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 years) 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 107±2420 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%.

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

# 1. 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**


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**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 reticulocyte count; erythropoiesis by peripheral erythroid progenitors cell cultures (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 cultures 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 haemolytic HCV patients and the HBV patients showed a significant reduction in BFUe number (HCV: 13.588 to 5.737/105 cells; HBV: 17.226 to 5.942/105 cells) with an increase in undifferentiated CFU-GEMM colony formation (HCV: 1.97 to 2.78/105 cells; HBV: 1.7 to 3.3/105 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.