was low. Further, the result needs to be verified by other laboratory diagnosis methods in case of Pott's disease.

OL-039 Can *Mycobacterium tuberculosis* DNA be detected in plasma/serum samples from tuberculosis patients?

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Background: Tests based on PCR have shown promise for the detection of *M. tuberculosis* in different clinical samples except plasma/serum. The purpose of this study was to know that whether *M. tuberculosis* DNA can be detected in plasma/serum samples.
Methods: 43 serums and 94 plasma were collected from 124 clinical diagnosed TB patients. Four different *M. tuberculosis* DNA extraction methods, including phenol-chloroform method, Qiagen kit, Omega kit and magnetic bead method were compared to get higher sensitivity. One quantitative fluorescent PCR designed by this study was used for the detection of *M. tuberculosis* DNA.
Results: The highest DNA extraction efficiency (52.8%) and the best reproducibility (CV = 26.7%) were seen in the magnetic bead method. And *M. tuberculosis* DNA can really be detected in some samples, and 39 of the 124 (31.5%) TB patients showed *M. tuberculosis* DNA positive in plasma/serum samples. Interestingly, 35.3% (12/34) smear negative cases demonstrate *M. tuberculosis* DNA positive.
Conclusion: In conclusion, this is the first study to report the existence of circulating *M. tuberculosis* DNA in plasma/serum from tuberculosis patients and showed that the detection of *M. tuberculosis* DNA may provide valuable information for the diagnosis of AFB negative TB patients.

OL-040 Rapid identification and molecular characterization of drug resistant *Mycobacterium tuberculosis* isolates circulating in China by multilocus PCR and electrospray ionization mass spectrometry

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Objective: The emergence of multidrug-resistant (MDR)-TB and more recently, of extensively drug-resistant (XDR)-TB is a real threat to achieve TB control and elimination. Quick detection of drug resistance is an urgent clinical need for personalized treatment to control MDR-TB or XDR-TB.
Methods: We used multilocus PCR and electrospray ionization mass spectrometry (PCR/ESI-MS) to determine the genotype and drug resistance profiles for 96 *Mycobacterium tuberculosis* isolates circulating in low and high endemic regions (Shanghai, n = 49; Chongqing, n = 47) in China.
Results: The mutation profiles obtained by the PCR/ESI-MS assay indicated that Principal Genetic Group 1 (PGG1) profile (87.5%) was dominant across the isolates tested in China. The results revealed that a cluster of 11 isolates with *katG* S315T & *inhA* promoter C-15T, *rpoB* S531L, and *embB* M306I in Chongqing, the high TB endemic region, which was not observed in Shanghai, the low endemic region. In addition, another resistance mutation profile with *katG* S315T & *inhA* promoter T-8C, *rpoB* D516G & P564R/A, was detected in 10 isolates in high endemic region in comparison to one in low endemic region. Drug-resistant gene mutations were more diversified in the low endemic region than in the high endemic region.
Conclusion: PCR/ESI-MS can provide another rapid and accurate laboratory diagnostic tool for antituberculosis drug resistance determination. This new technique has potential to facilitate rapid determination of MDR-TB in China, allowing timely guidance for individualized treatment.

Free Paper Presentation 6: Hepatitis B
Saturday, July 16, 2011, 15:30–17:00
Meeting Room 310

PL-006 HBV infection modeling and numerical simulation for anti-HBV infection personalized combination therapy

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Background: Some chronic HBV (CHB) patients have normal ALTs and hepatic injuries. Chinese herbal medicine and nucleosides combination treatments may be effective for some of such kind patients. It needs to develop an anti-HBV infection treatment model to interpret the mechanism for curable CHB patients with near normal liver functions.
Methods: A 57 years old male chronic HBeAg positive patient (nucleosides-native) received Chinese Herbal Medicine (CHM 15–23 ingredients, 450–600 g) treatment two times daily for 24 weeks, switched to CHM + Adefovir Dipivoxil for 53 weeks, then switched to CHM + Entecavir for 21 weeks. A new differential equation model is introduced to describe the dynamics of anti-HBV infection treatment, in which a *term* is in charge of killing virus rather than infected hepatocytes.
Results: His HBeAg got seroconversion at week 98. The numerical simulation of the model and his HBV DNA, ALT are shown in following figure.
Conclusions: Analysis shows that a treated CHB patient with infective number $R_0 < 1$ will eventually be cured. Large value of that *term* makes patient's virus be eventually cleared without damaging hepatocytes.
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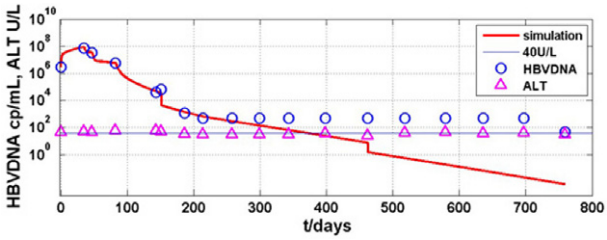

OL-041 Evaluation of serum level changes of sCD26 & sCD30 before and after treatment with interferon among naive chronic hepatitis B patients

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Propose: To evaluate the role of serum levels of sCD26 and sCD30 in predicting the outcome of therapy with IFNα among naïve chronic hepatitis B patients.

Methods: Among 33 naïve chronic hepatitis B patients, the serum level Changes of sCD26 and sCD30 before, 1 and 3 months after the start of therapy with IFNα were evaluated using sandwich enzyme-linked immonosorbant assay. The success Rate of treatment with IFNα was also obtained in these patients.
Result: sCD26 serum level changes before the start of therapy till one month ($P = 0.001$) and three months ($P < 0.001$) after the start of therapy were related to success rate of therapy. sCD30 serum level changes were not related to treatment success.
Conclusion: Using changes of serum level of sCD26 might be useful in predicting the outcome of therapy in naïve chronic hepatitis B patients undergoing treatment with IFNα. More studies with longer follow up time in this topic are recommended.

OL-042 HBV particles preferably induce Kupffer cells to produce TGF-β1 over pro-inflammatory cytokines

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Background: Kupffer cells and related cytokines are believed to play a critical role in liver fibrosis. However, it is not clear what role Kupffer cells play in HBV-related fibrogenesis.
Methods: Primary rat Kupffer cells were cultured with different titers of HBV particles purified from the sera of chronic hepatitis B patients. The concentrations of TGF-β1, IL-6, IL-1 and TNF-α in the culture supernatant were measured every 24 hours for 7 days. The mRNA and protein levels of these cytokine in Kupffer cells were also analyzed by quantitative real-time PCR and Western blot, respectively.
Results: Kupffer cells maintained normal morphology and function throughout the 7-day HBV treatment. TGF-β1 secreted by Kupffer cells under stimulation with HBV at 6 Log IU mL^{-1} increased 5.38±4.54- and 7.75±4.27-fold by Days 3 and 7, respectively ($p < 0.01$). Western blotting showed the expression of TGF-β1 in Kupffer cells exposed to high titer HBV increased from 1.80±0.20- to 2.42±0.46-fold by Days 3 and 7, respectively ($p < 0.01$). In contrast, Kupffer cell expression and secretion of proinflammatory cytokines (IL-6, IL-1 and TNF-α) were unchanged throughout the experiment.
Conclusion: HBV could preferably stimulate Kupffer cells to produce profibrogenic/anti-inflammtory cytokine TGF-β1 over proinflammatory cytokines IL-6, IL-1 and TNF-α. This in *vitro* study may partly explain why overt liver fibrosis still presents in chronic HBV infection with minimal or even in the absence of necroinflammation.

OL-043 HBV DNA change between HBeAg positive and negative patients with chronic hepatitis B

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Background: Spontaneous decrease of HBV DNA among chronic hepatitis B (CHB) patients who were not treated with antivirus drugs had been reported. The aim of this study was to compare the HBV DNA change between HBeAg positive and negative CHB patients.
Methods: Dynamic change of HBV DNA level was observed for 12 weeks in 171 cases with admission HBV DNA level above 10,000 copies/mL. All patients had never been treated with antiviral or immunoregulatory drugs during this period. HBV DNA detection were carried out every two weeks. HBV DNA baselines, minimum level and changing degree of HBV DNA were compared respectively between the two groups. All the data were analyzed by software SPSS 13.0, *t*-test was used to compare means and difference was significant statistically when $P < 0.05$.
Results: Among HBeAg positive patients (n = 83, 48.5%), HBV DNA baseline, minimum level and changing degree of HBV DNA were (7.44±1.32) copies/ml, (3.59±0.99) copies/ml and (3.84±1.39) copies/ml, respectively; Among HBeAg negative patients (n = 88, 51.5%), HBV DNA baseline, minimum level and changing degree of HBV DNA were (7.26±0.96) copies/ml, (3.93±1.18) copies/ml, (3.35±1.070) copies/ml respectively. HBV DNA baselines had no significant difference statistically ($t = 0.759$, $P = 0.449$); minimum level of HBeAg positive patients was lower than that of HBeAg negative patients ($t = -2.020$, $P = 0.045$); changing degree of HBV DNA of HBeAg positive patients was greater than that of HBeAg negative patients ($t = 2.363$, $P = 0.027$).
Conclusion: Among patients with chronic hepatitis B underwent spontaneous HBV DNA decrease, HBeAg positive patients were more likely to have a greater HBV DNA change and lower minimum.

OL-044 'Dinucleotide-pattern' G→A hypermutations in the pre-core 5′-GGGG tetrad of HBe negative hepatitis B virus (HBV) variant

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Objective: Liver-APOBEC enzyme mediated G→A hypermutation has been reported in HBV genome that prefers 5′GGGG tetrad substrate as an antiviral-innate immune mechanism. We therefore, intended to analyze the occurrence of G→A hypermutations in the in the 5′GGGG tetrad of HBV pre-C coding sequences from HBeAg negative patients.
Methods: Six HBeAg seronegative hepatitis B patients with chronic liver disease were studied that fulfilled the inclusion criteria: presence of chronic hepatitis: persistence of HBsAg and anti-HBeAb seropositivity for at least 12 months, HBV DNA seropositivity, liver ALT level >1.5× upper limit of normal, and seronegativity for HCV and HDV. Viral DNA were extracted from the patient's sea and subjected to PCR-amplification, using pre-C/C specific primer sets followed by direct automated sequencing. The nucleotide sequences of HBe negative HBV variants were analyzed with that of wild type HBV, using the on line DNA multi-alignment program.
Results: The pre-C nucleotide sequence analysis of the six HBe negative viral variants showed classical G1896A mutations in 3 samples. Of these, one viral sequence showed an additional G1897A substitution, representing a 'dinucleotide-pattern' hypermutation resulting in pre-C stop codon (U*GG* → U*AA*) in the 5′GGGG tetrad. In another sample, a second G1899A substitution was also identified in the same tetrad stetch, but in the next codon (UGGGG*C* → UAGG*AC*).
Conclusion(s): (1) The pre-C 5′GGGG stretch appears as a hot-spot for G→A stop codon-mutations in the HBe negative chronic hepatitis B patients, and (2) This 'dinucleotide-pattern' G→A hypermutation are likely to be introduced by the host-APOBEC enzymes.