercial sex workers, 29.87% among liver clinic attendees, 0.81% in blood donors and 4.2% among voluntary HBV testing participants. No sample tested hepatitis C positive. Of the 99 HBV positive samples successfully amplified and sequenced, 90 (89.6%) belonged to HBV genotype A and 9 to genotype D. Majority (8 of 9) of the D genotype were drawn from patients attending clinics in Eldoret.
CONCLUSIONS: Hepatitis B may be on the rise among low risk populations and is a major contributor to liver clinic attendance in the country's referral hospitals. Hepatitis B vaccinations among the adult populations need to be intensified. North Rift region may be experiencing a different type of HBV infections than the rest of the country. Hepatitis C prevalence among the general population may currently be scarce a phenomenon but there is a need to heighten surveillance.

P37

Association of IL-18 and CD24 gene polymorphism with risk of HBV infection in Indian population

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PURPOSE OF THE STUDY: The genetic polymorphism of IL-18 gene in the promoter region at positions -607, -137 and CD24 gene at positions P170 & P1527 has been studied to study the relationship between genetic polymorphism and risk of HBV infection within North Indian population.
METHODS: Genotyping of IL-18 and CD24 gene was performed in 100 HBV patients and 100 control subjects by using PCR-Restriction fragment length polymorphism (PCR-RFLP) method. Genotypes, alleles and haplotype frequencies at each position of IL-18 gene were statistically analyzed by using SHEsis software.
SUMMARY OF RESULTS: At position -607, CC, CA and AA genotypes and at position -137, GG, GC and CC genotypes of IL-18 gene and at position P170, CC, CT, TT genotypes and P1527, TG/TG, TG/del and del/del genotypes of CD24 gene were observed in patients and control objects. There were no significant differences in the allele frequency and genotype distribution at position -607 and -137 between patients with chronic hepatitis B and the control subjects. The frequencies of -607C/-137C haplotypes in hepatitis B patients were significantly lower than in the normal subjects ($\chi^2 = 9.574$, $p = 0.001 < 0.05$; odd ratio (95% CI) 0.368 (0.192–0.706) and -607A/-137C haplotypes were higher in hepatitis B patients than normal subjects ($\chi^2 = 6.528$, $p = 0.010 < 0.05$; odd ratio (95% CI) 0.368 (2.189–4.033), respectively. There was no statistical significant difference in the allelic and genotype frequency at position P170 between patients and the control subjects. But there was higher genotype frequency at position P1527 TG/del ($p = 0.009 < 0.05$; odd ratio (95% CI) 0.10 (0.013–0.812) and allele frequency at P1527del ($p = 0.012 < 0.05$; odd ratio (95% CI) 0.219 (0.061–0.781) in control subjects as compared to infected patients.
CONCLUSION: It is concluded that -607A/-137C in the promoter of IL-18 gene may play a role in the development of HBV infection and -607C/-137C haplotypes may be associated with protection from HBV. The genotype P1527 TG/del and P1527 del allele in CD24 gene may be a protective genetic susceptibility factor for HBV infection.

P38

Relationship between TLR3, Th1/Th2 cytokine and responsiveness to hepatitis B vaccine of infants born to HBsAg positive mothers

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PURPOSE OF THE STUDY: To investigate the relationship between the levels of TLR3 and Th1/Th2 cytokine and the responsiveness to hepatitis B vaccine of infants born to HBsAg positive mothers. To evaluate whether perfecting the levels of TLR3, Th1/Th2 cytokine in the cord blood mononuclear cells (CBMCs) stimulated by PolyI: C in vitro can improve the responsiveness to hepatitis B vaccine of infants.
METHODS: Two hundred and ninety-seven neonates born to HBsAg positive mothers were enrolled. All received hepatitis B vaccine and were followed up until they were 1 year old. The levels of anti-HBs, TLR3 and Th1/Th2 cytokine (IL-2, IL-4, IL-6, IL-10, IL-12 and IFN-γ) in the sera of infants were detected by CLIA, FCM and ELISA, respectively. Forty umbilical venous blood of neonates were collected and CBMCs were cultured in vitro, then each of CBMCs was assigned to three groups, including PolyI: C+HBV and HBV stimulating groups, and blank control. Finally, the quantitative detection of the levels of TLR3 and Th1/Th2 cytokine in the supernatant were performed.
SUMMARY OF RESULTS: The rate of non-/low-responsiveness was 18.18% (54/297). The levels of TLR3 of non-/low-responsiveness infants were lower than that of high-responsiveness but the differences were not significant

(P0.05). The levels of IL-12 of non- and low-responsiveness infants were significantly higher than that of high-responsiveness, and the levels of IL-6 were significantly lower than that of high-responsiveness (P0.05). Of 40 neonates with CBMCs, there were 28 high-responsiveness and 12 non-/low-responsiveness infants when they were 1 year old, separately. Compared with the control, decreased levels of TLR3 in CBMCs were observed in HBV stimulating group, while increased levels were found in PolyI:C+HBV stimulating group, with no significant difference (P0.05). The levels of Th1/Th2 in both non-/low-responsiveness and high-responsiveness infants decreased in HBV stimulating group compared with their controls, while significantly increased levels were found in PolyI:C+HBV stimulating group, compared with it in HBV stimulating group (P0.05).

CONCLUSION: The level of TLR3 was higher in high-responsiveness infants born to HBsAg positive mothers, and those with higher level of IL-12 and lower IL-6 were more likely to be non-/low-responsiveness to hepatitis B vaccine. PolyI:C may increase the expression levels of TLR3 and Th1/Th2 cytokine in CBMCs of infants with non-/low-responsiveness and high-responsiveness to hepatitis B vaccine.

P39

Coumarin labeling of hepatitis B virus

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Hepatitis B virus (HBV) contains three envelope proteins: large (L), middle (M) and small (S) with distinct functions in assembly, secretion and infectivity processes. Labeling the viral envelope proteins could represent a useful tool for the investigation of the mechanism of early steps of HBV infection and also for the screening of new viral entry inhibitors. Our previous studies have demonstrated that a recombinant M protein containing enhanced green fluorescent protein (EGFP) tag in N-terminal region (EGFP.M) was incorporated into HBV envelope and secretion-competent. However the proper secretion of HBV virions was achieved only in the presence of S protein. To overcome this we took a different approach and we constructed a mutant M protein (MN3) containing a 13 amino acids specific sequence for PRobe Incorporation Mediated by Enzyme (PRIME) labeling using the blue fluorophore coumarin. The results demonstrated that the recombinant MN3 protein is efficiently expressed, folded and secreted in human hepatoma cells. Transcomplementation experiments showed that the incorporation and secretion of fluorescently labeled recombinant MN3_HBV virus does not affect either HBV replication or HBsAg secretion as revealed by qPCR and ELISA experiments, respectively. Further studies regarding the infectivity features of the newly developed recombinant MN3_HBV virus are needed.

ACKNOWLEDGEMENTS: This work was supported by the Sectoral Operational Programme Human Resources Development (SOP HRD), financed from the European Social Fund and by the Romanian Government under the contract number POSDRU/159/1.5/S/137390 and by Romanian Academy Project 3 of the Institute of Biochemistry.

P40

Multidisciplinary approach to the trans-activation of human Hepatitis B Virus by body burden dioxin or dioxin-like polychlorinated biphenyls

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BACKGROUND: Human viruses evolved into novel target genes for host cell dioxin receptor (AhR/Arnt) transcriptional complex (1) since our discovery of trans-activation of HIV-1 by 1.0 nM 2,3,7,8-TCDD (dioxin), the most potent xenobiotic with extremely long half-life in humans. Later, up-regulation of cytomegalovirus (CMV) in human cells was shown with 0.3 pM dioxin (2), lower than current background level in the general population (2–6 pg/kg). However, the mechanism of viral trans-activation by dioxin was revealed after "dioxin response elements" (DRE) were computationally identified in viral promoters: a single DRE in HIV-1 and 8–10 DRE in CMV (3). Here, we compile experimental, in cilico, epidemiological, and medical prospective data, all concerning effects of dioxin on the HBV.

MATERIALS AND METHODS: Production of the HBV in HepG2 cells was determined using plaque assay, viral DNA – by hybridization and PCR. A computational search for DRE in HBV genes performed by the SITECON (4).

RESULTS: SITECON identified 2 to 4 promoter DRE in the HBV. Juxtaposing the DRE numbers with experimental results suggested that the HBV might be upregulated by dioxin at concentrations lower than 1.0 nM and higher than 0.3 pM shown for the HIV-1 and CMV, respectively. If fact, the lowest effective concentration was 70 pM. That is in the range of bodily dioxin level of some overexposed population groups in the Arctic and Vietnam consuming dioxin-contaminated seafood, resulted in high prevalence rates of chronic hepatitis B and HCC. The same defines increased mortality from cirrhosis and HCC among PCB-exposed 'Yucheng' patients in Taiwan, which is the HBV-endemic area.

CONCLUSIONS: All the above provide evidence for activation of DRE-containing HBV with sub-nanomolar dioxin, and allow exploring inhibitors of HBV-driven malignancy among the AhR antagonists or modifiers of AhR/Arnt complex binding to viral DRE.

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P41

Study of mutations in hepatitis B complete viral genome in chronic hepatitis B patients, from Southeast of Caspian Sea, Iran

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PURPOSE OF THE STUDY: Hepatitis B virus (HBV) represents a major health problem worldwide, developing cirrhosis and hepatocellular carcinoma (HCC) in some cases. Mutations in the HBV surface, precore and basal core promoter and polymerase regions has been observed frequently associated with the virus replication, vaccine and immune escape mutations, changes in HBeAg serum level, drug resistance and clinical outcomes, respectively. This study aimed to investigate mutation in these regions of chronic HBV patients (CHB) in Golestan province, Northeast of Iran.

METHOD: This cross sectional study is done on 65 CHB patients (HBsAg positive for more than 6 months), which were under lamivudine treatment, HBV serological marker HBeAg were measured. HBV-DNA was extracted and PCR was performed using specific primers for four regions including S gene, precore, basal core promoter which overlaps with X gene and polymerase region. Positive PCR products were subjected to automated sequencing. Alignment was applied using reference sequence from Gene Bank database with AB033559 accession number.

SUMMARY OF RESULTS: Results showed that 11% of our patients had mutations in the ''a'' determinant of surface gene, one case with G145R mutation was detected, which is called "Escape Mutation" and is reported for the first time from Iran, 20% and 21.5% showed A1762T and G1764A substitutions in basal core promoter region, respectively, which plays an important role in progression to HCC and cirrhosis, and 34% had G1896A mutation that caused stop codon at 28th amino acid of precore region and is the cause of HBeAg negativity in HBV chronic patients. Serological tests showed that HBeAg negativity was 98% among those with G1896A mutation. Mutations at the YMDD and FLLAQ motifs in the polymerase gene of HBV detected in 12 of 65 patients (18.46%) which were under treatment with lamivudine.

CONCLUSION: Mutations in basal core promoter, precore, polymerase and surface regions of the HBV genomes in this Iranian CHB population is very important to predict the outcome of infection. The administration of antiretroviral drugs especially is very useful for predicting the efficiency of HBV vaccines and is very useful for prevention of cirrhosis or HCC, which is needed the cohort study on this population.

P42

Possible interaction of HBx protein with ubiquitin-related proteins encoded by gene promoter regions hypermethylated in chronic hepatitis B infection

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BACKGROUND: Despite the existence of successful antiviral therapies, hepatitis B virus (HBV) remains a major global health concern that accounts for about 50% of hepatocellular carcinoma (HCC) cases worldwide (1,2). HBV genome encodes hepatitis B x (HBx), which is a transcriptional activator and oncogenic protein. HBx protein has been labelled an epigenetic deregulating agent that uses its oncogenic ability to induce promoter hypermethylation of various cellular genes contributing to tumorigenesis. HBx protein was shown to bind ubiquitin proteins to protect itself from degradation. It is possible that the HBx protein disrupts the normal physiological functions of ubiquitin proteins to achieve its transactivation roles (3–5). The reported mechanisms underlying the regulation of ubiquitin proteins and their physiological role in HBV-induced HCC are still not fully elucidated. The aim of the current study was to identify the HBV-induced hypermethylated promoter regions of the genes encoding the ubiquitin-related proteins that may interact with HBx protein.

MATERIALS AND METHODS: Previously, we successfully identified the presence of DNA hypermethylation in a cohort of HBV infected patients using high-throughput technology. Based on the same data, literature-based search was used to identify the genes that encode ubiquitin-related proteins that may interact with HBx protein.

RESULTS: The ubiquitin-related genes including UBX domain protein 1 (UBXN1), Ligand of Numb protein X 2 (LNX2), Proliferation associated 2G4 (PA2G4) and Thyroid hormone receptor interactor 12 (TRIP12) were identified.

CONCLUSIONS: UBXN1, PA2G4, TRIP12 and LNX2 genes play important roles in cellular signalling pathways such

as ubiquitination, DNA repair and transcription. Being localised in the cytoplasm and mitochondria of HBV infected hepatocytes, it is possible that HBx protein manipulates the entire body of cellular signalling pathways for viral survival and propagation through hypermethylation. This detrimental effect would connect HBV infection to malignant transformation by inducing uncontrolled cell growth, proliferation and disrupting apoptosis. Involvement of the identified genes in ubiquitination and other pathways suggests their critical role in the development of cancer (3–5). Further investigations including gene expression studies of the identified genes are required. These findings will be useful in identifying important diagnostics tools and treatment possibilities.

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P43

Cytotoxic T-lymphocytes and CD4 epitope mutations in pre-core/core region of hepatitis B virus in chronic hepatitis B patients in Northeast of Iran

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BACKGROUND: Hepatitis B virus (HBV) is vulnerable to a high number of mutations. Especially mutations within epitopes recognized by sensitized T cells may influence the re-emergence of the virus. This study was designed to investigate the mutation in immune epitope regions of HBV pre-core/core among chronic HBV patients of Golestan province, Northeast of Iran.

MATERIALS AND METHODS: In this cross-sectional study of 120 chronic HBV patients, HBV-DNA was extracted from plasma and PCR was performed, using specific primers. Direct sequencing and alignment of pre-core/core region were applied using reference sequence from Gene Bank database (Accession Number: AB033559).

RESULTS: Our results showed 27 amino acid changes, 9 (33.33%) in CD4 and 2 (7.40%) in cytotoxic T-lymphocytes (CTL) epitopes. Sixteen other mutations (59.25%) were seen out of these regions.

CONCLUSIONS: It has been demonstrated that CTL escape mutations are not commonly observed in pre-core/core sequences of chronic HBV carriers in our area. It could be concluded that most of the amino acid substitutions occur in different immune epitopes other than CTL and CD4.

Other

P44

The efficacy of hepatitis B vaccination program in Upper Egypt: flow cytometry and the evaluation of long-term immunogenicity

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BACKGROUND AND AIMS: To evaluate the efficiency of hepatitis B vaccine via evaluating antibody against hepatitis B surface antigen (anti-HBs) levels and hepatitis B surface antigen (HBsAg) specific memory T-lymphocytes.

DESIGN AND SETTING: The study was conducted in a tertiary care setting. This study included 440 vaccinated persons during infancy. Group I: 6–10 years old; Group II: 10–14 years old; Group III: 14–17 years old; Group IV: 17 years old. The serum samples were screened for hepatitis B virus (HBV) markers. Cytokines secretion by HBsAg-specific memory CD45RO+CD4+T cells was measured after in vitro culture using flow cytometry.

RESULTS: The mean titer of anti-HBs was higher in group I when compared with others ($p = 0.000$ for each). Interferon gamma (IFN-γ) and interleukin 4 (IL-4) secreted by memory CD4+T cells were positive in all with anti-HBs 100 mIU/mL, while positive in 87% and 75% of participants with anti-HBs <10 mIU/mL and positive in 73% and 32% of participants with absent anti-HBs. The percentage of cells secreting IFN-γ and those secreting IL-4 were higher among participants with serum anti-HBs >100 mIU/mL than those having 10 mIU/mL or absent ($p < 0.001$ for each).

CONCLUSIONS: Anti-HBs positivity decreased with time since childhood vaccination. Breakthrough infections are

rare in vaccinated persons. Hepatitis-B vaccine appears to be efficient in controlling HBV infection after childhood immunization. Flow cytometry is a useful tool to assess the long term persistence of T cell memory after childhood vaccination.

HEPATOCELLULAR CARCINOMA

P45

Model of end stage liver disease score and hepatocellular carcinoma

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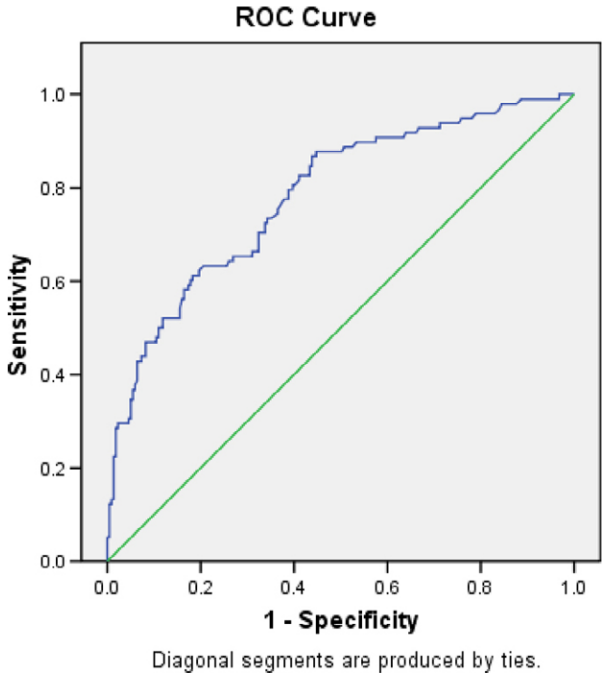
BACKGROUND: Hepatocellular carcinoma (HCC) is the most common primary hepatic malignancy worldwide (1). It is the first cause of cancer mortality in Egypt (2) due to the heavy burden of chronic HCV (14.7%). Among cirrhotic patients, 1–4% per year will develop HCC (3). HCC patients evaluated for liver transplantation are often given exceptional MELD score, giving them a priority for liver transplantation. We aimed at determining the MELD score in HCC patients, its correlation with TNM tumour stage and tumour size. Also, we aimed at determining a cut off value of MELD score above which chronic HCV (CHC) cirrhotic patients have high chance to develop HCC.

MATERIALS AND METHODS: The study included 98 patients with CHC and HCC (group I) and 219 patients with CHC without HCC (group II). CHC was diagnosed by ELISA for HCV Antibody and serum HCV RNA.HCC diagnosis was based on EASL criteria i.e. focal hepatic lesion with arterial phase enhancement and washout in portal and delayed phases, obtained by contrast enhanced abdominal CT and or MRI. HCC was staged according to the seventh edition TNM tumour staging system. MELD score was calculated using the following formula: MELD score = 10 * ((0.957*ln (Creatinine)) + (0.378*ln(Bilirubin)) + (1.12*ln (INR))) + 6.43.We used the MELD score calculator of the iLiver application of the EASL. We computed ROC curve for MELD score concerning the prediction of HCC. Stratum specific likelihood ratio (SSLR) was calculated as the proportion of diseased subjects (HCC) with a test result in a given range divided by the proportion of non-diseased subjects (non HCC) with a test result in the same range (4).

RESULTS: MELD score was significantly higher in group I than group II. The score was 9.71 ± 4.08 in group I versus 5.61 ± 3.25 in group II ($p \leq 000$). In group I the MELD score ranged from 0.7 to 20.33. There was significant positive correlation between MELD score and TNM tumour stage ($r = 0.312$, $p = 0.002$) but the correlation was insignificant as regards the tumour size ($r = 0.041$, $p = 0.687$). The distribution of TNM tumour stage in group I was as follows: stage I represented 19.3%, stage II represented 25.5%, stage IIIa represented 19.3%, stage IIIb represented 18.3%, stage IIIc represented 1%, stage IVa represented 8% and stage IVb represented 7%. The cut off value of MELD score above which there was a high risk of HCC development was ≥5.74. The area under the curve (AUC) was 78.3%, sensitivity was 87.8%, specificity was 56%, positive predictive value (PPV) of 46.7%, negative predictive value (NPV) of 91%, accuracy of 65.3% and positive likelihood ratio (LR) of 1.96. The SSLR for HCC presence by MELD score was 0.21 in score <5, 0.97 in score from 5 to 10 and 4.57 in score >10.



CONCLUSION: MELD score has significant positive correlation with TNM tumour stage in HCC cases. CHC patients with MELD score >10 have SSLR for HCC presence of 4.57 and are in need for closer follow up.

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P46

Hepatitis B virus might be transcriptionally activated in Southeast Asian inhabitants due to effect of food contaminated with dioxins or polychlorinated biphenyls

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In this study, medico-epidemiological and molecular virological data were combined to evaluate high frequencies of chronic liver diseases and hepatocellular carcinomas (HCC) in people groups accidently exposed to dioxins or dioxin-like coplanar polychlorinated biphenyls (PCB). Mass poisoning by PCBs with multifold increase of bodily PCB compared to general population in Taiwan was named Yucheng disease (1). Its major symptoms: development of fatty liver and increased mortality from chronic liver disease, cirrhosis and HCC (2). Similarly, human liver cancer incidence was significantly elevated in Northern Vietnam provinces exposed to dioxin during Vietnam War (3). However, when people in Northern Japan were poisoned with PCB, they developed Yosho disease, the major symptoms of which were dermal and ocular lesions (1,4). Comparing medical data with the epidemiological facts, it is noteworthy that Taiwan is HBV-endemic area, and the HBV is a main (67%) cause of HCC, a #1 cause of death in Taiwan (5) while Japan is human hepatitis C (HCV)-endemic area where HCV carriers consist 71%, while HBV carriers – only 1.4% of all inhabitants of PCB-contaminated regions. The above differences reported for Yusho in Japan and Yucheng in Taiwan might be explained by our pioneer findings (6,7) that HBV gene possesses active dioxin-response elements (DRE) in its promoter region, while there is no DRE identified in HCV genome. So, elevated bodily concentrations of dioxin-like coplanar PCB in affected population groups in Taiwan and Northern Vietnam might trigger activation of HBV via AhR-Ahrt transcriptional pathway in infected human cells. Such HBV activation may lead to inflammation and subsequent chronic hepatitis and HCC.

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TRANSPLANTATION AND VIRAL HEPATITIS

P47

Hepatitis C: sustained virological response after liver transplantation

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BACKGROUND: Hepatitis C has universal recurrence after liver transplantation and treatment with interferon and ribavirin is challenging (1,2).

PURPOSE OF THE STUDY: This study aims at describing patient survival, rates of hepatitis C recurrence and factors associated with sustained virological response (SVR) to treatment 5 years after liver transplantation.

METHODS: Retrospective analysis of medical records of patients with hepatitis C positive serology and PCR who underwent liver transplantation from 2004 to 2009 at the Hospital of the State University of Campinas-Brazil. Excluded from the analysis were patients co-infected with hepatitis B virus, patients who used alcohol or illicit drugs after surgery and those whose survival was <1 month.

RESULTS: During this 6-year period there were 195 liver transplantation procedures, 91 due to hepatitis C. Fifty-six patients who met the inclusion criteria were analyzed. The patients were mostly male (71.4%), median age of 51, median BMI 26, median MELD (without adjustment) 18, 64.2% Child-Pugh C. Survival at 1 and 5 years post-transplant was 93.7% and 89.4% respectively. Liver biopsy was performed in 41 (73.2%) patients, 55.3% demonstrating hepatitis C recurrence, median 13 months post-surgery. Twenty-four patients were treated with interferon and ribavirin (12.5%) or pegylated interferon and ribavirin (87.5%) for a median duration of 68 weeks. Two patients were still on treatment during data collection. 56.5% patients had genotype 1 and 43.5% had genotype 3 infection. Fourteen (63.6%) patients achieved SVR, 88.8% genotype 3 and 41.6% genotype 1. Patients who achieved SVR were treated for a longer period (median 79 weeks versus 33.5 weeks). Anemia was present in 75% of cases, 62.5% requiring erythropoietin and 70.8% ribavirin dose reduction. Sixteen patients (66.6%) presented neutropenia, managed with filgrastim (54.1%) and interferon dose reduction (50%). Follow up time was 86 months among patients who achieved SVR, but only 62 months for those who didn't. Twenty-two (39.3%) patients presented at least one rejection episode, associated to hepatitis C therapy in only five cases (22.7%).

CONCLUSIONS: Despite many challenges due to adverse events, therapy with interferon and ribavirin can cure 63.6% patients treated. In resource limited settings, without access to new interferon-free therapies, interferon-based treatment remains a viable option.

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P48

Sofosbuvir-containing regimens are better than NS3 protease inhibitors-containing regimes for genotype 1b hepatitis C in liver transplant recipients

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AIM: To compare efficacy and safety sofosbuvir (SOF)-containing regimens and telaprevir (TPV) or simeprevir (SIM)-containing regimes for treatment genotype 1b HCV in liver transplant recipients.

MATERIALS AND METHODS: We analyzed six cases of antiviral treatment (AVT) with pegylated interferon (PI), ribavirin (R) and TPV for 48 weeks; three cases of AVT with PI/R/SIM for 24 weeks; two cases of AVT with PI/R/SOF for 12 weeks and four cases of SOF/SIM (3) for 12 weeks or SOF/R/daclatasvir (DAC) therapy for 24 weeks.

RESULTS: Three male and six female patients with mean age 52 (95% CI 43.5; 59.6) year were treated with PI/R/TPV or PI/R/SIM after 28 (95% CI 9.3; 46.1) months after cadaveric liver transplantation. The mean log10 viral load was 6.2 (95% CI 5.3; 7.3) IU/ml. Two patients had cirrhosis and 3 – advanced liver fibrosis. Only four patients were naïve; 3 pts relapsed after PI/R course and 2 pts had null response (NR) for previous PI/R. The AVT was started with 4–8 weeks lead-in in 4 cases. Only two out of nine patients achieved sustained virologic response (SVR). Two patient relapsed after full course completion, in one case we could see breakthrough and in three cases null response occurred. The last patient discontinued AVT with TPV at week 6 due to severe rash. All our patients who received PI/R/TPV, developed significant cytopenia and had to use erythropoietin [5], filgrastim [4], eltrombopag [2] and blood transfusion [1]. Antibiotic-treated infections [2] and citalopram-treated depression [1] also occurred. Two courses of PI/R/SOF were induced for two persons with severe recurrent hepatitis and cirrhosis. Both were NR for PI/R and fail to achieve SVR for PI/R/TPV. The complete virologic and biochemical response was achieved in both cases for 12 week AVT. Another NR for PI/R/SIM patient with recurrent hepatitis A4F3 (METAVIR) was treated with SOF/R/DAC for 24 weeks. He also achieved fast and complete virologic and biochemical response. Among 3 naïve patients which recieved SOF/SIM for 12 weeks, one relapsed and two other achieved SVR. Only one patient with cirrhosis had to use erythropoietin simultaneously with PI/R/SOF.

CONCLUSION: In liver transplant recipients antiviral treatment regimens containing SOF are more effective and safe than ones containing TPV or SIM either with or without PI/R.

VIRAL HEPATITIS E

P49

Towards understanding pathogenesis of hepatitis E viremia in pregnant Egyptian women

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BACKGROUND: Hepatitis E virus is enterically transmitted viral pathogens. Pregnant women have potential risks of marked morbidity and mortality when they get infected.

AIM: In the present study, we investigated hepatitis E viremia and its correlation with immunoglobulins G and M (IgG and IgM) as determined by serologic testing in pregnancy.

MATERIAL AND METHOD: One hundred and twenty five pregnant patients were enrolled in this study. Complete virological profiles were performed for each woman including hepatitis C virus IgG, Hepatitis B surface antigen (HBsAg) and hepatitis E virus (HEV) IgG and IgM. Moreover, specific detection of HEV –RNA was screened by nested PCR.

RESULTS: HEV IgG was the most common' viral marker among the studied hepatotropic viruses (19.2%) followed by antibodies (Ab) for HCV (17.6%) and lastly HBsAg (9.6%). Hepatitis E virus was responsible for 8% of acute hepatitis as detected by the presence of viremia and/ or positive IgM in pregnant women. While other hepatitis viruses B and C was responsible for 17% of acute hepatitis in pregnancy. Seven patients (5.7%) had mixed viral hepatitis.

CONCLUSION: From this study, we can conclude that HEV infection is a common viral infection among pregnant women in Egypt. It is associated with remarkable morbidities during pregnancy, though not as severe as previously reported. There is viremia associated with the presence of immunoglobulin G. The question arises whether hepatitis E viremia represent a re-infection state or persistence viremia. A further large-scale prospective survey of HEV infection among pregnant women in Egypt should be conducted to evaluate the cost-effectiveness of antenatal HEV screening in Egypt.