and erythrocyte ribavirin levels in predicting SVR in HCV genotype-1 patients undergoing peginterferon + ribavirin treatment.

Material and methods: 30 HCV genotype-1 patients (22M/8F mean age 45,2±13,6 yrs) undergoing a standard treatment schedule (Peg-IFN 180 mcg weekly+ribavirin 1000 or 1200 mg daily, according to bw) were included in the study. Plasma and erythrocyte ribavirin levels were evaluated in all patients at week 12. At week 24 ribavirin levels were re-assessed in those obtaining EVR. Ribavirin concentration was evaluated by high performance liquid chromatography employing 3-methyl-cytidine as internal standard.

Results: Twenty-five patients (82%) obtained EVR, while sixteen (53%) achieved SVR. There was no difference among EVR and non EVR patients in terms of serum and erythrocyte ribavirin concentration at week 12. At week 24, EVR patients obtaining SVR exhibited higher levels of ribavirin in serum and lower in erythrocytes, in comparison with non SVR patients (serum 14,1±10,5 vs 5,9±4,1 μM; $p<0.02$; erythrocyte 1072±420 vs 1793±903 μM; $p<0.02$). When [serum ribavirin]/[erythrocyte ribavirin] × 100 ratio was compared, the difference was enhanced (1,6±1,5 vs 0,4±0,34; $p<0.01$). ROC curve analysis identified a cut-off for [serum ribavirin]/ [erythrocyte ribavirin] × 100 ratio in predicting SVR of 0,6, with a NPV of 80% and a PPV of 85%, while those related to EVR were 100% and 63%, respectively.

Conclusions: [serum ribavirin]/[erythrocyte ribavirin] × 100 ratio at week 24 seems to be an 85% indicator of SVR in genotype-1 patients obtaining EVR. Whether drug adjustment, according to this parameter, might be useful to increase SVR remains speculative.

I. Viral hepatitis 4. HCV

OC.16.4

ERYTHROID DIFFERENTIATION IS SUPPRESSED BY RIBAVIRIN DURING COMBINATION THERAPY WITH PEGYLATED INTERFERON-α2A IN CHRONIC HEPATITIS C: AN IN VITRO AND IN VIVO STUDY

G.M. Prati [1], A. Aghemo [1], L. Ronzoni [2], M.G. Rumi [1], S. Monico [1], A. Colancecco [2], P. Grillo [3], M. Viganò [1], M. Cappellini [2], M. Colombo [1]

[1] First Division of Gastroenterology, Fondazione IRCCS Ospedale Maggiore Policlinico, Mangiagalli e Regina Elena. University of Milan, Milano; [2] Department of Internal Medicine, Fondazione IRCCS Ospedale Maggiore Policlinico, Mangiagalli e Regina Elena. University of Milan, Milano; [3] Unit of Epidemioloy, Department of Preventive Medicine, Fondazione IRCCS Ospedale Maggiore Policlinico, Mangiagalli e Regina Elena, Milano

Background and aim: Anemia during Pegylated Interferon (PegIFN)/ Ribavirin (Rbv) therapy is attributed to the haemolytic effect of Rbv concentrated into erythrocytes. However, biomarkers of haemolysis are not altered in parallel in all patients. Aim We therefore compared the pattern of haemolysis and erythropoiesis in patients receiving PegIFN alone or combined with Rbv.

Material and methods: 18 patients with chronic HCV-2 infection consecutively receiving PegIFN-α2a 180 mcg/week plus Rbv 800 mg/day for 24 weeks were compared to 10 patients with chronic hepatitis B, consecutively receiving PegIFN-α2a 180 mcg/week for 48 weeks. Haemolysis was investigated by serum LDH, haptoglobin and reticolocyte count; erythropoiesis by peripheral erythroid progenitors cell coltures (BFUe and CFU-GEMM) and gene expression of gamma-globin and GATA2 by quantitative real-time PCR. The effects of PegIFN-α2a and Rbv added at day 0 and 7 to blood coltures obtained from healthy volunteers on erythroid progenitor cellular growth, cell differentiation and gene expression of glycoforin A were also investigated.

Results: A sharper and faster decrease of Hb, indicative for haemolysis was seen in 3 (11%) HCV patients, only (Hb decrease at week 4: 3.40 vs 1.55 g/dL, $p=0.01$), with an increase of BFUe at week 4 of therapy (7.723 to 25.0/105 cells) as a likely response to peripheral haemolysis. At week 4 the 15 non hemolytic HCV patients and the HBV patients showed a significant reduction in BFUe number (HCV: 13.588 to $5.737/10^5$ cells; HBV: 17.226 to $5.942/10^5$ cells) with an increase in undifferentiated CFU-GEMM colony formation (HCV: 1.97 to $2.78/10^5$ cells; HBV: 1.7 to $3.3/10^5$ cells) indicative of inhibition of erythroid differentiation by PegIFN/Rbv, confirmed also by increased expression of primitive erythropoiesis specific genes like gamma-globin (5.1 fold) and GATA2 (4.69 fold). In vitro analysis showed that both PegIFN and Rbv inhibit cell proliferation and differentiation with a 50% reduction of cellular growth and 47% of glycoforin A expression vs control, confirmed by cell morphology analysis.

Conclusions: These observations suggest suppression of erythroid differentiation to cause anemia during PegIFN/Rbv therapy, while Rbv itself may cause myelosuppression.

I. Viral hepatitis 4. HCV

OC.16.5

LIVER FIBROSIS IS INDUCED BY INSULIN RESISTANCE BY ENHANCING THE DUCTULAR REACTION THAT UNDERGO EPITHELIAL-MESENCHYMAL TRANSITION IN CHRONIC HEPATITIS C

G. Svegliati-Baroni [1], G. Faraci [1], S. Saccomanno [1], L. Fabris [2], M. Cadamuro [3], M. Strazzabosco [4], E. Bugianesi [5], A. Benedetti [1], G. Marchesini [6]

[1] Gastroenterology, Polytechnic University of Marche, Ancona; [2] University of Padua, Surgical and Gastroenterological Department, Padova; [3] Celiver, Bergamo; [4] Yale University, New Haven, Ct, USA; [5] Gastroenterology, University of Turin, Torino; [6] Clinical Dietetics, University of Bologna, Bologna

Background and aim: The mechanisms for progression of liver injury towards hepatic fibrosis in chronic hepatitis C (HCV) are unknown. Insulin resistance (IR) is an independent predictor of HCV-related fibrosis. A ductular reaction (DR) occur during chronic liver injury and represents a potential profibrogenic mechanism by the recruitment of myofibroblasts and by undergoing epithelial-mesenchymal transition (EMT). Aim of the study was to evaluate: a) the relationship between IR, the degree of liver injury and DR; b) the ability of DR to stimulate fibrogenesis in HCV.

Material and methods: 78 consecutive HCV patients underwent liver biopsy and IR evaluation by the oral glucose insulin sensitivity (OGIS) test. Liver injury was determined by Ishak's score, and immunohistochemistry perfomed for citokeratin-7 (CK7, a marker of DR), αSMA (marker of activated hepatic stellate cell, HSC), and S100A4 (EMT marker).

Results: IR (OGIS<9.8 mg/kg/min) was associated with aggregate hepatic inflammatory activity ($p<0.01$) and its individual components of piecemeal necrosis ($p<0.05$), confluent necrosis ($p<0.05$), and portal inflammation ($p<0.005$), as well as the degree of hepatic fibrosis ($p<0.001$). In the portal tract, CK7-positive cells appeared as elements of the DR (Ø < 6 um) or intermediate hepatobiliary cells (IHC, Ø <40 m), while at the interface they also showed a stellate-like fibroblastic appearance. DR was observed in all HCV, while IHC were detected only in 38% of cases (grading > 8 and staging > 3). IR correlated with an expansion of DR ($p < 0.02$) and a larger number of IHC ($p < 0.05$). DR and IHC were associated with the total grading, HSC activation (αSMA expression), and hepatic staging ($p < 0.001$). S100A4 expression was well evident in elements of the DR. By dual immunofluorescence and morphometry, S100A4/CK7 co-localization significantly increased with the progression of liver injury ($p < 0.05$). By multivariate analysis, IR and total inflammation were the only independent predictors of hepatic fibrosis.

Conclusions: In conclusion: a) IR determines a higher degree of necroinflammatory liver injury in HCV; b) this leads to enhancement