

nofovir (~2 fold), a 3 to 6 fold resistance to ADV, but remains sensitive to entecavir. The association of the rtA181T/V+N236T mutations increased the level of resistance to all the tested drugs as compared to the rtA181T/V mutation alone, except for entecavir. Conclusion: The rtA181T/V substitution is associated to a decreased susceptibility to both LAM and ADV. These data emphasize the clinical relevance of genotypic and phenotypic analysis in the management of antiviral drug resistance to tailor antiviral therapy to the virologic situation. This knowledge may help to design new treatment algorithm depending on the profile of mutations in patients failing antiviral therapy.

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IMPACT OF HEPATITIS B GENOTYPE ON TREATMENT RESPONSE: A META-ANALYSIS OF CONTROLLED AND UNCONTROLLED TRIALS

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Background/Aim: The significance of hepatitis B virus (HBV) genotype response to treatment is not well established. The aim of this study was to examine treatment response by genotype in patients with chronic hepatitis B HBeAg-positive infection. **Methods:** Studies were retrieved from MEDLINE between 1966 and 2006, abstracts of scientific meetings, and review of bibliographies of retrieved studies. Included treatments were peginterferon (with ribavirin in one study), lamivudine, telbivudine and entecavir. The primary end points were anti-HBe seroconversion, sustained loss of HBeAg, and undetectable HBV DNA. We used a DerSimonian and Laird random effects model to pool data. **Results:** We identified 9 randomized controlled trials (3407 patients) and 5 retrospective trials (439 patients) with genotypic response rate data. Anti-HBe seroconversion rates (95% CI) by genotype were 49% (40-57) for A, 35% (31-40) for B, 26% (23-29) for C, 23% (15-35) for D with any treatment in 8 studies of 1531 patients; 54% (43-64) for A, 31% (25-39) for B, 29% (25-34) for C, 27% (15-43) for D with peginterferon; and 48% (28-68) for A, 40% (32-47) for B, 21% (17-26) for C, 19% (6-42) for D with lamivudine. Sustained loss of HBeAg rates (95% CI) by genotype were 43% (36-51) for B and 28% (22-36) for C with any treatment in 5 studies of 343 patients. Undetectable HBV DNA level rates (95% CI) by genotype were 48% (39-58) for A, 48% (44-52) for B, 50% (46-54) for C, 42% (36-47) for D with any treatment in 8 studies of 1775 patients; and 44% (38-51) for B and 43% (38-48) for C with lamivudine. Statistically significant increased likelihood of response occurred for anti-HBe seroconversion for A vs D (OR 3.0, CI 1.4-6.6); HBeAg loss for B vs C (OR 2.3, CI 1.5-3.8), and undetectable HBV DNA for C vs D (OR 2.5, CI 1.6-3.9) and A vs D (OR 1.7, CI 1.1-2.8). **Conclusions:** Our analysis supports significant genotype-specific responses to treatment in chronic hepatitis B HBeAg positive infection. Genotype A appeared to have the highest response rates. When comparing response rates across different trials, the proportion of patients with each genotype should be examined.

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BIOINFORMATIC APPROACHES FOR THE IDENTIFICATION OF POTENTIAL TENOFOVIR ANTIVIRAL RESISTANCE

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Background: Tenofovir (TFV) and lamivudine (LMV) are currently being used for the treatment of patients with chronic hepatitis B as well as patients co-infected with HBV and HIV. Resistance to TFV

has been associated with a HBV mutation at rtA194T plus LMV resistance mutations. While results obtained from in vitro phenotypic assays used to measure LMV resistance typically correspond to clinical observations, this is not the case for TFV or adefovir (ADV). Mutations associated with treatment failure for TFV or ADV have been found in the HBV polymerase gene isolated from patients experiencing virological breakthrough, but in vitro phenotypic assays only show small differences in antiviral susceptibility. **Aims:** To characterise unique mutations and novel clusters of mutations in the reverse transcriptase (rt) gene of HBV by their association with specific antiviral treatment and in particular, mutations associated with TFV treatment. **Methods:** The HBV polymerase gene was amplified by PCR and sequenced from patient samples pre- and post- antiviral therapy. A software program that detects specific HBV mutations (SeqHepB) linked to a database, was used to identify all mutations relative to HBV genotype (A-H) reference sequences. Mutations detected from samples collected pre- and post- LMV treatment were evaluated by pair-wise analysis. The samples were also examined relative to HBV mutational data, increases in viral load and time post treatment. Novel associations between these parameters were examined using queries developed within the database. **Results/Discussion.** Evaluation of the system with the samples from LMV resistant patients successfully identified the primary LMV resistant mutations at rtM204I/V as well as a number of the compensatory mutations at rtL80I, rtV173L, rtL180M, rtT184G/S/A/I and rtS202I. Using the same approach for samples from patients treated with TFV, unique mutations at codons rtT184G/A and rtY/W257H/G were identified. Mutations at codon 184 have also been previously identified with both LMV and/or entecavir resistance. **Conclusion:** Bioinformatics approaches are useful in identifying and linking novel mutations that may be potentially associated with resistance to existing and new antiviral treatments. While the effect of the mutations on the drug sensitive phenotype would still need to be evaluated in vitro, such testing is time consuming, relatively restricted and there is a substantial delay between identification of a particular mutation and confirmation of resistance in vitro. Thus, bioinformatic analysis can provide a rapid alternative method for evaluating the clinical associations of mutations in the HBV genome.

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LOW RATES OF GENOTYPIC RESISTANCE TO ADEFOVIR IN LAMIVUDINE RESISTANT PATIENTS TREATED WITH ADEFOVIR-LAMIVUDINE COMBINATION THERAPY FOR 3 YEARS

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Background and Aims. The risk of developing genotypic and clinical resistance to adefovir and virological breakthrough in lamivudine resistant patients treated long-term with a combination of adefovir and lamivudine, is unknown. **Patients and Methods.** 145 lamivudine resistant patients with chronic hepatitis B (84% HBeAg neg, 73% cirrhotics) were treated for more than 1 year (median: 30 months, range 12-65) with 10 mg/daily of adefovir added to ongoing lamivudine. HBV DNA was assessed every two months by Versant 3.0 and drug resistance was assessed by INNO-LiPA HDR V2 assay in viremic patients every year. **Results.** Serum HBV-DNA became undetectable in 99/145 (68%), 70/92 (76%) and 43/52 (83%) patients after 1, 2 and 3 years, respectively. None of the patients who achieved undetectable HBV DNA or maintained persistently detectable viremia, showed >1 log rebound of HBV DNA compared to on-treatment nadir. Moreover, genotypic resistance for rtN236T was not identified in any patient. By converse, the rtA181T/V mutation was found in 4 (3%), 1 (1%), and 1 (2%) serum samples at 1, 2 and 3 years, respectively (overall, 6 patients, 4%). All the 5 patients with the rtA181T mutation, but

not the one with the rtA181V, had a mixed viral population with the wild-type sequence rtA181A. The rtA181T/V mutants were already detected at baseline in 3 patients, as a mixed viral population with rtA181A. Despite the presence of HBV viral strains with rtA181T/V mutations, serum HBV DNA became undetectable in 4 of 5 patients during 6-12 months of follow-up. Overall, after 3 years of combined therapy, de-novo emergence of rtA181T mutation and persistence of > 4 log copies/ml of serum HBV DNA were observed in 1 patient (0.7%), only. By converse, lamivudine-associated resistance mutations were confirmed in 93% of the samples, the pattern being similar to baseline. Conclusions. Adefovir and lamivudine combination therapy is an effective strategy in lamivudine-resistant patients, since it minimizes the risk of genotypic resistance to ADV and prevents both virological rebound and clinical resistance up to 3 years.

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ASSESSMENT OF QUANTITATIVE SELECTIVE REAL-TIME PCR FOR QUANTIFICATION OF LAMIVUDINE AND ADEFOVIR RESISTANT HEPATITIS B VIRUS AND COMPARISON WITH SEQUENCE ANALYSIS AND INNO-LIPA HBV DR V2 LINE PROBE ASSAY Julien Lupo¹, Sylvie Larrat¹, Marie-Noëlle Hilleret^{2,3}, Jean-Pierre Zarski^{2,3}, Jean-Marie Seigneurin¹, Patrice Morand¹; ¹Laboratory of Virology, CHU de Grenoble, Grenoble, France; ²Department of HepatoGastroEnterology, CHU de Grenoble, Grenoble, France; ³INSERM unit 548, CEA Grenoble, Grenoble, France

Background and aim: Antiviral-resistant hepatitis B virus (HBV) mutations have become an increasing problem in the treatment of chronic hepatitis B. To improve patient management, sensitive and robust assays that can early detect antiviral resistant HBV are required. The amplification refractory mutation system (ARMS) technology was used to detect lamivudine and adefovir minor resistant strains carrying rtM204V/I and rtN236T mutations respectively. This technique was then compared with 2 other genotyping methods with regard to their ability to detect minor variants. Methods: ARMS is a selective amplification by real time PCR of a variant strain. Discrimination between wild type and mutant is possible by using a primer-template mismatch at the 3' end of the primer. A common forward primer was used with different reverse primers which were specific for each resistance mutation. The total viral load was determined using a common reverse primer. Four plasmids were constructed: one built with wild type virus and the others carrying the mutations of interest. Sensitivity, specificity and reproducibility of ARMS technique were evaluated on mixtures of plasmids in variable proportions. This technique was then compared with InnoLiPA DR v2 line probe assay (Innogenetics, Gent, Belgium) and with a home-brew sequencing assay. Results: Specificity and reproducibility of this ARMS assay were consistent with its use in routine. Our technique allows the detection of 0.1% of mutant strain for a total viral load of more than 104 plasmid copies. For smaller populations, the ability to discriminate variants and wild type plasmids was lower and was related to the sensitivity of the technique (10 copies/reaction). With direct in-house sequencing, 10% of viral mutant strains could be detected for a total viral load of more than 500 plasmid copies. With line probe assay, minor variants were detected till a proportion of 5% including with very low viral load. These results were confirmed in clinical samples. Conclusions: Our results show that INNO LiPA HBV DR v2 assay is adapted to detect antiviral resistance mutations at an early stage in patients with low viraemia, but only mutations already described are detected. Direct sequencing is less sensitive but remains the best approach to identify new patterns of mutations. Among the 3 techniques, ARMS is the most sensitive to detect a very low percentage of mutated strains when total viral load is over 104 copies. Moreover this technique can be used to detect other mutations. However design of primers and PCR optimization are required for each new mutation.

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PREDICTIVE VALUE OF HBV DNA LEVELS AT FREQUENT TIME POINTS DURING EARLY AND MAINTENANCE PHASE OF 5-YEAR LAMIVUDINE AND MUTATIONAL PROFILES OF REVERSE TRANSCRIPTASE (RT) AND SURFACE (S) GENES Man-Fung Yuen, Danny Ka-Ho Wong, James Fung, John Chi-Hang Yuen, Ching-Lung Lai; Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong, Hong Kong

Background The optimal time points and levels of HBV DNA during initial therapy with lamivudine (LAM) to predict the 5-year outcome are unknown. Aims To identify 1) the optimal time and HBV DNA levels during early treatment phase associated with good response (GR) (HBV DNA level < 2000 copies/mL); 2) the mutational profiles after 5-year LAM treatment. Patients and Methods HBV DNA levels at baseline, week (wk) 0,2,4,8,16,24,32 and yearly till 5 years were measured by VERSANT® HBV DNA 3.0 assay (Bayer HealthCare LLC, NY) in 74 Chinese chronic hepatitis B patients on continuous LAM treatment. Determination of parts of the nucleotide sequence of RT and S genes by TRUGENE® HBV Genotyping Kit (Bayer HealthCare LLC) were completed in 50 patients. Results 20 (27%) patients had GR at year 5. Table shows the percentages of patients achieving GR at 5 years according to the cut-off HBV DNA levels of 4 logs at different early time points. Compared to patients with HBV DNA levels still > 4 logs at wk 12, patients with HBV DNA level < 4 logs had significantly higher rate of HBeAg seroconversion (20.3 vs. 90%, p<0.001), ALT normalization (51.5% vs. 100%, p=0.008) and lower chance of YMDD mutations (62.8% vs. 0%, p=0.002) at year 5. The corresponding figures as assessed at wk 24 are 18.6% vs. 73.3%, p=0.001; 48.3% vs. 92.3%, p=0.007; 62.5% vs. 20%, p=0.03 respectively. The sensitivity and specificity of using this cut-off level for determination of GR at 5 years are 50% and 100% for wk 12, and 60% and 96.2% for wk 24. Mutations of YMDD motif with M204I/V were associated with L180M and V173L (both p<0.05). Because of the overlapping of RT and S genes, corresponding S mutations with E164D (with V173L), I195M (with M204I), W196L/M/S/V/X (with M204V)(all p<0.05) were observed. These S mutants decrease the anti-HBs binding affinity (Torresi et al., 2002). 2 patients had the vaccine escape S mutation G145R/A at baseline, while 6 developed this mutation during LAM treatment. Conclusions HBV DNA level < 4 logs copies/mL at wk 12 during LAM treatment had excellent predictive value for long-term outcome. GR only occurred in 15.6% of patients who failed to achieve this target. Only 4.5% of patients with HBV DNA levels > 4 logs at wk 48 achieved GR at year 5. Potential impact of RT mutations associated with S mutations affecting anti-HBs binding affinity should be addressed.

Weeks

HBV DNA (copies/mL)	2	4	8	12	16	24	32	48
< 4 logs	100% (6/6)	100% (6/6)	87.5% (7/8)	100% (10/10)	82.4% (14/17)	80% (12/15)	75% (12/16)	60% (18/30)
> 4 logs	20.1% (14/68)	20.1% (14/68)	19.7% (13/66)	15.6% (10/64)	10.5% (6/57)	13.6% (8/59)	13.8% (8/58)	4.5% (2/44)

% signifies positive (second row) and negative (third row) predictive values of using HBV DNA of 4 logs as a cut-off level for GR at 5 years

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