ELISA, and then genotypes and subgenotypes was identified on four modes by using genotype-specific-primer PCR and nested polymerase chain reaction-restriction fragment length polymorphism (nested PCR-RFLP) and 5 cases of identified genotypes were randomly selected for directly sequencing their S gene to verify the accuracy of genotype-specific-primer PCR.
**Result:** 1. 57 samples were tested for the positive of HBV genotype,of which exist 34 genotype B,12 genotype C and 11 genotype B+C mixed, Genotype B is more prevalent in our group; There were 37 HBV subgenotype positive in identified genotype samples, of which exist 24 subgenotype Ba, 12 subgenotype Cs and 1 subgenotype Ce, Ba was main subgenotype in the group. 2. The HBV infection factors such as age,gender and marital status was not statistically signifycant except nation ($p$=0.0001).
**Conclusion:** The Result show: 1. Genotype B and subgenotype Ba were the predominant strains in the minority groups of Yunnan province. 2. Nation and the positive rate of HBsAg showed correlatitive, but age, gender and marital status existed no difference.

## PP-076 Diagnostic values of transforming growth factor (TGF)-β1 and TGF-β1 mRNA in HBV-related hepatocellular carcinoma

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**Background:** To investigated the characteristics of TGF-β1 and TGF-β1-mRNA expression, their relationship with HBV replication, and their diagnostic values for HCC.
**Methods:** Total RNAs were extracted from HCC tissues and their matched non-tumor tissues, or from PBMCs in HCC patients. TGF-β1 mRNA were amplified by RT-PCR and confirmed by sequencing. TGF-β1 expression was examined by immunohistochemistry. The clinical characteristics were analyzed between TGF-β1 and HBV replication. Diagnostic values of TGF-β1 and TGF-β1 mRNA were investigated.
**Results:** The incidence of TGF-β1 expression was 83.3% in HCC, 43.3% in their surrounding tissues, 94.7% in HBV-DNA-positive group, and 63.6% in HBV-DNA-negative one, respectively. TGF-β1 expression were associated with the degree of HCC differentiation and the statue of HBV replication, but neither to size nor to number of tumors. Circulating TGF-β1 level or incidence of TGF-β1 mRNA was significantly higher in HCC group than any group of patients with benign liver diseases, with higher sensitivity (89.5%) and specificity (94.0%) for HCC diagnosis when TGF-β1 level (> 1.2 μg/L). No significant correlation was found between TGF-β1 expression and AFP levels or tumor sizes. Combined TGF-β1 level and serum AFP could raise the detection rate up to 97.4%.
**Conclusion:** The abnormal expression of TGF-β1 was associated with degree of HCC differentiation and HBV replication. Both of TGF-β1 and TGF-β1-mRNA could be used as sensitive biomarkers for diagnosis and prognosis of HBV-related HCC.

## PP-077 Variation and significance of $CD4^+CD25^+$ regulatory T cells in chronic hepatitis B patients who complicated with hepatic steatosis

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**Background:** Hepatic steatosis is common in chronic hepatitis B (CHB) patients. The aim of this study was to investigate the effect and significance of hepatic steatosis to $CD4^+CD25^+$ regulatory T cells (Tregs) in CHB patients.
**Methods:** Tregs in peripheral blood and expression of Foxp3 in liver tissue were examined by flow cytometry and immunohistochemistry respectively. HBV markers, alanine aminotransferase (ALT) and HBV DNA levels were measured in each subject.
**Results:** ALT, HBV DNA and the inflammatory grade (G) of liver tissue in patients who had CHB plus hepatic steatosis were similar to CHB group; The percentage of Tregs in peripheral blood of patients with CHB plus hepatic steatosis was lower than that in CHB patients but higher than control group, which was coincided with the change of Foxp3 positive Tregs in liver tissue; The percentage of Tregs in high ALT group was higher compared with low ALT group; The inflammatory grade of liver tissue in 14 patients was $G_2$, in other 16 patients was $G_3$, Foxp3 positive Tregs in the latter group were more than those in the former group; The percentage of Tregs was not associated with HBV DNA level.
**Conclusion:** Tregs in peripheral blood and liver tissue of patients with CHB plus hepatic steatosis are fewer compared with CHB patiens, and Tregs have a close relation with hepatic inflammation. The decreased Tregs may lead to a lower suppression of inflammatory response, further more, the progression of CHB would be accelerated.

## PP-078 Effect of PDTC on NF-κB in the inflammatory injury and inducible nitric oxide in experimental fulminant hepatic failure

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**Objective:** to investigate the significance of the inhibition of NF-κB in D-galactosamine + Lipopolysaccharide-induced fulminant hepatic failure.
**Methods:** 60 Wistar rat were divided randomly to model group and PDTC group, which has 30 rats respectively. The serum and liver tissue were maintained. Hepatic tissue was observed by Routine HE staining; The levels of serum ALT and TB were detected by automatic biochemical analyzer. The changes of serum NO were detected by using nitrate reductase assay. The expression of NF-κB and iNOS mRNA were detected by RT-PCR.
**Results:** 1. The inflammation and necrosis were reduced in PDTC group. 2. Except for 8 hour, the ALT levels were lower than that of the model group ($P<0.05$). The TB level were lower than the model group at 4, 12, 24 hour, and the difference had statistical significance ($P<0.05$). NO of the serum in model group were lower than that of model group at 2, 8, 12 hour, and the difference had statistical significance ($P<0.05$). 3. At 4, 8 hour, the expression of NF-κB mRNA in PDTC group was lower than model group at corresponding different time. And the difference had statistical significance ($P<0.05$). At 4 and 8 hour, the expression of iNOS mRNA in PDTC group was lower than that of model group at corresponding different time ($P<0.05$).
**Conclusion:** NF-κB plays an important role in the hepatic inflammation of D-GalN+LPS-induced fulminant hepatic failure. PDTC can attenuate hepatic inflammation and necrosis through the inhibition of NF-κB.

## PP-079 Risk factors for the prognosis in chronic hepatic failure and construction of the prognostic model

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**Background:** To investigate the correlation between the risk factors and prognosis of chronic hepatic failure and try to construct the prognostic model.
**Methods:** All 28 patients with chronic hepatic failure were di-

vided into improved group and exacerbation group. The following clinical data and laboratory examinations were studied: ascites, spontaneous bacterial peritonitis (SBP), alimentary tract hemorrhage, hepatocerebral disease, hepatorenal syndrome (HRS), prothrombin time (PT), prothrombin time activity (PTA), International ratio (INR), blood coagulation factor V (FV:C), fibrinogen (FIB), D-dimer (D-D), total cholesterol (TC), total bilirubin (TBIL), albumin (ALB), prealbumin (PRE-A), alanine aminotransferase (ALT), Blood urea (BUN), serum creatinine (SCR), blood platelet (PLT). Analyze the index above with SPSS computer software (SPSS version 12.0) by logistic regression for univariat and multivariant analysis.
**Results:** Using logistic regression analysis, we found that: 1. Ascites, SBP, hepatocerebral disease, PT, PTA, INR, FV:C, D-D, TC, TBIL, PRE-A were significantly different between the two groups ($P<0.05$) in the univariat analysis; 2. TC, FV:C and ascites were significant risk factors for the prognosis of chronic hepatic failure ($P<0.05$) in the multivariant analysis
**Conclusion:** TC, FV:C and ascites are valuable in predicting patients with chronic hepatic failure. A prognostic model: $Y = 1/[1 + \exp(-s)]$, $s = 191.13 - 34.81*FV:C - 34.68*TC - 35.22*ascites$ (Y means the possibility of patient's death), was constructed by logistic regression analysis.

## PP-080 Application of CD3 gating to dection of HBV MHC-pentamer

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**Objectives:** To observe differences in specificity and stability of specific cytotoxic T cells (CTL), and select the best experimental conditions suitable for detection of HBV-specific CTL.
**Methods:** After HLA-A0201 and HLA-2402 allele-positive HBV-infected persons were screened by high-resolution SSP-PCR; PBMC of them were stained by different PE-labelled HBcAg epitope peptide complex, PerCP Cy5.5-labelled mouse anti-human CD3 monoclonal antibody and APC-labelled mouse anti-human CD8 monoclonal antibody respectively. Except for T lymphocytes, other cells which may be combined with Pentamer were removed by CD3 gating, comparing different kinds of added order of three reagents and by the multi-parameter flow cytometry analysis.
**Results:** Frequencies of epitope-specific CTL from HBV-infected persons who did not match HLA alleles were significantly lower than those of matched HLA alleles, $P<0.05$; Using the method that MHC-Pentamer and test samples were coincubated for 20min before adding CD3-PerCP Cy5.5 and CD8-APC which were coincubated 20min later, frequencies of HBV-specific CTL were higher than that of other groups, $P<0.05$; Frequencies of HBV-specific CTL from CHB were significantly lower than that of AHB, $P<0.05$.
**Conclusion:** If CTL epitopes and HLA alleles were matched, ratio of specific CTL can be improved; Both CD3 monoclonal antibody and CD8 monoclonal antibody can prevent MHC-Pentamer from combinating with the TCR, thereby frequencies of specific CTL were reduced; Inadequate specific CTL of CHB may be one important reasons for chronic infection.

## PP-081 Analysis of influential factors of prognosis of patients with acute-on-chronic hepatitis B liver failure after lamivudine treatment

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To evaluate the efficacy of lamivudine treatment in patients with acute-on-chronic liver failure (ACLF) and study the impact of HBV DNA load and its related factors on the prognosis of patients with acute-on-chronic liver failure (ACLF) after lamivudine treatment.
**Methods:** One hundred and thirty ACLF patients were treated with lamivudine and the influential factors on mortality of patients were studied by univariate and multivariate analysis.
**Results:** The mortality (50.0%) of patients in lamivudine group with MELD score from 30 to 40 was lower than that (86.1%) of control group ($\chi^2=23.319$, $P=0.000$). The mortality of patients with high virus load group (72/100, 72.0%) was higher than that of low virus load group (15/30, 50.0%) ($\chi^2=5.046$, $P=0.025$). For patients with MELD score from 30 to 40, by week 4 the mortality of patients with HBV DNA undetectable or more than 2 $\log_{10}$ decline (2/13, 15.4%; 18/40, 45.0%) was lower than that of patients with less than 2 $\log_{10}$ decline (18/23, 78.3%) ($\chi^2=15.328$, $P=0.002$). In multivariate analysis, in patients with MELD scores 30-40, treatment method ($P=0.004$), pretreatment HBV DNA load ($P=0.009$), decline of HBV DNA load during therapy ($P=0.014$) and encephalopathy ($P=0.019$) were independent predictors of mortality; for MELD scores above 40, only MELD score ($P=0.015$) was independent predictive.
**Conclusions:** Lamivudine treatment significantly decreases the 3-month mortality of patients with MELD score 30-40, and a low viral load pre-treatment and quick decline of HBV DNA load are good predictors for the survival of lamivudine treatment.

## PP-082 A Cis-acting regulatory variation of estrogen receptor-α and the association with HBV-related liver diseases

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**Background & Aims:** We have reported that the genetic variation at the ESR1 locus influences susceptibility to persistent HBV infection and HBV-related liver disease. However, further studies are needed to determine whether the polymorphism (IVS1-401) located in intron 1 is a regulatory polymorphism and clarify its functional molecular mechanisms for this association with chronic HBV infection and HBV-related diseases.
**Methods:** In a hospital-based case-control cohort composed of 359 HBV-related acute liver failure patients, 464 HBV-related liver cirrhosis patients and 469 HBV asymptomatic carriers, three polymorphisms (IVS1 T-401C, T29C, A11940G) of *ESR1* gene were analyzed using the polymerase chain reaction-restriction fragment length polymorphism assay. Functional analyses were conducted to verify the biological significances of the associated genetic variation.
**Results:** We found that the polymorphisms, T-401C and T29C, was associated with susceptibility to HBV-ALF (dominant model; odds ratio, 1.73; $P=0.000$) and HBV-LC (dominant model; odds ratio, 2.07; $P=0.000$). Functional analyses show that IVS1 T-401C polymorphism alters the binding affinity of c-Myb and regulates ESR1 expression. The disease-susceptible allele, -401C, has stronger ability to regulates ESR1 expression.
**Conclusions:** ESR1 IVS1 T-401C polymorphism is a cis-regultory variant and -401C allele contributes to the risk of occurrence of HBV-related liver failure and cirrhosis by binding c-Myb and altering ESR1 exression